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Irradiated sewage sludge for application to cropland

Results of a co-ordinated research project organized by the Joint FAO-IAEA Division of Nuclear Techniques in Food and Agriculture

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FOREWORD

Modern urban societies produce large volumes of sewage, which are transported through a network of underground sewers to wastewater treatment plants, where one or more stages of physical, biological and chemical treatment are imposed. Considerable tonnages of aerobically, and sometimes anaerobically, digested sludge are produced, and treated or untreated effluent is discharged to lagoons, waterways or the ocean. The disposal of sewage sludge is a major issue for municipal authorities. There are increasing legislative restrictions in many countries on disposal methods (e.g. incineration, landfill, composting) including surface application to agricultural land.

Sludge can either be viewed as a dangerous waste requiring expensive disposal procedures, or it can be seen as a resource for possible use in agriculture as a soil conditioning agent and a source of plant nutrients. Untreated sewage sludge presents a public-health hazard as it contains human pathogens, including bacteria, viruses and other harmful organisms. Although it has been demonstrated that an appropriate dose of gamma-irradiation can eliminate human parasites and bacterial pathogens from sewage sludge, there is still public concern about the presence of viruses, as well as heavy metals and toxic organic compounds from industrial sources that could enter the food chain if sludge is applied to croplands. More information is also needed on the value of sludge as a source of plant nutrients, expressed in terms of fertilizer equivalence. In this regard, isotopic labelling techniques have a unique role to play in estimating the contribution of sewage sludge to crop nutrition.

As a result of recommendations formulated at a Consultants Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and the IAEA Division of Physical and Chemical Sciences, 5–9 December 1994 (IAEA-TECDOC-971, Sewage Sludge and Wastewater for Use in Agriculture) the Joint Division implemented a Co-ordinated Research Project on the Use of Irradiated Sewage Sludge to Increase Soil Fertility and Crop Yields and to Preserve the Environment between 1995 and 2000. The overall objective was to assist national institutes from Member States to develop management practices for the efficient use of sewage sludge as an organic fertilizer for increasing and sustaining crop production and soil fertility in an environmentally sound manner.

Twelve contract holders from Argentina, Bangladesh, China, Egypt, India, Indonesia, Malaysia, Pakistan, Portugal, Romania, Sweden, and Thailand, and five agreement holders from Austria, Germany, Japan, the United States of America, and the United Kingdom participated in the project. The first Research Co-ordination Meeting (RCM) was held 10–14 July 1995 in Vienna (S. Kumarasinghe, Project Officer), the second RCM 14–18 September 1996 in Cairo, Egypt (C. Hera, Project Officer), the third RCM 22–26 June 1998 in Oeiras, Portugal (P.M. Chalk, Project Officer), and the fourth RCM was held 20–24 September 1999 in Serdang, Malaysia (P.M. Chalk, Project Officer).

This technical publication contains the manuscripts prepared by the project participants and edited by A.R.J. Eaglesham, Ithaca, New York. The IAEA Officer responsible for this publication is P.M. Chalk, Soil and Water Management & Crop Nutrition Section, Vienna.

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SUMMARY

The specific objectives of the project were:

- To characterize irradiated and non-irradiated sewage sludges in terms of physical and chemical properties, contents of pathogenic organisms, and toxic organic compounds;
- To quantify the benefits of sewage sludge in terms of increasing crop yields, and improving soil physical properties and chemical fertility, including macro- and micro-nutrient availability;
- To assess the extent of contamination of soils and crops by sludge-derived heavy metals.

Sludge characteristics

Physical and chemical properties

The physical consistency of the sludges varied considerably, from slurries to dried cake. The extent of processing carried out at the wastewater-treatment plant (primary, secondary, tertiary), and the degree of drying of the final sludge largely determines its physical characteristics. Dewatering of sludge is important in terms of the economics of disposal, because transport costs are a major component. Costs of dewatering can vary greatly depending on the method used. Arid and semi-arid areas have a distinct advantage in this regard (e.g. Egypt); sludge can be spread on sand beds and dried naturally.

The elemental composition of sludges was also very variable in terms of carbon and major nutrients: 6–44% C, 0.45–4.3% N, C/N 2–20/1, and 0.4–3.6% P. The relative contributions of domestic and industrial sources of wastewater to the total sewage flow and the extent of processing at the wastewater treatment plant are major determinants of the chemical composition. Stabilizing additives (e.g. lime) also have pronounced effects on chemical characteristics, including pH.

In addition to pathogens, sewage sludges contain a complex mixture of organic contaminants. Some of these compounds and their derivatives are highly toxic, even at low concentrations. Polychlorinated biphenyl (PCB) congeners (Romania), pesticide residues (Argentina) and thirteen polycyclic aromatic hydrocarbons (PAHs) (Egypt) were identified in moist sludge. Higher concentrations of PAHs were present in moist compared with dry sludge. Preliminary studies in Germany, Austria, Argentina, and Egypt indicated that toxic organic chemicals were degraded by irradiation, even at rather low doses. Doses of 2 to 10 kGy of gamma-irradiation reduced total PAH concentrations of moist sludge by 53 to 75%, respectively.

Biological properties

Radiation dosages (gamma only, no electron beam) varied from 2 to 25 kGy, with the majority between 3 and 6 kGy. The survival of pathogenic organisms in sewage sludge was investigated, i.e. total bacterial counts, total and faecal coliforms, *Ascaris* ova, and viruses.

The effectiveness of irradiation in pathogen elimination was influenced by sludge characteristics, including pathogen density and moisture content. Irradiation was more effective under moist conditions. Pathogen elimination followed first-order kinetics with respect to dose, i.e. the original pathogen density determines the number remaining after irradiation at a given dose:

$$C = C_o.e^{-k.d}$$

where

C is the final bacterial count,
C_o is the initial bacterial count,
K is a rate constant,
and d is the dose (kGy).

Irradiation was effective in reducing total bacterial counts, but the response was organism-specific. A reduction of three orders of magnitude in total bacterial counts may be adequate to ensure safety from pathogens. A consistent observation was that a dose of 2 to 4 kGy reduced total coliforms by one order of magnitude; 4 to 6 kGy, three orders of magnitude reduction; and >6 kGy, more than three orders of magnitude reduction. Addition of irradiated or non-irradiated sludges did not significantly alter total coliforms in soils having high background counts; in soils with low background counts, total and faecal coliforms were one to two orders of magnitude lower when irradiated sludge was applied compared with an equivalent application of non-irradiated sludge.

Ascaris ova in non-irradiated sludge were generally below limits of detection; in spiked samples, 5 kGy (Bangladesh) or 2 kGy with an 8-week incubation (Argentina) eliminated ova. Viruses were consistently detected in non-irradiated sludge; at 3 kGy dose, only 10% of samples were virus-positive; rota virus was not detected when the absorbed dose was ≥6 kGy.

Crop response to sludge application

Yield

Positive yield responses to incremental rates of sludge addition were obtained in a majority of experiments. In infertile soils, yield responses were obtained with low to moderate rates of sludge addition, whereas significant increases in yield were not obtained on sites having high fertility or large fertility gradients, even at high rates of application. The regression of relative yield increase with irradiated (y) vs. non-irradiated sludge (x) was linear and highly significant ($R^2 = 0.97$; $y = 1.1105x - 2.671$), showing that, on average, a greater yield response was obtained with irradiated sludge. The relative yield increment was linearly related to the amount of N applied in the sludge. The yield response with the highest rate of sludge was generally less than that obtained with the recommended rate of fertilizer N.

Macronutrient (N and P) uptake

Irradiation increased N (but not P) availability in sludge and increased crop response to sludge addition. The N response to sludge addition varied according to site fertility, being equivalent to 20 to 80% of the recommended N fertilizer rate on sites of low to medium fertility, depending on the sludge application rate. The proportional contribution of sludge to crop nutrition, as determined by isotope (^{15}N and ^{32}P) dilution and non-isotope (difference) methods, was therefore very variable. The residual N value of a single sludge application was small, but the residual effects were cumulative with annual additions. Sludge addition decreased the proportional dependence of a pasture legume (sub clover) on biological N_2 fixation as determined by ^{15}N-dilution, the effect being dependent on sludge application rate. Sludge was a good source of P, particularly on soils of high P-fixation capacity.

Micronutrient uptake

Many of the experiments showed increased concentrations of Zn and Cu in the soil and in the crops. However, although yield responses to sludge application were not specifically attributed to amelioration of micronutrient deficiencies, the possibility remains that this is one of several beneficial effects of sludge on plant nutrition and, hence, crop yields.

Heavy metals in sludges, soils and crops

The heavy-metal concentrations in the sludges were generally low to moderately low. Occasionally, increased concentrations of Cd, Pb, and Ni were measured in sludge-amended soils. Increased concentrations of Cd and Ni in crops in sludge-amended soils were occasionally measured, but, consistent with previous experience, concentrations of Pb and Cr did not increase. Unacceptable concentrations of metals in crops were found only for Cd, which is known to be the main limiting factor in the food chain due to its mobility and ease of plant uptake. The lowest EU limits were used as the reference, since the EU limits refer to soils with pH values > 6.0, and soil pH was < 6 in some experiments, increasing the bioavailability of heavy metals. Nevertheless, there was some doubt about the reliability of the Cd data due to uncertainty of the detection limits of the analytical methods used, and the suitability of the International Certified Standard samples supplied to participants in terms of the concentration ranges covered.

Metal loadings were calculated in some experiments to permit simple sustainability modelling, i.e. based on knowledge of annual sludge-application rates, soil pH (losses are unlikely in neutral or alkaline soils), soil bulk density, and depth of sludge incorporation, the time taken to reach maximum allowable soil concentrations of heavy metals was estimated. For example, for extremely high annual application rates of 40 or 80 tons sludge/ha, maximum permissible soil concentrations of heavy metals in Egyptian sandy soils were predicted within a few years, emphasizing the fact that it is unwise to apply sludge at such high rates in order to satisfy the N and P requirements of crops on infertile soils.

Conclusions

A moderate dose of gamma-irradiation is an effective means of eliminating human pathogens from sewage sludge, thus removing one of the principal concerns regarding recycling of this waste. However, it was concluded that there is a paucity of information, and therefore much less certainty, in regard to the presence of viruses and toxic organic compounds in irradiated sludge, due to methodological limitations including extraction procedures and detection methods for the wide range of viruses and organic compounds that may be present.

The beneficial effects of sludge application on the yields of crops grown on infertile soils, even at low to moderate rates of application, were clearly demonstrated in the many experiments performed in this CRP with a variety of crops grown under variable soil and climatic conditions. A number of factors are integrated in yield responses, including the amelioration of macro- (especially N and P) and micro-nutrient deficiencies. However, sludge should be regarded as an organic supplement to the use of manufactured fertilizers. Sludge cannot replace fertilizer, because the high annual rates of application needed to supply sufficient macronutrients to crops will create a heavy-metals hazard. In situations where heavy metal concentrations in sludge destined for land application are a concern, consideration could be given to the production of ornamental or industrial crops (e.g. lubricant oils) as an alternative to food crops.

Another factor integrated in the yield responses of crops to sludge application, especially in coarse-textured soil, is improved soil physical properties, including, inter alia, reduced bulk density and enhanced water-holding capacity. However, there are reports that sludge application can create negative physical effects such as surface sealing, which reduces infiltration of water into the soil. This aspect requires further study and definition.

During the early 1990s when this CRP was conceived, the future prospects for irradiation of sludge and municipal wastewater on a commercial scale looked promising. Several facilities were in operation and others were in various stages of planning or construction [1,2]. Today, the situation has changed dramatically with several facilities in developed countries closed down (e.g. the ^{60}Co and ^{137}Cs sources at Geiselbullach, Germany). In another case, construction was suspended at an advanced stage of plant development (the ^{60}Co facility at Tucuman, Argentina). Today, only one industrial plant remains operational (^{137}Cs source, Baroda, India).

Despite the present pessimistic outlook for the wider application of irradiation technologies to disinfect sewage sludges and wastewaters, application of sludge to agricultural land remains an attractive alternative to other disposal methods, including landfill, incineration, and dumping into water bodies, which themselves pose various threats to the environment. Indeed, some of these disposal methods are prohibited by legislation in some developed countries. Thus, the imperative to disinfect sludge prior to land disposal remains, and irradiation is the best method currently available to ensure public-health safety. The most pressing need in developing countries is to develop national or regional guidelines for loading limits of sludge based on sludge characteristics, type of crops to be grown, soil properties, and climatic factors.

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EVALUATION OF IRRADIATED SEWAGE SLUDGE AS AN INDUSTRIAL CROP FERTILIZER USING NUCLEAR TECHNIQUES

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Abstract

Irradiated sewage sludges were evaluated as fertilizers for ryegrass and sugarcane, which is the main industrial local crop. Sludges were analysed chemically, and experiments showed that irradiated sludge is a good source of nitrogen (N) and phosphorus (P). Nitrogen-15 and ^{32}P were used to assess uptake of these nutrients from sludge. Four annual sugarcane crops produced good yields with moderate applications of irradiated sewage sludge, better than with the equivalent rate of non-irradiated sludge.

1. INTRODUCTION

This project, titled "Irradiation Treatment of Sewage Sludges for Agriculture Reuse in Argentina," was focused, inter alia, on the disinfection of anaerobically digested sludges after primary treatment of urban wastewaters. As part of this project, a sewage-sludge irradiation plant is under construction next to the wastewater treatment plant at Tucuman City, 1,300 km from Buenos Aires, in northwestern Argentina [1]. Several factors contributed to the selection of Tucuman: it sits on the Sali, a mountain river that usually dries up in winter and sometimes is severely polluted; the surrounding area is agricultural with little heavy industry, therefore, heavy-metal concentrations in the sludges were expected to be low; costs of transportation of sludges to arable land are minimized.

Agriculture in the Tucuman area includes sugarcane (*Saccharum officinarum)*, citrus (mainly lemons), and horticulture crops. Sugarcane is by far the most important. It occupies 80% of the cultivated area. Tucuman province produces about 12 Mt of ground cane, more than 50% of the total production in Argentina. Variability in production results from changing meteorological and commercial-market conditions. The chief product is sugar, for home consumption and export; alcohol production is important also. Therefore, a chief objective of the project was to evaluate irradiated sludges as fertilizer for sugarcane.

Sugarcane, which originated in Asia, is grown between 35°N and S, in rain-fed and irrigated conditions. Optimum growth is achieved with mean daily temperatures between 22 and 33°C, and a minimum of approximately 20°C. For ripening, however, temperatures in the range of 20 to 10°C are desirable, since they reduce vegetative growth rate and increase sucrose accumulation. A long growing season is essential for high yields, between 9 months, with harvest before winter frost, and 24 months in Hawaii. It is about 10 months at Tucuman. The planted crop is normally followed by two to five ratoon crops, and in certain cases up to eight crops are grown. Flowering is influenced by day-length, water and N supplies, but, because it has a deleterious effect on sucrose content, it is prevented or non-flowering varieties are used. Sugar yield depends on cane tonnage, sugar content of the cane, and cane quality. Cane tonnage at harvest is usually between 50 and 150 t/ha.

Sugarcane does not require a particular soil type, but it should be well aerated, with a total available water content of 15% or more, and pH about 6.5. Soil salinity may adversely affect crop yields. Nitrogen (N) and potassium (K) requirements are high, whereas phosphorus (P) requirement is relatively low. At maturity, the N content of the soil should be as low as possible for good sugar recovery, particularly with a moist, warm climate.

At Tucuman, sugarcane is fertilized with urea as a supply of N; organic amendments like sugar-industry residues (filter cake) are rare or experimental [2]. Recommended fertilizer rates have been determined with field experiments with various cultivars [3]; it is 90 kg N/ha for the variety selected for this research, locally identified as TUC 77-42.

There are few reports in the literature related to sewage-sludge and biosolids fertilization of sugarcane [4,5]. In Hawaii, negative effects were obtained [5]. Some data exist also on utilization of filter cake and vinasse [6,7]. No data were found on irradiated sewage sludge use with sugarcane.

Experiments were performed to evaluate irradiated sludges as sources of N and P and effects on and sugarcane yields, between 1995 and 1999. Other aspects, also important in agricultural reuse of biosolids—micronutrients, effects on soil characteristics, heavy-metal and organic-compound contamination—were studied, but are not described in detain here.

2. SEWAGE-SLUDGE CHARACTERIZATION

Sewage sludges were obtained from the wastewater treatment plant of Tucuman City, which serves a population of about 400,000 inhabitants (60% of total). The process is a primary treatment of the wastewaters, after which the clarified liquid effluents are chlorinated and dumped into the Sali River. The concentrated sludges are stabilized in anaerobic digesters. The digested sludges are discharged with variable frequency (less in winter because the digesters have no heating system) into sandy drying beds until completely dried. Approximately 80,000 m^3/day of wastewaters (0.02% suspended solids) enter the plant, and the sludges leave the digesters at an average rate of 140 m^3/day (8% s.s.). A complete chemical screening was performed monthly during the first year of research to evaluate seasonal variations [8]; basic parameters are shown in Table I, with minimum and maximum values for the year.

When the irradiation plant begins operations, the digested sludges will enter directly. The radiation process will be accomplished in batches of 6 m^3 for about 30 min each (at maximum radioactivity charge) to achieve the required dose for pathogen elimination, 3 kGy absorbed gamma radiation.

Currently, the sludges used for experiments are being irradiated at the semi-industrial irradiation plant at Ezeiza Atomic Centre. Sludges are received dry, and are ground, fractionated, and packaged before irradiation. Particle size should be sufficiently small for adequate incorporation in the soil.

3. NITROGEN BIOAVAILABILITY

3.1. Mineral N in sludges and in sludge-amended soils

Percent-N (semi-microKjeldahl) values were determined for liquid sludges from the anaerobic digesters of the treatment plant, and for the sludge after evaporation in an open receptacle and also after drying at the plant (Table II). Losses of N due to evaporation or washing by rainfall and percolation through the sand during drying were observed, most of it as NH_3. Mineral forms, NH_4^+ and NO_3^-, were analyzed by Bremner's microdistillation method.

Analyses of NH_4^+ and NO_3^- have demonstrated that irradiation of liquid or dried sludges causes releases of NH_4^+. Literature data on releases of NH_4^+ indicate a negative overall effect of irradiation on the N availability in sludge [9]. However, it has been observed that amendments with relatively low rates of irradiated sludge increased NO_3^- in the soil due to nitrification, in comparison with similar rates of non-irradiated sludge [10].

Table I. Characteristics of wastewater

	Mean	Range
Total solids (%)	8.1	7.2–10.5
Volatile solids (%)	41	38–55
pH	7.45	7.02–7.88
BOD (g/L)	12.5	5.0–17.5
COD (g/L)	56.7	53.1–60.4
Alkalinity (g $CaCO_3$/L)	4.7	3.6–6.6
TKN (g/L)	2.25	2.06–2.60
Ortho-P (g/L)	0.26	0.20–0.30

Table II. Nitrogen values for sludge

	Liquid sludge	Dried sludge
TKN	33.2 mg/g	19.0 mg/g
NH_3, non-irradiated	12.9 mg/g	0.336 mg/g
NH_3, irradiated	13.8 mg/g	0.833 mg/g
Increase	+0.900 mg/g	+0.497 mg/g
%TKN release	2.7%	2.6%

3.2. Greenhouse ^{15}N experiment with ryegrass.

The experiment was carried out in pots, each containing 1 kg silty loam soil from Tucuman, uniformly labelled with 20 mg N/kg with ^{15}N at 10.3% a.e. as $(NH_4)_2SO_4$. Basal applications of P (50 mg P/kg soil) and K (100 mg K/kg soil) were made. After 10 days of equilibration, five treatments were installed with five replicates of each: 1, control; 2, urea at 100 mg N/kg; 3, sewage sludge at the equivalent of 100 mg N/kg; 4, irradiated sewage sludge at the equivalent of 100 mg N/kg; and 5, irradiated sewage sludge at the equivalent of 200 mg N/kg soil. Ryegrass (*Lolium multiflorum*) was sown uniformly.

Two cuts were made and the plants collected, oven-dried, and finely milled. Total dry matter, %N and ^{15}N-abundance values were determined for all replicates. Percent N derived from labelled fertilizer, (%Ndff), total N in the plants (N yield), the uptake of N from each fertilizer source (N-fertilizer yield) and % utilization of applied N were calculated (Table III).

Percent utilization of applied N was higher with irradiated than with non-irradiated sludge (Table III). Increased application of irradiated sludge did not result in greater utilization of N; instead. it had an inhibitory effect, as indicated by lower total biomass [11].

3.3. Discussion

Despite losses of N, dried sludge was an important source of N. Increased NO_3^- concentration in the soil indicated that N was mineralized as a result of irradiation. However, extra application of irradiated sludge did not further increase the NO_3^- level.

Table III. Nitrogen data, ryegrass

Treatment	%Ndff	%N	N yield (mg/pot)	Fert. N utilized (mg/pot)	% Utilization
1 Control	22.5	4.83±0.09[a]	43.4	–	–
2 Urea	10.0	4.84±0.07	45.6	5.7	5.7
3 Sewage sludge	18.7	4.81±0.07	34.1	1.3	1.3
4 Irrad. sludge (100 N)	18.1	4.99±0.09	47.4	2.1	2.1
5 Irrad. sludge (200 N)	17.7	4.87±0.08	50.8	2.4	1.2

[a] Standard error.

The ^{15}N data confirmed that N availability from irradiated sludge was higher than from non-irradiated sludge (60% higher in this experiment).

4. PHOSPHORUS BIOAVAILABILITY

4.1. Phosphorus analyses of the sludges

The sludges were analysed for total, hydrolyzable, and extractable P using different extraction methods followed with the widely used phosphomolybdate colorimetric reaction [12]. The nitric-perchloric acid extraction method was used to assay total P; the sulphuric acid and persulphate method was used for hydrolyzable orthophosphate P; and for extractable P, the soil-extraction method (Bray and Kurtz No. 1 technique) was used. Different samples of sewage sludges in liquid and dried forms, before and after irradiation, were tested. No irradiation effects were observed (Table IV).

4.2. Phosphus-32-dilution technique in the greenhouse experiment.

In a greenhouse experiment, the ^{32}P-dilution technique [13] was used with irradiated and non-irradiated sludge to assess and compare P availability. Five treatments, each of five replicates, were applied to ryegrass (*Lolium multiflorum*) in pots contain 1-kg aliquots of “labelled” soil: 1, without additional P fertilization (control); 2, with a chemical source of P at a rate of 40 mg P/kg soil; 3, non-irradiated sewage sludge equivalent to 40 mg P/kg soil (approximately 180 g of sludge); 4, the same as 3 with irradiated sludge; and 5, irradiated sewage sludge equivalent to 80 mg P/kg soil (360 g). Phosphorus-32-labelled phosphoric acid solution was produced at the Ezeiza Nuclear Reactor. Specific activity was 4.16 µCi/mg P totalling 25 µCi/pot at the start of the experiment. After equilibrating for a week, fertilizers were applied and ryegrass was sown. Amendments of N (100 mg/kg soil) and K (50 mg/kg soil) were made.

Plant samples were collected in two cuts at 20 and 33 days, oven-dried, ashed at 550°C, and completely dissolved in 10 mL 2 *M* HCl. The radiactivity of the solutions was measured by Cerenkov radiation in a Packard Tricarb 2700 TR scintillation counter. Percent P was assayed with the standard colorimetric technique.

Values for percent P derived from labelled fertilizer, %P in dried green matter, total P/pot, the yield from each source in mg P/pot, and the %P utilized from the total mass of P added with the unlabelled sources are shown in Table V. Irradiation had no significant effects.

Table IV. Phosphorus values for sludge

Sludge type	Total P	Ortho-P	Bray-Kurtz 1-P
	(ppm)		
Dried	9,200±200	3,910±280	232±6
Irradiated dried	9,300±300	3,470±390	222±19
Liquid	8,400±400	3,700±170	214±6
Irradiated liquid	8,500±350	3,920±360	208±9

Table V. Phosphorus data, ryegrass

Treatment	%Pdff	%P	P yield (mg/pot)	Fertilizer P yield (mg/pot)	% utilization
1 Control	8.4	0.34±0.03	9.86± 0.9	–	–
2 Chem. fertilizer	3.1	0.48±0.03	15.1± 0.8	0.80	2.00%
3 Non-irrad. sludge	2.4	0.48±0.01	13.2± 2.2	0.79	1.98%
4 Irrad. sludge (40P)	2.5	0.45±0.06	13.7± 2.8	0.81	2.02%
5 Irrad. sludge (80P)	1.4	0.53±0.02	20.4± 1.5	1.43	1.78%

4.3. Phosphorus analyses of soil and leaves of experimental sugarcane

Soil and plant samples were collected from the sugarcane experiment after four annual applications of sludge. Samples from plots were pooled for the control, the minimum sludge application rate (3 t/ha) and the maximum (10 t/ha). The soil samples were separated into three depth layers: 0 to 10 cm, 10 to 20 cm, and 20 to 30 cm. Total P and extractable P (Bray and Kurtz No. 1) were analysed in soil by the methods already described, as well as total P in leaves (Table VI).

Sugarcane is known to respond to P when the soil level is below 6 ppm (Bray-K 1); in the Tucuman area, it probably responds also between 6 to 9 ppm, but with no further response with higher concentrations, [3,14]. The main effect is on leaf development, even if the soil is not deficient and there is no increase in production. The results in Table VI show an accumulation in soil, mainly with maximum rate of sludge application, without evidence of uptake by the plant.

4.4. Discussion

It was confirmed that irradiation had no effect on the chemical forms of P, or on P availability in sludge. Sewage sludge is a good source of P. There was a >30% increase over the control in P yield in the pot experiment, and it also increased the P content in sugarcane. But larger amounts of sludges (> 10 t/ha) caused accumulation in soil, an undesirable effect in the long term.

Table VI. Phosphorus data, sugarcane

Treatment	Depth	Total P in soil	Extract. P in soil	Total P in leaves
	(cm)		(ppm)	
Control	0–10 cm	587±0.5	7.3± 0.5	1,340±44
	10–20 cm	568±2.4	6.9± 0.2	
	20–30 cm	585±2.8	7.0± 0.3	
S.S. min. rate	0–10 cm	697±1.6	9.9± 0.5	1,530±170
	10–20 cm	670±3.0	8.3± 0.2	
	20–30 cm	653±2.6	8.1± 0.3	
S.S. max. rate	0–10 cm	847±2.9	19.4± 0.5	1,360±90
	10–20 cm	792±1.8	10.9± 0.6	
	20–30 cm	765±6.6	9.2± 0.6	

5. SUGARCANE YIELD

5.1. Procedures

Sugarcane yields are usually evaluated in 10-m-long plots of seven furrows, with an inter-row spacing of 1.6 m. For this experiment, plot size was restricted to 3.2 m^2 because of limited amounts of sludge transported to Buenos Aires for irradiation. The experiment had eight treatments, with four replicates in a randomized design: 1, control without fertilization; 2, urea at 90 kg N/ha, the recommended rate; 3, sewage sludge at the equivalent of 100% of the recommended N rate; 4, sewage sludge at the equivalent of 200% N; 5, sewage sludge at 300% N; 6, irradiated sewage sludge at 100% N; 7, irradiated sewage sludge at 200% N; 8, irradiated sewage sludge at 300% N. The recommended rate of 90 kg N/ha or 9 g N/m^2 is contained in approximately 1 kg of sludge; 3.5 t/ha of sludge is equivalent to the 100% rate.

The soil, a Typic Hapludolls (USDA), was analysed previously and the parameters were: pH, 6.52; organic matter, 2.64%; TKN, 0.14%; P, 7.0 ppm (Bray Kurtz No. 1); K, 2.25 mEq/100g; TOC, 1.53%; and C/N, 10.9.

Variety TUC 77-42 had been planted the year before, and showed good uniformity in its second ratoon crop at the time of the first applications, in the spring (September) of 1995. Four experiments, examining annual applications and harvests, were carried out in 1996, 1997, 1998, and 1999. Rainfall record was normal for 1996 and 1997, and was in excess in 1998 and 1999 although insufficient for crop effects. Plots were hand-weeded regularly; no herbicides were used.

At harvest, canes were cut manually and total weight (biomass) recorded for each plot, as well as stalk numbers. Fresh biomass determinations were made separately for stalks, green leaves, and senesced leaves, then subsamples were weighed, dried, and re-weighed. Aliquots of dried material were stored for further analyses. Only total production parameters are presented here.

5.2. Results

Yield values for 1997, 1998, and 1999 are shown in Table VII; statistical comparisons between treatments were made for the 3-year means. The irradiated sludge had more-positive effects on cane yields that did the non-irradiated.

6. OTHER ASPECTS

Routine industrial analyses (Brix, Pol, and Purity index) were made to verify that sugarcane biomass increase did not adversely affect the quality of the product.

An important consideration is the effect of sludge application on soil characteristics. The main parameters were analysed year by year after harvest. After four years of applications, changes in soil parameters were minor. No significant effects on water-holding capacity were found, although sludge has a high water retention. Only slight differences in pH were obtained. Not even organic matter increased consistently, contrary to expectations. Some exchangeable cations were increased, which might have effects in the long term.

Table VII. Sugarcane yields in tons of cane/ha

Treatment	1997	1998	1999	Mean[a]
1 Control	51.2	53.7	47.1	50.7±3.3
2 Urea	67.5	75	85.6	76.0±9.1
3 Non-irrad. sludge (3.5 t/ha)	47.5	57.8	58.1	54.5±6.0
4 Non-irrad. sludge (7 t/ha)	64.7	63.1	69.7	65.8±3.4
5 Non-irrad. sludge (10 t/ha)	87.5	95.3	85.9	89.6±5.0
6 Irrad. sludge (3.5 t/ha)	70.6	69.5	71.6	70.6±1.1
7 Irrad. Sludge (7 t/ha)	81.5	92.2	88.7	87.5±5.4
8 Irrad. Sludge (10 t/ha)	–	108	101	104±4.9

[a] By one-way ANOVA and Student-Newman-Keuls multiple comparisons, significance values of treatment differences were as follow: 3 vs. 6 $P<0.01$, 4 vs. 7 $P<0.001$, 5 vs. 8 $P<0.05$.

Heavy-metal content of the sludges was evaluated year by year, i.e. Cd, Cr, Cu, Ni, Pb, and Zn. There were no significant increases during the first three years. After the fourth year of application, sugarcane plants were analysed in plants. Only two heavy metals were observed with slightly higher concentrations in cane juice, but not in leaves. Even so, the amounts were far below regulation limits.

Analyses for toxic organic compounds are pending.

7. CONCLUSIONS

The experiments confirmed that irradiated sewage sludge is a good source of N and P for sugarcane, comparable to chemical fertilizers. Whereas at the rate equivalent to the recommended N dose, non-irradiated sludge had no effects, irradiated sludge increased yield. A rate of 7 t/ha of non-irradiated sludge had an effect similar to that of 3.5 t/ha of irradiated sludge.

It is important to minimize sludge application to soil, due to possible undesirable accumulation of toxicants and changes in soil characteristics. Therefore, irradiated sludge is recommended, because whereas irradiation did not increase inorganic toxicants or their bioavailability, it increased the bioavailability of N.

The results suggest that large amounts of sludge had no additive effects on crop yield and there was evidence of negative effects. This finding might explain variability in responses to sludge obtained in different countries.

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USE OF SEWAGE SLUDGE IN AUSTRIA – I. A COMPREHENSIVE CASE STUDY*

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Abstract

The possibilities and basic requirements for long-term agricultural utilization of sewage sludge were studied at the wastewater treatment plant (WWTP) at Mödling. The complete process was considered, from the origin of the wastewater to sludge production and utilization. The following chief components of this large-scale 3-year program, are discussed: systems analysis and modelling of the catchment area, sewage-sludge production and quality, experiments on sewage-sludge composting, and demonstration field experiments. The systems analysis provided the background information and demonstrated that the studied catchment area is predominantly residential with evenly distributed small and medium-sized businesses. Mathematical modelling resulted in a ranking list of suspects as polluters of wastewater and, hence, of sewage sludge. This list was qualitatively verified, although satisfactory correlation of measured and predicted loads is pending. Sewage-sludge analysis showed that its quality meets current, but not scheduled, legal requirements. Experiments were performed to improve the structure and the stabilization level of sewage-sludge composting. Additionally, demonstration field trials were run for a period of 3 years, with comparisons of sewage sludge, compost, and mineral fertilizers. The results showed no distinct differences in terms heavy metals in the soil or taken up by plants. It was shown that sewage sludge and/or compost utilization can be integrated into agricultural practices. However, additional improvements in sewage-sludge quality will be necessary if adverse effects are to be avoided in the long term. The only practicable, feasible means of improving sewage sludge is to improve wastewater quality.

1. INTRODUCTION

Sewage sludge, a product of biological treatment of wastewater, can be used as a fertilizer and to improve the structure and organic content of soil. It contains nutrients, including nitrogen (N) and phosphorus (P), and humic substances. The nutrients should be recycled for crop production as a component of sustainable development. However, sewage sludge also contains pollutants produced by industry and other societal activities. Sludge utilization in agriculture, therefore, requires reducing the concentration of, e.g., heavy metals and organic toxicants. From a practical point of view, improvement of sludge quality mainly requires improvement in wastewater quality. This will entail not only regulation and application of technical solutions, but also motivation of treatment-plant operators, farmers, and of the population as a whole.

The Mödling case study not only emphasized measurements in the WWTP catchment area, but also dealt with the sewage-treatment system, the sludge-treatment system (e.g. dewatering, composting) and practical aspects of agricultural use. The complete process, from the origin of the wastewater to sludge production and utilization, was considered. This program has the potential to be beneficial only with general acceptance of sewage-sludge utilization by all relevant population groups, i.e. farmers, consumers, and plant operators. Therefore, the main scientific and technical results were disseminated in a special information program that will be discussed elsewhere. The results of the main aspects of the program, carried out over the past 3 years, are described here: systems analysis and modelling of the catchment area, sewage-sludge production and quality, experiments on sewage-sludge composting, and field demonstration trials.

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*Modified from [1].

2. METHODS

2.1. Survey of, and measurements in, the catchment area

A survey of the catchment area was performed by collecting demographic data: numbers of inhabitants and households, characteristics and distribution of businesses and industry, the sewer system, and water consumption. The bases were determined for published data on effluent concentrations specific to various industries and household and storm waters. To ensure general applicability, broad concentration bands rather than fixed values must be used.

The area was subdivided to correspond to sewerage subsystems. This subdivision was needed so that a specific economic activity (e.g. rural, industrial, mixed) or type of sewer system (combined or separate) would predominate in each subsystem. At the outflow points of the subareas, wastewater samples were collected; forty-four samples in total were taken over the 3-year period. All were taken over 24 h as flow-proportional combined samples, and wastewater flow rates were measured. The samples were analysed for common wastewater parameters [e.g. chemical oxygen demand (COD), 5-day biochemical oxygen demand (BOD_5), N, P, etc.), heavy metals (Cd, Cr, Co, Cu, Hg, Ni, Pb, Zn), and organic pollutants [adsorbed organic halogens (AOX), phenol, polycyclic aromatic hydrocarbons (PAHs)]. All survey data obtained were inserted into a database and correlated with a geographical information system.

2.2. Mathematical analyses

The main objective of the mathematical analyses was to establish a basis for introducing a wide palette of measures for wastewater-quality improvement. The goal is to find the most significant polluters of wastewater in the catchment area (or, rather, a representative fraction thereof) by using relatively inexpensive and generally available background information. Reducing the negative effects of these polluters provides the most immediate opportunity for improving wastewater quality and, hence, sewage-sludge quality.

Wastewater emissions were considered from a static point of view. Although the emission concentrations of all units connected to the sewage system are time-dependent quantities, one can replace them with appropriate time-averages [2] since only cumulative effects over relatively long time periods were relevant for the study. The possible methods for screening the essential polluters derived from the types of the information available:

(i) branch characteristic emission concentrations,
(ii) total wastewater amounts for the sub-areas per fixed period,
(iii) substance loads of the measured substances,
(iv) and pure-water amounts consumed by the separate emitters.

We assumed that the amounts in item (iv) are equal to the wastewater amounts released by the separate emitters. Differences in the total amount of wastewater released in the catchment area are defined in terms of wastewater of diffuse emitters, i.e. storm water, etc. The subsequent analysis was carried out by mapping emission-concentration bands onto a normal (Gaussian) distribution of the substance's emission-concentrations for the separate (branch) emitters. Other distribution types give rise to similar results, but are bound to require substantial increases in the corresponding computational efforts. The general background of the mathematical analysis is rooted in the abstract balance equation:

$$\begin{array}{c} \textit{a priori} \\ \text{(derived from literature data)} \\ \textit{substance-load} \end{array} = \begin{array}{c} \textit{a posteriori} \\ \text{(measured)} \\ \textit{substance-load} \end{array} \qquad (1)$$

This equation is to be interpreted probabilistically, and is a linear equation with random coefficients [3]. Its precise mathematical meaning is that the overlap of the densities of the random variables' distributions occurring on the left-hand and right-hand sides, respectively, has to be non-negligible. There are two possibilities for practical application of Eq (1) depending on the initial information used: the first is based on the data in items (i), (ii), and (iii) (functional or in a simplified version linear inequalities, independent of any particular information about the separate emitters at the expense of restricted applicability to small catchment areas); the second uses the data in items (i) and (iv) and is a simpler statistical calculation, providing the possibility of detecting a systematic error in either the literature data or the measurement procedure. A final step in both methods is the production of a ranking list of the most serious branch polluters. The technical details differ, but both procedures are based on the (experimentally verified) a-priori distribution.

2.3. Sewage sludge

The Mödling WWTP (100,000 population equivalents) produces 1,800 tons dry sewage sludge per year. The excess sludge is pre-dewatered by screw sieves and dewatered by chamber-filter presses to a dry matter (DM) content of approximately 28%. During dewatering, polyelectrolytes and ferric chloride are added. The sewage sludge was analysed for pH ($CaCl_2$), conductivity, DM, total carbon (C_t), $CaCO_3$, organic C, ignition loss, N_t, ammonium, nitrate, P_t, K_t, extractable P and K, Ca, Mg, Mn, Na, heavy metals (Cd, Cr, Co, Cu, Hg, Ni, Pb, Zn), AOX, PCBs, PAHs, organochlorine pesticides, and polychlorinated dibenzodioxin/polychlorinated dibenzofuran (PCDD/PCDF). Additionally, sanitary aspects (i.e. presence of *Enterobacteriaceae*, Salmonella, and *Ascaris*) were examined. These analyses were performed at intervals required by regulations [4] and, for practical reasons, altogether twenty-four samples were analysed over 3 years (at least four samples per year).

Table I. Maximum permissible values in sewage sludge for utilization in agriculture

Parameter	Unit	NÖ Klärschlammverordnung (1994)	
		class III	class II
Zn	mg/kg DM	2,000	1,500
Cu	mg/kg DM	500	300
Cr	mg/kg DM	500	50
Pb	mg/kg DM	400	100
Ni	mg/kg DM	100	25
Co	mg/kg DM	100	10
Cd	mg/kg DM	8	2
Hg	mg/kg DM	8	2
Radioactivity	Bq/kg DM	7,400	7,400
AOX	mg/kg DM	500	500
PCBs (each congener)	mg/kg DM	0.2	0.2
PCDD/PCDF	ng TE[a]/kg DM	100	100
Sanitary conditions[b]:			
Enterobacteriaceae	1/g FW[c]	1,000	1,000
Salmonella	1/g FW	0	0
Ascaris	1/g FW	0	0

[a]Toxicity equivalent. [b]For grassland only. [c]Fresh weight.

Table II. Maximum permissible values (mg/kg DM) in soil for sewage sludge utilization [4]

Parameter	Value
Zn	200
Cu	60
Cr	100
Pb	100
Ni	50
Co	–
Cd	1.5 (1.0[a])
Hg	1.0

[a] pH <5.

The results obtained were compared to limits prescribed by regulation, which distinguishes three quality classes. Class I is defined as common regional soil concentrations, and the maximum permissible values of classes II and III are given in Table I. The limits of class III will be valid up until 2004. Subsequently, sewage-sludge quality must meet class-II limits.

2.4. Composting of sewage sludge

Composting is one method of stabilizing dewatered sewage sludge, e.g. to decrease readily degradable organic compounds that cause odour problems during field treatment. Applied composting experiments were performed with sewage sludge and straw. To achieve cost efficiency, we chose an open-pile method. The experiments were performed over a period of 16 to 17 weeks. One experiment had to be stopped after only 5 days because of ammonia emissions [5]. Sewage sludge was mixed with barley straw (ratio 6:1) giving to a C/N ratio of 20 to 35 [6] and lined in open piles on a covered area adjacent to the WWTP. The material was mixed every 2 to 4 weeks with a turning machine. Initially, all parameters described above were analysed for the sewage sludge, and heavy-metal content of the straw was analysed once (mg/kg DM): Zn 4.1, Cu 2.7, Cr 7.5, Pb 0.4, Ni 5.8, Co 0.8, Cd 0.032, and Hg 0.018, i.e. very low values compared to the sewage sludge (see Table IV). Temperature was measured continuously throughout the experiment at the top, medium, and bottom areas of three different profiles. The compost produced was analysed for the same parameters as for the sewage sludge. The results obtained were examined in terms of limiting values prescribed by regulation.

2.5. Field-demonstration trials

The objective of the field experiments was to investigate the utilization of sewage sludge in agricultural practice, to measure changes, if any, in soil composition, and to observe possible effects on crop yield and quality. Negative effects of heavy metals and other pollutants were assessed on one hand, and beneficial effects on soil physical properties and plant nutrition [7] on the other. The trials were performed on a field adjacent to the WWTP at Mödling. The soil is classified as a Chernozem with an average ground-water depth of >2 m. Regulations permit an application of 2.5 t DM/ha/yr of sewage sludge of quality classes III or II [4]. The soil-concentration limits (Table II) were not exceeded. The test field was divided into four plots, each of which was treated differently: Plot A, mineral NPK fertilizer; B, sewage sludge at 2.5 tons DM/ha; C, composted sewage sludge at 2.5 tons DM/ha; D, control without treatments. Treatments were applied in 1994 (spring), 1995 (spring), and

1995 (autumn). The crop rotation consisted of maize (1994), spring barley (1995), and winter rape (1995/1996). Pesticide applications were kept to the necessary minimum.

The initial soil sampling was in November 1993, separately for the topsoil (0–0.25 m) and the subsoil (0.25–0.50 m) for each plot. Parameters analysed were: pH ($CaCl_2$), extractable P and K [calcium-acetate-lactate (CAL) method], exchangeable Ca, K, Mg, Na, soil texture, lime content, heavy metals and Ca, K, Mg, P in an aqua-regia extract, heavy metals extracted with 1 *M* ammonium acetate, AOX, PCBs, PCDD/F, PAHs, pesticides; and, in topsoils, C_t, N_t and organic-matter content. In 1994, 1995, and 1996, topsoil samples were taken and analysed for the same parameters as were the initial samples. An additional subsoil sampling was performed in 1996.

Harvested crops were analysed for As, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb, and Zn as well as AOX, PCBs, PCDD/F, PAHs and organochlorine pesticides.

3. RESULTS AND DISCUSSION

3.1. Survey and measurements in the catchment area

A permanent population of 55,000 in 23,000 households is served by the sewerage system of the Mödling WWTP. One of Europe’s largest shopping centres (100,000 visitors per day, 3,200 employees) is also served by the treatment plant. In total, some 4,190 businesses were documented in the catchment area, 186 of which are production companies. The others are engaged in the service and crafts sectors. Trade is involved in 22% of the enterprises. Sewage-sludge-relevant activities, metal processing, medicine (e.g. hospitals, dentists), ceramics, glass, painting, etc., were found in approximately 200 instances, corresponding to 3% of all business enterprises.

The measured concentrations in subareas are related to the wastewater quality of the areas that are defined by respective administrative units. The common wastewater parameters (e.g. COD, BOD_5, TOC, TKN) showed low mean concentrations compared to usual municipal wastewater. Mean heavy-metal concentrations were in the expected ranges for Hg, Ni, Pb and Zn (0.3, 34, 4.9, 230 μg/L, respectively), whereas the measured concentrations for Cr, Cd, Co, and especially Cu (13.2, 0.5, 0.6, 46 μg/L respectively), were lower than those described in the literature. Only a few data points were available for sewage concentrations of organic pollutants. It seems that the mean results obtained for phenol, AOX, and PAH (84, 72, 0.17 μg/L, respectively) were in the usual ranges [8]. A general feature of all analysis data was their substantial deviation with respect to the mean.

3.2. Mathematical analyses

The subareas in which measurements were performed were relatively large. Pure-water consumption by individual emitters was readily available, therefore, we used the second of the mathematical methods described above. For most of the measured substances, we encountered an insufficient overlap of the literature data with those stemming from the measurements. We observed an overall tendency for predicted loads to far exceed those measured. Under these circumstances we followed two parallel strategies. On the one hand, the literature data were fundamentally reassessed and compared with legal requirements. On the other, overall reduction re-scaling of the literature concentrations was implemented. The latter procedure permitted preliminary ranking lists. Such a list is given in Table III. It contains the impact coefficients (in percent) of the most significant emitters for the listed parameters. We define an impact coefficient to be the load of a separate emitter (with respect to a fixed substance) divided by the total load calculated for the catchment area. We have verified qualitatively the predictions of the preliminary ranking lists by means of long-term sewer deposits.

3.3. Sewage sludge

Table IV represents the results of analyses of the sewage sludge from the Mödling WWTP over a 3-year period. The sewage sludge fully met the class-III quality requirements decreed in Lower Austria. Only one measurement exceeded the upper limit, i.e. that of Cd. In most cases, the class II requirements for Cr, Pb, Ni, and Cd were not met. Numbers of *Enterobacteriaceae*, as an indicator of sanitary aspects, in all cases exceeded the limits for grassland utilization (Table I).

The parameters describing nutrient contents were in the usual ranges. Dry matter content varied from 21% to 43% due to maintenance work on the filter press or dosing with lime during pressing.

3.4. Composting of sewage sludge

Analysis data for the compost compared with sewage sludge showed an expected increase in dry matter (>50% DM) and loss of N and total C (of ca. 30%). A mineralization effect on N was observed in terms of increased nitrate concentration (0.3% DM) compared to ammonium (0.1% DM). There were no significant changes in heavy-metal concentrations in the transformation from sewage sludge to compost. An expected dilution caused by mixing with straw seemed to be compensated by the loss of mass. Significant changes, in both directions, were obtained in concentrations of organic pollutants. Therefore, no general statement on a trend is possible. Improvement in hygienic quality was demonstrated.

3.5. Demonstration trials

Pollutant concentrations in soil were influenced by small differences in soil characteristics as well as by variations in sampling and analyses. Some of these were obviously caused by spatial variability in soil parameters. To adjust or compensate for these local differences, the obtained heavy-metal concentrations (aqua-regia extraction) were related to annual average values for all plots and were compared with the results of other years. Table V shows the respective results from 1993 (initial analysis) to 1996.

With the exception of a few values, the plots showed no distinct differences in heavy-metal concentrations. Trends could not be deduced and, in fact, were not to be expected. For some elements (e.g. Cd, Cr, Cu, Mo, Zn) the control plots showed lower concentrations throughout. Therefore, higher-heavy-metal concentrations in other plots were not related to treatments. This is in agreement with other results [9] that showed that even long-term applications of sewage sludge did not necessarily result in significant increases of heavy-metal concentrations in German topsoils.

Extractable (ammonium acetate) heavy metals and micro-elements showed significant variations over the observed years (data not shown) that could not be related to treatments. The analysed values did not reach regulation limits [10]. Organic pollutants showed less variation in soil and no trends in response to treatments. The average crop yields over the 3-year period, relative to the control set at 100%, were as follows: mineral fertilizer, 121±25%; sewage sludge, 130±34%; compost, 130± 35%; Although treatment effects were not significant, there was a trend to higher yields.

The quality of the harvested rape crops was not influenced by sewage sludge or compost. Heavy-metal contents (1996) were generally low, confirming that the investigated site lies in a non-polluted area. No clear effects of sewage-sludge or compost treatments were observed, which was also true for the crops grown in 1994 and 1995. Heavy-metal concentrations were in the usual ranges [11], and additional root uptake could be excluded. The calculation of transfer factors [12] showed that plant availability of heavy metals is very low in this Chernozem. Dioxins and organochlorine pesticides were below detection limits. Plant-tissue contents of PAHs were typical for non-polluted areas [13,14].

Table III. Preliminary ranking list of the most significant industrial polluters in the catchment area

Company	Code	Type	Subarea	Cd	Cr	Cu	Hg	Ni	Pb	Zn
							(%)			
König & Bauer	1	i[a]	M. Enzersdf.	4.07	1.99	0.39	0.00	1.46	0.61	0.32
Aichelin Industrieofenbau	2	i	Mödling	3.99	3.49	0.27	0.00	0.90	1.02	0.17
Worthington GmbH.	3	i	Brunn	2.04	3.53	0.05	0.00	0.49	0.03	0.04
Knorr Bremse GmbH.	4	ii[b]	Mödling	1.79	1.51	0.10	0.00	0.35	0.35	0.06
Rosendahl Maschinen	5	i	Brunn	1.26	2.44	0.03	0.00	0.30	0.02	0.02
Czech Reinlufttechnik	6	i	Brunn	0.90	1.79	0.02	0.00	0.21	0.01	0.02
Zelisko	7	i	Mödling	0.71	0.59	0.04	0.00	0.13	0.15	0.02
Heuduschek Ernst	8	i	Brunn	0.68	1.35	0.02	0.00	0.16	0.01	0.01
EVN AG	9	NK[d]	M. Enzersdf.	0.66	0.26	0.06	0.00	0.22	0.08	0.04
SEW-Eurodrive GmbH.	10	i	Brunn	0.59	1.19	0.01	0.00	0.14	0.01	0.01
Wieser Maschinenbau	11	i	Mödling	0.53	0.44	0.03	0.00	0.10	0.11	0.02
Tamussino GmbH	12	i	Mödling	0.40	0.33	0.02	0.00	0.08	0.17	0.01
Höbart Rolf-Dieter	13	i	Mödling	0.37	0.31	0.02	0.00	0.07	0.08	0.01
Maschinglasindustrie AG	14	iii[c]	Brunn	0.36	0.29	0.33	0.81	1.62	1.56	1.41

[a] Metal processing. [b] Metal processing and ceramics. [c] Glass industry. [d] Not known.

Table IV. Quality of sewage-sludge from Mödling WWTP, 1993 to 1996

Parameter	Unit	n	Mean	Std. dev.	Minimum	Maximum
Dry matter	%	24	28.2	4.5	20.8	43.2
Total C	% DM	22	28.5	3.9	21.2	37.4
Ignition loss	% DM	22	55.4	9.1	34.5	67.1
Total N	% DM	22	4.01	1.11	0.84	5.60
Ca (as CaO)	% DM	22	6.7	5.5	3.4	23.5
Mg (as MgO)	% DM	22	0.917	0.239	0.510	1.35
Total P (as P_2O_5)	% DM	22	5.30	0.73	3.67	6.58
Total K (as K_2O)	% DM	22	0.537	0.121	0.316	0.720
C/N ratio	–	15	7	1	6	9
Zn	mg/kg DM	24	1056	180	804	1450
Cu	mg/kg DM	24	253	37	161	324
Cr	mg/kg DM	24	72	17	37.3	106
Pb	mg/kg DM	24	121	26	73	185
Ni	mg/kg DM	24	35.3	8.1	23.3	53.0
Co	mg/kg DM	24	4.3	0.9	2.3	6.2
Cd	mg/kg DM	24	4.6	3.3	2.2	16.9
Hg	mg/kg DM	24	2.1	0.7	1.0	4.2
AOX	mg/kg DM	14	339	88	173	480
PCB	µg/kg DM	12	155	88	72	401
PAH (sum acc. to WHO)	µg/kg DM	12	4,278	1,848	3,19	6,178
PCDD/PCDF (sum)	ng TE/kg DM	12	5.3	3.1	2.7	11.9
Enterobacteriaceae	1/g FM	14	>260,100	>295,235	<200	>1,000,000
Salmonella	1/g FM	15	0	0	0	positive
Ascaris	1/g FM	15	0	0	0	0

Table V. Heavy-metal concentrations (%) in topsoil relative to annual average values of all plots

Element	Mineral fertilizer 1993/94/95/96	Sewage sludge 1993/94/95/96	Compost 1993/94/95/96	Control plot 1993/94/95/96
As	98/100/96/98	100/95/88/100	98/102/91/99	104/103/125/103
Cd	100/97/112/100	111/109/112/109	104/109/92/103	85/85/84/89
Co	103/98/99/95	94/93/96/99	103/107/112/104	94/102/93/102
Cr	100/94/107/96	104/104/105/102	111/117/102/113	86/85/86/89
Cu	97/98/104/102	104/101/105/107	104/104/96/94	95/97/96/97
Hg	102/105/108/110	107/97/113/106	83/88/63/78	107/109/117/106
Mo	118/108/108/102	118/94/97/112	91/108/99/102	72/89/97/85
Ni	106/101/101/98	95/92/98//102	101/106/108/105	98/102/94/95
Pb	101/101/100/92	90/107/109/108	113/98/88/89	96/95/103/111
V	101/99/98/96	101/94/100/99	103/106/105/102	96/101/97/102
Zn	98/99/106/95	104/103/106/108	102/105/92/100	97/93/96/97

4. CONCLUSIONS AND SUMMARY

4.1. Survey and measurements in the catchment area

The survey demonstrated that the studied catchment area is predominantly residential without particularly distinct elements. With few exceptions, the industrial enterprises are small or medium in scale, and evenly distributed through the densely populated part of the area. Overall measurements were, in general, lower or equal to those described in the literature.

4.2. Mathematical analyses

Mathematical modelling procedures are potentially powerful, but their applicability is determined by the quality of the input data. Currently, the data for branch-specific emission concentrations are generally inadequate. A critical reassessment on regional, national, and continental levels would dramatically improve the background for theoretical predictions.

4.3. Sewage sludge and compost

The sewage sludge and compost fully met present legal requirements. However, their quality was such that thresholds will be exceeded in the next decade. This, together with the principles of sustainable development, point to the necessity for improvement in the quality of the sewage sludge in the long term. For sewage sludge application to grassland, sanitary bacteriological parameters must be improved, e.g. by composting. It should be also noted that the use of sewage sludge as fertilizer is often unacceptable because of odour problems, also soluble by composting.

4.4. Demonstration field experiments

The treatments showed no distinct effects on soil concentrations in terms of total content or extractable fractions, and no recommended limits were exceeded. The average yields of treated plots were not significantly different from the controls. However, a tendency to higher yields on treated plots was deduced. The quality of the harvested crops was influenced neither by sewage sludge nor compost. These results show that the short-term effects of agricultural utilization of sewage sludge and compost are similar to those of commonly used mineral fertilizers.

4.5. Summary

It was shown that sewage sludge and/or compost can be integrated into agricultural practices. However, an additional improvement in sewage-sludge quality will be necessary if negative effects are to be avoided in the long term. The only feasible way of improving sewage sludge is to improve wastewater quality. This requires identifying polluters and establishing adequate measures for abating their negative effects. Mathematical modelling and strategically designed catchment-area surveys can be helpful tools towards these goals.

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USE OF SEWAGE SLUDGE IN AUSTRIA – II. N-AVAILABILITY AND HEAVY-METAL UPTAKE[*]

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Abstract

Results are presented from a 3-year experiment with large pots in the field, evaluating the effects of sewage sludge (sterilized by γ-irradiation or not sterilized) on the growth of oil-seed rape and on uptake of heavy metals and of N (using the ^{15}N-dilution technique). Mobile fractions of Cd, Cu, and Zn increased significantly in the substrate due to sewage-sludge application. However, heavy-metal transfer into the plants did not show a clear trend. Rape growth was clearly enhanced in the first and third years due to sewage-sludge applications. Average utilization of sewage-sludge N by rape decreased from 7.4% in the first year to 1.8% in the second to 1.1% in the third year, resulting in an overall utilization of 10% of sewage-sludge N. Irradiation of the sludge had no significant effects on the investigated parameters.

1. INTRODUCTION

The application of sewage sludge to agricultural land has been a subject of intensive discussion for decades. Sewage sludge, a product of biological treatment of wastewater, can be used as a fertilizer or at least to improve the structure of the soil through additional supply of organic matter. Although there is general agreement that sewage sludge has positive effects on agricultural soils, there is considerable concern over accumulation of heavy metals and other pollutants, and over sanitary aspects [2–4]. In this respect, soil acts both as a sink for pollutants and as source for their transfer into the food chain. Wilcke and Döhler [5] calculated that the upper limits for Zn and Ni in agricultural soils in Germany will be reached within 600 to 800 years of continuous application of sewage sludge containing these metals at the values set for sewage sludge. To achieve sustainable soil management with respect to the use of organic wastes and residues, it is important to gain additional information on, i) the plant availability of nutrients contained in sewage sludge, ii) the mobility and accumulation of heavy metals introduced into soil, and iii) the sanitary aspects of sewage-sludge application.

The objectives of this work were: i) to quantify the N availability from sewage sludge under realistic climatic conditions, ii) to quantify the accumulation of heavy metals in soil fertilized with sewage sludge and their potential uptake into plants, and iii) to evaluate the impact of γ-irradiation (sterilization) on N availability.

2. MATERIALS AND METHODS

2.1. Soil

The experiment was started in 1995 with large pots, each containing 30 kg of a soil/quartz sand substrate (1:1), which were sunk into the ground at the level of the surrounding experimental field at the Austrian Research Centre, Seibersdorf. The pots had a diameter of 42 cm and a height of 40 cm. Each treatment was replicated four times. The soil used in the experiment is a Chernozem from Seibersdorf. Chemical and physical parameters were determined according to Blum et al. [6] (Table I). The experimental soil had a pH of 7.8, a high lime content (29%) and a high organic matter content (4.9%). Texture can be classified as loamy silt. The high organic matter content made dilution of the soil with quartz sand necessary in order to amplify treatment effects.

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[*] Modified from [1].

Table I. Chemical and physical characteristics of the soil and applied sewage sludge (dry weight basis)

Parameter	Soil	Sewage sludge		Parameter	Soil	Sewage sludge	
		1995	1996			1995	1996
pH ($CaCl_2$)	7.78	6.06	6.24	CEC[c] (mEq/100g)	18.3	–	–
$CaCO_3$ (%)	29	2.6	4.8	N_t (%)	0.10	5.30	5.43
Organic matter (%)	4.87	55.9	57.8	NH_4–N (mg/kg)	–	11,900	2,300
K_2O (%)	–	0.68	0.65	NO_3–N (mg/kg)	–	2,500	<100
K_2O (CAL[a], mg/kg)	230	3790	4290	Cd (mg/kg)	0.19	3.2	2.2
P_2O_5 (%)	–	6.26	5.6	Cr (mg/kg	14.3	70.6	37.3
P_2O_5 (CAL, mg/kg)	460	2,660	460	Cu (mg/kg)	14.0	243	222
Sand (%)	18	–	–	Hg (mg/kg)	0.042	1.7	1.3
Silt (%)	58	–	–	Ni (mg/kg)	12.9	34.4	27.8
Clay (%)	24	–	–	Pb (mg/kg)	16.0	106	79
EC[b] (mS/cm)	–	3.93	4.34	Zn (mg/kg)	39.3	1,160	880

[a]Calcium acetate lactate extract. [b]Electrical conductivity. [c]Cation exchange capacity.

2.2. Sewage sludge

The sludge originated from a sewage-treatment plant at Mödling, Lower Austria. Its main characteristics are shown in Table I. In the experimental years 1995 and 1996 the sludges originated from different samplings, therefore, characteristics differed slightly (Table I). The most important parameters, total N and organic-matter content, were similar. Heavy-metal concentrations did not exceed limits specified in Lower Austrian regulations.

Pathogenic bacteria were present. For the experiment, part of the sludge was sterilized by γ-irradiation in the ^{60}Co-irradiation plant of the Austrian Research Centre, Seibersdorf, with an applied radiation dose of 28.5 kGy. The application of an equivalent of 7.5 t/ha of sewage sludge introduced considerable amounts of N (398 kg N/ha in 1995, 407 kg N/ha in 1996) and of organic matter (4,193 kg/ha in 1995, 4,335 kg/ha in 1996) into the substrate. The amounts applied were three times what may be legally used in agriculture in Austria, in order to amplify the effects.

2.3. Pot experiment

The treatments in the 1995 experiment are shown in Table II. On August 8, air-dried soil was mixed with the dried and ground sewage sludge, quartz sand and mineral fertilizer N in a concrete mixer. Each pot received 30 kg of mixture. The experimental oil-seed rape crop was sown on August 10 (twenty seeds/pot) and green plants were harvested on September 19, 1995. In order to test N-availability in the sewage sludge in the second year, no additions were made to Treatments 1 to 3 (Table II). To test a possible cumulative effect, irradiated sewage sludge was applied in Treatment 5 (7.5 t/ha) and non-irradiated sewage sludge to Treatment 6 (7.5 t/ha) in 1996; $(^{15}NH_4)_2SO_4$ was added in 1996 to Treatments 4, 5 and 6. Rape was sown on April 22, 1996 (twenty seeds/pot). The green rape was harvested on August 13, 1996. In the last year of the experiment (1997) no further sewage sludge was applied. All pots were treated with $(^{15}NH_4)_2SO_4$. To optimize the isotope-dilution method, the N-application was split into two portions of 25 kg N/ha (May 25 and July 12, 1997) and the solution was applied with a 50 mL syringe to avoid immediate gaseous losses. The rape was sown on May 16, 1997, (twenty seeds/pot) and harvested on July 28, 73 days after sowing.

Table II. Pot experiment treatments, 1995

Treatment (n = 4)	Sewage sludge (irradiated)	Sewage sludge (non-irradiated)	$(^{15}NH_4)_2SO_4$[a]	$(NH_4)_2SO_4$
1	–	–	50 kg/ha	–
2	7.5 t/ha	–	50 kg/ha	–
3	–	7.5 t/ha	50 kg/ha	–
4	–	–	–	50 kg/ha
5	7.5 t/ha	–	–	50 kg/ha
6	–	7.5 t/ha	–	50 kg/ha

[a] 4.820 at% ^{15}N excess

2.4. Analysis

Plant biomass was determined after cutting and drying. The samples were then milled and dried again for 24 h at 105°C. Approximately 2 g of the material were accurately weighed and wet-digested (20 mL HNO_3 and 4 mL $HClO_4$). Heavy-metal contents were analysed by means of atomic absorption spectroscopy (AAS, Perkin Elmer) and inductively couple plasmaspectrometry (ICP, Perkin Elmer Plasma II). Sub-samples were transferred to the IAEA laboratories for N and ^{15}N analysis with a mass spectrometer (Micromass Sira 9) in combination with an elemental analyser (Carlo Erba 1500).

In 1995, 1996, and 1997 (autumn) composite samples of the substrate from the four replicates of each treatment were collected. The substrate samples were air-dried and passed through a sieve (mesh size 2×2 mm). Subsamples were transferred to the IAEA laboratories for ^{15}N analyses. Heavy-metal contents were analysed after wet digestion under reflux using aqua regia by means of AAS and ICP. Mobile fractions of Cd, Cr, Cu, and Zn in soil were additionally determined in the 1996, using 1 *M* ammonium acetate [6,7] as a mild neutral extractant.

2.5. Calculations

Nitrogen uptake by oil-seed rape derived from mineral fertilizer (Ndff) was calculated by isotope dilution:

$$\%Ndff = \left[\frac{\%^{15}N\,a.e.\ in\,rape}{\%^{15}N\,a.e.\ in\ fertilizer} \right] \times 100 \tag{1}$$

Percent N derived from unlabelled sewage sludge (Ndfss) was obtained by:

$$\%Ndfss = \left[1 - \frac{\%^{15}N\,a.e.\ in\,rape\,treated\ with\ {}^{15}N\,and\,sewage\,sludge}{\%^{15}N\,a.e.\ in\,rape\,treated\ with\ {}^{15}N} \right] \times 100 \tag{2}$$

Percent N derived from soil (Ndfs) was calculated as a difference:

$$\%Ndfs = 100 - (\%Ndff + \%Nfss) \tag{3}$$

Statistical differences between means were analysed using the WinStat 2.0. programme.

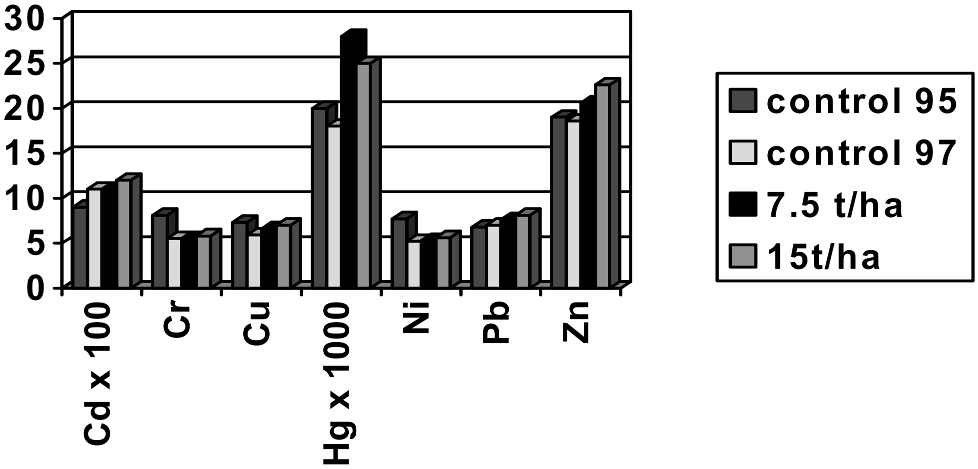

FIG. 1. Heavy-metal contents of substrate samples (mg/kg).

3. RESULTS AND DISCUSSION

3.1. Soil

Figure 1 shows the results of the aqua-regia extraction of composite samples of substrate collected in 1995. The heavy-metal concentrations reached approximately 50% of the initial values determined in the soil samples (Table I), reflecting the 1:1 dilution with quartz sand. No distinct differences were observed between mineral-N-treated pots (Treatments 1, 4) and those that received 7.5 t/ha of sewage sludge (Treatments 2, 3, 5, 6). No effects of the heavy-metal input as sewage sludge could be deduced. Therefore, it was decided to measure only the most relevant heavy metals in the 1996, but to keep all replicates and determine mobile fractions.

Although we analysed eight replicates per treatment, no statistically significant differences between the treatments were observed for Cd, Cr, Cu, or Zn. This result was not expected, because large amounts of sewage sludge had been applied (up to 15 t dry matter/ha) and the inputs of heavy metals were quite high relative to the original soil content. For example 0.64 mg Cd, 12.8 mg Cr, 55 mg Cu and 242 mg Zn were added to the pots receiving an equivalent of 15 t/ha sewage sludge within two years. These values represent 22%, 6%, 26%, and 41% of the total substrate content, respectively. The substrate was analysed again in 1997 and the previous findings confirmed; a tendency to even smaller heavy-metal values was observed. The fact that increases in heavy metal concentrations could not be detected may have had two causes.

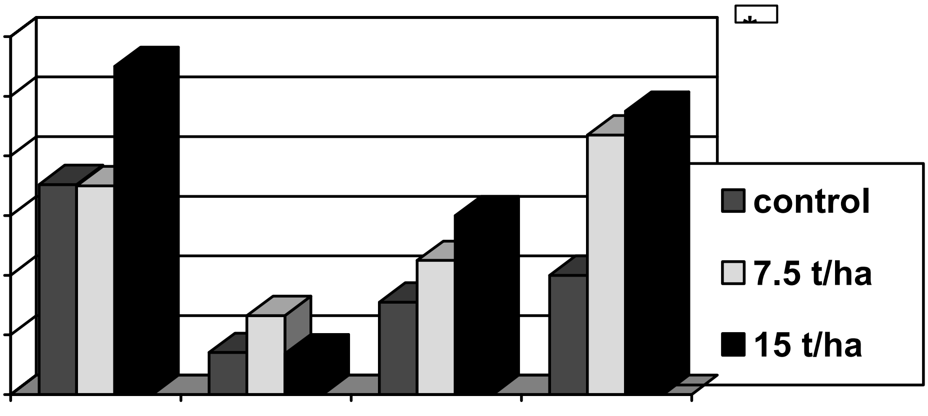

FIG. 2. Mobile heavy-metal fractions in the substrate (ammonium acetate, µg/kg), 1996.

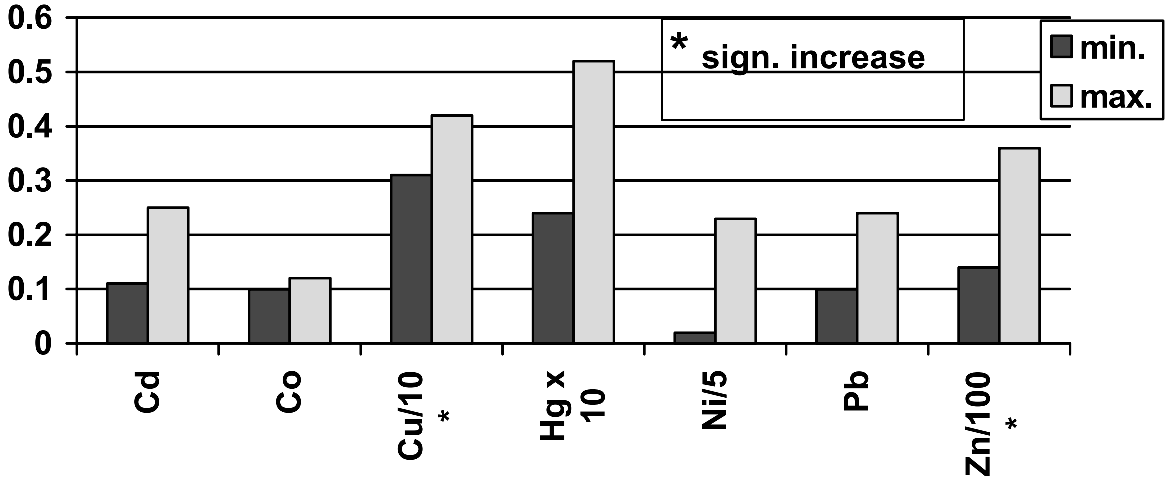

FIG. 3. Ranges of heavy-metal concentrations in oil-seed rape samples (mg/kg DW) 1995.

The first is the uncertainty in heavy-metal determination, which is reflected by standard deviations sometimes of 10% or more, and incomplete extraction of heavy metals by aqua regia. Gerzabek [8] reported that acid extraction of soils yielded only 69%, 49%, 76% of total contents of Cd, Cr, Cu, and Zn, respectively. A second reason could be losses of mobile heavy-metal fractions from the pots. Such an effect was observed in a field pot trial by Kamel [9]; losses of heavy metals from experimental soils reached 17% (Zn) of initial values within 550 days. In our experiment, conditions for leaching of heavy metals were more favourable than in field conditions due to the increase in hydraulic conductivity of the substrate by the presence of quartz sand; part of the observed effect, therefore, may result from the experiment itself. Nevertheless, it has to be taken into account that the mobile heavy-metal fraction in sewage sludge generally is larger than in uncontaminated soils. This is supported by the mobile heavy-metal data for the second experimental year (1996, Fig. 2). The portion extracted by ammonium acetate ranged between 0.015% (Cr) and 7.3% (Cd) of the aqua-regia-extracted contents. The less-mobile element, Cr, showed no response to the sewage-sludge treatments, whereas concentrations of Cd, Cu, and Zn in the ammonium-acetate extract increased significantly: by factors of 1.9 and 2.4, respectively, between control pots and those receiving an equivalent of 15 t/ha sewage sludge. It can be concluded that the ammonium-acetate extraction is a potentially suitable and sensitive method to examine increases of heavy-metal mobility due to the application of organic residues. Similar trends of increasing mobile heavy-metal fractions due to sewage-sludge application were reported by Kumazawa [10].

3.2. Heavy metals in oil-seed rape

Figure 3 shows ranges of concentrations of heavy metals in the plants in 1995. Comparison with values in the literature [11] indicates that none of the investigated elements exceeded ranges frequently observed in plants. In most cases, the measured concentrations were close to the lower limits observed in nature. The high pH of the substrate did not favour heavy-metal availability to the plants, which could be the main reason for the observed low levels of uptake. Only a few differences were found between the mineral-fertilized pots and those amended with sewage sludge. Copper concentrations in the plants (1995) were slightly, but significantly, increased by a single sewage sludge treatment of 7.5 t/ha. In 1996, small but significant differences between treatments were measured for Cd and Cr. The 7.5 t/ha sewage-sludge treatement showed the lowest values in both cases, although the mobile fractions of Cd and Cr were similar in the mineral-fertilizer and 7.5 t/ha sewage-sludge treatments. The 15 t/ha treatment was not significantly different from the control.

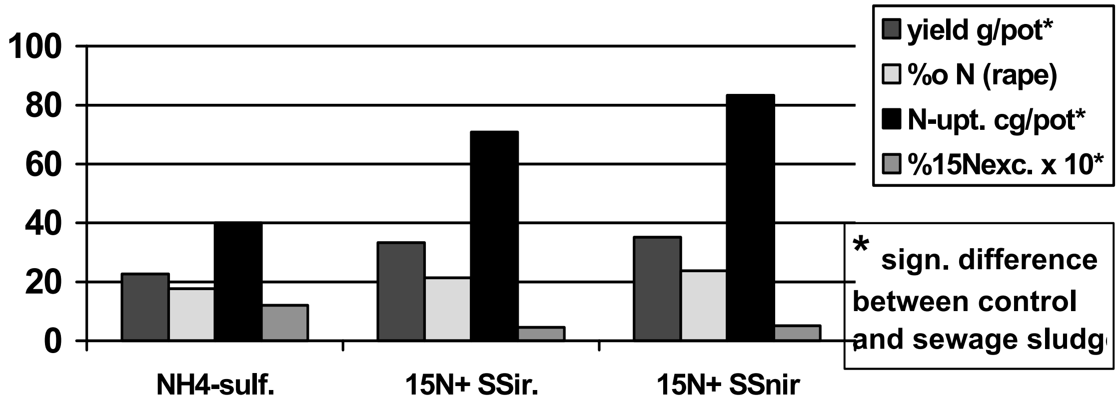

FIG. 4. Oil-seed rape yields and N-uptake, 1995.

According to the literature, the application of organic manure and residues can reduce heavy-metal uptake by crops. This is explained by the fact that even quite small quantities of organic matter applied to soil through compost [12] or sewage sludge [4,13] can increase the adsorption capacity of the soil and thus diminish heavy-metal uptake through formation of stable organo-metallic complexes. The lack of a diminishing effect on Cd and Cr uptake in the 15 t/ha treatments (1996) might be a bias of the experimental set-up in that year. The 7.5 t/ha treatment was the only one not receiving ammonium sulphate as a mineral N source. Ammonium is known to reduce the pH in the rhizosphere [14] and thus may have increased heavy-metal availability in the 15 t/ha treatment. In 1997, again there was no clear effects in terms of heavy-metal concentrations in the plants in response to the treatments; higher (Cu, Hg, Zn) and lower (Cd, Ni) concentrations of heavy metals were observed, compared to the control. Thus, no final conclusion on the impact of sewage sludge on heavy-metal uptake by rape plants can be deduced from our study, although quantities of sludge applied were much larger than in normal practice. These findings generally are supported by results from a 3-year field trial in Austria on a similar Chernozem soil in which no clear impact of repeated sewage-sludge treatments on heavy-metal concentrations in field crops was observed [15]. In future, it may be important to consider the nutritional status of the plants and the processes taking place in the rhizosphere for improved prediction of heavy-metal availability.

3.3. Oil-seed rape yield and N uptake

Yields in 1995 were significantly enhanced by sewage-sludge applications (Fig. 4). Average rape shoot dry matter in sewage-sludge treated pots was 150% higher than in pots treated with mineral N solely; differences between irradiated and non-irradiated sludge were not statistically significant. The positive effects on growth can be attributed to the plant nutrients in the sewage sludge, especially N, P, and K (Table I). Mineral N provided by the equivalent of 7.5 t/ha sewage sludge exceeded N from $(^{15}NH_4)_2SO_4$ by a factor of 2.2.

Due to large variations among replicates, the differences in %N in rape shoots between treatments were not significant; however, the N uptake per pot was significantly enhanced by the sewage sludge application. Sewage-sludge-treated rape exhibited a 1.9-fold higher N-uptake as compared to the control. Differences between irradiated and non-irradiated sludge treatments were not statistically significant in terms of N uptake.

Nitrogen in above-ground plant biomass originating from $(^{15}NH_4)_2SO_4$ was highest in the control pots (Fig. 5), as was the utilization of N from $(^{15}NH_4)_2SO_4$. The ammonium fertilizer contributed approximately 10% to the N-uptake by rape treated additionally with sewage sludge.

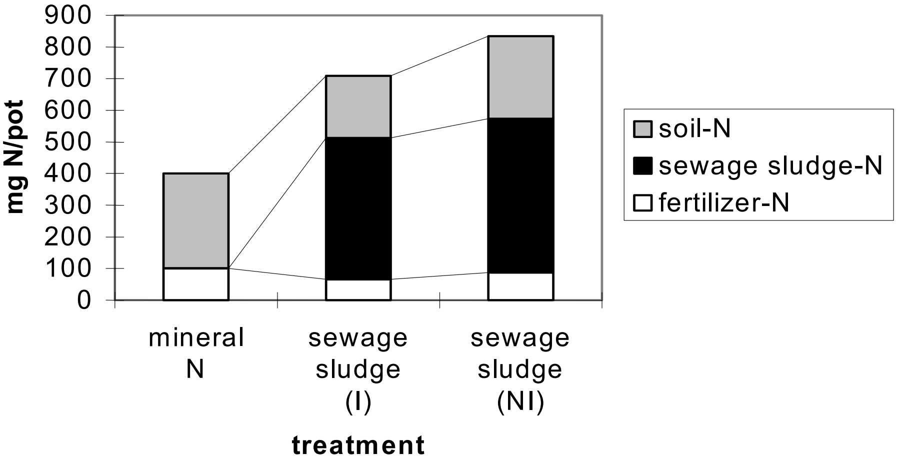

FIG. 5. Contributions of N-sources to N-uptake by rape plants (1995); I: irradiated, NI: not irradiated.

The largest portion of N in these plants originated from sewage sludge (61% on average) (Fig. 5). Sewage-sludge N partially substituted both for soil N and for fertilizer-N uptake, and accounted for the increase of the total N-uptake as compared to the control.

Within 40 days, rape was able to use 7.4% (mean of irradiated and non-irradiated sewage sludge) of total N or 27% of the mineral N in the sewage sludge. Similar N-utilization obtained by the ^{15}N-dilution technique has been reported for other crops (Table III). According to Smith [4] the sewage sludge used in our experiment can be classified as having high mineralizable N (total N >5%, C:N <8). Paul and Beauchamp [16] reported ^{15}N-recoveries by maize plants from $(^{15}NH_4)_2SO_4$ and from dairy-cattle slurry of 29% and 15%, respectively. Considering the short period of growth in our experiment, the latter value is consistent with our findings.

The results in 1996 differed significantly from those obtained in 1995 (Fig. 6). Due to wet soil conditions, the plants developed poorly after incomplete germination. Therefore, no significant differences were obtained for yields. Plant-N contents were higher in Treatments 4, 5, and 6 due to additional N-supply as ammonium fertilizer and sewage sludge. Nitrogen uptake did not differ significantly among treatments. The ^{15}N-enrichment in rape biomass showed no statistically significant differences as a result of losses of labelled N through leaching and/or volatilization. This explanation is supported by extremely low utilization of labelled ammonium sulphate, 2 to 3.5%, obtained from the isotope-dilution calculation, 2.4- to 6-fold lower than in 1995.

Table III. Literature values for utilization of N from sewage sludge in the first year of application

Crop	Country	%N utilization	Source
Sugarcane leaves	Argentina	7.9–19%	Magnavacca et al. [17]
Maize (above-ground biomass)	Malaysia	5.8–7.8%	Ishak et al. [18]
Wheat	China	1.7–15%[a]	Calculated from Jiang et al. [19]
Oil-seed rape	Austria	7.1–7.7%	This study

[a] Obtained by using the difference method.

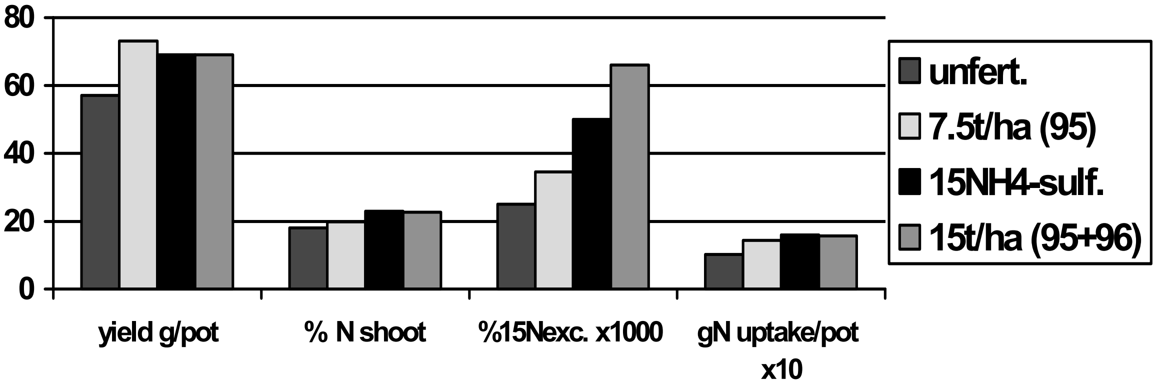

FIG. 6. Oil-seed rape yields and N-uptake, 1996.

The contribution of ammonium sulphate N to the N-uptake of rape in 1996 was a factor of 6 to 24 lower as compared to the results in 1995 (Fig. 6). High losses of N by leaching and a factor of 6 lower mineral N fraction in sewage sludge applied in spring 1996 (Table I) could be the explanation for the lack of effect of sewage sludge on plant yield and N-uptake. Similar problems with the isotope method used in the present experiment were reported by Jiang et al. [19] and Azam et al. [20]. The latter authors reported a trend of increasing ^{15}N content in plants grown on sewage-sludge-treated plots. This trend (not significant) was also obtained in Treatments 1, 2, and 3, suggesting that sludge-treated pots kept a higher portion of fertilizer N. The input of organic matter to the soil probably increased the microbial biomass, which biologically immobilized some of the mineral N. Negative effects of heavy metals on soil microbial activity can be observed only in long-term studies [4], and thus were not likely to be seen in our experiment.

In 1997, the isotope-dilution method again was successful, and there were clear effects of sewage-sludge additions made in previous years. Rape shoot yields were, in some cases, significantly higher in the sewage-sludge treated pots as compared to the control (Fig. 7). Nitrogen uptake also clearly showed a residual effect of sewage-sludge addition in 1995 (Treatments 2 and 3) and 1995 plus 1996 (Treatments 5 and 6). Nitrogen uptake into rape-shoot biomass increased from the control pots to those that received sewage sludge in 1995 (7.5 t/ha) to the 15 t/ha treatment. The increase of N-contents in rape shoots was significant only for the repeated sewage-sludge treatments (5 and 6). No significant differences were observed between irradiated and non-irradiated sewage sludge for N-uptake and N-content.

The contribution of fertilizer N to N-uptake was nearly constant across treatments in 1997 (Fig. 8). The N-utilization from ammonium sulphate (average: 33%) was clearly higher than in 1995, which can be attributed to the split application in 1997. In the solely ammonium-sulphate-treated pots, fertilizer was the main source of plant N. The pots treated in 1995 with 7.5 t/ha sewage sludge provided approximately the same amounts of N from ammonium sulphate and soil. Nitrogen uptake from soil was partly substituted by sewage-sludge N in 1995 and 1996. The average contribution of sewage-sludge N was 11% and 29% for the 7.5 t/ha and 15 t/ha treatments, respectively. The high N-portion originating from sewage sludge observed for the 15 t/ha treatment as a residual effect supports the exceedingly high values of approximately 61% detected in 1995 shortly after the first sewage-sludge application.

The absolute N-utilization from sewage sludge was quite low and did not exceed 1.5%, distinctly lower than in the first year (Fig. 8). Using the difference method, the following average N-utilizations from the sewage sludge applications were calculated: 7.4% (first year), 1.8% (second year), 1.1% (third year). In total, approximately 10% of sludge-N_t was taken up by the rape plants during the 3-year period.

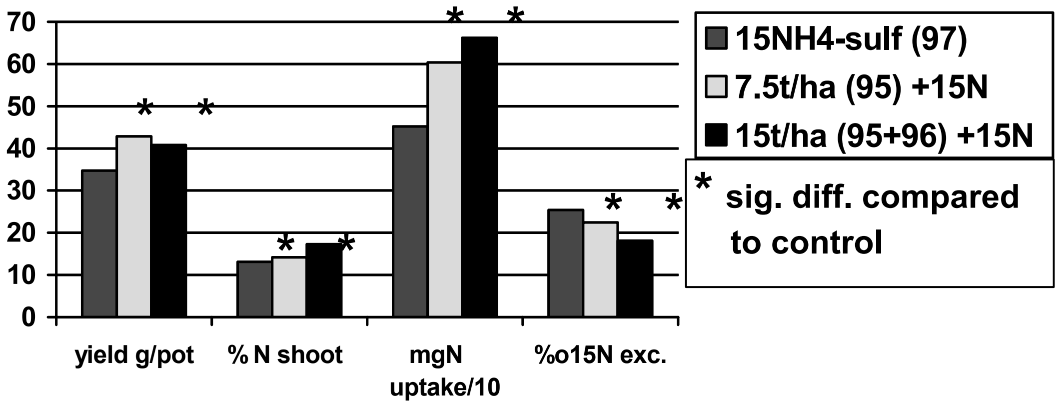

FIG. 7. Oil-seed rape yield and N-uptake, 1997.

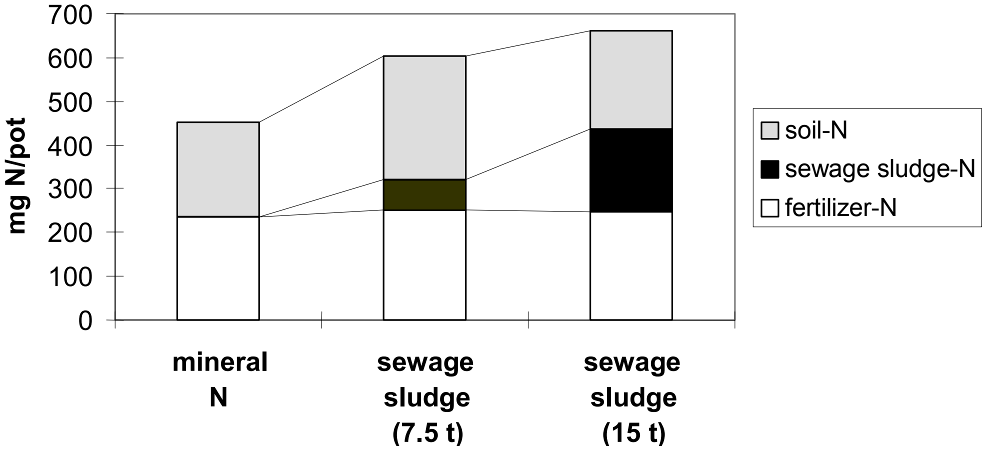

FIG. 8. Contribution of N-sources to N-uptake by oil-seed rape, 1997.

4. CONCLUSIONS

- Heavy-metal concentrations in the substrate (aqua-regia extraction) did not change significantly after addition of equivalents of 7.5 and 15 t/ha dry sewage sludge. Mobile fractions of Cd, Cu, and Zn measured in 1996 increased significantly in the substrate due to sewage-sludge application. However, no clear trends were observed in heavy-metal uptake by oil-seed rape, due to the sewage-sludge treatments.
- Dry-matter yield of rape and N uptake were significantly enhanced by sewage sludge in the first and third years. In 1996, rape yields, N contents and ^{15}N-enrichment in biomass showed no statistically significant differences among treatments due to high losses of N during spring and slow plant development. Yield increases and enhanced N-uptake in the third year can be interpreted as residual effects of the sludge applied in previous years.
- Irradiation of sewage sludge did not have any significant effects on rape-biomass production, N availability in the sewage sludge or heavy-metal uptake by plants.
- The ^{15}N-dilution method can be distinctly improved by splitting the applications of the labelled fertilizer.

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ISOTOPE-AIDED STUDIES ON THE EFFECTS OF RADIATION PROCESSED SEWAGE SLUDGE ON CROP YIELDS AND BIOAVAILABILITY OF HEAVY METALS

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Abstract

Field experiments using ^{15}N and a greenhouse experiment using ^{32}P were carried out to study the effects of application of non-irradiated and irradiated sewage sludges on crop yields, contributions of N and P from sludge, accumulation of heavy metals in soils and crops, and improvement in soil-fertility status. Gamma-irradiation (5 kGY) of sewage sludge reduced total bacterial counts and almost eliminated hazardous pathogenic bacteria, indicating the possibility of safe use of irradiated sludge in crop production. Progressive increases in wheat yields were observed with higher rates of sewage-sludge application, and relatively more with irradiated sludge. Highest wheat yield was produced with irradiated sludge applied at the equivalent of 400 kg N/ha, almost identical to the yield obtained with 100 kg N/ha as urea. On average, applications of irradiated sludge produced 7.2 to 19.5% more yield than did non-irradiated sludge treatments. Nitrogen-15 and ^{32}P studies helped in quantifying the amounts of N and P contributed by sewage sludge and utilized by wheat. The highest sludge-N yields varied from 9.3 to 11 kg/ha. The highest sludge-P yield was 11 mg/pot with the treatment receiving 400 kg N equivalent of irradiated sludge. For an accurate estimate of uptake of N and P from applied sources, the isotope method was more reliable than the indirect method. Although metal concentrations showed increasing trends in terms of plant and post-harvest-soil contents due to continuous use of sludge, the values were much below permissible limits according to the literature. Marginal improvement in soil-fertility status was observed in respect to organic matter content. Our findings thus indicate good prospects for environmentally safe utilization of irradiated sewage sludge as an organic fertilizer for increased and sustainable crop production.

1. INTRODUCTION

Sewage sludge is a nutrient-rich organic by-product of municipal wastewater treatment. Its disposal is a serious problem in many countries due to rapid urbanization. In recent years there has been a growing interest in applying sewage sludge to agricultural land. High levels of N, P and organic matter make it excellent as a fertilizer and soil conditioner. Its use is reported to enhance crop productivity [1]. Integrated nutrient and fertilizer management thus offers the unique opportunity to maximize crop yield, sustain agricultural production, and solve the disposal problem. On the other hand, sewage sludge is considered to be an environmental pollutant because it is likely to contain toxic heavy metals and hazardous pathogenic organisms that may adversely affect plants and, via the food change, animals and humans [2]. There are reviews on various aspects of the application of sewage sludge to agricultural land as fertilizer, its phytotoxic effects on plant and its content of pathogenic organisms.

The risk of using sewage sludge as fertilizer on agricultural land can be mitigated by pre-treatment with gamma-irradiation to reduce the numbers of micro-organisms [3]. Since sewage sludge has a high nutritional value, recycling it both as a source of essential plant nutrients and as a soil conditioner has received considerable attention.

As some limiting factors are involved in the land-application of sewage sludge, its proper management is important for economic and environmental reasons. The use of isotope and radiation techniques can serve as valuable tools in undertaking such studies.

Under a Co-ordinated Research Project organized by the Joint FAO/IAEA Division, the present work was undertaken to assess sewage sludge (non-irradiated and irradiated) as a source of N and P for crops (using ^{15}N and ^{32}P tracer techniques), as a contributor of heavy metals, as a source of organic matter for improvement in soil fertility status, and as a source of pathogenic organisms. The overall objective was to develop environmentally benign practices for managing sewage sludge as an organic fertilizer for improving soil fertility and increasing and sustaining crop production .

2. MATERIALS AND METHODS

Moist sewage sludge (semi-solid) was collected from a wastewater-treatment plant situated 15 km from Dhaka, the capital city of Bangladesh. The sludge was air-dried for a month, milled, processed, and irradiated at a dose rate of 5 kGy (500 kRad) in a ^{60}Co gamma irradiator. Microbiological analyses of non-irradiated and irradiated sludge and soil were performed at the Environmental Microbiological Laboratory of Dhaka University, following standard methods [4,5]. The physico-chemical analyses of soil and sewage sludge (non-irradiated and irradiated) were carried out following standard analytical procedures.

2.1. Core experiment using ^{15}N, 1995-96, 1996-97 and 1997-98

Experiments were conducted at the experimental field of the Bangladesh Institute of Nuclear Agriculture, Mymensingh. The experimental soil is a dark-gray floodplain (Haplaquept) with sandy-loam texture. The experiment comprised ten treatments: 100 kg N/ha as urea (T_1), 20 kg N/ha as urea (T_2, control), sewage sludge equivalent (eqv.) to 100 kg N/ha (T_3), 200 kg N eqv./ha (T_4), 300 kg N eqv./ha (T_5), 400 kg N eqv./ha (T_6), (T_3–T_6 with non-irradiated sewage sludge), 100 kg N eqv./ha (T_7), 200 kg N eqv./ha (T_8) , 300 kg N eqv./ha (T_9), 400 kg N eqv./ha (T_{10}), (T_7–T_{10} with irradiated sludge). Nitrogen-15-labelled urea (10% a.e) was applied at 20 kg N/ha in Treatments 2–10. Unit plot size was 4×3 m, of which 1×1 m was delineated by G.I. sheeting as the isotope subplot. The experiment had an RCB design with four replications. The test crop was wheat (*Triticum aestivum* L. cv. Kanchan); it was harvested at maturity. Plant samples collected from ^{15}N subplots were oven-dried at 70°C and ground for ^{15}N analysis and chemical analysis of N, P, micronutrients, and heavy metals.

2.2. Isotope Analysis

Plant samples from isotope subplots were analysed for ^{15}N and total N at the IAEA Soils Laboratory at Seibersdorf, Austria. Percent N derived from fertilizer (%Ndff), fertilizer-N-yield and percent N-recovery were calculated using the ^{15}N data. Percent N derived from sewage sludge (NdfSS) and sludge N yield were estimated as follows:

$$\%NdfSS = \left[1 - \frac{^{15}N\, a.e. with\, sludge}{^{15}N\, a.e. without\, sludge} \right] \times 100$$

$$Total\, N\, derived\, from\, sludge(kg\, N/ha) = \left[\frac{\%NdfSS \times Total\, N\, in\, crop}{100} \right]$$

2.3. Chemical Analyses

Soil and plant samples were chemically analysed using standard techniques; heavy-metal contents were determined by atomic absorption spectroscopy (AAS). Data were statistically analysed following standard methods.

2.4. Residual effects of sewage sludge, 1995–96 and 1996–97

To study residual effects of sewage sludge after the harvest of wheat (the first crop), seeds of summer mung bean (*Vigna radiata* L. cv. Binamung-2) were sown in the first year on the same layout as a part of a wheat-mung bean-fallow cropping sequence. In the succeeding year, a mutant variety of rice (*Oryza sativa* L. cv. Binashail) was planted on the same layout as part of a wheat-fallow-rice cropping sequence. Recommended doses of P, K and S were applied as basal fertilizers. Both crops were harvested at maturity and grain- and straw-yield data recorded.

2.5. Nitrogen fixation by summer mung bean

An experiment was laid out to estimate biological N_2 fixation (BNF) by summer mung bean using the ^{15}N-dilution technique. Finger millet (*Elucine coracana* L. cv. Titas) was the non-fixing reference crop. The experiment had an RCB design with four replications of each treatment, as follows.

— T_1, no sewage sludge, ^{15}N-labelled urea (10% a.e.) at 20 kg N/ha in the ^{15}N subplot.

— T_2, 50 kg N ha^{-1} from irradiated sewage sludge (50% of the recommended N-rate) with ^{15}N-labelled urea (10% a.e.) at 20 kg N ha^{-1} in the ^{15}N subplot.

— T_3, 100 kg N ha^{-1} from irradiated sewage sludge (the recommended N-rate) with ^{15}N-labelled urea (10% a.e.) at 20 kg N ha^{-1} in the ^{15}N subplot.

Estimates of BNF (N derived from the atmosphere, Ndfa) by mung bean were made by the isotope-dilution method [6]:

$$\%Ndfa = \left[1 - \frac{\%Ndff_{fixing\ crop}}{\%Ndff_{non-fixing\ crop}}\right] \times 100$$

$$Amount\ of\ N\ fixed = \frac{\%Ndfa \times total\ N_{fixing\,crop}}{100}$$

where

Ndff is N derived from fertilizer.

2.6. Non-isotope experiments, 1996–97, 1997–98 and 1998–99

Experiments without ^{15}N were also conducted with the same objectives as those for the isotope experiment using the same treatments, in order to compare contributions of N from sludge treatments. The N contributions from soil, fertilizer, and sewage sludge were calculated by the indirect (conventional) method, using data from the no-fertilizer (control), N-fertilized control, and sewage-sludge treatments.

2.7. Greenhouse experiment using ^{32}P

During the wheat-growing season, a greenhouse experiment was conducted using the dark-grey floodplain soil (Haplaquept) to assess contributions of P from applications of different rates of irradiated and non-irradiated sewage sludge, using ^{32}P as tracer.

Three-kg aliquots of soil were added to each pot and 8.4 µCi ^{32}P as carrier-free orthophosphate were applied at the rate of 40 kg P/ha, i.e.60 mg P/pot (Treatments T_2–T_{10}, see below), according to the treatment combinations. Nitrogen and K were applied from urea and KCl, respectively, There were ten treatments with four replications for each treatment, and the experiment was laid out in an RCB design. The treatment descriptions are in Table I.

Seeds of the test crop, wheat, were sown and seedlings were thinned to four per pot. Irrigation was sparingly provided. The plants were harvested at 65 days of growth, washed, and oven-dried at 70°C for chemical analysis. The dry-matter yields were recorded, and plants were milled and digested with a diacid mixture for total and radiochemical analyses of P. Total P and ^{32}P were determined colorimetrically and by liquid scintillation counting, respectively. The %Pdff, fertilizer P yield, and percent recovery of P were calculated using ^{32}P data.

Table I. Treatment descriptions

Designation	Treatment	Designation	Treatment
T_1	No-P, 20 kg N/ha urea, 80 kg K_2O/ha	T_6	NIS equivalent to 400% N requirement + 40 kg P/ha
T_2	40 kg P/ha 100 kg/N ha, 80 kg K_2O/ha	T_7	IRS equivalent to 100% N requirement + 40 kg P/ha
T_3	NIS[a] equivalent to 100% N requirement + 40 kg P/ha	T_8	IRS equivalent to 200% N requirement + 40 kg P/ha
T_4	NIS equivalent to 200% N requirement + 40 kg P/ha	T_9	IRS equivalent to 300% N requirement + 40 kg P/ha
T_5	NIS equivalent to 300% N requirement + 40 kg P/ha	T_{10}	IRS equivalent to 400% N requirement + 40 kg P/ha

[a]Non-irradiated sewage sludge. [b]Irradiated sewage sludge.

Table II. Chemical characteristics of non-irradiated (NIS) and irradiated sewage sludge (IRS) and the experimental soil

Parameter	NIS	IRS	Soil	NIS	IRS	Soil	NIS	IRS	Soil
	1995–96			1996–97			1998– 99		
pH (1:5 soil/sludge: $CaCl_2$)	4.7	4.6	6.7	5.3	5.2	6.2	5.7	5.4	6.3
Water-holding capacity (%)	–	–	45	–	–	50	–	–	51
CEC (mEq %)	8.3	9.3	10.5	9.3	9.0	12.2	9.7	9.4	12.7
Organic C (%)	6.9	7.1	0.65	14.5	14.0	0.89	14.6	13.8	0.95
Total N (%)	0.87	0.95	0.09	1.68	1.70	0.09	1.52	1.48	0.09
Bray II extractable P (mg/kg)	496	431	9.3	246	239	16	251	237	21
Heavy metals (mg/kg)									
Aqua–regia extract									
Zn	420	419	21	689	685	50	698	688	53
Cu	408	423	19	256	252	28	258	253	28
Mn	–	–	–	258	253	46	271	263	49
Pb	188	192	14	156	145	15	157	145	16
Ni	–	–	–	137	130	4	141	129	4
Cd	1.3	1.5	0.25	5	3	*[a]	6	4	2
DTPA extract									
Zn	334	340	9	120	117	10	124	118	11
Cu	110	99	8	15	13	8	16	14	10
Pb	39	42	2	3	4	3	4	3	3
Ni	–	–	–	23	19	2	25	21	3
Cd	–	–	–	0.85	0.92	*	1.0	1.0	*

[a]Below detectable limit.

3. RESULTS AND DISCUSSION

3.1. Chemical analysis of sewage sludge and soil

Physico-chemical data for the non-irradiated (NIS) and irradiated sewage sludges (IRS), and for the experimental soil (initial) are presented in Table II. The sludge was acidic, with pH values ranging from 4.6 to 5.7. Soil pH was 6.3 to 6.7 for all 3 years. Water-holding capacity of the soil showed increases with the application of sewage sludge, from 45 to 51%. CEC values for the sewage sludges were similar in all the 3 years, whereas the CEC values for soil increased with time from 10.5 to 12.7 mEq%. Organic C and total N values of the sludges were higher during the second and third years, whereas soil N showed no changes. Soil organic C increased. Although sludge N was higher, soil N did not show any change during the period of study. The extractable P content in sludge was quite high in the first year, but decreased is succeeding years, soil P (Bray-II method) increased during the later years of the experiment.

Aqua-regia-soluble micronutrients and heavy metals in the sludges recorded fairly high values; differences in heavy-metal content between non-irradiated and irradiated sludges were unremarkable. The soil contents of micronutrients and heavy metals were relatively low and, in certain cases, Cd content was below detectable limits. In general, applications of sewage sludge did not significantly affect the physico-chemical properties of the soil.

Table III. Microbiological analysis of non-irradiated (NIS), irradiated sewage sludge (IRS) and initial and post-harvest soils

Year/parameter	NIS	IRS	Initial soil	Post-harvest soil	
				NIS-treated	IRS-treated
1995–96					
Total bacteria (MPN/g DM, 37°C)	1.2×10^{6}	4.0×10^{3}	1.9×10^{5}	1.8×10^{6}	2.0×10^{3}
Total coliforms (MPN/g DM, 37°C)	3.0×10^{4}	Nil	1.5×10^{3}	1.5×10^{6}	< 1
Faecal coliforms/g DM	1.0×10^{2}	Nil	0.5×10^{2}	<1	Nil
E. coli/g DM	5.6×10^{3}	Nil	1.0×10^{3}	3.5×10^{3}	<1
Salmonella/g DM	Nil	Nil	Nil	Nil	Nil
Ascaris ova/g DM	Nil	Nil	Nil	Nil	Nil
1997–98					
Total bacteria (MPN/g DM, 37°C)	5.0×10^{5}	2.0×10^{3}	2.8×10^{3}	4.5×10^{3}	2.8×10^{3}
Total coliforms (MPN/g DM, 37°C)	0.7×10^{2}	Nil	0.28×10^{2}	0.67×10^{2}	<1
E. coli/g DM	4	Nil	2	4	Nil
Salmonella/g DM	Nil	Nil	Nil	Nil	Nil
Ascaris ova/g DM	Nil	Nil	Nil	Nil	Nil
1998–99					
Total bacteria/g DM	5.3×10^{5}	1.6×10^{3}	1.1×10^{3}	1.5×10^{5}	1.1×10^{2}
Total coliforms/g DM	2.4×10^{2}	Nil	0.20×10^{2}	1.7×10^{2}	<1
Faecal coliform/g DM	1.0×10^{2}	<1	<1	<1	Nil
E. coli/ g DM	3.7×10^{2}	Nil	<1	1.5×10^{2}	<1
Salmonella/g DM	2.0×10^{2}	Nil	Nil	0.8×10^{2}	Nil
Ascaris ova/g DM	4.0×10^{2}	Nil	Nil	Nil	Nil

Table IV. Effects of non-irradiated and irradiated sewage sludge on the yields of wheat grain and straw (isotope experiments)

Treatment	1995–96		1996–97		1998–99	
	Grain	Straw	Grain	Straw	Grain	Straw
	(kg/ha)					
T_1	2,091	4,820	2,493	3,380	3,040	4,076
T_2	397	1,857	1,106	1,508	900	1,499
T_3	236	909	1,077	1,538	1,486	2,122
T_4	398	1,394	1,296	1,738	1,698	2,911
T_5	855	1,783	1,752	2,130	2,475	3,160
T_6	1,040	2,009	2,084	2,665	2,938	4,051
T_7	200	1,073	1,280	1,558	1,665	2,339
T_8	493	1,360	1,743	2,188	1,812	2,649
T_9	913	1,796	2,025	2,488	2,630	3,374
T_{10}	1,383	2,638	2,373	2,800	3,015	4,111
$LSD_{0.05}$	113	196	111	187	102	142

Table Va. Influence of non-irradiated and irradiated sewage sludges on total dry matter yield, total N, and ^{15}N-derived parameters in wheat, 1995–96

Treatment	Total DM (kg/ha)	%N	Total N (kg/ha)	Fert. N yield (kg/ha)	%Ndff[a]	%Ndfs[b] (%NdfSS[c])	%N recovery
T_1	6,911	0.84	58.2	12	22	78	13
T_2	2,254	1.0	23.1	3.8	16	84	19
T_3	1,085	0.76	8.10	1.4	17	83	7.0
T_4	1,792	0.69	12.4	1.6	13	87 (3.8)	7.9
T_5	2,638	0.96	25.1	4.4	17	82.6	22
T_6	3,049	0.98	30.0	4.2	14	86 (2.4)	21
T_7	1,273	0.84	10.7	1.8	17	83	8.9
T_8	1,852	0.90	16.7	3.1	18	82	15
T_9	2,709	1.1	29.4	4.5	16	84 (0.1)	23
T_{10}	4,021	0.91	36.5	6.5	18	81.9	32
$LSD_{0.05}$	2,573	0.11	4.7	0.94	NS	NS	4.1

Nitrogen derived from [a]fertilizer, [b]soil, [c]sewage sludge.

3.2. Microbiological analysis

The total bacterial density counts (MPN/g dry matter at 37°C) were considerably lower in the irradiated sludge (Table III). Correspondingly, IRS-treated post-harvest soils showed very low total bacterial counts, and, in same cases, the micro-organism counts were almost nil. The irradiation of sewage sludge effectively eliminated pathogens. In the opinion of radiation scientists 3 to 5 kGy are sufficient to completely inactivate hazardous pathogens in sludge [7].

3.3. Core experiments using ^{15}N, 1995–98

3.3.1. Grain and straw yields

The highest wheat yield was recorded in 1997–98, and the lowest was in 1995–96 (Table IV). Sludge application had little effect on wheat growth initially, indicating that nutrients in the sludge, particularly N, were mineralized slowly and became available for uptake only after a period of time.

The highest wheat yield was obtained with the 400% N-equivalent rate of irradiated sludge (T_{10}), which was identical to that obtained with 100 kg N/ha from urea (T_1). In general, IRS produced higher wheat yields than did NRS, which is attributable to inactivation of hazardous pathogens and growth inhibitors due to irradiation [8].

3.3.2. Total N and ^{15}N-derived parameters in wheat

3.3.2.1. Total and fertilizer N in wheat, 1995–96

Total N content in wheat varied from 0.69 to 1.1% in various treatments—the highest being recorded in T_9 (Table Va). Total N yield in wheat was highest in T_1 (58.2 kg N/ha). The IRS contributed more N to the total N yield, which was attributed to higher dry matter production. Nitrogen-15-aided studies indicated that fertilizer N yield in wheat and %Ndff were highest in T_1 (100 kg N/ha as urea). Although %Ndff ranged between 13 and 22, the fertilizer-N yield was relatively low. This indicates that most of the N in the wheat was derived from the soil. In the first year of the experiment, neither NRS nor IRS contributed towards total N yield in wheat except in T_4 and T_6, which received NRS at 100 and 200 kg N/ha, respectively. Fertilizer-N recovery by wheat varied between 7.0 and 32%—the highest being recorded in T_{10}, the highest rate of IRS.

3.3.2.2. Total N, fertilizer N and sludge N in wheat, 1996–97

Nitrogen uptake increased with the rates of NRS and IRS in the 1996–97 season (Table Vb). Total N yields were higher in IRS-treated plots—the highest being recorded in T_{10} (50 kg /ha). The application of 100 kg N ha^{-1} from urea in T_1 plots, however, produced the highest total N yield (60.1 kg/ha). Nitrogen-15-aided studies indicated that Ndff and fertilizer N yield were highest in T_1 receiving 100 kg N/ha as urea. The IRS, in general, contributed more N to total N yield, which was attributed to higher dry-matter production. Although %Ndff in various sludge-treated plots ranged between 9.8 and 15.9 on a whole-plant basis, the fertilizer N yields were, in general, relatively low, indicating that most of the N in the wheat was derived from the soil. There were increases in NdfSS and sludge-N yield with increases in rates of sludge application. The lower rates of application both for IRS and NRS contributed less N to the total-N pool in wheat; T_5 and T_6 contributed only 6.0 and 7.3 kg N/ha, respectively. Higher rates of IRS contributed more, i.e. 7.8 and 9.3 kg/ha in T_9 and T_{10}, respectively.

Percent recoveries of ^{15}N by wheat varied from 13 to 40 (Table Vb). Higher N recoveries were recorded with IRS treatments. The effects of irradiation of sludge in terms of crop yield in relation to N status of sludge-amended field soils is seldom reported. As stated above, increases in crop yields and N uptake are thought to be due to inactivation of pathogenic microorganisms and growth inhibitors due to the irradiation of sludge [8].

3.3.2.3. Total N, fertilizer N, and sludge N in wheat, 1997–98

Total N yield of plants increased with increasing rates of non-irradiated and of irradiated sludge (Table Vc). Similar to previous seasons, the total N yield in wheat was higher in irradiated sludge treated plots; the highest was recorded in T_{10} (82.5 kg/ha), similar to that recorded with 100 kg N/ha from urea (T_1, 78.3 kg/ha).

Nitrogen-15-aided studies showed that Ndff and fertilizer-N yield were highest in T_1. Otherwise, fertilizer-N yield and Ndff were similar across treatments, with no significant differences. The fertilizer-N yields were, in general, low; most of the N in the wheat was derived from the soil. The sludge-N yield showed an increasing trend with increasing rates of application of non-irradiated and irradiated sludge. There were no significant differences in %NdfSS or sludge-N yield between the non-irradiated and irradiated sludge-treated plots, which varied with treatment from 10 to 13% and 4.3 to 11 kg/ha. The %N recovery by wheat was higher in irradiated sludge-treated plots. Treatment T_1, receiving 100 kg N as urea, recorded the highest N recovery (52%). Recovery varied from 10 to 34% of the 20 kg ^{15}N labelled urea in treatments T_2 to T_{10}.

Total and fertilizer-N values in wheat gradually increased and were highest in 1997–98. This indicates that N bound in sludge material was gradually mineralized and became available for uptake.

Table Vb. Influence of non-irradiated and irradiated sewage sludge on total N and ^{15}N-derived parameters in wheat, 1996–97

Treatment	Plant part	%N	Total N yield (kg ha)	%Ndff[a]	Fert. N yield (kg/ha)	%NdfSS[b]	Sludge N yield (kg/ha)	%N recovery
T_1	Grain	1.99	49.6	47	23.4	–		
	Straw	0.31	10.5	43	4.50	–		
	Total	1.02	60.1	46	27.9	–	–	28
T_2	Grain	1.62	17.9	13	2.27	–		
	Straw	0.22	3.32	12	0.41	–		
	Total	0.88	21.2	13	2.68	–	–	13
T_3	Grain	1.94	20.9	16	3.28	10	2.13	
	Straw	0.31	4.77	16	0.65	6.1	0.29	
	Total	0.98	25.7	15	3.93	9.3	2.42	20
T_4	Grain	1.76	22.8	16	3.74	11	2.58	
	Straw	0.28	4.87	15	0.74	5.8	0.28	
	Total	0.91	27.7	15	4.48	10	2.86	22
T_5	Grain	1.83	32.1	9.3	2.98	17	5.50	
	Straw	0.29	6.18	13	0.78	8.2	0.51	
	Total	0.98	38.2	9.8	3.76	16	6.01	19
T_6	Grain	1.72	35.8	12	4.19	18	6.52	
	Straw	0.31	8.26	12	0.96	9.5	0.78	
	Total	0.93	44.1	12	5.15	17	7.30	26
T_7	Grain	1.87	23.9	14	3.45	13	3.06	
	Straw	0.31	4.83	16	0.75	6.7	0.32	
	Total	1.01	28.8	15	4.20	12	3.38	21
T_8	Grain	1.60	27.9	12.9	3.60	14	3.98	
	Straw	0.31	6.78	13.8	0.94	8.1	0.55	
	Total	0.88	34.7	13.1	4.54	13	4.53	23
T_9	Grain	1.82	37.9	12.0	4.55	18	6.98	
	Straw	0.32	7.96	13.3	1.06	9.7	0.77	
	Total	1.00	45.9	12.2	5.61	17	7.75	28
T_{10}	Grain	1.74	41.3	16.0	6.61	20	8.42	
	Straw	0.31	8.68	15.3	1.33	9.8	0.85	
	Total	0.97	50.0	15.9	7.94	19	9.27	40

Nitrogen derived from [a]fertilizer, [b]sewage sludge.

Table Vc. Effects of non-irradiated and irradiated sewage sludge application on total N and ^{15}N-derived parameters in wheat, 1997–98

Treatment	Plant part	%N	Total N yield (kg/ha)	%Ndff[a]	Fert. N yield (kg/ha)	% NdfSS[b]	Sludge N yield (kg/ha)	%N recovery
T_1	Grain	1.97	64.8	66.2	42.76	–	–	
	Straw	0.37	13.8	67.1	9.23	–	–	
	Total	1.03	78.3	66.4	51.99	–	–	52
T_2	Grain	2.12	17.9	8.59	1.54	–	–	
	Straw	0.34	5.83	8.67	0.51	–	–	
	Total	1.10	24.9	8.19	2.05	–	–	10
T_3	Grain	1.62	29.7	7.10	2.10	13	3.82	
	Straw	0.71	7.08	7.55	0.53	6.8	0.48	
	Total	1.02	41.8	6.29	2.63	10	4.30	13
T_4	Grain	2.36	37.8	8.57	3.41	13	5.09	
	Straw	0.37	10.6	6.65	0.70	7.8	0.82	
	Total	1.09	50.3	8.17	4.11	12	5.91	21
T_5	Grain	2.10	52.1	8.72	4.53	15	7.65	
	Straw	0.33	10.5	7.63	0.80	7.9	0.83	
	Total	1.11	62.6	8.52	5.33	14	8.48	27
T_6	Grain	2.14	62.7	7.67	4.80	14	8.90	
	Straw	0.24	13.4	6.95	0.93	7.7	1.03	
	Total	1.10	76.1	7.53	5.73	13	9.93	29
T_7	Grain	1.95	32.4	8.45	2.73	13	4.05	
	Straw	0.33	7.74	6.35	0.49	8.1	0.63	
	Total	0.99	39.9	8.08	3.22	12	4.68	16
T_8	Grain	2.16	39.1	7.81	3.05	13	4.93	
	Straw	0.43	11.3	7.94	0.89	8.5	0.96	
	Total	1.13	50.4	7.82	3.94	12	5.89	20
T_9	Grain	2.10	55.1	7.68	4.23	13	7.22	
	Straw	0.38	12.9	7.43	0.96	8.7	1.12	
	Total	1.13	68.0	7.63	5.19	12	8.34	26
T_{10}	Grain	2.10	63.5	8.10	5.14	14	9.14	
	Straw	0.46	19.0	9.15	1.73	9.2	1.75	
	Total	1.20	82.5	8.33	6.87	23	10.9	34
$LSD_{0.05}$	Grain	NS	5.7	2.8		NS		
	Straw	NS	4.3	3.1	–	NS	–	–
	Total	NS	8.4	–		–		

Nitrogen derived from [a]fertilizer, [b]sewage sludge.

3.4. Plant uptake of P and heavy metals

3.4.1. 1995–96

The highest P contents in grain and straw were recorded in treatments receiving the lowest rates of non-irradiated and irradiated sludge, i.e. T_3 and T_7 (Table VIa). The application of higher rates of irradiated sewage sludge in most cases decreased the P content in both grain and straw of wheat.

Heavy-metal concentrations in wheat showed variable results. Zinc content was lowest in T_1, which received 100 kg N from urea, whereas the lower rate of 20 kg N/ha (T_2) increased the Zn content in wheat grain and straw considerably (Table VIa). Sludge application also increased the Zn. Copper concentrations both in grain and straw differed significantly among the various treatments, but did not follow any trend. Manganese and Fe contents gave similar results. The Cd concentration in wheat grain remained mostly below detectable limits and in straw it varied from 0.20 to 0.5 mg/kg. The Pb content was, in general, higher in straw than in grain. The levels of heavy metals in wheat straw, in response to application of sludge, were consistent with numerous published reports.

3.4.2. 1997–98

The P levels in wheat grain and straw did not vary significantly among sewage-sludge treatments (Table VIb). The highest P contents were recorded in T_5 and T_6. Significant increases in Zn concentrations both in grain and in straw were observed with various sludge treatments. High Cu contents were recorded in grain and straw with higher rates of sewage-sludge application. The Cd concentrations in wheat grain ranged between 0.02 to 0.03 mg/kg, and in some cases remained below detectable limit by AAS. Concentration of Pb in grain and straw also were low. These data are consistent with observations that heavy-metal removal in harvested crops and by leaching is generally very small [9,10].

3.4.3. 1998–99

The P content in grain was higher in non-irradiated sludge-treated plots (Table VIc). As in previous years, P concentration in grain was higher than in straw. Zinc concentration was highest in grain with treatment T_{10} (66.0 mg/kg) followed by T_6 (63.2 mg/kg), which received 400 kg N equivalent of irradiated and non-irradiated sludge, respectively. Irradiation of sludge did not consistently affect the Zn and Cu content in grain or in straw. Concentrations both of Cd and of Pb were higher compared to previous years, indicating accumulation of heavy metals due to continuous applications of sludge in the same field for several years. Although the Ni content in grain and straw was more or less similar for all the treatment plots as in the previous years, it remained well below the reported toxic values. Reports in the literature indicate Ni values between 10 and 100 ppm in most species [11].

Table VIa. Concentrations of P and heavy metal concentrations in grain and straw of wheat as affected by sewage sludge, 1995–96

Trt.	Grain							Straw						
	%P	Zn	Cu	Mn	Fe	Cd	Pb	%P	Zn	Cu	Mn	Fe	Cd	Pb
		(mg/kg)							(mg/kg)					
T_1	0.47	18.0	7.9	26.0	106	*[a]	*	0.08	9.0	8.2	11.9	157	*	*
T_2	0.51	41.6	10.	32.0	71.2	*	*	0.15	21.4	5.5	13.8	194	*	*
T_3	0.58	50.8	10	43.3	61.0	0.12	0.25	0.24	30.8	5.9	17.9	240	0.20	1.5
T_4	0.52	57.4	9.5	39.0	24.3	0.52	0.50	0.18	41.4	4.5	15.9	221	0.25	1.5
T_5	0.53	54.0	9.0	31.6	31.5	*	*	0.18	32.8	6.3	14.3	215	ND	2.0
T_6	0.47	45.6	7.3	41.4	35.8	*	0.25	0.11	47.9	6.9	10.7	188	ND	1.3
T_7	0.58	66.2	13	39.9	37.0	*	0.20	0.24	21.6	9.7	14.5	270	0.5	0.5
T_8	0.52	52.7	11	39.2	106	*	*	0.17	45.8	8.4	18.6	227	0.25	0.50
T_9	0.49	53.0	7.7	28.6	56.0	–	0.50	0.14	52.9	11	12.2	171	0.25	0.25
T_{10}	0.48	60.5	11	28.5	49.3	–	0.55	0.10	57.3	7.9	17.7	244	0.5	1.5
$LSD_{0.05}$	0.03	8.9	1.7	6.9	21.8	–	–	0.03	7.3	1.9	NS	34.7	–	–

[a] Below detectable limit.

Table VIb. Concentrations of P and heavy metal concentrations in grain and straw of wheat as affected by sewage sludge, 1997–98

Treatment	Grain					Straw				
	%P	Zn	Cu	Cd	Pb	%P	Zn	Cu	Cd	Pb
		(mg/kg)					(mg/kg)			
T_1	0.33	33.6	6.8	*[a]	*	.08	10.4	7.0	*	*
T_2	0.35	35.2	10.1	*	*	0.13	15.4	6.9	*	*
T_3	0.33	40.9	10.5	0.2	0.20	0.14	20.5	6.0	0.3	0.5
T_4	0.35	48.8	9.6	0.5	0.20	0.12	21.8	5.4	0.3	1.6
T_5	0.37	50.6	10.2	*	0.25	0.15	25.0	6.3	0.4	1.0
T_6	0.37	52.0	10.6	0.4	0.25	0.15	30.0	6.0	0.5	1.5
T_7	0.32	42.2	10.8	*	*	0.13	31.3	5.6	*	1.0
T_8	0.34	43.0	11.9	*	0.30	0.14	27.4	7.2	0.2	0.5
T_9	0.34	53.2	12.3	0.3	0.32	0.13	25.2	7.0	0.3	1.0
T_{10}	0.36	50.0	12.3	0.4	0.35	0.14	29.0	7.4	0.4	0.2
LSD$_{0.05}$	NS	3.8	1.4	–	–	NS	3.4	NS	–	–

[a] Below detectable limit.

Table VIc. Concentrations of P and heavy metal concentrations in grain and straw of wheat as affected by sewage sludge, 1998–99

Treatment	Grain						Straw					
	%P	Zn	Cu	Cd	Pb	Ni	%P	Zn	Cu	Cd	Pb	Ni
		(mg/kg)						(mg/kg)				
T_1	0.49	48.0	5.5	.035	0.16	1.18	0.18	31.5	11.5	0.20	0.21	1.20
T_2	0.35	44.8	7.3	.030	0.16	1.20	0.10	30.8	10.3	0.22	0.14	1.28
T_3	0.45	54.6	7.7	.047	0.23	1.25	0.14	36.2	11.7	0.32	0.62	1.34
T_4	0.50	54.8	9.8	.040	0.46	1.29	0.14	41.9	12.5	0.49	0.96	1.38
T_5	0.52	48.1	7.3	.036	0.66	1.34	0.12	44.6	9.5	0.44	1.25	1.42
T_6	0.56	63.2	7.8	.050	0.82	1.39	0.16	47.8	12.8	0.50	1.02	1.45
T_7	0.46	51.2	7.2	.037	0.50	1.21	0.11	36.3	10.8	0.40	0.73	1.30
T_8	0.44	58.3	7.0	.050	0.48	1.32	0.13	45.1	11.7	0.45	0.90	1.43
T_9	0.48	58.8	5.4	.048	0.56	1.36	0.15	45.6	10.6	0.47	0.97	1.46
T_{10}	0.45	66.0	6.9	.045	0.72	1.40	0.17	46.5	10.9	0.46	0.86	1.48
LSD$_{0.05}$	0.05	3.2	NS	NS	NS	NS	0.03	5.2	1.9	NS	NS	NS

3.5. Post-harvest soil analyses

3.5.1. 1995–96

The data on chemical analyses of soils collected immediately after the harvest of wheat are presented in Table VIIa. There was no change in soil pH due to the various treatments with urea and sludge. Plots treated with sewage sludge (non-irradiated and irradiated) showed higher levels of organic C in the top soil compared to initial values. Total-N content increased progressively with the rate of irradiated sewage sludge. Available P contents were similar in soils of various treatment plots, showing no consistent effect of sludge application.

Heavy-metal concentrations in post-harvest soil generally showed no significant differences due to treatment. Available Zn was higher in soil receiving irradiated sludge. It has been reported that heavy metals are more mobile in acid soil, and become less so when the pH is raised [12]. Application of higher rates both of non-irradiated and irradiated sludge contributed significantly towards the total Zn content in soil. The available Cu content was higher in treatment T_6 with 200 kg N from non-irradiated sludge. Similar higher values were also obtained in all treatments with irradiated sludge. The available Cd contents increased with the higher rates of sludge. Available Pb content also increased with sludge application.

Table VIIa. Chemical properties of post-harvest soil, 0–30 cm depth, 1995–96

Treatment	pH^a	Org. C	%N	Av. P	Zn^b	Cu^b	Pb^b	Cd^b
		(%)		(mg/kg)				
T_1	6.6	0.49	0.074	7.5	7.2	4.6	4.8	0.08
T_2	6.7	0.48	0.068	6.6	6.9	4.5	4.6	0.08
T_3	6.8	0.60	0.070	8.1	8.4	4.7	4.2	0.09
T_4	6.8	0.55	0.051	7.5	9.9	4.9	3.3	0.07
T_5	6.9	0.58	0.054	7.9	9.5	5.4	5.1	0.09
T_6	6.7	0.62	0.052	8.1	11.8	5.7	4.9	0.06
T_7	6.7	0.51	0.052	8.0	9.6	5.9	5.1	0.09
T_8	6.7	0.58	0.054	7.6	11.2	5.5	4.8	0.12
T_9	6.8	0.59	0.058	8.4	13.2	6.0	4.8	0.09
T_{10}	6.8	0.64	0.066	8.0	12.1	6.5	5.0	0.10

^{a}1:5 soil:$CaCl_2$. ^{b}0.1 *N* HNO_3 extractable.

Table VIIb. Chemical properties of post-harvest soil, 0–30 cm, 1997–98

Treatment	pH^a	Org. C (%)	%N	Av. P	Zn^b	Cu^b	Pb^b	Cd^b	Ni^b
				(mg/kg)					
T_1	5.5	0.72	0.09	8.10	3.00	4.1	0.81	0.01	0.50
T_2	5.8	0.83	0.12	9.50	9.20	7.4	0.90	0.03	0.55
T_3	5.5	0.76	0.11	10.8	12.6	6.2	0.87	0.02	0.62
T_4	5.5	0.76	0.12	11.2	17.5	10.2	0.81	$*^c$	1.20
T_5	5.8	1.10	0.17	12.0	10.4	7.1	0.91	*	1.05
T_6	5.4	1.20	0.20	14.0	18.0	5.2	1.9	0.01	1.21
T_7	5.7	0.88	0.20	10.6	31.4	6.8	1.7	0.03	0.70
T_8	5.5	1.10	0.20	11.5	18.4	6.6	1.7	*	0.68
T_9	5.6	1.30	0.20	12.6	24.6	11.2	1.7	0.05	1.20
T_{10}	5.6	1.30	0.30	14.5	26.1	12.0	1.8	0.05	1.20

^{a}1:5 soil:$CaCl_2$. ^{b}DTPA extractable. ^{c}Below detectable limit.

Table VIIc. Chemical properties of post-harvest soil, 0–30 cm, 1998–99

Treatment	pH[a]	Org. C	%N	Av. P	Zn	Cu	Pb	Cd	Ni
		(%)		(mg kg)					
T_1	6.7	0.92	0.10	12.2	5.2	4.6	0.65	0.047	0.72
T_2	6.7	0.88	0.05	8.5	5.6	4.2	0.72	0.060	0.60
T_3	6.5	1.08	0.09	10.3	10.5	8.5	0.80	0.14	1.62
T_4	6.8	1.08	0.11	10.2	14.8	9.7	0.78	0.17	1.85
T_5	6.6	1.10	0.11	11.5	16.6	10.8	1.52	0.20	2.80
T_6	6.6	1.48	0.16	14.2	20.3	11.2	2.20	0.32	2.00
T_7	6.6	1.08	0.09	9.3	12.2	7.6	1.20	0.26	0.90
T_8	6.7	1.11	0.11	13.3	14.8	7.0	1.25	0.14	1.05
T_9	6.7	1.11	0.12	12.4	22.0	9.2	2.42	0.18	2.15
T_{10}	6.7	1.20	0.14	15.1	18.8	10.5	2.40	0.20	2.38

[a] 1:5 soil : $CaCl_2$. [b] DTPA extractable.

3.5.2. 1997–98

Chemical analyses of soil collected immediately after the harvest of wheat are presented in Table VIIb. There were no significant differences in soil pH due to treatments with urea and sludge. Organic C increased with rates of sludge application, and ranged from 0.72 to 1.32%. The increasing trend of organic C was recorded with the higher rates irrespective of being irradiated or not. Total N% increased progressively with the increase in the rates of irradiated sewage sludge. The highest amount of P (14.5 mg/kg) was recorded with T_{10}.

Metals extractable with diethylenetriaminepentaacetic acid (DTPA) from post-harvest soil showed that available Zn increased with the higher rates of non-irradiated and of irradiated sludge, and varied between 3.0 to 26 mg/kg. The available Cu content was highest in T_{10} receiving 400 kg N equivalent rate as irradiated sludge; higher values were also obtained in some of the treatments receiving non-irradiated sludge. The available Pb content varied little among treatments. Cadmium content was generally low, and in some cases remained below detection limit. The Ni concentrations in post-harvest soil was influenced by sludge application, and was highest in T_6 (1.21 mg/kg).

3.5.3. 1998–99

Table VIIc shows the chemical-analysis data for the soil collected immediately after the harvest of wheat. Differences in pH due to sludge applications were non-significant. Organic C increased with sludge application, but there were no significant difference among the treatments. The highest organic C (1.48%) was, however, recorded in treatment T_6. The highest total %N was also recorded in T_6 (0.16%). Higher amounts of P (14.2 and 15.1 mg/kg) were recorded with treatments T_6 and T_{10}, which received 400 kg N equivalent of non-irradiated and irradiated sludge, respectively.

DTPA-extractable Zn in post-harvest soil increased with higher rates of sewage sludge, ranging from 10.5 to 22.0 mg/kg. The Cu content showed a similar trend, and was, in general, higher in non-irradiated sludge treatments. Available Pb content was higher in treatments with higher rates both of non-irradiated and of irradiated sludge. In contrast to previous years, Cd content was relatively high during 1998–99 and could be detected in soils of all the treatment plots. Nickel concentration in the soil was also influenced by sludge application; it was highest with T_5.

Table VIII. Residual effects of urea N, and non-irradiated and irradiated sewage sludge on yields of mung bean and rice

Treatment	Mung bean, 1995–96		Rice, 1996–97	
	Grain	Straw	Grain	Straw
	(kg/ha)			
T_1	668	1,802	2,638	3,712
T_2	501	1,648	2,505	3,540
T_3	485	1,621	2,904	4,045
T_4	596	1,749	3,140	4,499
T_5	610	1,759	3,453	4,973
T_6	622	1,768	4,017	5,240
T_7	506	1,612	2,688	3,786
T_8	592	1,749	3,218	4,589
T_9	609	1,767	3,534	5,277
T_{10}	631	1,800	4,045	5,362
$LSD_{0.05}$	41.1	NS	141	293

During the period of study (1995–99) changes in pH values were insignificant. Organic C, total N, and available P were low in the first year, and gradual increases in organic matter and, to some extent, in N and P status were observed. Although DTPA-extractable metals increased with sludge application, the trends varied with year.

Although there was progressive accumulation of Zn, Cu, Cd, Pb, and Ni in plants as well as in the sludge-treated post-harvest soil, the contents were within permissible levels [13].

3.6. Residual effects of sewage sludge

3.6.1. Summer mung bean, 1995–96

The residual effects of application of non-irradiated and of irradiated sewage sludge on the seed yield of summer mung bean are presented in Table VIII. A significantly higher yield (668 kg/ha) was recorded in T_1, i.e. urea at 100 kg N/ha applied to the preceding wheat crop.

A seed yield of 632 kg/ha was recorded with T_{10}, statistically the same as that with T_1 (Table VIII). The seed yield, although lower, increased with rates of both non-irradiated and irradiated sewage sludge applied in the preceding wheat crop. The other agronomic parameters of mung bean were not significantly affected by treatment.

3.6.2. Rice, 1997

The grain and straw yields of rice (Table VIII) indicated a significant carry-over effect of sewage sludge under the sequential cropping pattern of wheat-fallow-rice. Although the highest grain yield (4,045 kg/ha) was recorded in T_{10}, which received 400 kg N/ha equivalent of irradiated sewage sludge, it was not statistically different from that of T_6, i.e. 400 kg N/ha equivalent of non-irradiated sludge. In terms of straw, the highest amount (5,363 kg/ha) was obtained with T_{10}. The grain and straw yields, in general, increased with rate of sewage sludge applied to the preceding wheat crop. The results thus indicate that sewage sludge had a residual effect as an organic amendment on one or two succeeding crops. Residual beneficial effects of sewage sludge on two succeeding crops have been reported before; the effects varied depending on soil type and the amount of sludge applied [14].

3.7. Optional experiment with summer mung bean, 1995–96

Total dry matter yield in N_2-fixing summer mung bean and non-fixing finger millet did not vary significantly in response to application of irradiated sewage sludge (Table IX). Total N yield in mung was higher in treatment T_2 receiving 50 kg irradiated sludge. The total N yield in non-fixing millet did not vary significantly among the three treatments. The ^{15}N-aided studies indicated that fertilizer N yield, %Ndff, %NdfS, and %N utilization were higher in millet than in mung bean. The %Ndff value in mung bean was highest without sewage-sludge, indicating little contribution by sludge towards total plant-N content. Most of the N in mung bean was derived from the atmosphere by fixation of N_2 in the root nodules, with the highest amount (89.5 kg N/ha) recorded with T_2, attributable to the presence of sludge materials in the complex soil system.

Table IX. Dry-matter yield, total and fertilizer N, Ndff, N-utilization in mung bean and the non-fixing reference millet and %Ndfa and amount of N_2 fixed by mung, 1995–96

Treatment	Total dry-matter yield	Total N-yield	Fert.-N yield	%Ndff[a]	Fert. N utiliz'n	%Ndfa[b]	N fixed
	(kg/ha)				(%)		(kg/ha)
T_1 Mung	2,334	77.8	1.0	1.3	5.0	92	71.5
Millet	3,721	46.0	7.5	16	37	–	–
T_2 Mung	2,682	97.1	1.19	1.2	6.0	92	89.5
Millet	2,909	35.0	5.8	16	29	–	–
T_3 Mung	2,149	75.3	0.69	0.91	3.5	93	69.8
Millet	3,056	35.4	4.4	13	20	–	–
LSD$_{0.05}$ Mung	NS	11.3	0.26	0.20	NS	NS	8.7
Millet	NS	NS	NS	2.4	6.3	–	–

N derived from [a]fertilizer, [b]air (i.e. BNF)

Table X. Yields of wheat grain and straw as affected by urea and non-irradiated and irradiated sewage sludge (non-isotope experiment)

	1996–97		1997–98		1998–99	
Treatment	Grain	Straw	Grain	Straw	Grain	Straw
			(kg/ha)			
T_1	3,232	4,073	2,807	3,825	2,348	3,996
T_2	1,116	1,311	1,077	1,345	1,016	1,637
T_3	1,360	1,950	1,492	2,538	1,255	2,023
T_4	1,596	2,067	1,600	2,598	1,738	2,938
T_5	2,208	2,607	2,267	3,555	2,080	3,508
T_6	2,648	2,967	2,449	3,602	2,890	4,090
T_7	1,452	1,867	1,608	2,461	1,282	2,136
T_8	1,632	2,333	1,710	2,593	1,890	3,212
T_9	2,280	2,907	2,265	3,570	2,134	3,630
T_{10}	2,832	3,366	2,512	3,672	3,035	4,327
LSD$_{0.05}$	228	305	144	245	219	200

Table XI. Total dry matter yield of wheat, N-uptake and N contribution from sewage sludge (non-isotope experiments)

Trtmnt	1996–97			1997–98			1998–99		
	Total DM	Total N	N from sludge	Total DM	Total N	N from sludge	Total DM	Total N	N from sludge
				(kg/ha)					
T_1	7,305	55.5	–	6,635	62.4	–	6,343	48.4	–
T_2	2,427	28.0	–	2,907	24.0	–	2,653	20.5	–
T_3	3,310	25.2	7.21	4,014	32.6	8.60	3,278	26.5	6.00
T_4	3,663	27.5	9.57	4,198	34.4	10.4	4,676	34.6	13.8
T_5	4,815	38.5	20.6	5,822	50.5	26.5	5,588	42.7	20.3
T_6	5,615	44.9	26.9	6,052	57.7	33.7	6,980	57.9	37.5
T_7	3,319	26.2	8.30	4,069	34.4	10.4	3,419	26.2	5.70
T_8	3,965	31.3	13.4	4,303	36.3	12.3	5,102	39.8	19.4
T_9	5,187	41.5	23.5	5,835	53.0	29.0	5,763	45.4	24.9
T_{10}	6,198	50.5	32.9	6,184	59.3	35.3	7,362	58.2	37.7
$LSD_{0.05}$	466	3.1	5.3	327	3.4	–	379	5.5	–

3.8. Non-isotope experiment, 1996–98 and 1998–99

The grain and straw yields of wheat obtained from several non-isotope experiments in 1996–97, 1997–98 and 1998–99 are presented in Table X. During 1996–97 and 1997–98 the highest grain and straw yields were recorded with treatment T_1 which received 100 kg N as urea, whereas in 1998–99 the highest yield was obtained with T_{10} followed by T_6, both of which received 400 kg N equivalent of irradiated and non-irradiated sewage sludge, respectively. The lower rates of sludge application in all years produced lower grain and straw yields that increased linearly with the increasing rates of sludge application. It is evident from the data that sludge application significantly increased wheat yield over time in the same field.

3.8.1. Total dry matter, total-N, and sludge-N yields in wheat

The data on total dry-matter yield, total-N uptake and N yield from sewage sludge calculated by the indirect method from the non-isotope experiment are presented in Table XI. Significantly higher total plant dry-matter yield and N uptake were observed with T_1 (100 kg N/ha as urea). The higher rates of sludge application, both irradiated and non-irradiated, also contributed considerably towards total N uptake in wheat. The sludge-N yield increased with the increase in rate of sludge application in all 3 years, the highest being recorded in 1998–99 (37.7 kg N/ha).

The values for N derived from sewage sludge calculated indirectly in these non-isotope experiments were considerably higher than those obtained using ^{15}N. Isotopic methods provide certain advantages and, as such, are generally regarded as more accurate and precise, and thus more reliable, for estimating nutrients from applied sources.

4. GREENHOUSE EXPERIMENT WITH ^{32}P

4.1. Total dry-matter yield and P contribution from sewage sludge

The total dry-matter yield of wheat and ^{32}P-derived parameters obtained from greenhouse experiment are presented in Table XII. The lowest dry-matter yield (9.6 g/pot) was obtained with treatment T_1 (no P) and the highest yield (14.2 g/pot) was observed in T_6 followed by T_{10} (13.6 g/pot), both of which received 400 kg N equivalent as non-irradiated and irradiated sewage sludge, respectively. Total-P yield, was highest in T_{10}, whereas fertilizer-P yield was highest in T_2, in which the recommended dose of 100 kg N/ha as urea and 40 kg P/ha from ^{32}P-labelled orthophosphate were applied.

Table XII. Influence of non-irradiated and irradiated sewage sludge on dry matter yield of wheat, total P, and ^{32}P-derived parameters, 1998–99

Treatment	Total DM (g/pot)	%P	Total P (mg/pot)	%Pdff[a]	Fert. P yield (mg/pot)	%PdfSS[b]	Sludge P yield (mg/pot)	%P recovery
T_1	9.60	0.15	13.9	–	–	–	–	–
T_2	10.1	0.16	15.9	15	2.4	–	–	4.1
T_3	12.5	0.16	20.0	8.6	1.7	44	8.8	2.9
T_4	13.0	0.16	21.0	8.9	1.9	40	8.4	3.1
T_5	12.3	0.16	19.9	8.8	1.7	43	8.3	2.9
T_6	14.2	0.16	22.1	9.7	2.1	37	8.3	3.5
T_7	11.6	0.15	17.1	8.9	1.6	43	7.6	2.6
T_8	13.6	0.16	21.8	8.6	1.9	40	8.8	3.1
T_9	12.4	0.16	19.5	9.0	1.8	42	8.1	2.9
T_{10}	13.6	0.18	24.3	9.2	2.2	44	10.8	3.7
$LSD_{0.05}$	2.3	NS	5.27	1.12	0.46	NS	1.21	0.78

P derived from [a]fertilizer, [b]sewage sludge.

The study using ^{32}P indicated that %PdfSS was highest in T_{10}, which received 400 kg N/ha equivalent of irradiated sludge. The %P-contribution from sewage sludge varied between 37 and 44%.

Significantly higher (10.8 mg P/pot sludge P yield was recorded in T_{10}, varying from 7.6 to 10.8 mg/pot. Irradiated sewage sludge contributed more towards the P pool. The P recovery was, in general, low and ranged between 2.6 and 3.7% with sludge treatments. The %P recovery did not follow any trend with respect to the rates of sludge application.

5. CONCLUSIONS

The results are summarized as follows:

- The irradiated sewage sludge and the sludge-treated soil showed minimum contamination with pathogenic organisms.
- Higher wheat yields were generally produced by application of irradiated compared with non-irradiated sludge.
- By application of 400 kg N/ha equivalent of irradiated sewage sludge, wheat yields were similar to those obtained with the 100 kg N/ha as urea. In some instances, however, there were no statistical difference between 400 kg N/ha equivalent of non-irradiated and irradiated sludge.
- The ^{15}N-aided studies showed that, in general, irradiated sewage sludge contributed more towards the N pool, which was attributed to higher plant dry-matter production. The values for N derived from sewage sludge calculated indirectly from the non-isotope experiment were relatively high and probably are not accurate. It is widely accepted that isotope methods are more precise and provide reliable estimates of availability of nutrients from applied sources.
- The study using ^{32}P also showed that sludge P yield was significant.
- The data on heavy-metal concentrations in plants and post-harvest soil, indicated some accumulation from repeated application of sewage sludge; however, concentrations remained below the permissible limits the literature.
- Marginal improvements in soil status with respect to organic matter and other soil properties were observed.
- Residual beneficial effects of sewage sludge were also observed by its application at higher rates in the third crop under a single cropping pattern.

Based on the results obtained, we conclude that the prospect exists in Bangladesh for safe utilization of irradiated sewage sludge for improving soil fertility and sustaining crop productivity without harming the environment.

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CHARACTERIZATION OF IRRADIATED SEWAGE SLUDGE AND ITS EFFECTS ON SOIL FERTILITY, CROP YIELDS AND NUTRIENT BIOAVAILABILITY

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Abstract

Our aim was to assess the effects of irradiation on sludge properties and subsequent effects on soil fertility and crop yields in a rice-wheat rotation, through laboratory, greenhouse, and field experiments. Irradiation increased the amount of dissolved organic matter (DOM) in sludge (especially of the small molecular-weight fraction) and the availability of N and P. There was a strong increase in N-mineralization in irradiated sludge in the initial 5 weeks of an 11-week incubation as indicated by increased NH_4^+. In the field experiment, yields of wheat and rice increased with increased application rates of sludge, the yields were higher in soil receiving irradiated sludge. However, the yields in soil receiving irradiated and non-irradiated sludge were 70 to 80% and 85 to 90%, respectively, lower than with fertilizer. By comparing the growth of the two crops in irradiated-sludge-amended soil, the yields of wheat were significantly higher than those of rice. Isotope studies in pots and the field showed an increase in efficiency of utilization of ^{32}P and ^{15}N, which explains higher yields in response to irradiated sludge. In addition, the increase in yields was due also to increasedorganic-N mineralization, the formation of large amounts of DOM, the breakdown of the large molecular-weight fraction of DOM, and increases in available nutrients following irradiation. The addition of sludge has long-term effects on fertility by increasing soil organic matter, total N and cation-exchange capacity. However, the economics of sludge irradiation need to be carefully considered for developing countries. Also, potential increases in mobility of heavy metals in soil following irradiated-sludge treatment, owing to increased DOM content, should not be overlooked, especially for coarse-textural soils, which may pose a threat to groundwater.

1. INTRODUCTION

In China, only 22% of total agricultural land is classified by the Soil Science Society of China as “high quality” and about 37% as “low quality”. It is estimated that about 80% of the arable land is deficient in nitrogen (N) and organic matter, 90% is deficient in phosphorus (P), 50% in potassium (K), and 50% is deficient in one or more trace elements. Therefore, recycling organic waste materials from municipal and agricultural sectors would play an important role in alleviating deteriorating soil quality. At present, the sewage sludge that has to be disposed of annually in China exceeds 300,000 ton of dry solid, and is expected to increase substantially in the next five to ten years in accordance with expected requirements for drinking water [1]. Both for practical and for economic reasons, interest in land utilization of sewage sludge has been increasing recently in China

Over the past 15 years, our work has indicated that sludge produced by most sewage-treatment plants in China can be classified as “clean” according to limits set in the United States. These sludges significantly improved soil physical, chemical, and biological properties by increasing soil-water-stable aggregates, soil organic matter, humus content, and N and P contents; they also enhanced soil respiration and total numbers of micro-organisms, and lowered soil bulk density. Crop yields following application of sludge can reach 80% of those obtained with chemical fertilizer in the first crop, on an equal-nutrient-input basis [2–5].

However, the microbial pathogen content, heavy metals, and poor physical structure of sludge constrains its wide application to farmland. One of the conventional methods of killing pathogens in sewage sludge is composting, which is time-consuming and does not eliminate parasites. An alternative, rapid and economic means of eliminating pathogen is irradiation [6].

Companella et al. [7](1989) noted that irradiation promoted decomposition of organic matter in sewage sludge and thus increased the mobility of heavy metals in sludge-treated soils due to release of low-molecular weight compounds. Pandya et al. [8] ascribed increases in crop yields to inactivation of growth inhibitors in sludge by irradiation. Obviously, it is very important to understand positive and negative effects of irradiation on sludge, to develop mechanisms for safer land-disposal.

Therefore, the purposes of this study were:

- to elucidate the effects of irradiation on sludge with emphasis on organic matter mineralization and soluble organic matter,
- to examine changes of crop yields and soil fertility through applying irradiated and non-irradiated sludge in a rice-wheat rotation system, and
- to explore the bioavailability of sludge-borne N, P, and metals as affected by irradiation.

2. MATERIALS AND METHODS

2.1. Sewage sludge

Sewage sludge was collected from a wastewater treatment plant at Suzhou Chengxi, located in eastern Jiangsu Province, where only domestic wastewater is treated. After air-drying in the greenhouse for 7 days, the sewage sludge was broken up and mixed thoroughly. Half was placed in plastic bags and subjected to irradiation at a dosage of 5 kGy. The chemical composition of the selected sludge is shown in Table I.

2.2. Field experiment

A field experiment was carried out on a paddy soil near Nanjing, Jiangsu Province, from 1995 to 1999. The soil was developed from a yellow-brown earth with a heavy loam texture. The total N in the soil was 15.7 mg/g. Seeds of wheat (*Triticum aestivum* var. Yanmai No. 3) were sown at the end of October each year and harvested in early June of the following year. Seedlings of rice (*Oryza sativa* L. var. Wuyujin No.33) were transplanted at the end of June each year and harvested at the end of October of the same year. Treatments are listed in Table II.

Treatments were randomly arranged in 25-m^2 (10×2.5 m) plots with four replications. Superphosphate and potassium chloride were applied to each treatment to supply P and K, 1995 to 1998. In the first year for the project, ^{15}N-labelled ammonia sulphate (10% atom excess) was used as tracer. It was dissolved in water for homogeneous application to 1.44 m^2 (13.5 g in 200 mL) of sub-plot. The same dosage of normal ammonium sulphate was applied around each subplot. In the CK1 treatment (i.e. fertilizer) the dosage of ^{15}N-labelled ammonia sulphate was 101.4 g/subplot (1.0% atom excess).

After crops were harvested, grain and straw were separated, dried at 70°C for 24 h, weighed, milled, and stored in plastic bags pending chemical analyses.

Table I. Chemical composition of sewage sludge used in the field experiment

OC	N	P	K	Ca	Mg	Zn	Cu	Ni	Pb	Cd	pH
(g/kg)					(mg/kg)						
357	43.0	9.40	4.25	24.1	3.97	1,980	129	228	89.2	0.85	6.13

Table II. Treatments for the field experiment

Treatment	1996	1997–1998	1999
CKo	20 kg ^{15}N/ha[a]	No fertilizer (control)	No fertilizer
CK1 (fert.)	150 kg^{15}N/ha[b]	225 kg N/ha as $(NH_4)_2SO_4$	Super-P at 90 kg P_2O_5/ha
ISS1	75 kg N/ha from ISS[c] +20 kg ^{15}N/ha	112.5 kg N/ha from ISS	ISS at 90 kg P_2O_5/ha
ISS2	150 kg N/ha from ISS +20 kg ^{15}N/ha[a]	225 kg N/ha from ISS	ISS 180 kg P_2O_5/ha
ISS3	225 kg N/ha from ISS +20 kg ^{15}N/ha[a]	337.5 kg N/ha from ISS	ISS 270 kg P_2O_5/ha
ISS4	300 kg N/ha from ISS +20 kg ^{15}N/ha[a]	450 kg N/ha from ISS	ISS at 360 kg P_2O_5/ha
NSS1	75 kg N/ha from NSS[d] +20 kg ^{15}N/ha[a]	112.5 kg N/ha from NSS	NSS at 90 kg P_2O_5/ha
NSS2	150 kg N/ha from NSS +20 kg ^{15}N/ha[a]	225 kg N/ha from NSS	NSS at 180 kg P_2O_5/ha
NSS3	225 kg N/ha from NSS +20 kg ^{15}N/ha[a]	337.5 kg N/ha from NSS	NSS at 270 kg P_2O_5/ha
NSS4	300 kg N/ha from NSS +20 kg ^{15}N/ha[a]	450 kg N/ha from NSS	NSS at 360 kg P_2O_5/ha

[a] 10% a.e. as $(NH_4)_2SO_4$. [b] 1.0% a.e. as $(NH_4)_2SO_4$.
[c] Irradiated sewage sludge. [d] Non-irradiated sewage sludge.

Soil samples were collected immediately after harvesting. Fifteen samples were taken from each plot, combined and mixed thoroughly. After being air-dried, the samples were ground to pass through a 20–100-mesh nylon screen, and stored in plastic bottles.

2.3. Pot experiment

This experiment was designed to explore the efficiency of utilization of P from sludge and fertilizer, using ^{32}P. Each pot held 1.5 kg soil. Phosphorus-32 solution was mixed with superphosphate or with irradiated or non-irradiated sludge, and incubated for some time in order that the ^{32}P would be completely adsorbed by sludge or fertilizer. The ^{32}P-labeled fertilizer and sludge were re-ground and mixed thoroughly, then applied to the pots. Wheat was sown on 28 April 1999 and harvested on 16 June 1999, after which the activity ^{32}P in the plants was measured. The efficiency of utilization of P was calculated from the biomass, activity ratio of ^{32}P in the plant, the dosage of ^{32}P in a given amount of P_2O_5 before application, and the application rate of P_2O_5. Seven treatments in triplicate were as follows: CK, zero treatment + ^{32}P; P1, superphosphate at 0.1 g P_2O_5/kg soil + ^{32}P; P2, superphosphate at 0.2 g P_2O_5/kg soil + ^{32}P; NS1, non-irradiated sludge at 0.1 g P_2O_5/kg soil + ^{32}P; NS2, non-irradiated sludge at 0.2 g P_2O_5/kg soil + ^{32}P; IS1, irradiated sludge at 0.1 g P_2O_5/kg soil + ^{32}P; IS2, irradiated sludge at 0.2 g P_2O_5/kg soil + ^{32}P.

2.4. Laboratory study

2.4.1. Incubation

Nitrogen mineralization in sewage sludge was studied anaerobically. Sewage sludge (irradiated and not) aliquots of 0.1 g were mixed with 10-g soil samples and placed in small plastic bottles each containing 10 mL deionized water, and incubated at 40ºC. Soil incubated without sewage sludge was used as the control. Three bottles of each treatment were extracted with 40 mL of 2.5 *M* KCl solution after 1, 3, 5, 7, 9, and 11 weeks of incubation, The extracts were filtered and analysed for N.

2.4.2. Preparation and characterization of dissolved organic matter

Irradiated and non-irradiated sewage sludges were extracted with deionized water at 10:1 (v/w) for 16 h in a reciprocal shaker at 200 rpm and 22ºC. The suspension was centrifuged at 12,000 rpm for 10 min and filtered through a 0.45-μm membrane to remove colloidal material. Dissolved organic matter (DOM) was analysed for pH and dissolved organic carbon (DOC).

The components of each DOM sample were separated according to molecular weight using dialysis bags (Spectra/Por 7) with nominal cutoffs of 1,000, 3,500, 8,000, 15,000, and 25,000 D. Twenty-mL aliquots of DOM were placed in each bag and dialyzed against 1 L of 0.01 *M* K_2SO_4 for 12 h at 4ºC with renewal of external solution twice, then the 0.01 *M* K_2SO_4 was replaced with deionized until completion. After dialysis, portions of the interior solution were withdrawn from each bag for DOC analysis. Distributions of DOM fractions of various molecular sizes were thus obtained.

2.4.3. Dissolved organic matter effect on metal adsorption at different pHs

The effects of pH on Cu adsorption in the presence and absence of DOM were examined to explore how DOM affects metal mobility in sludge-treated soil. Aliquots of 0.4 g soil were shaken for 2 h with 20 mL of solution containing 0.01 *M* KCl (to maintain ionic strength), and 40 mg/L of Cu in a centrifuge tube with or without the addition of 200 mg C/L as DOM. The pH was adjusted to various levels ranging from 2 to 10. After the suspensions were centrifuged and filtered, Cu in the filtrate was determined. The quantity of Cu sorbed was calculated from the difference between the initial and equilibrium concentrations.

2.4.4. Chemical analyses

Total N in plants was determined by the semi-micro Kjeldahl method with steam distillation. Nitrogen-15 in soil and plant samples was determined by mass-spectrometry. Soil pH was determined after suspension in water (1:1). Cation-exchange capacity (CEC) was obtained by exchange with NH_4OAC at pH 7. Soil total organic carbon (TOC) was determined by the Walkley and Black wet dichromate oxidation method. Total P was determined by H_2SO_4-$HClO_4$ digestion and analysed by the molybdenum blue-colour method. Organic N in sludge was separated into total hydrolyzable N, amino-acid N, amino-sugar N, ammonia N, and acid-insoluble N by using 6 *M* heated HCl hydrolysis. Total organic C and Cu were determined by TOC analyser (TOC-5000A, Shimadzu) and flame atomic absorption spectrophotometry (Varian Spectra AA-20).

Table III. Irradiation effect on physicochemical properties of non-irradiated (NSS) and irradiated (ISS) sewage sludge

	Total			DOC	Available	
	OC	N	P		N	P
		(%)		(mg/100g)	(mg/kg)	
NSS	23.7	3.57	1.23	900	4,190	370
ISS	23.6	3.52	1.18	1,680	4,820	450
ISS/NSS	0.99	0.99	0.97	1.86	1.15	1.22

Table IV. DOM fractions of molecular weights (D) in irradiated/non (ISS/NSS) sewage sludge

	<1,000	1,000–3,500	3,500–8,000	8,000–15,000	15,000–25,000	>25,000
	(%)					
NSS	44	10	0.21	0.12	2.0	44
ISS	40	13	0.32	9.7	1.0	36

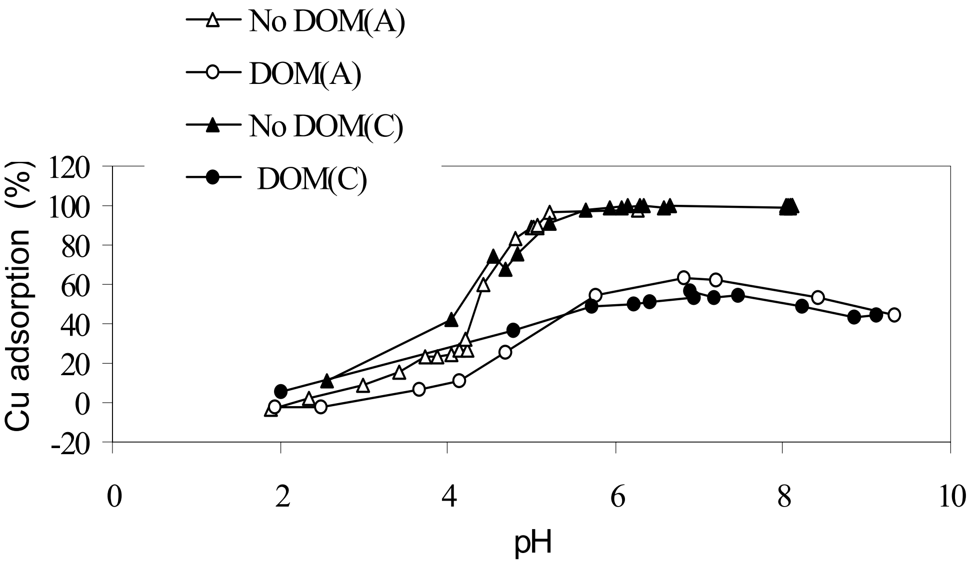

FIG. 1. Effect of pH on Cu adsorption onto acidic (A) and calcareous (C) soils with or without the addition of 300 mg C/L of sludge DOM.

3.RESULTS AND DISCUSSION

3.1. Irradiation effects on sewage sludge

Although the amounts of total organic C, N, and P in sludge were not altered by irradiation, available N and P were increased by 15% and 22%, respectively (Table III). Dissolved organic matter was increased by 86%. It has been documented that DOM in terrestrial ecosystems play an important role in the mobility and translocation of many soil elements, such as N, P, Fe, Al, and trace metals by formation of soluble metal-organic complex [9,10].

The molecular-weight characteristics of DOM from irradiated and non-irradiated sludge are in Table IV. Most of the DOM was in the smallest (<1,000 D) and largest molecular-weight fractions (>25,000); most of the intermediate fractions comprised less than 10% of the total. Irradiation increased the 8,000 to 15,000 D fraction and decreased the >25,000-D fraction. Changes in DOM fraction-distribution are likely to affect behaviour of metals.

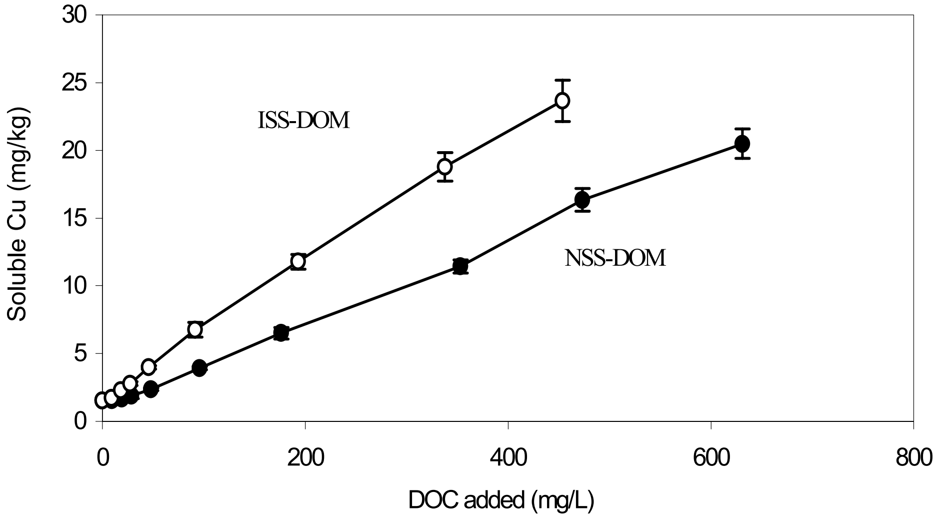

FIG. 2. DOM effect on Cu release of contaminated soil.

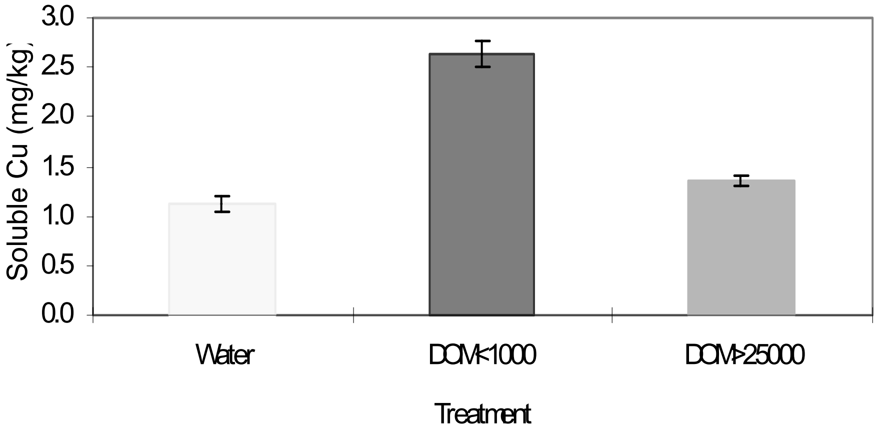

FIG. 3. Effect of DOM fractions (100 mg C/L) from non-irradiated sludge on Cu release in contaminated soil.

Indeed, Fig. 1 shows that sludge-borne DOM inhibited Cu adsorption in the pH range 2 to 10. The inhibition was especially clear with the higher pH values, which implied that DOM could bind with Cu more readily and strongly in alkaline conditions. However, at pH >6.8, Cu adsorption unexpectedly decreased in the presence of sludge DOM for both soils.

Dissolved organic matter derived from irradiated sludge more strongly mobilized soil Cu than did DOM from non-irradiated sludge (Fig. 2). This may result from the increase in the 8,000 to 15,000 D fraction or from the decrease in the higher molecular-weight fraction in irradiated sludge; lower molecular-weight fractions of DOM usually exhibited higher affinity for metal, resulting in greater dissolution of metal in soil (Fig. 3).

Total acid-hydrolyzable N in sludge was increased slightly by irradiation (Table V). Amino-acid N, amino-sugar N and ammonia N were increased by 12%, 36%, and 63%, respectively. The amount of hydrolyzable unknown-N of relative complicated structure was reduced by 22%, indicating that irradiation of sludge increased the available-N pool, resulting in more N for plant growth.

Alteration of sludge organic-N forms affects the mineralization of sludge N (Fig. 4). The amount of N mineralized in irradiated-sludge-treated soil was much higher than that with non-irradiated sludge, especially in the initial 5 weeks after its application. It was concluded that irradiation improved the mineralization of organic N in sludge due to alternation of organic N forms, and possibly due to destruction of complex stable organic-N compounds. It was deduced from these results that irradiation can increase the bioavailability of nutrients in sludge and, thus, possibly increase crop yields.

3.2. Effect of irradiated sewage sludge on crop yield

Field data for seven crops showed that irradiation of sludge increased yields; and both non-irradiated and irradiated sludges enhanced yields relative to CK0 (Fig. 5a, b, c, d). For the 1999 experiment (Fig. 5a), wheat grain yield was increased by 3 to 8% and straw by 7 to 15% for sludge-borne P_2O_5 applications of 0 to 360 kg/ha. For the sludge-born N input experiments from 1995 to 1998 (Fig. 5b, c, d), increases in yields with irradiated sludge were greater for wheat than for rice. Compared to non-irradiated sludge, irradiated sludge increased wheat yields 10 to 30% (mostly 20–30%), whereas increases in rice yields were mostly less than 10%.

Table V. Organic-N forms (mg/g) in non-irradiated (NSS) and irradiated (ISS) sewage sludge

	Total acid-hydrolyzable N	Organic forms		Hydrolyzable	
		Amino-acid N	Amino-sugar N	Ammonia N	Unknown N
NSS	27.9	10.5	5.60	2.42	9.38
ISS	30.2	11.2	7.62	3.95	7.36

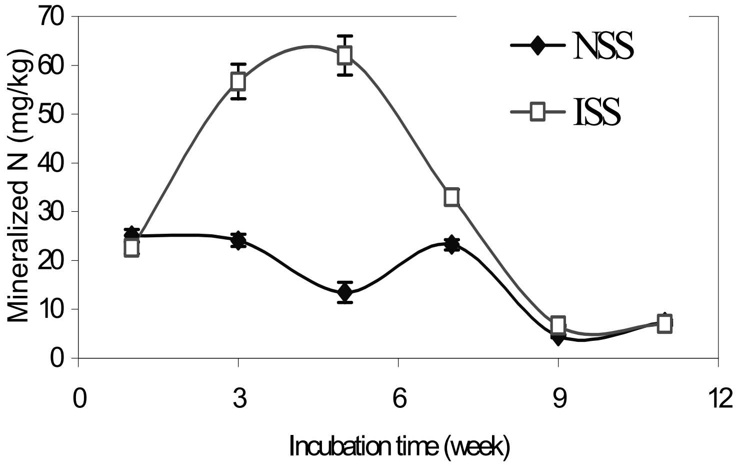

FIG. 4. Mineralization dynamics of sludge-borne N under waterlogged condition.

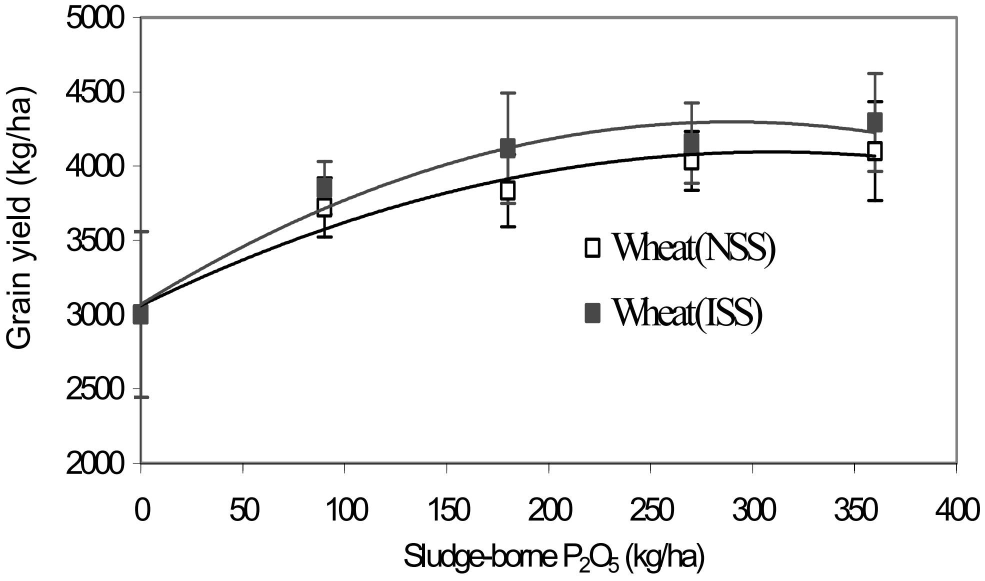

FIG. 5a. Wheat yield in 1999 following application of different rate of sludge-borne phosphorus.

It is noteworthy that for the same input of N or P, mineral fertilizer usually resulted in higher crop yields compared to sludge (Fig. 6a, b). Yields of rice and wheat obtained following the application of non-irradiated sludge and irradiated sludge were about 70 to 80% and 85 to 90% of the yields with fertilizer, respectively, indicating that levels of available N and P provided by sludge through organic matter minerilization were less than those provided directly by fertilizer.

The correlation between crop yield and inputs of sludge-born N or P can be described as nonlinear equations for the two crop for 1995 to 1999 (Table VI). The calculated highest yields can be obtained from these equations for each crop treated with irradiated and non-irradiated sludge. Generally speaking, the maximum yields for rice and wheat can be reached with lower inputs of N or P from irradiated than from non-irradiated sludge. Moreover, the calculated maximum yields were usually higher with irradiated-sludge-treated plots than those for non-irradiated-sludge plots. Once more it is confirmed that irradiated sludge can increase crop yields, consistent with changes of physicochemical properties of sludge due to irradiation.

3.3. Fate of N and P in irradiated and non-irradiated sludge

The contributions of fertilizer N in the different treatments to the rice-soil system, obtained by using ^{15}N, are shown in Table VII. The data indicate that application of sludge reduced recovery of labelled N by rice. Recovery of fertilizer N by rice was highest (29%) in four treatments (CK0, fertilizer, irradiated and non-irradiated sludge). The application of irradiated sludge led to the smallest recovery rate of labelled N by rice (20%), which implies that more N was taken up from irradiated sludge. On the other hand, in comparison with fertilizer alone, 40 to 50% of fertilizer N in the sludge-treated pots remained in the soil. Consequently, fertilizer N losses from surface soil in non-irradiated and irradiated sludge treatments were decreased by 47% and 29%, respectively. This implies that more N was lost if N was provided only as fertilizer, whereas the combination application of sewage sludge with fertilizer decreased N losses, which is of importance for preservation of the environment.

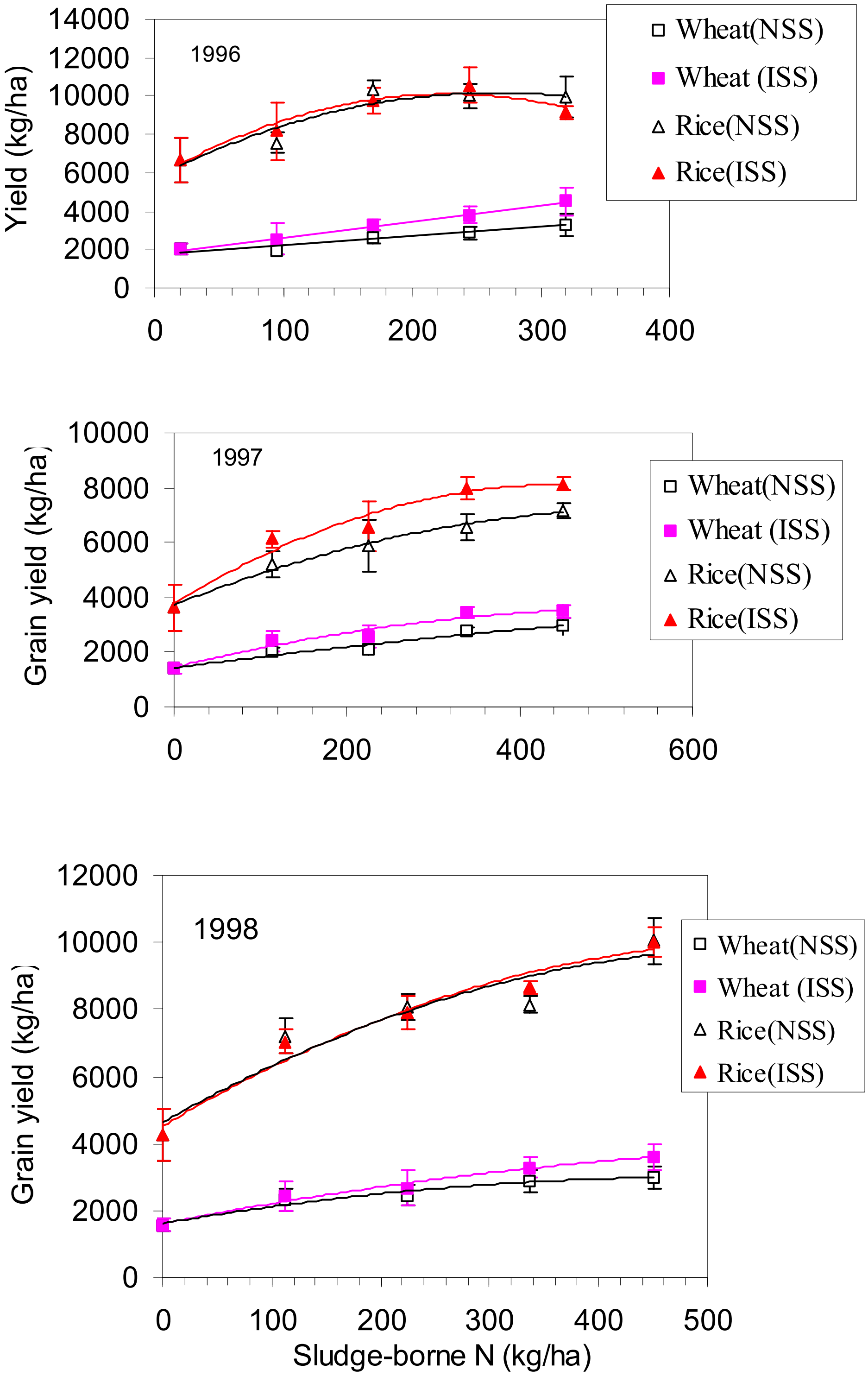

FIG. 5b, c, d. Crop yield obtained with various inputs of N derived from irradiated (ISS) or non-irradiated (NSS) sewage sludge.

There were no significant differences among treatments in terms of N concentration in rice or wheat for 1995 to 1998, partly due to the "dilution effect" (Table VIII). However, the amounts of N absorbed by wheat or rice exhibited obvious differences among the ten treatments. On the basis of the same N input, the relative amounts of N uptake by rice and wheat were fertilizer>irradiated sludge>non-irradiated sludge. A positive correlation between application rate of sludge-born N and the N taken up was found in the field experiment (Fig. 7a, b). Furthermore, N taken up by wheat and rice from irradiated sludge was consistently greater than that from non-irradiated sludge. This indicated that bioavailability of N derived from irradiated sludge was higher than from non-irradiated sludge, directly affecting crop yields (Table IX).

In a pot experiment, ^{32}P was used to explore utilization efficiencies of sludge-borne P and fertilizer-P (Fig. 8). It was noted that application of sludge increased P-utilization efficiency from about 12% in the fertilizer treatment to 17% for non-irradiated sludge-amended soil and 23% for irradiated sludge. In other words, P-utilization efficiency was significantly improved through application of sludge. This may be due to the reduction in soil-P adsorption following application of sludge.

3.4. Effects of sludge and fertilizer on soil fertility

Table X shows selected physicochemical properties of soils following application of sludge and fertilizer. The various treatments caused no significant changes in pH. Addition of sludge significantly increased the organic C and nutrient contents and cation-exchange capacity as compared to the control soil with or without fertilizer treatment. Fertilizer treatment did not cause any significant effect on the physicochemical properties studied except for N and organic C contents, which increased following the addition of fertilizer. Hence, addition of sludge has long-term effects on soil quality by increasing organic matter as well as nutrient availability.

Table VI. Regression equations for crop yield (Y) vs. application rate of sludge-borne N (X) and calculated maximum yield

Crop/sludge	Equation	R^2	Ymax (kg/ha)
1996			
Rice-NSS[a]	$Y=-0.0627X^2+33.328X+5742$	0.891	10,170
Rice-ISS[b]	$Y=-0.0845X^2+38.512X+5710$	0.944	10,100
Wheat-NSS	$Y=4.624X+1764$	0.928	–
Wheat-ISS	$Y=8.309X+1811$	0.996	
1997			
Rice-NSS	$Y=-0.0112X^2+12.515X+3721$	0.990	7,217
Rice-ISS	$Y=-0.0212X^2+19.204X+3778$	0.966	8,127
Wheat-NSS	$Y=-0.0016X^2+4.1211X+1429$	0.958	3,614
Wheat-ISS	$Y=-0.0068X^2+7.6856X+1442$	0.954	4,082
1998			
Rice-NSS	$Y=-0.0163X^2+18.454X+4629$	0.912	9,852
Rice-ISS	$Y=-0.0168X^2+19.258X+4523$	0.965	10,042
Wheat-NSS	$Y=-0.0056X^2+5.5665X+1613$	0.968	2,995
Wheat-ISS	$Y=-0.0042X^2+6.2756X+1620$	0.981	3,964
1999			
Wheat-NSS	$Y=-0.0108X^2+6.6833X+3061$	0.956	4,095
Wheat-ISS	$Y=-0.0146X^2+8.4767X+3070$	0.956	4,500

[a]Non-irradiated sewage sludge. [b]Irradiated sewage sludge.

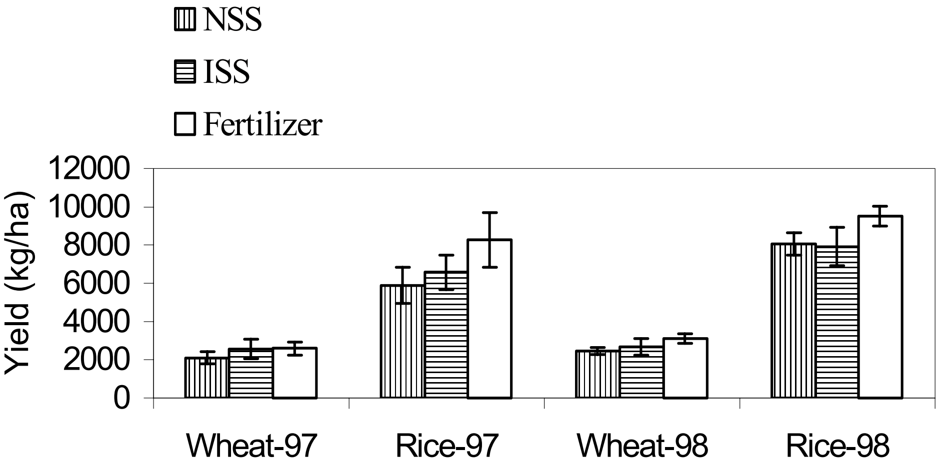

FIG. 6a. Effect of the origin of N [fertilizer, irradiated (ISS) and non-irradiated (NSS) sewage sludge] on crop yield at the same N input of 225 kg/ha

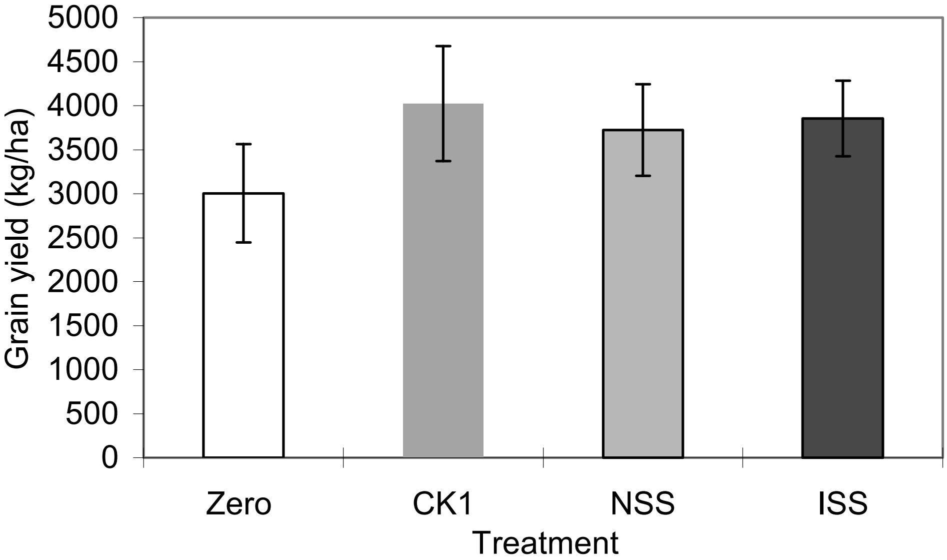

FIG. 6b. Wheat grain yield following application of 90 kg P_2O_5/ha of various origin.

Table VII. The fate of fertilizer ^{15}N in the rice-soil system, 1996

Treatment	Recovered by plant	Remained in soil	Lost from surface soil
	(%)		
Control (CK0)	20.8 ± 3.9	38.5 ± 9.0	40.7
+ Fertilizer	29.1 ± 6.2	17.6 ± 4.3	53.3
+ NSS[a]	23.8 ± 4.5	48.1 ± 3.4	28.1
+ ISS[b]	20.0 ± 2.4	41.9 ± 6.8	38.1

[a] Non-irradiated sewage sludge. [b] Irradiated sewage sludge.

Table VIII. Nitrogen concentration of rice and wheat (mg N/g)

Treatment	1996		1997		1998	
	Grain	Straw	Grain	Straw	Grain	Straw
Rice						
Control (CK0)	14.93±0.21	32.43±0.06	17.58±0.49	2.74±0.33	16.63±0.17	2.63±0.17
Fertilizer	15.2±0.48	2.40±0.07	16.05±0.90	2.41±0.24	15.68±0.29	2.67±0.24
NSS[a]	15.34±0.63	2.61±0.09	17.39±0.25	3.13±0.27	15.69±0.58	2.83±0.21
ISS[b]	15.13±0.28	2.51±0.10	16.79±0.40	2.42±0.07	16.07±0.63	2.75±0.17
Wheat						
Control (CK0)	10.68±0.42	4.43±0.063	10.43±0.33	4.54±0.10	10.73±0.33	4.81±0.34
Fertilizer	11.45±0.87	4.40±0.07	11.10±0.43	6.15±0.15	11.02±0.61	5.08±0.36
NSS	10.91±0.38	4.61±0.09	10.14±0.38	4.83±0.42	10.42±0.22	5.01±0.21
ISS	10.63±0.19	4.51±0.10	10.70±0.36	5.48±0.56	10.38±0.12	5.27±0.21

[a] Non-irradiated sewage sludge. [b] Irradiated sewage sludge.

Table IX. Correlation coefficients (n=10, $r_{0.01}=0.734$) between crop yield and N uptake

	1996	1997	1998
NSS[a]	0.995	0.969	0.984
ISS[b]	0.991	0.947	0.991

[a] Non-irradiated sewage sludge. [b] Irradiated sewage sludge.

Table X. Selected physicochemical properties of soils receiving sludge and fertilizer

Treatment	After the 2nd growing season			After the 6th growing season			
	OC	N	pH	OC	N	CEC	pH
	(mg/g)			(mg/g)		(cmol/kg)	
CK0	14.4a	1.66b	6.96a	12.6c	1.51c	17.75b	6.92a
Fertilizer	14.9a	1.60b	6.81a	14.6b	1.79b	17.97b	6.70a
NSS2[b]	15.1a	1.66b	6.89a	15.0ab	1.89a	18.56a	6.86a
ISS2[c]	14.8a	1.73a	6.90a	15.3a	1.85ab	19.84a	6.79a

[a] Numbers in a column followed by the same letter are not significantly different.

[b] Non-irradiated sewage sludge. [c] Irradiated sewage sludge.

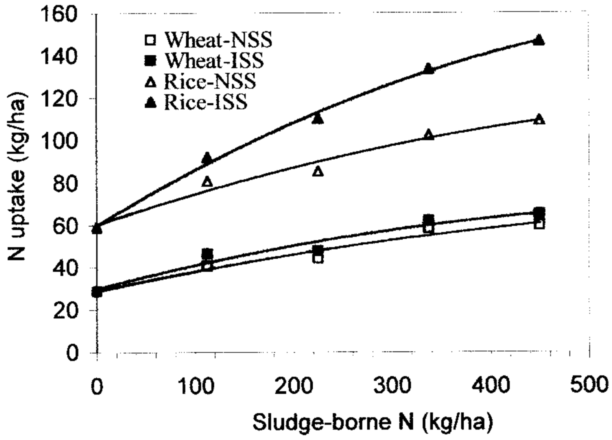

FIG. 7a. Correlation between N uptake by the crop and application rate of sludge-borne N in 1997.

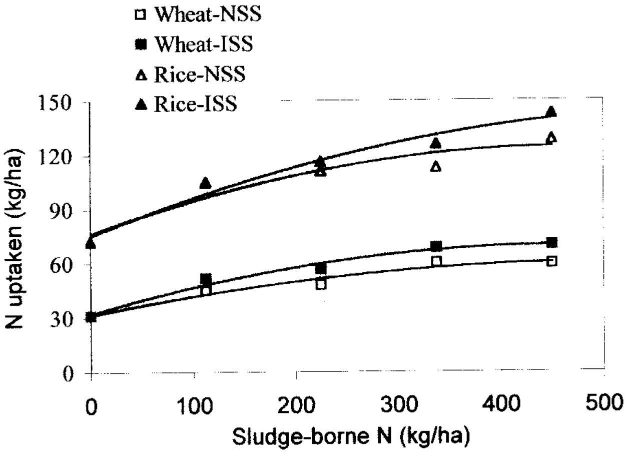

FIG. 7b. Correlation between N uptake by the crop and application rate of sludge-borne N in 1998.

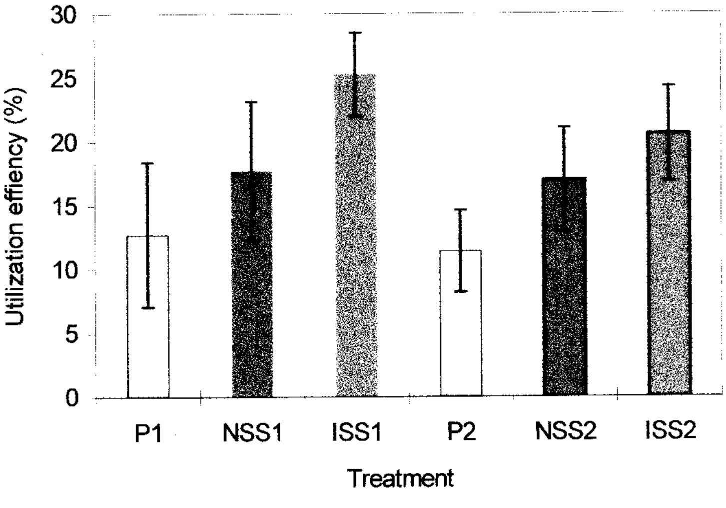

FIG. 8. Utilization efficiency of sludge-borne P by using ^{32}P.

4. CONCLUSIONS

Irradiation had positive effects on the availability of sludge-borne N and P, which, in turn, enhanced crop yields. Yields with irradiated sludge were significantly higher than with non-irradiated sludge. However, yields with irradiated sludge were lower than with mineral fertilizer. Increases in yields in irradiated-sludge-treated soil can be attributed to increased mineralization of inorganic N, changes in the chemical forms of organic N, the formation of large amounts of DOM, the breakdown of large molecular-weight fractions of DOM, and increases in available nutrients. Sludge, with or without irradiation, increased soil fertility as compared to the control, but no significant difference was noted between the two treatments. Moreover, the use of sludge with inorganic N could reduce losses of fertilizer N and mitigate environmental pollution. Therefore, irradiated sludge would have much better effects on soil physicochemical properties as compared with raw sludge.

However, the economics of sludge irradiation would need to be carefully considered before applying this technology in developing countries. On the other hand, the potential increases in the mobility of heavy metals in soil following irradiated-sludge treatment owing to the increase in DOM contents should not be overlooked, especially for coarse-textured soils, which could pose a threat to groundwater quality.

ACKNOWLEDGEMENTS

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IRRADIATED SEWAGE SLUDGE FOR INCREASED CROP PRODUCTION – I. PATHOGENS AND POLYCYCLIC AROMATIC HYDROCARBONS

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Abstract

Samples of raw sewage and sewage sludge, and of fruits and nuts were collected from El-Gabal El-Asfar farm for determination of their pathogen contents. The dominant viable bacterium in the sludge and sewage water was *Escherichia coli*. Bacteria were present in the water phase in much lower counts than in the sludge. Fruits taken from orchards irrigated with raw sewage showed no detectable internal contamination with bacteria, parasites, or viruses. Gamma irradiation reduced pathogen density significantly. The *E. coli* counts in sludge decreased from 10^9 CFU/L to nil with a dose of 6 kGy, and from 10^6 CFU/L to nil at a dose of 1 kGy for sewage water. The vegetative forms of unicellular parasites disappeared at 6 kGy and 1 kGy for sludge and sewage water, respectively. The the dose required for 90% reduction in bacterial count ($D_{10)}$) was 0.67 kGy and 0.17 kGy for sludge and sewage water, respectively. It was concluded 6 kGy is appropriate for sludge, whereas the water phase requires only 1 kGy for decontamination, probably due to the dilution effect. The effect of gamma radiation on degradation of toxic organic pollutants in dry and moist sludges was investigated. Thirteen polycyclic aromatic hydrocarbons (PAHs) were identified in sludge. Non-irradiated moist sludge showed total PAHs of 29 mg/kg, whereas dry sludge contained 5.4 mg/kg. Gamma irradiation reduced PAH content by 53 to 75% for the moist sludge, and 26 to 63% for the dry sludge for doses of 2 to 10 kGy, respectively.

1. INTRODUCTION

In Egypt, 5.24×10^6 m^3/day of wastewater were produced in 1998, and 6.83×10^6 m^3/day are expected in 2002 according to the Ministry of Housing, Utilities and Urban Communities [1], with further increases predicted due to population growth. Such large volumes of wastewater should be considered as a resource rather than a waste. It could be recycled as irrigation, which would be especially useful in Egypt where shortage of water limits agricultural expansion [2]. Also, use of sewage sludge in agriculture as an organic fertilizer is an attractive recycling option, as incineration, land filling, and ocean dumping all result in various forms of environmental degradation. However, sewage sludge contains pathogenic organisms that must be eliminated before it can be safely utilized.

Bacterial pathogens in wastewater comprise salmonellas causing enteric fever, food poisoning and enteritis; shigellas causing bacillary dysentery; brucellas causing Malta (undulant) fever; clostridia causing tetanus and botulism; spirochaetes (*Leptospira* spp.) causing leptospirosis; mycobacteria causing tuberculosis and similar diseases, *Escherichia coli* and other enterobacteria causing enteritis, diarrhea, and pyogenic infections. *Escherichia coli* is the main indicator of degree of water contamination [3–5].

Parasitic pathogens in wastewater are divided into helminthic and protozoic. Species of *Ascaris*, *Anchylostoma*, *Taenia*, and *Schistosoma* cause infections of roundworm, hookworm, tapeworm and bilharziasis. Unicellular protozoa, namely *Entamoeba* causing amoebic dysentery and *Giardia* causing giardiasis, are abundant both in vegetative and cystic forms [3,6].

The list of viruses includes enteroviruses, adenoviruses, rotovirus and reovirus, although it is difficult to recover indigenous viruses from sludge [3].

Several ameliorative processes are available for treatment of raw sewage at conventional wastewater plants, all of which are designed to reduce biological and chemical hazards. Aerobically digested sewage sludge may undergo further treatment before disposal on land, including anaerobic digestion, composting, liming, and thermal disinfection [7,8], while effluent is often chlorinated. These methods aim at making the final discharge biologically and chemically safe. However, not all harmful pathogens in sludge are destroyed; enteric viruses and parasite eggs may not be affected.

Gamma irradiation is used on a commercial scale to disinfect food and medical products, and can also be used to decontaminate sewage sludge and effluent. The treatment of wastewater and sludge with ionizing radiation as a means of disinfection has generated much interest worldwide [3,9]. The use of ionizing radiation (gamma radiation) has proven to be more effective than traditional methods of decontamination [10,11]. The dosage of ionizing radiation required for inactivation may vary with pathogen type and moisture content. Usually, greater doses are required to inactivate ova, mycobacteria, and viruses. Coliform bacilli are unsurpassed in assessing inactivating doses, using the most probable number [12]. Due to difficulties in recovering indigenous viruses from sludge, assessment of the effect of irradiation on their viability is, therefore, hindered, and a model system is used [13].

Sewage sludges also contain a complex mixture of organic contaminants, including polycyclic aromatic hydrocarbons (PAHs). Some of these compounds and their derivatives are highly toxic, even at low concentrations. Irradiation produces high-energy excitation and ionization of these molecules, and the formation of free radicals that create strong oxidation conditions in the sludge matrix [14]. Very little is known about the presence of various PAHs in sewage sludge or the effect of gamma irradiation on their persistence.

The objectives of this investigation were to:

- determine the lowest effective radiation dose for pathogen elimination in sludge and wastewater, determine internal fruit contamination by pathogens, and
- identify PAHs in moist and dry sludge and determine the effect of gamma irradiation on their persistence.

2. MATERIALS AND METHODS

Samples of raw sewage, sewage sludge, fruits and nuts were collected from El-Gabal El-Asfar farm, where raw sewage from the greater Cairo area has been used to irrigate orchards for over 80 years.

2.2. Sampling and sample preparation

Samples of raw sewage were collected at the farm inlet in sterilized plastic bottles. Samples of sludge (both moist and air-dried) were collected from six locations within various drying beds; at each location, six sub-samples were collected and mixed to form composite samples. The dried sludge was crushed to pass through a 2-mm sieve. Dry and moist sludge samples were packed into 0.5-kg plastic bags, which were sealed using an electric sealer.

Ripe citrus fruits (mandarin, sour orange and navel orange) and pecans were collected from six locations in the farm. The fruits and nuts were thoroughly washed with sterile distilled water in the laboratory. Fruits were squeezed for juice and nuts were hashed after shell removal.

2.3. Irradiation

Samples of raw sewage and sludge were sent directly to the irradiation facility, at the National Centre for Research and Radiation Technology (NCRRT), Nasr City, Cairo. Samples were exposed to gamma radiation (^{60}Co) in the Indian experimental-scale gamma-radiation unit. Treatments included a control (non-irradiated) and irradiation at 1 to 10 kGy in 1-kGy increments. All treatments were duplicated.

2.4. Pathogen assays

2.4.1. Bacteria

Samples were processed using standard methods [6]. Bacteria were identified according to Collee et al. [15] with API 20E species confirmation [16,17].

Coliform viable counts were performed on all treatments. Presumptive counts were calculated per unit volume. Each 50-g sample of sludge was suspended in 1 L of sterile distilled water [5]. Confirmatory most probable number (MPN) counts were made at 44ºC using fluid MacConkey medium in a modified Eijkman test [5,6].

2.4.2. Parasites

Parasite ova, vegetative forms, and cysts were identified systematically and morphologically [6].

2.4.3. Viruses

Rotavirus presence was determined by an ELISA kit for direct detection of antigens (Diamedix Corp., Miami).

2.5. Polycyclic aromatic hydrocarbons

Sludge samples were mixed with anhydrous sodium sulphate and sea sand. The mixture was packed in a glass column and extracted with hexane/acetone (2/1, v/v). The crude extract was rotary-evaporated to 10 mL and cleaned up by gel-permeation chromatography. The concentrated extracts were subjected to further clean-up steps using a column of aluminium oxide and silica gel. Finally, GC/MS screening analysis was performed in full-scan mode combined with an NIST-library search in order to identify the chief pollutants. The extraction procedure was based on the principles of the DFG (German Research Association) S19 method.

3. RESULTS

Sewage sludge and sewage water contained several harmful pathogens of medical importance (Table I).

3.1. Efficiency of gamma irradiation in removing pathogenic microorganisms

3.1.1. Bacteria

Bacteria were present in sludge in very high numbers. The quantitative viable counts of coliform bacilli showed significant reductions as the irradiation dose increased (Fig. 1). The viable counts were 10^9, 10^9, 10^9, 10^5, 10^3, 10^2 CFU/L for 0, 1, 2, 3, 4, and 5 kGy, respectively. The initial count for the sludge (10^9 CFU/L) was completely eliminated at 6 kGy (Table II).

Table I. Pathogens of medical importance found in raw sewage and sludge

Bacterium	Parasite	Virus
E. coli	*Ascaris* egg	Rotavirus
Klebsiella	*Ancylostoma* egg	
Proteus	*Taenia* egg	
Salmonella	*Ascaris* worm	
	Faschiola worm	
	Entamoeba (c&v)[a]	
	Giardia (c&v)	

[a] Cyst and vegetative forms.

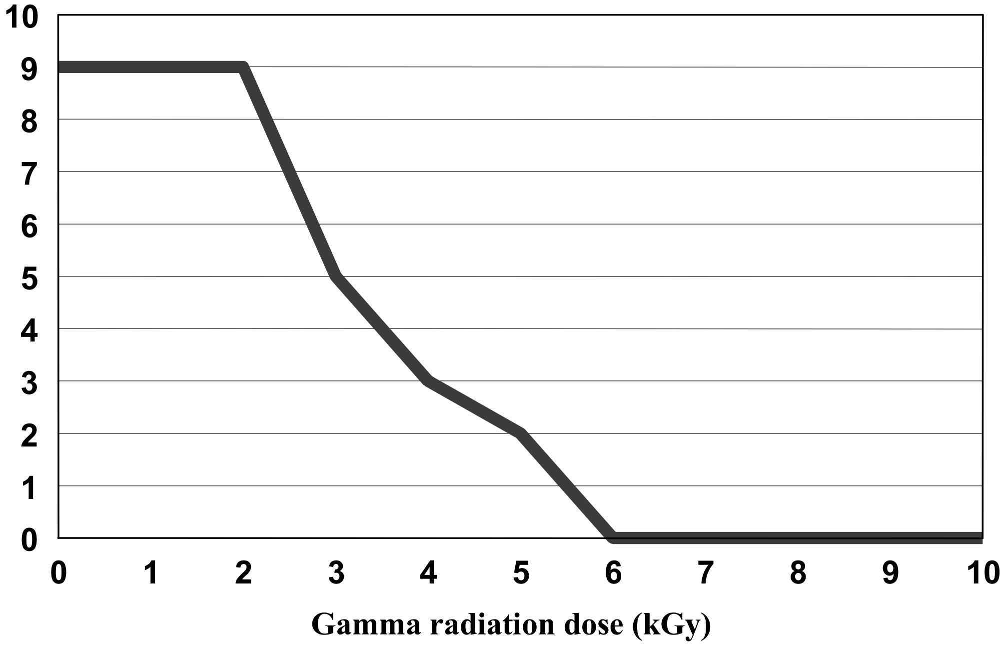

FIG. 1. Total viable counts (log_{10} CFU/mL) of coliforms in sewage sludge as affected by radiation.

Bacteria were in much lower numbers in raw sewage and required a much lower dose for elimination; the initial count (10^6 CFU/L) was completely eliminated by 1 kGy (Table II). Bacteria in wastewater tend to settle with the solid material and are, therefore, concentrated in the sludge [3]. Bacterial counts in sewage water represented $\times 10^{-3}$ of the counts in sludge. The D_{10} for sludge, which could be considered the target dose, was 0.67 kGy. An interesting observation was that treatment with 1 or 2 kGy showed apparent increases in viable counts suggesting either treatment failure or even enhancement of growth due to inadequate treatment.

3.1.2. Parasites

The vegetative forms of unicellular parasites (*Entamoeba* and *Giardia*) were killed at 6 kGy and 1 kGy dose for sludge and raw sewage, respectively, within the dose range of 1 to 10 kGy that was applied (Table II). Therefore, vegetative forms of unicellular parasites were eliminated by the same radiation doses that were effective for coliform bacilli. Hence, the decline in the vegetative forms of unicellular parasites had the same value as an index of treatment efficiency as the decline in coliforms.

3.1.3. Viruses

Rotavirus was detected in all samples. No differences were observed between irradiation doses. Since it is difficult to recover indigenous viruses from sludge, assessment of the effect of irradiation on the viability of viruses was impossible.

Table II. Viability assessment and ionizing radiation effectiveness using coliform bacilli and unicellular vegetative protozoa as quantitative indices

Type of waste	Coliform bacilli			Unicellular vegetative protozoa
	Initial count (CFU/L)[a]	Disinfecting dose (kGy)	D_{10} (kGy)	Disinfecting dose (kGy)
Sludge	10^9	6	0.67	6
Raw sewage	10^6	1	0.17	1

[a] 50 g/L content.

Table III. Effect of γ-irradiation on polycyclic aromatic hydrocarbon degradation

Dose (kGy)	Moist sludge (mg/kg)	Reduction (%)	Dry sludge (mg/kg)	Reduction (%)
0	29.0	–	5.5	–
2	13.7	53	4.0	26
4	6.8	77	2.4	56
6	6.1	79	3.1	43
8	10.1	65	2.8	47
10	7.2	75	2.0	63

3.2. Contamination of fruits and nuts

Neither citrus juice nor hashed pecans contained streptococci, vegetative or cystic parasites, or rotavirus; total coliform counts were nil. Preliminary data showed possible contamination with microorganisms, which was eliminated by proper cleaning of external surfaces. Any health risk was from external rather than internal contamination.

3.3. Polycyclic aromatic hydrocarbons

The following toxicants were identified: naphthalene, acenaphthylene, acenaphtene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benzo(b)fluoranthene, benzo(a)pyrene, lendeno(1,2,3-

cd)pyrene, dibenzo(ah)anthracene and benzo(ghi)perylene. Non-irradiated moist sludge showed total PAHs of 29 mg/kg, whereas dry sludge contained 5.4 mg/kg. Gamma irradiation reduced PAH content (Table III). Magnitude of decline varied according to the applied dose; 77, 79, 65 and 75% for the moist sludge, and 26, 56, 43, 47 and 63% for the dry sludge for irradiation doses of 2, 4, 6, 8, and 10 kGy, respectively . Thus, utility of gamma irradiation was demonstrated for decreasing toxic PAHs

4. DISCUSSION

The total coliform count is regarded as an index of sanitary condition of water [12]. Coliform bacilli (e.g. *E. coli*) represent a quantitative index that confirms and denotes the efficiency of treatment by the reduction in viable count [18]. Indeed, *E. coli* not only represent a major cause of diarrhea for all ages, but is also the main indicator of faecal pollution [18].

The literature describes a range of proposed doses of radiation, 2.5 kGy to 1 MGy with different end points, for eradication of coliforms; sterility may be reached at 2.5 MGy [19]. On the other hand, an absorbed dose of a few kGy was found sufficient to inactivate coliforms and salmonellas in liquid sludge [3].

The disappearance of vegetative forms of unicellular parasites is an indicator of the efficiency of treatment. Brandon [3] found that a dose of 1.5 kGy was sufficient to prevent embryonation in more than 99.9% of *Ascaris* eggs added to liquid sludge. He also found that a dose of 1.0 MGy was sufficient to ensure the inactivation of *Ascaris* eggs naturally present in digested sludge filter cake and in composted sludge. Those results conflict with previous indications that 1.0 MGy failed to prevent embryonation of naturally occurring *Ascaris* eggs.

Yeager and O’Brien [20] showed that an absorbed dose of 1 to 1.5 kGy resulted in a two-orders-of-magnitude reduction in *Ascaris* ova in sewage sludge. Similar results were obtained by Horak [21] who found no viable ova at doses >1.1 kGy. Lower doses were found to be effective by Capizzi et al. [22]; no viable ova were found after exposure to 0.75 kGy (D_{10} at 0.39 kGy). At a meeting of radiation scientists, Pikaev [9] concluded that 3 to 5 kGy of irradiation was adequate for complete inactivation of pathogens in sewage sludge.

5. CONCLUSIONS

Doses of 1 and 6 kGy were sufficient for disinfection of raw sewage and sludge, respectively, received at El-Gabal El-Asfar farm from the greater Cairo area. The lower dose required for raw sewage disinfection is mainly due to the lower initial contamination level (10^3 CFU/L) compared with sludge (10^9 CFU/L). D_{10} values of 0.67 kGy and 0.17 kGy were obtained for raw sewage and sludge, respectively.

No internal contamination of fruit or nuts by bacteria, parasites, or viruses was detected. Although this investigation demonstrated that total PAHs in sewage sludge were reduced by gamma irradiation, further studies are required on individual PAHs before firm conclusions can be drawn, especially in view of sparse and sometimes conflicting information in the literature.

Gamma irradiation offers the possibility for safe disposal of sludge on agricultural land, with possible benefits in terms of soil properties and crop yields (Part II of this series) and crop nutrition through the supply of macronutrients (Part III) and micronutrients (Part IV). Nevertheless, the hazard of non-nutrient heavy metals in sludge needs to be evaluated (Part V).

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IRRADIATED SEWAGE SLUDGE FOR INCREASED CROP PRODUCTION – II. EFFECTS ON SOIL PROPERTIES AND TOMATO YIELDS

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Abstract

Irradiated and non-irradiated sewage sludge from El-Gabal El-Asfar Farm near Cairo, was applied to sandy and calcareous soils at rates of 20, 40, 60, and 80 t/ha. Treatments with and without a basal fertilizer dressing were included for comparison. Soil organic matter, water-holding capacity, cation-exchange capacity, electrical conductivity, and concentrations of available N, P, and K increased under sludge treatments. However, total carbonate, soil pH, and bulk density decreased with increasing application rates of sludge. The changes were higher in the sandy than in the calcareous soil. There was no significant difference between irradiated and non-irradiated sludge treatments in any of the measured soil properties. The concentrations of available N, P, and K were significantly correlated with organic matter content in both soils. Improved soil fertility due to sludge addition was reflected in higher yields of tomato and enhanced fruit quality.

1. INTRODUCTION

Egypt has an arid environment; competition for water among urban, industrial, and rural sectors causes demand to exceed supply. About 90% of the available water supply is used for irrigated agriculture. The rapidly expanding population creates the need for increased food production, which may be met either from greater productivity on existing farmland or expansion into the sandy and calcareous soils of the desert. Expansion of cultivated land will require external inputs of water, organic matter, and plant nutrients. Water is considered to be the main constraint to expansion, with reuse of urban wastewater providing a potential solution. The production of wastewater in greater Cairo alone is estimated to be 5 million m^3/day, sufficient to irrigate 208,000 ha/year. In addition, sludge generated from the treatment of wastewater amounts to 150,000 t/year, with potential use as an organic fertilizer.

The agricultural use of sludge provides an alternative to other disposal options, such as river or ocean dumping, incineration, and landfill. Sewage sludge contains organic matter, nutrients (N, P, S), and micronutrients (Zn, Cu, Fe, Mn) that are essential for plant growth and are often deficient in sandy [1] and calcareous [2] soils. Therefore, land application of sludge can help to remedy nutrient deficiencies [3] and increase yields [4]. In addition, it is reported that application of sludge can improve soil physical properties, although the evidence is less clear than effects on soil chemical properties.

Sewage sludge generated in wastewater-treatment plants through conventional primary and secondary treatment processes generally undergoes further stabilization before its disposal on land. Several methods are available, including anaerobic digestion, composting, liming, and thermal disinfection [5–9]. These methods aim at making the sludge biologically and chemically safe.

Recently, the use of ionizing radiation has proven to be effective in hygenization of sewage sludge [10,11]. Gamma irradiation of sewage sludge, in addition to removing harmful pathogenic organisms, can lead to changes in physical and chemical properties [12]. Our aim was to evaluate the effects of γ-irradiation on sludge properties and to examine the use of sewage sludge, irradiated and non-irradiated, as a chemical and physical ameliorant for calcareous and sandy soils.

2. MATERIALS AND METHODS

2.1. Sampling and preparation of sewage sludge

Raw sludge was collected from drying beds at El-Gabal El-Asfar farm, where sewage from the greater Cairo area is used to irrigate orchards. Samples were collected from six locations within various drying beds; at each location six sub-samples were collected and mixed to form composite samples. The dried sludge was crushed to pass through a 2-mm sieve.

2.2. Irradiation of sludge

Each sludge sample was divided into two equal portions for irradiated and non-irradiated treatments, and sealed into 5-kg plastic bags using an electric sealer machine. Every eight bags were inserted into a cardboard carton with dimensions 45×45×90 cm. Irradiation was performed at the National Center for Research and Radiation Technology using the industrial-scale facility, by exposure of the cartons to a ^{60}Co source of type JR-6500. The cartons were irradiated in a conveyor system to a dose of 600 krad (6 kGy) at a dose rate of 20.5 rad/sec. The temperature during irradiation was 37°C. Radiachromic film FW160 was used to monitor the absorbed dose.

2.3. Treatments and experimental design

Two field experiments were conducted, for two consecutive years. One experiment was located at the Egyptian Atomic Energy Authority Experimental Farm, Anshas (1.8% sand; texture class, sand), and another was set up on a calcareous soil in the Sinia desert (2.6% sand; texture class, loamy sand). Irradiated and non-irradiated sewage sludge was incorporated into the soil to 25 cm depth. Treatments were arranged in a randomized complete block design. Each block contained ten treatments (Table I) and each treatment was replicated four times.

Tomato seedlings (cv. GS) were purchased from a local nursery and transplanted into each treatment plot of area 10 m^2. They were planted in rows 1 m apart with 0.5 m between seedlings. Thus each treatment contained forty plants. Tensiometers were installed to monitor soil-moisture tension. Plants were irrigated using a drip system according to evapotranspiration demand. There were no visible symptoms of stress throughout the growing period.

Table I. Treatments applied in field experiments on sandy and calcareous soils

Treatment	Sludge rate (t/ha)	Fertilizer rate (kg/ha)[a]
Control	0	0
Fertilizer	0	N, 286; P_2O_5, 143; K_2O, 167
Non-irradiated sludge	20	0
	40	0
	60	0
	80	0
Irradiated sludge	20	0
	40	0
	60	0
	80	0

[a] N, ammonium sulphate; P, superphosphate; K, potassium sulphate.

2.4. Plant and soil sampling

Tomato fruits were collected as they ripened and were weighed for yield determination. Soil samples (0–25 cm depth) were collected at the end of the growing season, from the middle of each plot to minimize boundary effects. An auger was used to obtain six sub-samples, which were mixed to form composite samples. The samples were air-dried, crushed to pass through a 2-mm sieve, and stored for analysis.

2.5. Analytical procedures

The pH, electrical conductivity (EC), organic matter (OM) and total carbonate content (TCC), of sewage sludge and soils, were determined using standard methods [13,14]. Total N in sewage sludge was determined after digestion in an H_2SO_4-$HClO_4$ mixture [13]. Soil water-holding capacity (WHC), bulk density (BD), clay content, and cation exchange capacity (CEC) were also determined by standard methods [13,14]. Available P was extracted with $NaHCO_3$ and measured as described by Olsen and Sommers [15]. Available K was extracted using 1 *M* neutral CH_3COONH_4 [16] and measured with an atomic absorption spectrometer (Perkin-Elmer 2830). Available N was extracted with 2 *M* KCl and analyzed colorimetrically [17]. Total sugars, total soluble solids, and acidity in fresh tomatoes harvested from the sandy soil were determined [18].

2.6. Statistical analysis

The data representing two years were subjected to analysis of variance, and LSD values and correlation coefficient (r) were determined using the MicroStat statistical analysis package.

3. RESULTS AND DISCUSSION

3.1. Sludge characteristics

The sewage sludge had a pH close to neutral, high organic-matter content, high total-N content, and high contents of P and K in bio-available forms (Table II). Similar results have been reported by others [19–21]. Sludge also contained soluble salts and carbonate. Differences in chemical properties between irradiated and non-irradiated sludge were not significant.

Table II. Chemical characteristics of raw sewage sludge from El-Gabal El-Asfar farm

Sludge properties	Irradiated sludge	Non-irradiated sludge
pH (1:2.5)	6.80	6.64
EC (dS/m, 1:2.5)	4.11	4.27
$CaCO_3$ (%)	3.01	3.25
Organic matter (%)	44.5	44.9
Total N (%)	2.00	2.10
C/N ratio	12.4	13.0
Available P (μg/g)	464	473
Available K (μg/g)	390	380

3.2. Soil properties

Both soils contained carbonate, and had pH values of 8.2 to 8.4 (control treatment, Table III). The soils were coarse-textured (sand and loamy sand) and were, therefore, of low organic-matter content, very low CEC, low WHC and high BD. The high organic matter content of sludge improved soil physical and chemical characteristics, including an increase in the WHC, a decrease in BD), and an increase in CEC, confirming previous findings [22–26]. Soil pH decreased with sludge addition, which is consistent with results reported by Aboulroos et al. [27]. The decrease in pH was greater for the sandy soil than for the calcareous soil, reflecting the higher buffering capacity of the latter due to higher content of clay and $CaCO_3$. A negative effect of sludge addition was an increase in soluble salt content of the soil as indicated by increased electrical conductivity, which agrees with results reported previously [26,27].

Table III. Physical and chemical properties of sandy and calcareous soils treated with irradiated and non-irradiated sewage sludge

Treatment	Sandy soil							
	Sludge rate (t/ha)	pH 1:2.5	OM (%)	$CaCO_3$ (%)	CEC (meq/100g)	EC (dS/m)	WHC (%)	BD (g/cm^3)
Control	0	8.15	0.43	2.38	2.18	0.65	11.2	1.67
Fertilizer	0	7.93	0.56	2.44	3.13	0.70	13.3	1.59
Non-irradiate sludge	20	7.57	1.03	2.13	3.80	1.83	14.3	1.54
	40	7.39	1.74	1.83	4.70	1.94	14.7	1.50
	60	7.27	2.38	1.65	6.53	2.10	15.3	1.45
	80	7.08	2.95	1.31	10.4	2.56	16.0	1.38
Irradiated sludge	20	7.74	1.26	2.24	3.18	1.69	13.8	1.49
	40	7.59	1.76	1.91	4.18	2.17	16.0	1.40
	60	7.38	2.17	1.58	6.03	2.61	18.3	1.38
	80	7.18	2.79	1.22	9.38	2.95	19.7	1.27
LSD$_{0.05}$		0.21	0.20	0.35	0.78	0.35	0.74	0.14
	Calcareous soil							
Control	0	8.40	0.36	50.3	4.04	3.58	27.1	1.43
Fertilizer	0	8.12	0.50	46.7	5.03	3.67	28.3	1.37
Non-irradiated sludge	20	8.02	1.24	45.6	6.55	4.83	30.0	1.30
	40	7.85	1.49	43.8	8.56	5.03	30.6	1.25
	60	7.66	1.90	42.9	11.0	5.48	32.4	1.21
	80	7.43	2.26	42.0	16.3	6.28	33.0	1.29
Irradiated sludge	20	7.89	1.08	47.5	6.00	3.40	28.3	1.29
	40	7.62	1.82	43.9	9.00	4.08	28.9	1.23
	60	7.47	2.48	41.9	11.2	5.13	29.5	1.17
	80	7.32	2.74	42.1	16.4	6.03	33.7	1.09
LSD$_{0.05}$		0.22	0.24	2.6	1.82	0.42	0.95	0.11

Table IV. Concentrations of available N, P and K in soils amended with irradiated and non-irradiated sludge

Treatment	Sludge rate	Sandy soil			Calcareous soil		
		N	P	K	N	P	K
	(t/ha)	(µg/g)					
Control	0	4.5	3.8	41.3	0.6	3.7	55.2
Fertilizer	0	12.2	6.0	55.5	0.8	9.1	68.7
Non-irradiated sludge	20	26.4	16.8	120	3.4	12.8	186
	40	43.3	29.8	249	8.1	23.4	334
	60	50.7	52.5	280	13.2	41.9	687
	80	77.2	75.4	384	27.5	60.0	913
Irradiated sludge	20	31.3	22.7	184	5.9	16.7	197
	40	42.4	38.4	256	11.4	26.0	329
	60	63.1	57.2	272	16.1	49.1	523
	80	88.7	79.4	390	24.4	63.5	833
$LSD_{0.05}$		12.8	6.9	55.4	3.6	7.2	85.6

3.3. Available N, P, and K

Increasing application rates of irradiated and non-irradiated sludge significantly available N, P and K in the soil compared with the control (Table IV). Similar results were obtained by Werner et al. [28]. At the highest rate of application (80 t/ha) the concentrations of available N, P and K were 32-, 7- and 12-fold higher than the control in the calcareous soil, and 7-, 13- and 7-fold higher in the sandy soil, respectively. The sandy soil contained much higher concentrations of available N and P than the calcareous soil, possibly due to the precipitation of P in unavailable forms and losses of N at the higher pH. Increases in major plant nutrients N, P, K Ca, and Mg with sludge amendment have been reported before [29]. Data in Table IV indicate that the concentrations of available N, P, and K were adequate to meet plant demands, and no deficiency symptoms were observed in the tomatoes. Highly significant correlations were found between concentrations of available N, P and K and organic-matter content (r = +0.700**, +0.956**, and +0.783**, respectively). No significant differences were found between irradiated and non-irradiated treatments in soil concentrations of available N, P and K.

3.4. Tomato yield and quality

Tomato yields increased with rate of application of irradiated and non-irradiated sewage sludge, with larger increases in the sandy soil (Table V). The lower yields in the calcareous soil may have been due to the higher content of $CaCO_3$ (up to 50%), resulting in precipitation of sludge-derived micronutrients (Cu, Zn, Fe, Mn) thus decreasing their availability for plant uptake. The increases in tomato yields were 1.7-, 2.7-, 4.0-, and 5.7-fold in the calcareous soil, and 2.0-, 3.5-, 5.3-, and 7.0-fold in the sandy soil for 20, 40, 60 and 80 t/ha respectively, averaged over irradiated and non-irradiated treatments.

Total sugar content, acidity and total soluble solids were higher in all treatments compared with the control (Table VI), and each increased as the sewage sludge application rate increased. There were no significant differences between irradiated and non-irradiated sludge treatments in their effects on fruit-quality values, which were within normal ranges as defined by Watt and Merrill [30]. Total sugars in fruits grown on soils amended with sewage sludge at 80 t/ha increased were by 1.6-fold. This was mainly due to growth enhancement and increases in chlorophyll content (data not shown), which increased photosynthesis.

Table V. Fresh weight yields of tomato in sandy and calcareous soils amended with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate	Yield	
		Sandy soil	Calcareous soil
	(t/ha)		
Control	0	2.3	3.3
Fertilizer	0	5.1	5.7
Non-irradiated sludge	20	10.0	9.7
	40	17.9	14.9
	60	26.7	21.5
	80	36.0	31.5
Irradiated sludge	20	10.8	9.5
	40	18.0	15.7
	60	27.0	24.3
	80	35.5	33.7
$LSD_{0.05}$		1.9	2.3

Table VI. Total sugar, acidity and total soluble solids (TSS) in tomato fruits grown on sandy soil treated with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (t/ha)	Total sugar (%)	Acidity (%)	Total sugar/ acidity	TSS (%)
Control	0	2.0	0.23	8.7	5.25
Fertilizer	0	2.3	0.28	8.2	5.00
Non-irradiated sludge	20	2.6	0.28	9.3	5.25
	40	2.8	0.33	8.5	6.25
	60	2.9	0.34	5.0	6.50
	80	3.1	0.38	8.2	6.75
Irradiated sludge	20	2.8	0.28	10.0	6.25
	40	2.9	0.35	8.3	6.50
	60	3.0	0.36	8.3	6.50
	80	3.1	0.38	8.2	7.00
$LSD_{0.05}$		0.18	0.11	—	0.29

4. CONCLUSIONS

Sewage-sludge application provided coarse-textured sandy and calcareous soils with organic matter, and thereby provided tangible benefits in terms of increased water-holding and cation-exchange capacities, reduced BD and increased availability of N, P and K. These results support its utilization as an organic fertilizer and physical ameliorant in coarse-textured soils. The improved fertility status due to sludge addition was reflected in higher yields of tomatoes and improved fruit quality. The only negative aspect observed was an increase in the soluble salt content in sludge-amended soils; however, this was insufficient to affect crop growth over two seasons. The benefits of sludge as a source of macronutrients (N, P) and micronutrients (Cu, Zn, Fe, Mn) are further explored in Parts III and IV, respectively, of this series. The potentially detrimental effect of sludge application as an entry point for non-nutrient heavy metals into the food chain is addressed in Part V.

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IRRADIATED SEWAGE SLUDGE FOR INCREASED CROP PRODUCTION – III. MACRONUTRIENT AVAILABILITY

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Abstract

Irradiated and non-irradiated sludge (~2% N) was applied to field plots at rates between 10 and 40 t/ha. An N-fertilizer treatment was applied at the recommended rate (286 kg N/ha) for tomato. Microplots were labelled with ^{15}N-enriched ammonium sulphate (20 kg N/ha) to investigate the availability of N from fertilizer, sludge and soil. Irradiated and non-irradiated sludge was applied to pots in a glasshouse experiment at rates between up to 80 t/ha. Pots were labelled with carrier-free $KH_2{}^{32}PO_4$ to investigate the availability of P from various sources to tomato. The application of sewage sludge to sandy soil increased dry matter and N yield of, and N recovery by, tomatoes. The increases were significantly higher in irradiated than in non-irradiated sludge treatments. Total N uptake varied from 46 to 87 kg/ha for the non-irradiated treatment and from 47 to 106 kg/ha for the irradiated treatment, for application rates of 10 to 40 t/ha, respectively. Recovery of N fertilizer by tomato in the absence of sludge was only 14%, whereas recovery values of 17 to 34% and from 21 to 39% of sludge N were obtained for non-irradiated and irradiated treatments, respectively. Percent N in tomatoes derived from sludge was 88 to 92% and from soil, 2 to 6%, depending on the application rate. Dry-matter production, plant-tissue P concentration and P uptake by tomato increased significantly as the sludge application rate increased up to 80 t/ha. Phosphorus uptake was more than two-fold above the control value, with the 80-t treatment. Percent P derived from sludge increased as the application rate increased, whereas%P derived from fertilizer decreased. The%P derived from sludge varied between 64 and 79%. Tomato plants took up a maximum of 2.5 to 3.3% of the P applied in sewage sludge. Phosphorus was mainly concentrated in the root tissue; 22 to 38% was translocated to the shoots under sludge application.

1. INTRODUCTION

Application of sewage sludge for agricultural land is recognized as a potentially useful means of disposal [1]. In addition to its high organic matter content, sludge contains N, P, K, S, Ca, Mg, and micronutrients essential for plant growth. The N concentration range in sludge is <0.1 to 18% [2], whereas P concentrations range from 0.54 to 3.2% [3]. Most of the P in sludge is in inorganic forms, mainly associated with Fe and Al oxides, and a small fraction, 2 to 5%, is in organic forms [4].

Sandy soils are found in a large proportion of arid and semi-arid areas of the world. They are low in organic matter and, therefore, lack adequate levels of organically bound nutrients such as N, P and S, have a low water-holding capacity, and are low in exchangeable bases. Nitrogen is often the most limiting nutrient for plant growth [5]. Therefore, sewage sludge provides special advantages for ameliorating sandy soils through enhancing organic matter content and hence chemical fertility and water-holding capacity, and, consequently, yield potential [6].

Nitrogen uptake by plants from an organic source is proportional to the amount of N mineralized from that source [7]. Nitrogen-mineralization capacity and crop-N requirement are the main factors determining the waste-application rate [8]. Few studies have been conducted to precisely evaluate sewage sludges as sources of N and P [9,10].

Numerous methods have been used to treat sludge in order to ensure a safe product for agricultural utilization, including aerobic and anaerobic digestion, composting, lime stabilization, and irradiation [11]. Süss et al. [12] observed that application of irradiated sludge produced higher crop yields than did non-irradiated sludge in a greenhouse experiment. Rosopulo et al. [13] reported higher N concentrations in plants grown on soils treated with irradiated sludge than with non-irradiated sludge, also in a greenhouse experiment. Companella et al. [14] found that irradiation of sewage sludge promoted the decomposition of organic matter and the release of low molecular-weight compounds. Wen et al. [8] reported that irradiation of sludge appeared to release NH_4^+. The effects of gamma irradiation on N uptake and utilization under field conditions have seldom been reported. In addition, little is known of the effects of gamma irradiation on the availability of P in sewage sludge

Therefore, my objective was to quantify the availability of N and P to tomato plants from irradiated and non-irradiated sewage sludge. An indirect ^{15}N-labelling technique [7] and an indirect ^{32}P-labelling technique were used to determine the proportional contributions of sludge to the N and P nutrition of tomatoes, grown in the field and glasshouse, respectively.

2. MATERIALS AND METHODS

2.1. Sampling, preparation and irradiation of sewage sludge

The procedures were described in Part II of this series.

2.2. Treatments and experimental design

Field plots were established on a sandy soil (pH, 8.2; clay, 1.8%; organic matter, 0.43%; $CaCO_3$, 2.4%; cation exchange capacity, 2.18 cmol(+)/kg at 0–25 cm depth), at the Egyptian Atomic Energy Authority Experimental Farm, Anshas, to evaluate the potential of sludge as a source of N for tomatoes. A basal fertilizer dressing was applied: superphosphate at 143 kg P_2O_5/ha and potassium sulphate at 167 kg K_2O/ha. Irradiated and non-irradiated sewage sludge was incorporated into the soil to a depth of 25 cm. Ten treatments (Table I) were arranged in a randomized complete block design (RCBD) with 1 m separating the four replicate blocks. Each plot was 10 m^2 in area, and 1.5 m^2 sub-plots were established within each for the application of ^{15}N-labeled fertilizer. ^{15}N-ammonium sulphate (Isotec, Miamisburg, Ohio) was applied in solution as a spray to the soil surface (Table I), and then incorporated into the top 25-cm soil layer. Tomato seedlings (cv. GS) were transplanted with a 1-m row spacing and 0.5 m between plants. Irrigation was applied using a drip system according to the evapotranspiration demand, not exceeding field capacity to avoid leaching of fertilizer N.

A pot experiment was established in a greenhouse to investigate the availability of P from sewage sludge to tomato plants using an indirect ^{32}P-tracer technique. Plastic pots were filled with 2-kg aliquots of sandy soil. A 10% solution of KH_2PO_4 was prepared and then labelled with carrier-free ^{32}P. Tomato plants were transplanted and the labelled P solution was applied to the soil. Each pot received 0.23 g labelled P with an activity of 2 mCi. Pots were arranged in a RCBD with ten treatments (Table I) and four replications.

Table I. Treatments in field and pot experiments

Treatment	N experiment (field)		P experiment (pot)	
	Sludge rate (t/ha)	^{15}N fertilizer rate[a] (kg N/ha)	Sludge rate (t/ha)	^{32}P fertilizer rate[b] (g P/pot)
Control	0	20	0	0
Fertilizer	0	286	0	0.23
Non-irradiated sewage sludge	10	20	20	0.23
	20	20	40	0.23
	30	20	60	0.23
	40	20	80	0.23
Irradiated sewage sludge	10	20	20	0.23
	20	20	40	0.23
	30	20	60	0.23
	40	20	80	0.23

[a] 20 kg N/ha, 10 atom% ^{15}N excess; 286 kg N/ha, 1 atom% ^{15}N excess. [b] 2 mCi/pot.

2.3. Plant sampling and analysis

Four tomato plants located in the centre of each of the ^{15}N-labelled sub-plots were harvested 40 days after planting, at maturity [15]. Plants were separated into leaves, stems, and roots, oven-dried at 60°C and dry weights recorded. The composite samples of the four plants were ground to pass through a 0.2-mm sieve using a micro-mill grinder. Tomato fruits were collected as they ripened and weighed for yield. Representative fruit samples were oven-dried at 60°C and dry weights recorded. Total N values of plant tissues were determined using the Kjeldahl procedure [16]. The ^{15}N-abundance values for plant samples were determined at the IAEA Soil Science Unit, Seibersdorf, using Dumas continuous-flow isotope-ratio mass spectrometry.

Plants in pots were harvested one month after sowing, and were separated into leaves, stem and roots. Plants were washed thoroughly with de-ionized water, soaked for 2 h in 0.1 *N* HCl and then for another 2 h in 2×10^{-3} *M* Na-EDTA solution to remove non-absorbed ^{32}P. Plant samples were dried and weighed and sub-samples were ashed at 500°C. Labelled P was determined by liquid scintillation counting (Packard Tri-Carb-2700 TR).

2.4. Calculations and statistical analysis

The percentage of N in plant samples derived from the fertilizer (%Ndff) was calculated according to Zapata [17]:

$$\%Ndff = \left[\frac{{}^{15}N\,enrichment_{sample}}{{}^{15}N\,enrichment_{fertilizer}}\right]\times 100$$

$$\%N\,recovery = \%Ndff \times \left[\frac{N\,uptake}{N\,added}\right]$$

The percentage of N in plant samples derived from the sewage sludge (%Ndfss) was calculated by ^{15}N dilution, according to:

$$\%Ndfss = \left[1 - \frac{{}^{15}N\,enrichment_{+sludge}}{{}^{15}N\,enrichment_{-sludge}}\right]\times 100$$

The percentage of N in plant samples derived from the soil (%Ndfs) in the sludge treatments was calculated as:

$$\%Ndfs = 100 - (\%Ndff + \%Ndfss)$$

The percentage of P in plant samples derived from the fertilizer was calculated according to Vose [18]. The percentage of P in plant samples derived from the sewage sludge (%Pdfss) was calculated by ^{32}P dilution, according to:

$$\%Pdfss = \left[1 - \frac{specific\ activity_{+sludge}}{specific\ activity_{-sludge}}\right]\times 100$$

Analysis of variance, Duncan's LSD and correlation coefficients (r) were determined using the MicroStat package.

3. RESULTS AND DISCUSSION

3.1. Dry matter yield

Significant increases in dry-matter production were obtained in response to sludge application (Table II). The maximum dry-matter yield, obtained with sludge applied at 40 t/ha, was significantly higher than those obtained in the control and with the other application rates. The fractional increases in total dry-matter production over the control were 23, 47, 75 and 118% for non-irradiated sludge and 25, 61, 92 and 135% for irradiated sludge, corresponding to 10, 20, 30 and 40 t/ha application rates, respectively. Irradiated sludge treatments provided higher dry matter yields than non-irradiated treatments (Table II).

3.2. Nitrogen

3.2.2. Nitrogen yield

There was a highly significant correlation (r = 0.957**; $P<0.01$) between total-N yield and dry-matter yield. The N yields were significantly higher in all sludge treatments compared with N fertilizer (Table III). Nitrogen yield increased significantly with increasing rates of sludge application, and was highest at 40 t sludge/ha, representing more than double the N yield obtained with fertilizer. On average, plants grown with irradiated sludge absorbed 14% more N than did plants grown with non-irradiated sludge.

Jiang et al. [19] reported that irradiation of sewage sludge by a gamma source at a dosage of 3 kGy promoted mineralization of organic N; wheat plants grown with irradiated sludge absorbed 26% more N than did those with non-irradiated sludge, seedling growth was enhanced, and grain yield was 27% higher as compared with non-irradiated sludge.

The mechanism by which irradiated sewage sludge provides more N to plants may be explained by the degrading effect of irradiation on organic N compounds and/or the possibility of radiation-induced inactivation of toxic compounds [20], thus stimulating plant growth and N uptake.

Table II. Dry matter yield of tomato plants in fertilizer and sludge treatments

Treatment	Sludge rate (t/ha)	Yield			
		Shoot	Fruit	Root	Total
		(kg/ha)			
Fertilizer	0	1,336	656	340	2,332
Non-irradiated	10	1,549	976	340	2,865
sludge	20	1,646	1,392	380	3,418
	30	1,729	1,920	420	4,069
	40	1,913	2,720	460	5,093
Irradiated	10	1,527	960	420	2,907
sludge	20	1,840	1,456	460	3,756
	30	1,899	2,144	500	4,543
	40	2,049	2,896	540	5,485
$LSD_{0.05}$		171	171	68	165

Table III. Uptake of N by above- and below-ground components of tomato plants in N fertilizer and sewage-sludge treatments

Treatment	Sludge rate	N fertilizer rate	N uptake			
			Shoot	Fruit	Root	Total
	(t/ha)	(kg/ha)				
Fertilizer	0	286	22.9	11.3	5.2	39.4
Non-irradiated sludge	10	20	23.9	16.4	5.2	45.6
	20	20	25.5	22.1	6.6	54.7
	30	20	25.4	31.3	7.4	64.8
	40	20	33.5	44.9	7.5	86.6
Irradiated sludge	10	20	24.1	16.6	6.3	47.0
	20	20	24.8	26.9	7.1	59.2
	30	20	27.0	39.0	9.1	75.4
	40	20	41.6	55.3	9.3	106
LSD$_{0.05}$			5.1	3.4	2.1	5.1

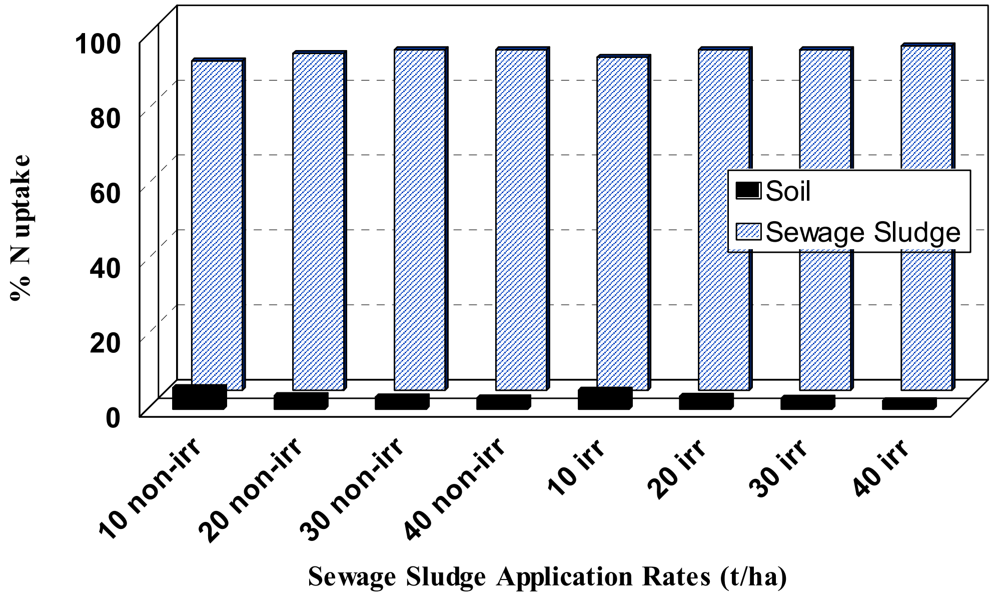

FIG. 1. Percentage of tomato N derived from soil and sewage sludge.

Gamma irradiation of ≥10 krad was reported to remove most of the toxic compounds contaminating sewage water, such as herbicides (monuron) and PCBs [21]. The low C/N ratio of the sludge used in this experiment (12.4 for irradiated and 13.0 for non-irradiated) would be expected to enhance mineralization of N. For example, a mineralization rate of approximately 10% was reported for sewage-sludge compost with a C/N ratio of 17 [22]. High C/N ratios in sludge can negatively affect N mineralization [23].

Table IV. Recovery of N from fertilizer and sludge

Treatment	Sludge rate (t/ha)	N recovery (%)
N fertilizer	0	14
Non-irradiated sludge	10	34
	20	22
	30	17
	40	18
Irradiated sludge	10	39
	20	25
	30	21
	40	25

Table V. Dry matter yield and P concentration in tomato and soil P in fertilizer and sludge treatments

Treatment	Sludge rate (t/ha)	Dry matter (g/pot)	Plant P (%)	Soil P	
				(g/g)	(% increase)
Control	0	2.00 ± 0.15[a]	0.15 ± 0.01	26.7 ± 1.5	—
Fertilizer	0	2.57 ± 0.33	0.26 ± 0.02	—	—
Non-irradiated sludge	20	2.60 ± 0.17	0.25 ± 0.02	37.3 ± 3.6	40
	40	2.90 ± 0.46	0.28 ± 0.02	64.0 ± 2.3	140
	60	3.50 ± 0.29	0.32 ± 0.02	64.8 ± 3.8	143
	80	3.50 ± 0.23	0.40 ± 0.03	71.9 ± 3.7	169
Irradiated sludge	20	3.00 ± 0.06	0.28 ± 0.02	47.3 ± 4.2	77
	40	3.52 ± 0.18	0.35 ± 0.02	64.3 ± 3.1	141
	60	3.83 ± 0.25	0.41 ± 0.02	75.8 ± 4.4	184
	80	3.88 ± 0.28	0.43 ± 0.03	77.7 ± 4.0	191
$LSD_{0.05}$		0.81	0.06	10.5	

[a] Mean ± standard error.

3.2.3. Nitrogen derived from sewage sludge and soil

There was a highly significant correlation (r = 0.945**; $P < 0.01$) between N derived from sewage sludge and dry matter yield. Percent of plant N derived from sewage sludge (%Ndfss) was very high, within the narrow range of 88 to 92% (Fig. 1). This indicates that most plant N was derived from sewage sludge regardless of the sludge-application rate. Although%Ndfss values were similar for irradiated and non-irradiated sludge (Fig. 1), the total N yields for irradiated sludge were greater than for non-irradiated (Table III), therefore the former contributed more N to the total N yield of tomato. The sandy soil utilized in this experiment was very low in total N. Therefore, as expected, the%N in tomato derived from soil was very low, in the range 2.2 to 5.7% (Fig. 1), emphasizing the need to add N in either an organic or mineral form to sustain crop production.

3.2.4. Recovery of N from fertilizer and sludge

Values for recovery of N from fertilizer by tomato plants were very low at only 13% (Table IV). This indicates that a large portion of the fertilizer N was lost, probably mainly by leaching as NO_3^-. Another possible mechanism of loss was by NH_3 volatilization, which is likely to have occurred since the pH of this sandy soil was 8.2. The data demonstrate that irradiated sewage sludge, when applied at high rates, could fully substitute for N fertilizer for tomato plants.

The ranges of sludge-N recovery were 17 to 34% for non-irradiated and 21 to 39% for irradiated treatments (Table IV). The average N recovery by tomato plants grown with irradiated sludge was higher than for those grown with non-irradiated sludge (27% vs. 23%, respectively). Similar ranges, 13 to 36% for sludge-N utilization by maize and sudan grass were reported by Dontsov et al. [24]. The average recovery of ^{15}N by wheat plants grown with irradiated sludge was 12%, higher than with non-irradiated sludge [19].

3.3. Phosphorus

3.3.1. Dry-matter production, plant and soil P

Dry matter accumulation by tomato plants was significantly increased as a result of sludge application (Table V). The increase was highest at 80 t/ha, i.e. 36 and 51% higher than the control for non-irradiated and irradiated treatments, respectively. There were also gradual increases in P concentration of tomato plants as the sludge application rate increased. Several metabolic processes in plants are affected by P deficiency [26]. Tomato plants grown in this sandy soil amended with 80 t/ha of sewage sludge, contained adequate P to satisfy nutritional requirements. The average available P concentrations in the sludge used in this experiment were 473 and 464 µg/g for non-irradiated and irradiated treatments, respectively.

The P concentration in the soil under investigation was very low at 0.003%. However, soil P concentration increased significantly as a result of applying sludge. The increase was higher for irradiated than for non-irradiated sludge. The increase reached 191% of the control with 80 t/ha irradiated sludge. Sludge application raised the soil-P content by 1.4-, 2.4-, 2.4- and 2.7-fold for non-irradiated sludge and by 1.8-, 2.4-, 2.8- and 2.9-fold for irradiated sludge, for 20, 40, 60, 80 t sludge/ha, respectively.

3.3.2. P uptake

Total P uptake increased from 6.60 mg/pot for the fertilizer treatment (KH_2PO_4 only) to 16.7 mg/pot at the 80 t/ha sludge-application rate + KH_2PO_4 (Table VI), increases of 2.5- and 2.1-fold over the fertilizer treatment in irradiated and non-irradiated sludge treatments, respectively. Terman et al. [26] found greater uptake of P by corn plants grown in pots containing municipal waste compost plus superphosphate than corn plants that received superphosphate alone. Phosphorus was concentrated mainly in the root tissue and only 22 to 38% was translocated to the shoots with sewage sludge application (Table VI). Absorption and translocation of P increased as sludge application rate increased. Hilmy et al. [27] found that 5 t/ha of irradiated sludge was equivalent to 90 kg P_2O_5/ha of triple superphosphate (TSP) based on dry-matter production and P uptake by corn; 20 t/ha of irradiated and non-irradiated sewage sludge was equivalent to almost 352 kg P_2O_5/ha of TSP based on dry-matter production.

3.3.3. Phosphorus in tomato derived from fertilizer and sludge

The highest value for Pdff in tomato plants was obtained with the fertilizer treatment (Table VI). The P derived from sludge was highest with 80 t/ha of irradiated sewage sludge (13.2 mg/pot). Percent P in tomato derived from sewage sludge increased as the sludge application rate increased (Fig. 2), whereas%Pdff in tomato as a whole decreased. The availability of fertilizer P would be expected to be low in the sandy soil (pH 8.2), whereas P availability from the organic sludge (pH 6.7) would be less likely to be affected by the alkaline pH. The percent P in tomato derived from sludge varied between 64 and 79% (Fig. 2).

3.3.3. Phosphorus recovery by tomato from sewage sludge

Tomato plants recovered from 2.5 to 3.3% of the P applied in sludge (Table VII). This is considered to be a good recovery, as it represents 25 to 33 kg P for each ton of sludge applied to the soil. Although the total P uptake increased from 6.6 to 16.7 mg/pot at 80 t/ha, the percent recovery from sludge was only ~2.0%. Coutinho et al. [28] found that the percentage P recovery by ryegrass from composted sewage sludge averaged 2.5%.

Table VI. Effect of fertilizer and sewage sludge on P uptake by tomato

Treatment	Sludge rate	P uptake				Pdff	Pdfss
		Leaf	Stem	Root	Total		
	(t/ha)	(mg/pot)					
Fertilizer	0	2.0	1.5	3.1	6.62	6.3	—
Non-irradiated sludge	20	0.4	1.1	5.1	6.60	2.0	4.2 ± 0.69[a]
	40	0.5	1.3	6.3	8.10	1.8	5.9 ± 0.52
	60	0.7	2.1	8.4	11.2	1.5	8.0 ± 0.69
	80	0.8	2.6	10.6	14.0	1.3	11.0 ± 0.86
Irradiated sludge	20	0.5	1.6	6.4	8.50	2.3	5.5 ± 0.57
	40	0.8	1.9	7.5	12.2	2.1	9.0 ± 0.29
	60	0.99	2.4	12.3	15.7	1.9	11.3 ± 0.75
	80	1.0	3.0	12.7	16.7	1.7	13.2 ± 1.27
$LSD_{0.05}$							1.88

[a] Mean ± standard error.

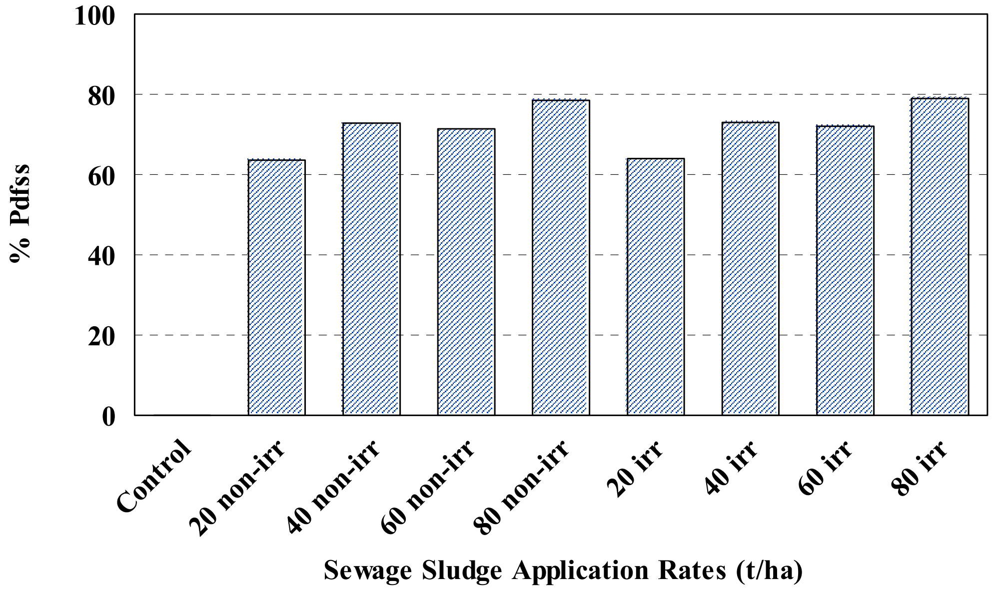

FIG. 2. Percentage of P in tomato derived from sewage sludge.

Table VII. P recovery by tomato from sewage sludge

Treatment	Sludge rate (t/ha)	Recovery (%)
Non-irradiated sludge	20	2.50
	40	1.76
	60	1.59
	80	1.63
Irradiated sludge	20	3.27
	40	2.68
	60	2.24
	80	1.96

4. CONCLUSIONS

Field and pot experiments demonstrated that sewage sludge could supply a large proportion of the N and P requirements of tomatoes grown on a sandy alkaline soil. Thus, sewage sludge can substitute for costly synthetic N and P fertilizers with consequent economic benefits. However, sewage sludge should not be regarded as a complete fertilizer replacement as the high rates applied in the present study are unlikely to be used in farmers' fields. The percentage recovery of N by tomatoes from sludge was greater than for fertilizer N. Thus potential losses of N from fertilizer due to leaching or ammonia volatilization are reduced, giving tangible environmental benefits.

Gamma irradiation of sludge not only eliminated pathogens (Part I of this series), but it increased the availability of N and N uptake by tomato. Tomato yields were higher with irradiated than non-irradiated sludge. Thus irradiation has beneficial effects both in the public health domain and as a process that increases the availability of N from a slow-release organic fertilizer source, and, hence, crop yield. The supply of plant micronutrients is an additional benefit that is explored in Part IV. In Part V, the potential hazard of heavy metals in sludge is examined.

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IRRADIATED SEWAGE SLUDGE FOR INCREASED CROP PRODUCTION –
IV. MICRONUTRIENT AVAILABILITY

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Abstract

Irradiated and non-irradiated sewage sludge, from El-Gabal El-Asfar Farm near Cairo, were applied to tomato (*Lycopersicon esculentum* cv. GS) grown in a calcareous and a sandy soil at rates of 20, 40, 60, and 80 t/ha. Unfertilized controls and basal-fertilizer treatments were included. Total concentrations of micronutrients (Cu, Zn, Fe, Mn) in sludge-treated calcareous soil were higher than those in the sandy soil, although DTPA-extractable micronutrient concentrations were lower. There were no significant differences between irradiated and non-irradiated sludge treatments in DTPA-extractable and total micronutrient concentrations for the calcareous or the sandy soil. The total micronutrient concentrations for the highest sludge application rate (80 t/ha) were 5,108, 125, 68.2, and 207 μg/g in the calcareous soil and 2,200, 74.8, 43.2, and 139 μg/g in the sandy soil for Fe, Mn, Cu, and Zn, respectively, whereas the DTPA-extractable micronutrient concentrations were 25.0, 6.2, 5.5 and 6.6 μg/g in the calcareous soil and 53.3, 10.1, 7.3 and 9.83 μg/g in the sandy soil, respectively. Highly significant differences were observed in total and available micronutrient concentrations in calcareous and sandy soils among the sludge-application rates. Micronutrient concentrations of tomato leaves and fruits increased with increasing application rates of irradiated and non-irradiated sludge, and were higher in the sandy than in the calcareous soil for the same treatment. Highly significant differences were observed among the sludge-application rates in terms of the concentrations of micronutrients in both leaves and fruits. However, there were no significant differences between the irradiated and non-irradiated sludge treatments in the micronutrient concentrations of leaves and fruits in either soil. Micronutrient uptake increased with increasing rates of application of sludge to the soil, more so in the sandy than in the calcareous soil. The amounts of Fe, Mn, Cu, and Zn taken up by fruits were very low compared with those added to the soil in sludge.

1. INTRODUCTION

The treatment of raw sewage from urban environments and the disposal of the effluent and sewage sludge from treatment plants are major challenges for municipal authorities worldwide, both from technical and from economic viewpoints. The twin goals of treatment and disposal are to reduce the hazard of the waste from human-health and environmental-pollution perspectives. There are various methods for disposal of sewage sludge, including dumping in waterways (rivers, seas, and oceans) and landfills, and incineration. However, these practices pollute surface water, groundwater, and the atmosphere, respectively. Land application of sewage sludge is an attractive alternative, especially in Egypt, where desert soils represent about 96% of the total area and are lacking in organic matter, essential nutrients, and the capacity to hold water.

The use of sewage sludge (biosolids) as an organic fertilizer or soil conditioner can provide organic matter and nutrients and improve water-holding and nutrient-retention capacities. These issues were addressed in Parts II and III of this series. Thus, the utilization of sludge on farmland is seen as beneficial for agriculture and as an environmentally neutral means of disposal [1,2]. However, sewage sludge contains pathogenic microorganisms [3] and heavy metals [4–7] which can be harmful to human health and the environment. These issues are addressed in Parts I and V of this series, respectively.

Primary- and secondary-treatment processes at conventional wastewater-treatment plants that produce aerobically digested sludge may be followed by anaerobic digestion, composting, liming and thermal disinfection to further stabilize the waste material [8–12]. However, the use of ionizing radiation has proven to be the most effective means of decontaminating sludge of microbes [13–14]. Gamma irradiation, in addition to removing harmful pathogenic organisms, can also alter physical and chemical properties [15]. For example, irradiated sludge was shown to promote higher tomato yields than equivalent rates of non-irradiated sludge (Part II of this series) and was also a better source of N (Part III).

Our aim with this investigation was to quantify the accumulation of micronutrients (Fe, Mn, Cu. Zn) in soil and their availability to tomato plants as a result of applying irradiated and non-irradiated sewage sludge to sandy and calcareous soils.

2. MATERIALS AND METHODS

2.1. Sampling, preparation and irradiation of sludge

These were described in Part II of this series.

2.2. Treatments, experimental design and plant and soil sampling

These were also described in Part II.

2.3. Analytical procedures

Total-content values of Fe, Mn, Cu, and Zn in the soil were determined after digestion with HF-$HClO_4$ [16]. Available Fe, Mn, Cu, and Zn were extracted in 0.005 *M* DTPA solution buffered at pH 7.3 [17]. Plant materials were digested with an HNO_3-H_2SO_4-$HClO_4$ mixture [16]. Iron, Mn, Cu, and Zn in digests and extracts were measured by atomic absorption spectrophotometry (Perkin-Elmer Model 2830).

2.4. Statistical analysis

Data were subjected to analysis of variance, and LSD and correlation-coefficient (r) values were calculated using the microcomputer MicroStat package.

3. RESULTS AND DISCUSSION

3.1. Micronutrient concentrations in sludge

Sewage sludge contained a high total concentration of Fe, with much lower concentrations of Zn, Mn and Cu (Table I). However, DTPA-extractable Zn was much higher in concentration than were the other micronutrients. Thus total concentrations were a poor index of availability. Similar results were obtained for irradiated and non-irradiated sludge (Table I). Chaney and Ryan [4] considered that sludge containing >2.000 µg/g Zn or >800 µg/g Cu should not be applied to agricultural land; the sludge used in this study contained lower concentrations of both.

3.2. Total micronutrient concentrations in soil

The total concentrations of Fe, Mn, Cu and Zn were generally higher in sludge-amended soils than in the controls (Table II). The increases in soils amended with irradiated sewage sludge (80 t/ha), were 1.6-, 1.7-, 4.8-, and 1.8-fold in the calcareous soil, and 1.5-, 1.7-, 3.7-, and 2.3-fold in the sandy soil for Fe, Mn, Cu and Zn, respectively. The soil concentrations of micronutrients increased as the sludge application rate increased. The increases were 1.5-, 1.6-, 5.0-, and 1.7-fold in the calcareous soil and 2.0-, 1.7-, 3.6-, and 2.2-fold in the sandy soil for Fe, Mn, Cu, and Zn, respectively.

Table I. Concentrations of micronutrients in sewage sludge collected from El-Gabal El-Asfar farm

Form	Micronutrient	Irradiated sludge	Non-irradiated sludge
		(µg/g)	
Total	Fe	5,881	5,607
	Mn	388	380
	Cu	219	203
	Zn	760	756
DTPA-extractable	Fe	76.6	71.3
	Mn	21.1	20.3
	Cu	69.2	65.4
	Zn	368	308

Table II. Total micronutrient concentrations in calcareous and sandy soils treated with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn
		(µg/g)							
Control	0	3,299	74.2	14.4	115	1,155	43.2	11.7	61.9
Fertilizer	0	3,424	80.7	13.7	118	1,125	42.9	12.2	64.4
Non-irradiated sludge	20	3,705	98.6	27.6	134	1,366	49.5	24.2	66.6
	40	4,615	104	35.1	149	1,505	52.6	25.2	95.0
	60	4,774	109	47.5	181	1,816	66.5	35.6	103
	80	4,906	121	66.6	196	2,060	71.6	37.9	144
Irradiated sludge	20	3,624	92.4	26.1	125	1,220	57.7	26.6	78.4
	40	4,765	109	38.6	140	1,540	62.1	29.0	98.0
	60	5,021	115	52.9	177	2,160	68.2	38.3	115
	80	5,108	125	68.2	207	2,200	74.8	43.2	139
$LSD_{0.05}$		599	15.0	7.9	13.8	539	16.4	4.2	18.3

There were no significant differences between irradiated and non-irradiated sludge for each treatment in total micronutrient concentrations either for the calcareous or the sandy soil (Table II).

The total concentrations of Fe, Mn, Cu and Zn in the calcareous soil were higher than in the sandy soil for equal application rates. At 80 t/ha, increases in micronutrient concentrations were 2.3-, 1.7-, 1.6-, and 1.5-fold in irradiated and 2.4-, 1.7-, 1.8-, and 1.4-fold in non-irradiated sludge treatments, for Fe, Mn, Cu, and Zn, respectively. This reflects the initial soil micronutrient concentrations.

Total micronutrient concentration is an important soil parameter because it determines the pool size. Many authors have suggested that excessive inputs of sludge should be avoided and maximum acceptable levels of some micronutrients in soil should be set, in order to avoid risks of water pollution and/or human health problems [7]. Tietjen [18] suggested the following as tolerable soil concentrations for Zn and Cu: 300 and 100 µg/g, respectively; concentrations of Zn and Cu in the sludge-amended soils were lower than these, even at the highest application rate (Table II).

3.3. **DTPA-extractable micronutrients in the soils**

The concentrations of DTPA-extractable Fe, Mn, Cu, and Zn in the soil (Table III) were much lower than the total metal concentrations. Only small proportions of the total micronutrients were in available forms. The percentage of the available metals in the calcareous soil (80 t/ha) were: 0.53, 5.4, 7.5, and 3.6 and in sandy soil were 3.1, 13.0, 17.2, and 6.1, for Fe, Mn, Cu and Zn, respectively. Generally, the available fractions of micronutrients followed the order Fe > Zn > Mn > Cu for both soils.

The increases in extractable micronutrients with 80 t/ha sludge compared with the controls, were higher in the sandy than the calcareous soil, although the total micronutrient concentrations were higher in the calcareous soil. The increases were 6.6-, 5.6-, 11.7-, and 11.2-fold in the calcareous soil and 10.3-, 7.8-, 10.4-, and 19.6-fold in the sandy soil, for Fe, Mn, Cu, and Zn, respectively. The available forms of these micronutrients were higher in the sandy soil than in the calcareous (2.4-, 1.4-, 1.4-, and 1.4-fold for Fe, Mn, Cu and Zn, respectively). This was due to the higher content of calcium carbonate in the calcareous soil, which resulted in the precipitation of these micronutrients [7].

There were no significant differences between soils amended with irradiated and non-irradiated sludge in their concentrations of DTPA-extractable metals. There were highly significant linear relationships between various soil properties (OM, $CaCO_3$, pH, CEC, clay, total micronutrient concentrations) and DTPA-extractable micronutrient concentrations (Table IV).

Many reviews on micronutrient reactions in soil are available [5,19–23] showing that several soil components (clay, OM, hydrous oxides, carbonates, phosphates) have the ability to bind micronutrient elements and thereby reduce their availability for plant uptake. Several different mechanisms are involved in the adsorption of metal ions: cation exchange, specific adsorption, organic complexation, precipitation and co-precipitation [23].

Table III. DTPA-extractable micronutrients in the soils treated with sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn
		(µg/g)							
Control	0	3.8	0.9	0.44	0.4	5.5	0.8	0.4	0.5
Fertilizer	0	3.8	1.1	0.47	0.6	5.2	1.3	0.7	0.5
Non-irradiated sludge	20	7.9	1.8	1.5	1.2	9.2	2.2	1.5	2.9
	40	12.6	2.2	2.7	3.1	17.4	3.4	3.5	4.7
	60	20.2	4.3	3.6	5.0	31.1	7.8	5.1	6.4
	80	26.4	6.5	5.0	6.2	54.0	9.3	6.7	8.8
Irradiated sludge	20	8.6	2.0	1.7	1.3	8.3	3.1	2.7	2.5
	40	13.1	3.0	2.8	3.4	20.4	3.8	3.7	4.6
	60	21.8	4.2	4.0	4.6	34.6	8.5	6.3	7.7
	80	25.0	6.2	5.5	6.7	53.3	10.1	7.3	9.8
$LSD_{0.05}$		1.0	0.4	0.4	0.6	5.8	0.8	1.0	0.5

Table IV. Correlation coefficients (r) for linear relationships between DTPA-extractable micronutrients and soil characteristics

DTPA-metal (μg/g)	OM (%)	$CaCO_3$ (%)	pH	CEC (mEq/100g)	Clay (%)	Total metal (μg/g)
Fe	0.760**[a]	–0.793**	–0.801**	0.810**	0.730**	0.710**
Mn	0.815**	–0.899**	–0.844**	0.989**	0.939**	0.780**
Cu	0.792**	–0.876**	–0.838**	0.978**	0.937**	0.902**
Zn	0.891**	–0.874**	–0.849**	0.980**	0.911**	0.910**

[a] $P<0.01$.

Table V. Micronutrient concentrations in tomato leaves grown on soils treated with sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn
		(μg/g)							
Control	0	169	37.2	4.2	35.5	147	41.2	6.2	36.9
Fertilizer	0	175	44.0	5.6	37.0	170	47.0	7.0	39.0
Non-irradiated sludge	20	208	44.1	13.4	43.1	216	57.1	16.5	51.1
	40	278	47.4	15.2	52.7	368	59.4	20.4	61.8
	60	340	57.5	16.8	60.3	415	66.4	22.5	69.4
	80	434	63.6	18.4	64.9	559	72.6	26.0	81.8
Irradiated sludge	20	214	42.2	13.0	44.3	233	61.1	19.1	54.2
	40	275	50.1	14.2	55.5	377	65.6	21.0	63.7
	60	363	59.3	16.0	63.9	491	70.9	23.9	69.8
	80	431	70.5	20.1	68.4	610	78.3	27.5	80.8
$\text{LSD}_{0.05}$		51.6	13.1	1.9	2.4	61.0	14.5	2.3	6.8

3.4. **Micronutrient concentrations in tomato plants**

3.4.1. Leaves

The concentrations of Fe, Mn, Cu, and Zn in tomato leaves, both in the calcareous and sandy soil, significantly increased as the application rates of sludge increased, consistent with the availability of these metals in the soils (Table V). There were no significant differences between irradiated and non-irradiated sludge treatments in terms of leaf micronutrient concentrations at any application rate. Also, highly significant correlation coefficients were found between leaf-micronutrient concentrations and the sludge-application rates in both soils. The leaf concentrations of Fe, Mn, Cu, and Zn at 80 t/ha of irradiated sewage sludge were higher than the control by 2.5-, 1.6-, 3.6-, and 1.8-fold in the calcareous soil and by 3.6-, 1.7-, 3.9-, and 2.1-fold in the sandy soil, respectively.

A comparison of the micronutrient concentrations in leaves shows higher values in plants grown in the sandy than in the calcareous soil. The relative values of the increase at the 80 t/ha application rate were 42, 11, 37, and 18% for irradiated sludge and 29, 14, 41, and 26 % for non-irradiated sludge for

Fe, Mn, Cu, and Zn, respectively. This might be due to the precipitation of these metals in carbonate forms [7]. Also, the data show that there were fewer soluble forms in the calcareous soil than in the sandy soil.

3.4.2. Fruits

Concentrations of Fe, Mn, Cu, and Zn in fruits increased as the sludge application rate increased (Table VI), and were similar in the irradiated and non-irradiated treatments. The concentrations of Fe, Mn, Cu, and Zn at the highest application rate (80 t/ha) were higher than the control by 1.3-, 1.9-, 3.4-, and 1.3-fold in the calcareous soil and 1.7-, 2.7-, 5.3-, and 1.4-fold for the sandy soil. Concentrations of Fe, Mn, Cu and Zn in the fruits were lower than in the leaves. At 80 t/ha of irradiated sludge, as a fraction of the leaf concentrations, those of the fruits were 27, 33, 36, and 35% in the calcareous soil and 25, 46, 44 and 46% in sandy soil, for Fe, Mn, Cu and Zn, respectively. The concentrations of these micronutrients were higher in the sandy soil than in the calcareous soil, and were within normal concentration ranges reported by several authors [23–26]. The correlation coefficients (r) between the metal concentrations in the leaves and fruits were highly significant ($r = 0.88^{**}$, 0.98^{**}, 0.92^{**} and 0.93^{**}) in the sandy soil and ($r = 0.73^{**}$, 0.80^{**}, 0.90^{**} and 0.83^{**}) in the calcareous soil, for Fe, Mn, Cu and Zn, respectively.

3.5. Total micronutrient uptake by tomato

The amounts of Fe, Mn, Cu and Zn taken up by fruits increased as the sludge application rate increased in both soils (Table VII). The increase was higher in the sandy soil than the calcareous soil. Although the total amounts of micronutrients added to the soil in sludge were high, the amounts taken up by tomato fruits were low. The percentage uptake from the total amount added to the soil was 0.06% for Fe, 0.16% for Mn, 0.1% for Cu, and 0.1% for Zn in the calcareous soil, and 0.09% for Fe, 0.27% for Mn, 0.16% for Cu, and 0.16% for Zn in the sandy soil.

Table VI. Micronutrient concentrations in tomato fruits grown on soils amended with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn
		(μg/g)							
Control	0	88.0	12.0	2.0	17.2	87.3	14.6	2.1	20.3
Fertilizer	0	90.0	12.5	2.1	18.6	90.2	13.3	2.3	26.0
Non-irradiated sludge	20	90.5	13.6	5.1	20.1	112	17.1	5.9	30.0
	40	94.8	16.1	6.0	21.3	120	21.3	7.8	31.4
	60	96.3	20.1	6.5	21.1	136	30.2	9.2	33.2
	80	109	22.0	7.1	23.4	147	36.4	12.0	36.9
Irradiated sludge	20	91.5	14.0	5.4	21.6	121	18.4	5.0	30.2
	40	100	17.9	6.0	22.8	130	25.1	7.8	29.3
	60	98.5	20.8	6.5	23.0	137	31.2	10.5	31.9
	80	115	23.2	7.2	24.2	155	36.3	12.2	37.2
$LSD_{0.05}$		19.0	7.5	0.90	2.30	25.3	7.80	1.37	2.60

Table VII. Total micronutrient content of tomato fruits grown on soils amended with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (kg/ha)	Calcareous soil				Sandy soil			
		Fe	Mn	Cu	Zn	Fe	Mn	Cu	Zn
		(g/ha)							
Control	0	23.1	3.1	0.5	4.5	16.1	2.7	0.4	3.7
Fertilizer	0	36.0	5.0	0.8	7.4	31.9	4.7	0.8	9.2
Non-irradiated sludge	20	56.9	8.6	3.2	12.6	72.5	11.1	3.8	19.4
	40	120	20.4	7.6	26.9	182	32.4	11.9	47.7
	60	162	33.9	11.0	35.6	290	64.4	19.6	70.8
	80	309	62.5	20.2	66.4	476	118	38.9	120
Irradiated sludge	20	69.3	10.6	4.1	14.4	104	15.9	4.3	26.1
	40	141	25.3	8.5	32.3	211	40.7	12.8	47.5
	60	191	40.4	12.6	44.7	296	67.4	22.7	68.9
	80	385	70.3	21.8	73.4	495	116	39.0	119

4. CONCLUSIONS

Application of sewage sludge for two consecutive years as a soil amendment provided micronutrients (Fe, Mn, Cu, Zn) essential for the growth of tomato plants. The use of irradiated sludge of the same or similar quality with respect to micronutrient concentrations and absence of harmful pathogens is recommended as an organic fertilizer, both for calcareous and sandy soils. The calcareous soil exhibited lower amounts of DTPA-extractable micronutrients at the same sludge application rate, and can, therefore, accept higher rates of sludge application, with the $CaCO_3$ content being the important factor. In addition to micronutrient elements, sludge contains other potentially harmful heavy metals (Cd, Co, Ni, Pb). The availability of these metals to tomato plants is examined in Part V of this series.

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IRRADIATED SEWAGE SLUDGE FOR INCREASED CROP PRODUCTION – V. HEAVY-METAL AVAILABILITY

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Abstract

Tomato plants (cv. GS) were cultivated in sandy and calcareous soils amended with irradiated and non-irradiated sewage sludge at application rates of 20, 40, 60, and 80 t/ha. The total DTPA-extractable amounts of Cd, Co, Ni, and Pb in soil increased with sludge-application rate. The total metal concentrations (μg/g) at 80 t/ha were 2.35 for Cd, 20.8 for Co, 26.8 for Ni, and 41.3 for Pb for the calcareous soil, and 1.25 for Cd, 16.7 for Co, 22.3 for Ni, and 25.2 for Pb for the sandy soil. Also, the DTPA-extractable concentrations (μg/g) were 0.21 for Cd, 0.32 for Co, 1.76 for Ni, and 4.02 for Pb for the calcareous soil, and 0.52 for Cd, 0.65 for Co, 2.27 for Ni, and 5.97 for Pb for the sandy soil. The total concentrations of Cd, Co, Ni and Pb for the sandy soil were lower than those for the calcareous soil at the same sludge application rate, but the DTPA-extractable metal concentrations were higher in the former. This result is attributed to the precipitation of these metals in carbonate forms in the calcareous soil. Highly significant positive correlations were found between the soil DTPA-extractable metal concentrations and OM, CEC, clay, and total metal concentrations. However, the correlation coefficients were negative with soil pH and $CaCO_3$ content. There were no significant differences between soils amended with irradiated and non-irradiated sewage sludge in total or available metal concentrations. The heavy-metal concentrations in leaves and fruits increased with sludge-application rate, and exhibited higher levels than the normal ranges. Leaves of plants grown in the sandy soil maintained higher heavy-metal concentrations than those in the calcareous soil. At the highest application rate (80 t/ha) the values were as follows: 0.19, 0.31, 0.39 and 0.24 μg/g in the calcareous soil and 0.23, 0.54, 0.71 and 0.43 μg/g in the sandy soil for Cd, Co, Ni, and Pb, respectively. The heavy-metal concentrations in fruits were 0.102, 0.210, 0.209, and 0.027 μg/g in the calcareous soil, and 0.189, 0.217, 0.380, and 0.059 μg/g in the sandy soil, respectively. Based on Cd content at the highest sludge-application rate (80 t/ha), maximum permissible quantities for human consumption were calculated as 41 and 22 kg fresh tomatoes/week from the calcareous and sandy soils, respectively.

1. INTRODUCTION

Land application of sewage sludge has attracted considerable attention over the past two decades. There have been many studies on the effects of sewage sludge on soils and crops [1–5]. Although the primary constituents—organic matter, P and N—are beneficial to crop growth, sewage sludge always contains potentially hazardous heavy metals. High loading with sewage sludge may increase the concentrations of such metals in crops, potentially affecting yields, soil fertility, and animal and human health. Nationwide surveys indicated that food crops in the USA were generally very low in metal content [6,7]. However, crops grown on sludge-treated soil can accumulate higher concentrations of metals than those grown on untreated soils [8,9]. Climatic, edaphic and agronomic factors affect the ability of a plant to absorb metal elements [10,11].

Hilmy et al. [12] stated that "metals in sewage sludge are generally present as mineral particulates and colloids, due to cation exchange, sorption, precipitation, complex formation and chelation." Gamma irradiation of sewage sludge can remove harmful pathogens and also change its physical and chemical properties [13]. Our objective was to evaluate the effects of various rates of irradiated and non-irradiated sewage sludge on the accumulation of heavy metal (Cd, Co, Ni, Pb) in tomato plants grown on calcareous and sandy soils.

2. MATERIALS AND METHODS

2.1. Sampling, preparation and irradiation of sludge

These were described in Part II of this series.

2.2. Treatments, experimental design and plant and soil sampling

These were also described in Part II.

2.3. Analytical procedures

Total contents of Cd, Co, Ni, and Pb in the soil were determined after digestion with HF-$HClO_4$ [14]. Levels of available Cd, Co, Ni, and Pb were determined by extraction in 0.005 *M* DTPA solution buffered at pH 7.3 [15]. Plant materials were digested in a HNO_3-H_2SO_4-$HClO_4$ mixture [14]. Cadmium, Co, Ni, and Pb in digests and extracts were measured by atomic absorption spectrometry (Perkin-Elmer Model 283).

2.4. Statistical analysis

Data were subjected to analysis of variance, and LSD and correlation coefficient (r) values were determined using the MicroStat package.

3. RESULTS AND DISCUSSION

3.1. Heavy metals in sewage sludge

In general, there were no significance differences between irradiated and non-irradiated sludge in total or available (DTPA-extractable) heavy-metal concentrations (Table I). Sewage sludge metal concentrations were in the following order: Pb > Ni > Co > Cd. Compared with metal concentrations reported for municipal sewage sludges in the United States [16], Europe [17] and Egypt [18], the concentrations in sludge from El-Gabal El-Asfar farm were within the normal ranges or lower. Also, Chaney and Ryan [19] considered that sludge containing >100 µg/g Ni, and 0.5% Cd/Zn should not be applied to agricultural land. The sludge used in this study contained lower amounts of these metals.

The Zn equivalents calculated for the irradiated sewage sludge were 31, 62, 93 and 124 µg/g for 20, 40, 60 and 80 kg sludge/ha, respectively, all lower than the critical concentration (250 µg/g). The metal equivalents in the calcareous soil calculated for the irradiated sewage sludge were 4.9, 9.7, 14.6, and 19.4 µg/g for the same application rates, respectively. According to the criteria proposed by United States Environmental Protection Agency [20] for sewage sludge application, these metal concentrations were below the critical limits and, therefore, presented no hazard.

Concentrations of plant-available metals, as determined by DTPA extraction, ranged from 0.94 for Cd to 6.14 µg/g for Pb, in the order Pb > Co > Ni > Cd, with no significant differences between irradiated and non-irradiated sludge. The fractions of total heavy metals in irradiated sludge extracted by DTPA were 2.2, 4.1, 6.3 and 18% for Pb, Ni, Co and Cd, respectively. This indicates that Cd was more soluble or available to plants than Co, Ni or Pb. Similar data were obtained by Badawy and Helal [18].

3.2. Heavy metals in soil

3.2.1. Total concentrations

Sludge application usually resulted in increases in total heavy-metal concentrations in the soils. The increase depended on the sludge application rate. The total Cd, Co, Ni, and Pb concentrations at 80 t sludge/ha compared with the control were 2.8-, 1.3-, 2.7-, and 7.8-fold for Cd, Co, Ni and Pb, respectively, in the calcareous soil, and 2.8-, 2.0-, 2.9-, and 6.2-fold in the sandy soil.

Comparing the concentrations of heavy metals in the present study at 80 t sludge/ha with the maximum permitted loadings in the soil, established by the United States Environmental Protection Agency [20] (i.e. (kg/ha) Cd 39, Co 100, Ni 420, and Pb 300) gives levels of 6.0, 21, 6.4, and 14%, respectively, for the calcareous soil and 3.2, 17, 5.2, and 8.3% for the sandy soil. The exact conversion depends on soil density and depth of sludge mixing [4]. Also, Tietjen [21] suggested the following tolerable concentrations (µg/g) for heavy metals in soil: 100 for Pb or Ni, 50 for Co, and 5 for Cd which were in excess of those obtained even with the highest sludge-application rate (80 t/ha) (Table II).

Table I. Concentrations of heavy metals in the sewage sludge

Form	Metal	Irradiated sludge	Non-irradiated sludge
		(µg/g)	
Total	Cd	6.9	6.8
	Co	33.5	34.2
	Ni	44.3	43.8
	Pb	278	284
DTPA-extractable	Cd	0.94	0.89
	Co	2.10	1.95
	Ni	1.81	1.87
	Pb	6.1	7.5

Table II. Total concentrations of heavy metals in soils treated with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Cd	Co	Ni	Pb	Cd	Co	Ni	Pb
		(µg/g)							
Control	0	0.84	15.8	10.0	5.29	0.45	8.50	7.80	4.07
Fertilizer	0	0.89	13.5	11.0	6.20	0.40	8.90	8.58	4.10
Non-irradiated sludge	20	1.02	16.1	14.0	18.2	0.60	9.40	10.8	9.98
	40	1.43	18.4	20.0	26.9	0.75	11.9	14.1	15.3
	60	1.84	19.6	23.8	40.0	0.95	13.2	18.8	20.3
	80	2.61	21.8	26.2	40.7	1.35	15.7	21.2	24.9
Irradiated sludge	20	1.10	16.4	15.0	18.0	0.60	8.90	11.6	9.20
	40	1.62	18.8	19.0	29.5	0.80	13.0	15.0	13.9
	60	1.83	19.4	21.8	40.7	0.99	14.0	17.9	20.0
	80	2.35	208	26.8	41.3	1.25	16.7	22.3	25.2
$LSD_{0.05}$		0.29	1.78	1.74	4.30	0.17	1.40	1.34	2.08

The total concentrations of Cd, Co, Ni, and Pb in the sandy soil were lower than those in the calcareous soil for the same treatments (Table II). The relative values were: 0.52 for Cd, 0.63 for Co, 0.79 for Ni, and 0.58 fold for Pb. This reflects the native values of these metals in these soils [22–24]. The relative total concentrations for calcareous and sandy soils were Cd < Co < Ni < Pb. Significant differences were found between the total heavy metals concentrations with increasing rate of sludge application. However, there were no significant differences between irradiated and non-irradiated sludge-amended soils in their concentrations of Cd, Co, Ni and Pb.

3.2.2. DTPA-extractable

Soil concentrations of DTPA-extractable Cd, Co, Ni, and Pb increased with sludge-application rate (Table III). Judged from previous work [3,18,23] the concentrations were within normal ranges found in non-polluted soils of Egypt. The concentrations of DTPA-extractable Cd, Co, Ni and Pb in sludge-amended soils were low in spite of the high total content of these metals, representing only 8.8, 1.5, 6.6 and 9.7% for Cd, Co, Ni and Pb, respectively, in the calcareous soil and 41, 91, 10 and 24% in the sandy soil at 80 t sludge/ha. The values of DTPA-extractable metals at the highest application rate (80 t/ha) compared with the control soil were: 19-, 5.2-, 21, and 8.6-fold for Cd, Co, Ni, and Pb, respectively, in the calcareous soil, and 47-, 13-, 32-, and 13-fold in the sandy soil.

Although, the total concentrations of heavy metals in the sandy soil were lower than those in the calcareous soil for the same sludge-application rate, the DTPA-extractable concentrations for the sandy soil were higher than the calcareous soil (2.5-fold for Cd, 2- for Co, 1.3- for Ni, and 15-fold for Pb) at 80 t sludge/ha. This is presumably due to precipitation as carbonates in the calcareous soil, thereby reducing their concentrations in the soil solution and hence their availability for plant uptake. Many investigators have shown that the carbonate content of calcareous and alkaline soils controls the solubility of Pb [25], Co [22], Ni and Cd [26] and Cd [27].

Table III. Concentrations of DTPA-extractable heavy metals in soils treated with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Cd	Co	Ni	Pb	Cd	Co	Ni	Pb
		(µg/g)							
Control	0	0.011	0.062	0.083	0.469	0.011	0.052	0.070	0.447
Fertilizer	0	0.013	0.060	0.080	0.682	0.011	0.064	0.083	0.679
Non-irradiated sludge	20	0.061	0.108	0.158	1.10	0.087	0.157	0.230	1.42
	40	0.078	0.132	0.301	1.99	0.103	0.258	0.550	2.10
	60	0.129	0.176	0.898	3.11	0.298	0.348	1.29	4.85
	80	0.200	0.277	1.67	4.18	0.503	0.620	2.45	5.85
Irradiated sludge	20	0.067	0.116	0.152	1.02	0.050	0.190	0.280	1.62
	40	0.088	0.139	0.402	2.05	0.145	0.208	0.620	2.13
	60	0.137	0.208	0.880	3.03	0.258	0.343	1.04	4.93
	80	0.207	0.321	1.76	4.02	0.515	0.653	2.27	5.97
$LSD_{0.05}$		0.025	0.039	0.094	0.358	0.087	0.060	0.195	0.714

Table IV. Correlation coefficients (r) for linear relationships between DTPA-extractable heavy metals (μg/g) and soil characteristics

Metal	OM (%)	$CaCO_3$ (%)	pH	CEC (mEq/100g)	Clay (%)	Total metal (μg/g)
Cd	0.934**	–0.770**	–0.870**	0.973**	0.980**	0.910**
Co	0.718**	–0.795**	–0.890**	0.975**	0.860**	0.912**
Ni	0.814**	–0.826**	–0.870**	0.984**	0.888**	0.934**
Pb	0.829**	–0.862**	–0.907**	0.902**	0.973**	0.864**

Table V. Heavy-metal concentrations in tomato leaves grown on soils amended with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Cd	Co	Ni	Pb	Cd	Co	Ni	Pb
		(μg/g)							
Control	0	0.013	0.040	0.088	0.014	0.011	0.039	0.055	0.016
Fertilizer	0	0.012	0.047	0.085	0.012	0.014	0.039	0.065	0.020
Non-irradiated sludge	20	0.046	0.135	0.138	0.120	0.061	0.204	0.241	0.153
	40	0.091	0.180	0.225	0.139	0.118	0.280	0.398	0.224
	60	0.142	0.225	0.315	0.165	0.167	0.369	0.533	0.285
	80	0.174	0.320	0.360	0.219	0.215	0.490	0.649	0.372
Irradiated sludge	20	0.050	0.124	0.174	0.132	0.088	0.262	0.311	0.181
	40	0.110	0.185	0.253	0.153	0.137	0.340	0.484	0.274
	60	0.156	0.245	0.328	0.180	0.185	0.407	0.614	0.356
	80	0.190	0.309	0.394	0.236	0.225	0.539	0.712	0.433
$LSD_{0.05}$		0.022	0.038	0.041	0.019	0.032	0.065	0.089	0.071

There were highly significant increases in the soil concentrations of Cd, Co, Ni, and Pb with increasing rates of application of irradiated and non-irradiated sewage sludge. However, there were no significant differences between irradiated and non-irradiated sewage sludge treatments in heavy metal concentrations. Also, highly significant positive correlation coefficients (r) were found between DTPA-extractable metals and soil OM, CEC, clay and total metal concentrations, and negative correlation coefficients were obtained for soil pH and $CaCO_3$ content (Table IV).

3.3. Heavy-metal concentrations in tomato plants

3.3.1. Leaves

The concentration of Cd, Co, Ni, and Pb in leaves increased with increasing application rates of irradiated and non-irradiated sewage sludge to calcareous and sandy soils (Table V) in the order Cd < Pb < Co < Ni. The increases in metal concentrations in leaves at 80 t sludge/ha compared with the control were 15-, 8-, 5- and 17-fold for Cd, Co, Ni, and Pb, respectively, in the calcareous soil, and 21-, 14-, 13-, and 27-fold in the sandy soil. The heavy-metal concentrations in tomato leaves cultivated in the sandy soil were higher than those in the calcareous soil, presumably due to precipitation as insoluble carbonates in the latter. Similar results have been obtained by other authors [24,28–31] who reported reduced concentrations of Pb, Cd, Ni, Zn, and Cu in tissues of plants grown in soils amended with limestone or calcium carbonate.

The concentrations of Cd, Co, and Ni in tomato leaves were within ranges previously reported to be normal. The intermediate concentration of Cd reported by Adriano [24] was <0.30 μg/g, whereas ranges for Co and Ni were reported by Chapman [32] to be 0.06 to 0.25 and 0.28 to 1.08 μg/g, respectively. Also, the concentrations of Pb in the leaves in this study were comparable with tomato leaves grown in uncontaminated soil [32]. Although all the data in the present study show that application of sewage sludge resulted in increased concentrations of Cd, Co, and Ni in tomato leaves, the concentrations were still lower than the toxicity levels for plants according to several sources [e.g. 33). Suggested permissible tolerance levels in agronomic crops are 10, 5, 50, and 3 μg/g for Cd, Co, Ni, and Cd, respectively.

Highly significant correlation coefficients were found between concentrations of Cd, Co, Ni and Pb in leaves and DTPA-extractable concentrations in soils (r = 0.864**, 0.938**, 0.839** and 0.910**, respectively). However, there were no significant differences between irradiated and non-irradiated sludge treatments with regard to the concentrations of Cd, Co, Ni and Pb in tomato leaves.

3.3.2. Fruits

The application of sewage sludge resulted in a substantial increases in the concentrations of Cd, Co, Ni, and Pb in tomato fruits (Table VI). Magnitude of the increases depended on the sludge application rate and soil type. The concentrations at the highest rate (80 t/ha) compared with the control were 20-, 8-, 5-, and 3-fold higher for Cd, Co, Ni, and Pb, respectively, for tomatoes grown on the calcareous soil, and 38-, 17-, 21- and 6-fold higher for those grown on the sandy soil. This reflects the effect of soil properties on heavy-metal solubility and hence accumulation in fruits. Tomato fruits exhibited lower concentrations of heavy metals as compared with leaves, i.e. 72, 62, 52, and 25% of the leaf concentrations for Cd, Co, Ni, and Pb, respectively. The translocation of metals from leaves to fruits followed the order Cd > Co > Ni > Pb.

Table VI. Concentrations of heavy metals in tomato fruits grown on soils amended with sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Cd	Co	Ni	Pb	Cd	Co	Ni	Pb
		(μg/g)							
Control	0	0.005	0.028	0.045	0.010	0.005	0.013	0.018	0.010
Fertilizer	0	0.005	0.028	0.048	0.010	0.008	0.014	0.015	0.010
Non-irradiated sludge	20	0.028	0.074	0.065	0.012	0.056	0.135	0.198	0.030
	40	0.052	0.098	0.098	0.015	0.087	0.156	0.250	0.038
	60	0.075	0.140	0.139	0.020	0.130	0.180	0.320	0.049
	80	0.102	0.189	0.210	0.024	0.173	0.208	0.356	0.055
Irradiated sludge	20	0.040	0.085	0.075	0.016	0.064	0.145	0.220	0.035
	40	0.071	0.120	0.112	0.018	0.109	0.169	0.260	0.041
	60	0.094	0.160	0.165	0.023	0.152	0.195	0.296	0.051
	80	0.102	0.210	0.209	0.027	0.189	0.217	0.380	0.059
$LSD_{0.05}$		0.020	0.024	0.029	0.003	0.030	0.019	0.032	0.005

The concentrations of Cd, Co, Ni, and Pb in tomato fruits were generally within the normal concentration ranges for plants, with the exception of Cd at the highest sludge application rate. The intermediate concentrations (µg/g) of Cd, Co, Ni, and Pb reported by Adriano [24] and Chapman [32] were 0.04 to 0.06 for Cd, 0.06 to 0.25 for Co, 0.15 for Ni and 1.19 for Pb. Thus, compared with these values, tomato fruits grown in soil amended with sludge at 80 t/ha contained 1.7 times as much Cd in the calcareous soil and 3.2 times as much Cd in the sandy soil. However, the concentrations of Pb were within the normal range for tomato fruits grown in unpolluted soil.

Highly significant correlation coefficients were found between concentrations of Cd, Co, Ni, and Pb in leaves and fruits (r = 0.965**, 0.944**, 0.970**, and 0.939**, respectively). There were no significant differences between the irradiated and non-irradiated sludge treatments in concentrations of Cd, Co, Ni, or Pb in tomatoes.

3.4. Metal content of fruit

The amounts of Cd, Co, Ni and Pb taken up by fruits increased as the application rate of sludge increased in both soils (Table VII). The increases were higher in the sandy soil than the calcareous soil, which is attributed to the role of $CaCO_3$ on precipitation of metals. The amounts of heavy metals taken up by fruits were very low compared with the total soil content. The percentage range was 0.02 to 0.07 for Cd (average 0.05%), 0.007 to 0.024 for Co (average 0.014%), 0.005 to 0.018 for Ni (average 0.010%) and 0.0001 to 0.0004 for Pb (average 0.0002%) for the calcareous soil, and 0.036 to 0.145 for Cd (average 0.090%), 0.013 to 0.026 for Co (average 0.020%), 0.015 to 0.034 for Ni (average 0.025%) and 0.0003 to 0.0008 for Pb (average 0.0006%) for the sandy soil.

Because accumulation of Cd, Co, Ni and Pb in tomato fruits increased as the sludge application rate increased, human consumption of such fruits represents a potential health hazard. The maximum permissible dietary intake amounts for these metals are: 60 µg Cd/person/day; 75 µg Pb/adult/day and 15 µg Pb/child/day; and 1.2 mg Ni/person/day [34,35].

Table VII. Uptake of metals by tomato fruit from soils amended with irradiated and non-irradiated sewage sludge

Treatment	Sludge rate (t/ha)	Calcareous soil				Sandy soil			
		Cd	Co	Ni	Pb	Cd	Co	Ni	Pb
		(g/ha)							
Control	0	0.001	0.007	0.012	0.003	0.001	0.002	0.003	0.002
Fertilizer	0	0.002	0.011	0.019	0.004	0.003	0.005	0.005	0.004
Non-irradiated sludge	20	0.018	0.047	0.041	0.008	0.036	0.087	0.128	0.019
	40	0.066	0.124	0.124	0.019	0.132	0.237	0.380	0.058
	60	0.126	0.236	0.234	0.034	0.277	0.384	0.682	0.105
	80	0.290	0.537	0.596	0.068	0.561	0.674	1.15	0.178
Irradiated sludge	20	0.030	0.064	0.057	0.012	0.055	0.125	0.190	0.030
	40	0.101	0.170	0.159	0.025	0.177	0.274	0.421	0.066
	60	0.183	0.311	0.320	0.045	0.328	0.421	0.640	0.110
	80	0.309	0.637	0.634	0.082	0.604	0.694	1.22	0.189

Calculating the maximum amount of tomato fruits for safe human consumption, it is clear that Cd is the limiting factor in determining the consumption rate. The maximum permissible amount of tomato fruits can differ in relation to the soil type and the sewage-sludge application rates, as follows: 110, 59, 45 and 41 kg fresh fruits/week for the calcareous soil and 66, 39, 28 and 22 kg fresh fruits/week for the sandy soil for irradiated sludge application rates of 20, 40, 60 and 80 t/ha respectively. These limits should not be exceeded to avoid impairment of kidney function, disturbance in Ca and P metabolism, and bone disease.

4. CONCLUSIONS

Application of irradiated and non-irradiated sewage sludge, even at 80 t/ha, did not raise the concentrations of Cd, Co, Ni, or Pb beyond international permissible levels for soils, tomato leaves, or fruits. Using the Zn-equivalent criterion for the sludge in this study, it was concluded that the heavy-metal concentration was lower than the critical limit (250 μg/g) presenting no hazard for use in agriculture. The calcareous soil can accept higher levels of sewage sludge application than the sandy soil before extractable metal concentrations exceed the maximum permissible limit, presumably due to precipitation of heavy metals as carbonates.

Gamma irradiation is recommended as a method for sewage-sludge disinfection. The irradiation treatment did not increase the extractable forms or plant uptake of the studied metals. The concentrations of Cd, Co, Ni and Pb in the fruit limit utilization in the human diet. Consumption should not exceed the maximum permissible quantity, which was calculated on the basis of the Cd content in tomato fruits at the highest sludge application rate (80 t/ha) as follows: 41 kg fresh tomato fruits/week in the calcareous soil and 22 kg fresh tomato fruits/week in the sandy soil.

The use of sludge-amended soils to grow ornamental plants or plants that can provide fiber, fuel wood or oilseeds for industrial purposes merits serious consideration as an alternative to growing crops for human consumption,. Another factor in designing a good agronomic package is the suitability of the cropping system to drip irrigation, which is necessary under Egyptian conditions.

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ORGANIC CONTAMINANTS IN SEWAGE SLUDGE AND WASTEWATER: THEIR METABOLIC FATE IN CROPS AND THEIR IMPACT ON FOOD QUALITY

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Abstract

Sewage sludges contain a complex mixture of organic contaminants. Therefore, data are needed on the persistence of sludge-derived organics in soils and their uptake by food crops. In this study, all compounds, even non-polar, were assimilated by intact plants and in-vitro systems. Uptake depended on the plant species and on the physico-chemical properties of the chemical. The main metabolites were polar conjugates with carbohydrates and amino acids. Polycyclic aromatic hydrocarbons (PAHs) were partly converted to oxygenated derivatives that are even more toxic. Depending on the plant species partly, large amounts of the chemicals and/or their metabolites were frequently incorporated into non-extractable (bound) residues. The association, and type of binding to cell-wall components, enable conclusions to be drawn about the bioavailability of these bound residues.

1. INTRODUCTION

The application of sewage sludge and biowastes to agricultural land is generally the most economical means of disposal, and provides the opportunity to recycle beneficial plant nutrients and organic matter for crop production. For instance, sewage sludges contain appreciable amounts of N and P and have significant inorganic-fertilizer-replacement value. Crop productivity can also be increased by improving soil physical properties through the organic matter contained in sludges and biowastes.

However, besides beneficial plant nutrients, these waste matrices also contain hazardous heavy metals and organic pollutants that may enter the food chain and are of great public concern. Compared with the voluminous information that is available on heavy metals, less is known about the environmental consequences of organic pollutants in sewage sludges and biowastes. The range of organic compounds known to exist in these matrices is extensive and diverse, and is potentially transferred to sludge- and compost-amended agricultural soils. From a review of literature, Drescher-Kaden et al. [1] reported that 332 organic pollutants, potentially hazardous to human or environmental health, were identified in sewage sludges in Germany, forty-two of which are regularly detected in sludge by researchers. To ensure the safe and beneficial use of sewage sludge in agriculture, appropriated directives are needed. Procedures and limiting values defined in such a directive would offer a rational basis for recycling sewage sludge in agriculture.

In order to satisfy these requirements, standardized tests have been developed. In addition to tests with intact plants under septic and aseptic conditions, in-vitro techniques, such as plant-cell suspension cultures or differentiated root cultures, have been used to study the phytotoxicity, metabolic fate, and persistence of xenobiotics in plants.

2. MATERIALS AND METHODS

2.1. Uptake of xenobiotics by plants from soil

To investigate the uptake of xenobiotic compounds by crops from soils, plants were grown in rectangular plexiglass pots, each containing 1.2 kg soil containing 5 or 10 ppm of selected ^{14}C-labelled contaminants. To follow the evolution of $^{14}CO_2$ or other labelled volatile compounds from degradation processes in the soil and to distinguish between uptake of the original compound and possible photosynthetic incorporation of released CO_2, the root and shoot spheres were separated by an airtight lid. The stems passed through openings sealed with silicon rubber. A circulating air stream allowed continuous sampling of gases from the root zone for subsequent analysis. The incubation period was 4 weeks.

2.2. Aseptically grown plants

Wheat (*Triticum aestivum* L. cv. Heines Koga), tomato (*Lycopersicum esculentum* L. cv. Money Maker), and garden orache (*Atriplex hortensis* L.) plants were aseptically cultured using previously described methods [2]. After 2 to 3 weeks of growth, the labelled test compounds were added to the nutrient solution. After 5 days of treatment, the plants were harvested and analysed.

2.3. Cell-suspension cultures

Cell-suspension cultures of monocots, *Triticum aestivum* L., *Hordeum vulgare* L., *Pennisetum americanum* L., and dicots such as *Glycine max* (L.) Merr., *Daucus carota* L., *Lycopersicon esculentum* L. were cultured as described [2,3] and used for metabolism tests during the last 48 h of the late-logarithmic phase of growth.

2.4. Root cultures

Transformation of *Lupinus hartwegii* Lindl. and *L. polyphyllus* Lindl. plants by *Agrobacterium rhizogenes*, produced root cultures on MS-medium. The metabolism tests were as described for cell culture except that the incubation period with ^{14}C-labelled xenobiotics was lengthened to 3 days.

2.5. Phytotoxicity and metabolism test

The phytotoxicity, uptake, and metabolic fate of xenobiotics were studied by standardized methods [4]. The bound or non-extractable residues were analysed by a sequential fractionation procedure [5].

3. ORGANIC POLLUTANTS IN SEWAGE SLUDGES AND BIOWASTES

Many of the organic compounds (>300) designated by the European Commission as "priority pollutants" due to their potentially toxic effects, are known to occur in sludge. In the specific context of wastewater and sewage sludge, this enormous number of organic chemicals at large in the environment can be subdivided into several groups (Table I). Of these, the halogenated aromatics, like PCBs, furans and dioxins, and polycyclic aromatic hydrocarbons (PAHs) are generally regarded as the most critical.

The survey of representative samples in Table I provides ranges of organic pollutants to be expected in sewage sludge. European sludges contain between 0.5 and about 10 mg PAH/kg, depending on the individual substance concerned, most of them arising from road and surface runoff. Comparable contents of PCBs were also found, though in exceptional cases of industrial pollution readings of 1,000 ppm and more have been recorded. In the past, abundant quantities of organochlorine pesticides were also common in sewage sludge. Today, only traces, well under 1 ppm, remain. More recently, phthalates have received the attention they deserve; up to several hundred ppm of these plasticizers have been found in sewage sludge.

Levels of over 1 g/kg dry matter have recorded for nonylphenol, a toxic decomposition product of alkylphenolpolyethoxylate detergents present in effluents. It is a compound of great environmental concern, especially since estrogenic effects have been reported in fish, birds, and mammals. Adverse effects have also been shown for germination and growth of various plant species.

Table I. Abundance and concentration ranges of the main hazardous compounds in sewage sludge

Compound	Abundance (%)	Range / Mean (mg/kg DM)
Chlorinated Hydrocarbons		
PCBs	100	0.05–1 / 0.5
Lindane	40–60	<0.01–0.07 / 0.02
p,p'- DDT + p,p'- DDE	30–60	<0.01–0.25 / 0.1
PAHs		
Fluoranthene	100	0.5–60 / 5
Benzo(a)pyrene	100	0.1–15 / 3
Benzo(b)fluoranthene	100	0.1–14 / 3
Phenolics		
Phenol	30–50	0.002–300 / 2
Pentachlorophenol	20-30	0.03–8500 / 0.2
Phthalates		
Di-(2-ethylhexyl)-phthalate	>95	2.4–320 / 80
Surfactants		
LAS	100	50–16,000 / 5,000
Nonylphenol	100	10–2,500 / 500

At present there is a great deal of interest in the long-term effects of applications of sewage sludge and compost to agricultural land. One group of organics, PAHs, are of concern because of their carcinogenicity/mutagenicity. In an experiment at Rothamsted Experimental Station [6] sludge was added to agricultural land from 1942 to 1961 and PAH content was analyzed in the archived sludges and in soil collected before, during, and subsequent to application. After the cessation of sludge addition, a gradual fall in total PAH was observed; however, the sludge-amended soil contained three-fold more PAHs than the control after 23 years. These data reveal relatively long-term residual effects of sludge on PAH content of soil, with higher molecular-weight aromatics appearing to be more persistent.

Analyses showed low-level presence of polychlorinated biphenyls (PCBs) in the soils treated with sewage sludge. Although PCBs are known for their persistence, the data suggest that they are gradually degraded in soil, unless the decreases can be attributed to relatively high volatility. Although the long-term experiments showed at least a five-fold increase in PCB content in the soils, the quantities were well below 1 ppm.

In addition to volatilization, microbial degradation has been shown to be an important loss-mechanism for many trace organic compounds in soil. Resistance to microbial degradation is dependent on the type, position, and degree, e.g. of the halogen residue. Significant degradation of di- and trichloro-PCDDs and PCDFs in soil have been measured, and the tetra- to octachloro congeners were found to be highly persistence.

Table II. Metabolism rates (%) of PCBs

PCB[a]	GM[b]	TR	HV	TA	PA	DC	LE	AH	BVa	BVe	CR	CQ
4	23	–	10	–	–	–	20	14	–	–	–	–
8	56	70	48	–	64	10	–	–	–	–	–	–
15	–	–	–	–	–	–	–	–	–	–	–	–
18	45	30	17	83	–	–	–	–	–	–	–	–
28	24	–	–	–	12	–	–	–	–	–	–	–
31	27	18	–	–	20	–	–	–	–	–	–	–
47	–	10	–	–	–	–	–	–	–	–	–	–
52	12	–	–	20	–	–	–	–	–	–	–	–
101	–	–	–	–	–	–	–	–	–	–	–	–
153	–	–	–	–	–	–	–	–	–	–	–	–

[a] 4 = (2,2'), 8 = (2,4'), 15 = (4,4'), 18 = (2,2',5), 28 = (2,4,4'), 31 = (2,4',5), 47 = (2,2',4,4'), 52 = (2,2',5,5'), 101 = (2,2'4,4',5), 153 = (2,2'4,4',5,5').

[b] *Glycine max, Trifolium repens, Hordeum vulgare, Triticum aestivum, Pennisetum americanum, Daucus carota, Lycopersicon esculentum, Atriplex hortensis, Beta vulgaris altissima, Beta vulgaris esculenta, Chenopodium rubrum, Chenopodium quinoa.*

4. UPTAKE AND METABOLISM BY PLANTS

Uptake, metabolism, and persistence of the hazardous xenobiotics listed in Table I were investigated.

4.1. Polychlorinated biphenyls

Polychlorinated biphenyls are industrial compounds that have been detected in almost every compartment of the global ecosystem. Although their production has ceased in most industrial countries, PCBs are still important environmental pollutants due to their persistence. In the present study we describe the metabolism of ten PCB congeners in cell-culture systems of twelve plant species. Recovery rates were calculated as compared to dead cells.

Decreases in PCBs, attributable to metabolism, were dependent on plant species and congener (Table II). Cultures of Leguminosae species, soybean (*Glycine max*) and clover (*Trifolium repens*), were able to metabolize the largest numbers of congeners with six and four, respectively. The three Poaceae species, *Pennisetum americanum*, *Triticum aestivum* and *Hordeum vulgare*, metabolized smaller numbers of different congeners, but some to a very high degree, e.g. PCB 18 by wheat cultures at a rate of 83%. Carrot and tomato cultures showed only limited capacity for metabolism of PCBs; each metabolized only one of the tested congeners with a maximum of 20%. Except for *Atriplex hortensis,* none of the tested Chenopodiaceae species (*Beta vulgaris altissima* and *esculenta, Chenopodium rubrum* and *C. quinoa*) were unable to metabolize any of the tested metabolites. *Atriplex* showed a decrease in PCB 4 of 14%. From this it is clear that the metabolic capacity is strongly dependent on the plant species; however, the limited number of tested species does not allow extrapolation to plant families.

With lower chlorination grade, a greater possibility for metabolism was observed, especially in PCBs without substituents in the ortho or meta positions. The penta- and the hexachlorinated PCBs, 101 and 153, were not metabolized by any of the tested cell cultures. Also, higher water solubility was related to metabolism, although PCB 15 (4,4') was an exception—it was not metabolized, despite of its low chlorination grade and its relatively high water solubility. This can be explained by its coplanar configuration, which sterically hinders attack by enzymes. The data obtained with cell cultures in the present study are consistent with those found for Paul's Scarlet rose [7].

There seems to be a relationship between the structural and physical properties of PCBs. Those that possess a free ortho-meta and meta-para position were metabolized best, whereas those with free ortho-meta or meta-para positions were metabolized by only some of the cultures and to a small extent. If these positions are substituted by chloro atoms, no metabolism was detected.

To investigate the metabolism of PCBs, ^{14}C-labelled 2-chlorobiphenyl (PCB 1), 2,2'5,5'-tetrachlorobiphenyl (PCB 52), and 3,3'4,4'-ztetrachlorobiphenyl (PCB 77) were applied to soybean cell cultures. Table III shows the distribution of radioactivity.

In all cases, the major amounts of radioactivity were detected in the cell extracts. Whereas for PCB1 and PCB 52 higher amounts were present in the methanol/water fraction, PCB 77 revealed higher amounts in the dichloromethane phase. The latter is due to the parent compound, whereas radioactivity in the methanol/water phase represents polar metabolites. Paul's Scarlet rose was the only plant culture that showed noteworthy metabolism rates. Analysis of the methanol/water phase gave several polar metabolites that yielded a number of products after hydrolysis with HCl. Based on co-chromatography with authenticated compounds, these products were identified hydroxylated PCBs. The main metabolites of PCB 1 were 2'-chloro-4-biphenylol and 2'-chloro-3-biphenylol while the very polar compound was 2'-chloro-3,4-biphenyldiol. For PCB 52, the 2,2',5,5'-tetrachloro-4-biphenylol was the main metabolite, and further monohydroxylated and dihydroxylated biphenyls were also detected. The tetrachlorinated PCB 77, which has a low water solubility (1.88×10^{-9} *M*), was metabolized by only three of the tested cultures, tomato (LE), lettuce (LS), and, to the greatest extent, Paul's Scarlet Rose (PSR). None of the other cultures metabolized PCB 77. Chromatographic separation showed that several polar compounds were formed. The GC/MS-analysis revealed that these metabolites were mono- and dihydroxy-compounds of PCB 77. The main metabolites identified were 2-hydroxy-3,3'4,4'-tetrachlorobiphenyl and 5-hydroxy-3,3'4,4'-tetrachlorobiphenyl. Furthermore a 6-hydroxy-3,3'4,4'-tetrachlorobiphenyl was been identified in PSR cultures.

Table III. Fractional distribution of radioactivity (%) after application of ^{14}C-PCB 1, ^{14}C-PCB 52, and ^{14}C-PCB 77

Fraction	PCB 1	PCB 52	PCB 77		
	GM[a]	TA	LE	LS	PSR
			(%)		
Medium	7	7	13	>1	6
Cell extract	58	69	81	95	81
Methanol/water phase	36	57	2	8	18
Dichloromethane phase	22	12	79	87	63
Nonextractable residue	10	8	1	1	2
Total recovery	75	84	95	97	89

[a] *Glycine max, Triticum aestivum, Lycopersicum esculentum, Lactuca sativa, Paul's Scarlet rose.*

Table IV. Fractional distribution of radioactivity after application of (3-^{14}C)-fluoranthene to cell cultures

Species	Cells			Medium	Recovery
	DCM phase	MeOH/H_2O phase	Bound residue		
			(%)[a]		
Artiplex hortensis	90	4.4	0.3	4.8	100
B. vulgaris altissima	71	1.2	2.2	4.3	79
B. vulgaris conditiva	91	2.7	0.8	3.9	99
Brassica napus	67	1.5	1.7	8.7	79
Daucus carota	85	1.9	1.1	4.1	92
Glycine max	75	4.2	4.5	8.7	92
Hordeum vulgare	82	5.3	0.4	4.2	92
Lactuca sativa	67	6.3	4.9	7.9	86
L. esculentum	72	15.0	0.7	5.4	93
Paul's Scarlet Rose	16	49.9	1.6	27.2	96
Tricitum aestivum	73	9.1	1.7	4.7	88

[a] Fraction of applied (3-^{14}C)-fluoranthene, means from three parallel experiments.

4.2. Polycyclic aromatic hydrocarbons

Environmental contamination with PAHs can be traced back mainly to industrial and household activities, and motor-vehicle exhausts. The pyrolysis and/or incomplete charring of organic substances at temperatures between 500 and 700°C releases more than 160 PAHs of varied composition. The distribution of PAHs occurs mainly as waste in water and air; rain washes them into surface waters and soil. The relatively large concentration of PAHs found in urban wastewater has raised the concern that agricultural utilization of sewage sludge might affect food quality and human health. Therefore, investigations were performed to measure accumulation of PAHs from urban composts and sludges in soils and plants.

4.2.1. Fluoranthene

One of the most abundant PAHs, fluoranthene, has been detected in air, water, soil, sediments and even in biota including humans. To assess the metabolism of this hazardous compound, cell-suspension cultures of various plant species were incubated in 1 ppm.

The data for the non-polar DCM-phase demonstrated that most of the applied activity remained as parent compound (Table IV). Only the cultures of lettuce, tomato, wheat and rose revealed significant turnover rates. Autoclaved cells were used as the control; they produced no metabolites, demonstrating absence of abiotic reactions. The cell extracts showing higher turnover rates were subjected to chromatography. Pattern of metabolites were similar for all cultures tested. Tomato and wheat produced two main metabolites and two others to a lesser extent, and the PSR culture formed only two peaks corresponding to the main metabolites. After HCl-hydrolysis and clean-up with GPC, the samples were separated by semi-preparative HPLC into two (PSR) or four (tomato) fractions. Because of their intensive fluorescence, it was possible to detect the metabolites very accurately without radioactive label, by comparing the peak patterns with those of corresponding radioactive samples.

Table V. GC-MS data for the metabolite fractions isolated by semi-preparative HPLC

Cell culture	HPLC-fraction (retention time window)	GC/MS retention index[a]	m/e (relative intensity)
PSR	fraction 3 (24–26 min)	2340	218 (100), 189 (50)
	fraction 4 (27–29 min)	2320	218 (100), 189 (62)
Tomato	fraction 3 (24–26 min)	2342	218 (100), 189 (50)
	fraction 4 (27–29 min)	2330 2358	218 (100), 189 (53) 218 (100), 189 (58)

[a]Base peak.

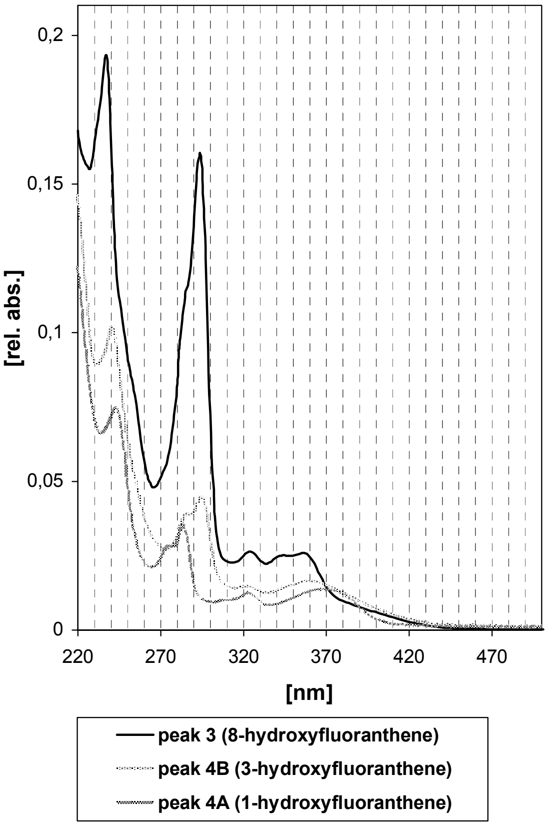

FIG. 1. UV/VIS spectra of fluoranthene metabolites.

To identify the metabolites, the concentrated fractions were analyzed by GC-MS (EI) and HPLC-DAD. On GC-MS analysis of HPLC-fractions 3 and 4 of PSR and tomato, mass spectra with a base peak of m/e 218 were detected (Table V). These base peaks could be attributed to the molecular ions [M^+] of monohydroxylated fluoranthene isomers. In addition, the mass spectra contained fragment ions of m/e 189 [M^+–CHO].

As the UV-VIS data of fluoranthenols are available in the literature [8,9], HPLC-DAD analyses were performed to confirm the formation of fluoranthenols and to identify the position of the OH-group. The spectra obtained are shown in Fig. 1.

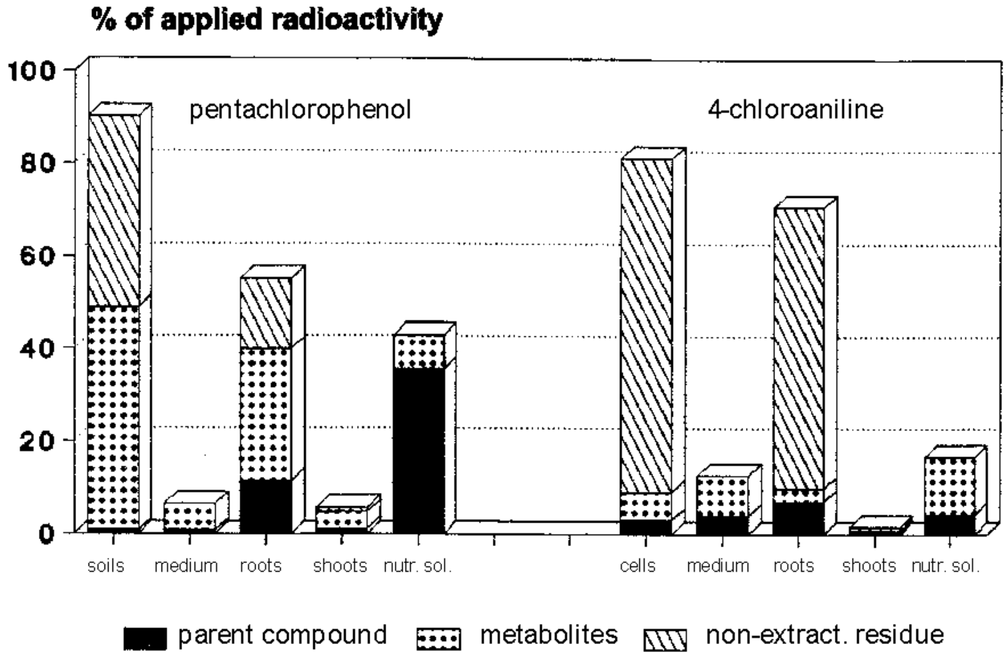

FIG. 2. Metabolism of pentachlorophenol and 4-chloroaniline in wheat-cell-suspension cultures and intact plants.

The comparision of the UV/VIS spectra with those of reference compounds confirmed that the metabolites formed in tomato cultures corresponded to 8-hydroxyfluoranthene and to 1-hydroxyfluoranthene and 3-hydroxyfluoranthene, respectively. In cultures of Paul's Scarlet rose only 8-hydroxyfluoranthene and 1-hydroxyfluoranthene were detectable.

4.3. Phenolic compounds and aromatic amines

4.3.1. Pentachlorophenol and 4-chloroaniline

Pentachlorophenol (PCP) and its salts have been used as wood protectants. On account of its antimicrobial, herbicidal and insecticidal properties, PCP has been used also as a herbicide in rice. 4-chloroaniline (4-CA) is a known degradation product of a variety of substituted phenylurea compounds that are used mainly as herbicides. The fate of 4-CA in soil and in soil microorganisms has been thoroughly studied [10]. A common feature of the metabolism of these compounds in soil and plants is a large proportion of nonextractable residues bound to high molecular-weight compounds such as humic substances in soil or lignin in plants.

The validity of extrapolating data obtained with cell-culture techniques to intact plants is still a matter of debate. In order to compare these two systems, cell-suspension cultures and wheat seedlings of the same cultivar were incubated with PCP and 4-CA. The metabolic fates of these compounds in the two plant systems are shown in Fig. 2.

The compounds were taken up and metabolized by both plant systems. The ^{14}C-label of both compounds was transported from the roots into the shoots of intact wheat plants. Cell cultures adsorbed pentachlorophenol very rapidly and formed high amounts of polar metabolites that were mainly associated with the cells. Of the radiolabel, 41% was converted (via the conjugate fraction) into the non-extractable residue fraction. The ^{14}C-label was bound mainly to lignin and to a high-molecular-weight hemicellulose fraction. Polar conjugates could also be extracted from roots and shoots, and PCP glycosides were predominant in the cultured cells. More than 16% of the total radioactivity from shoots and roots was found as bound residues. These were fractionated into several cell-wall components, yielding a patern similar to that in cell cultures.

In cell cultures, 72% of 4-CA was detected in the bound-residue fraction. Further studies showed that this high proportion of ^{14}C-label was associated mainly with the pectin and lignin fractions of the cell wall. 4-chloroaniline was also rapidly absorbed by the roots of wheat plants and metabolized to polar conjugates. The radioactivity of the bound-residue fraction reached more than 61%, which is comparable to the fraction found in cell cultures.

4.4. Surfactants

4.4.1. Nonylphenol

4-nonylphenol (4-NP) is a persistent product of the degradation of alkylphenolpolyethoxylates (non-ionic surfactants) that has been of public concern since aquatic toxicity was proven in the 1980s [11]. Recently, new discussions on possible bans or plans of some countries to introduce environmental quality standards on the compound have been triggered by the finding that nonylphenols are weakly estrogenic [12,13]. 4-NP occurs in sewage sludges and effluents of water-treatment plants. The application of sludge to agricultural land and the use of the associated wastewater in irrigation may lead to uptake and metabolism of 4-NP in crop plants. Another source of plant contamination may be the use of nonylphenols in the formulation of pesticides. Presence of nonylphenol and its derivatives in crop plants may have important implications for food quality. Therefore, toxicity, uptake, and metabolism in plants must be studied in order to estimate possible risks emerging to animals and/or humans.

In the presented study, the toxicity, uptake, and metabolism of 4-n-NP in intact plants and in-vitro systems were investigated. Besides in-vitro systems, intact plants were examined under aseptic conditions and in soil/plant-systems. To study species-specific metabolism, cell cultures of fourteen species were tested for 48 h with 4-*n*-nonyl-U^{14}C-phenol at a non-toxic concentration of 1 mg/L (4.5×10^{-3} m*M*). The results are documented in Table VI.

Cell cultures of all the plant species took up 4-*n*-NP and metabolized it. No $^{14}CO_2$ was trapped in NaOH from the gaseous phase, indicating that the cells did not degrade 4-*n*-NP to CO_2. Except for *Glycine max*, the major proportion of the radioactivity was recovered in the soluble cell extract and, except for *Chenopodium quinoa,* the major proportion of radioactivity was detected in the methanol/water phases. This indicates that the metabolites were more polar than the parent compound, which was extracted into the dichloromethane-phase. Figure 3 shows HPLC separation of the metabolites in some of the crude cell extracts and the same extracts after acid hydrolysis.

After hydrolysis, two HPLC peaks appeared that could be identified as 4-NPs with one or two hydroxyl groups in the side chain. The position of the OH-groups varied. Table VII shows the composition of the 4-NP metabolites.

The analysis of fraction [a] (Fig. 3) by ESI/MS/MS gave several dihydroxylated 4-NPs (positions of the OH-groups in the aliphatic side-chain of 4-NP at the C-atoms 5,7; 3,7; and 4,7) which were conjugated with glucose and glucuronic acid. Fraction [b] contained a compound isomeric with the dihydroxylated compounds in fraction [a], with OH-groups in positions 4 and 8 of the aliphatic side-chain. Fraction [c] was heterogeneous, comprising mono- and dihydroxylated derivatives, (position of the OH-groups at C-atom(s) 7-H_2O, 4,7; 5,7; 4,8) which were conjugated with glucose and organic acids, e.g. malonic acid. Fraction [d] yielded a dominant MS signal corresponding to monohydroxylated 4-NPs (position of the OH-group at C-atoms 4, 5, 6, 7, or 8 of the aliphatic side-chain), conjugated with a glucuronosyl-glucose moiety [14].

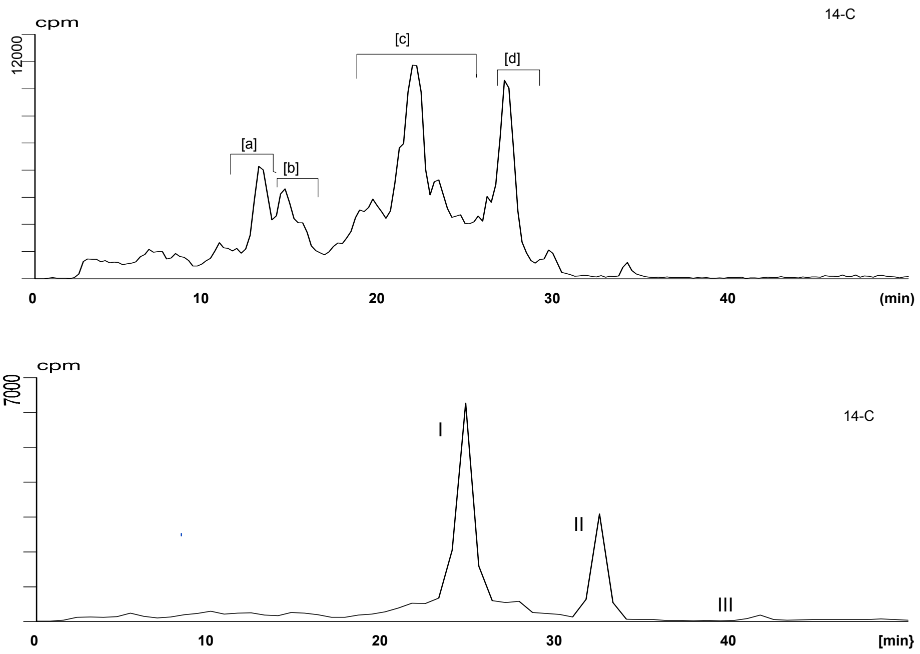

FIG. 3. HPLC traces of a crude extract (upper) and after acid hydrolysis (lower).

Table VI. Distribution of radioactivity (%) after application of 4-n-nonylphenol to cell-suspension cultures of various plant species

Species	Cells				Medium		Recovery
	DCM-phase	Methanol/ water phase	Bound residue		DCM phase	Methanol/ water phase	
Lupinus polyphyllus	5.4[a]	63	26		5.1	1.8	100
Lupinus hartwegii	8.8	77	12		0.78	1.7	100
Glycine max	6.2	25	320		(39.1)[b]		102
Daucus carota	13	33	22		14	11	93
Lactuca sativa	3.7	19	38		0.57	16	77
L. esculentum	0.00	87	6.6		0.00	5.7	99
Atriplex hortensis	43	45	4.8		4.7	1.6	99
Chenopodium quinoa	71	7.9	2.5		7.5	0.45	89
Chenopodium rubrum	14	71	3.8		2.4	3.5	95
B. vulgaris esculenta	24	57	12		0.00	5.6	98
B. vulgaris altissima	0.00	69	2.0		0.00	5.5	76
Hordeum vulgare	20	53	6.5		(3.5)[b]		83
Pennisetum americanum	0.00	64		10	2.8	6.2	83
Triticum aestivum	2.1	61		16	(5.00)[b]		83

[a] Fraction of applied ^{14}C-4-nonylphenol, average values for five combined parallel experiments.

[b] Medium not separated into two phases.

Table VII. 4-nonylphenol metabolites formed by wheat cells

Fraction	Glucose	Glucuronic acid	Position of OH-groups of 4-NP
[a][a]	++[b]	+[c]	5,7 (70%)[d]; 3,7 (20%); 4,7 (10%)
[b]	++	+	4,8 (80%)
[c]	++	–[e]	7,-H_2O (5%); 4,7 (40%); 5,7 (25%); 4,8 (30%)
[d]	++	+	4 (50%); 5 (10%); 6 (15%); 7 (20%); 8 (3%)

[a] Fractions according to Fig. 3. [b] Main component. [c] Further component. [d] Relative amount (%) of a compound in the actual fraction. [e] Not detected.

Table VIII. Fractional distribution of radioactivity in aseptically grown plants following application of 4-n-nonylphenol for 4 days

	Lycopersicon esculentum[a] (n=9)	*Triticum aestivum*[a] (n=18)	*Atriplex hortensis*[a] (n=70)
		(%)	
Medium	5.0 ± 1.1	52 ± 4.7	5.6 ± 2.0
Dichloromethane-phase	0.7	26	3.6
Methanol/water-phase	4.3	26	2.0
Shoot	4.2 ± 0.9	1.1 ± 0.5	3.2 ± 0.4
Dichloromethane-phase	0.1	ne[b]	0.1
Methanol/water-phase	1.9	ne	0.5
Non-extractable residue	2.2	0.4	2.6
Root	59 ± 5.1	27 ± 3.1	78 ± 5.3
Dichloromethane-phase	13	4.3	6.2
Methanol/water-phase	11	6.5	7.7
Non-extractable residue	35	16	64

[a] Plants were analysed in three replicates of three plants for *L. esculentum*, in three replicates of plants for *T. aestivum*, and in seven replicates with ten plants for *A. hortensis*. After the first fractionation in medium, shoot and root, extracts of the respective plants were combined for phase separation and bound-residue determinations. Therefore, standard deviations are not given for these values. [b] Not examined.

The metabolism of ^{14}C-4-n-NP in aseptically grown plants is documented in Table VIII. Plants grown in nutrient medium containing ^{14}C-4-n-NP incorporated the compound and metabolized it. The amounts taken up differed depending on the plant species. No mineralization to $^{14}CO_2$ took place. As the systems were sterile, this confirmed that plants do not mineralize 4-*n*-NP. The presence of radioactivity in shoots proves that 4-*n*-NP or its metabolites were transported from the root. *Atriplex* showed high uptake rates, which enabled a further extraction according to the described method. This extract was compared with an extract from *A. hortensis* cell-suspension cultures obtained in a previous study [15]. Thin-layer chromatography gave a similar pattern of radioactively labelled compounds with Rf-values of 0.30, 0.40, 0.60, and 0.75 (with the compound at an Rf-value of 0.75 being the dominating peak in both systems). This indicated that metabolism in cell-suspension cultures and intact plants is qualitatively identical. Quantitatively, in aseptically grown plants, the non-extractable residue constitutes the major fraction showing radioactivity.

In soil-plant transfer studies, the mineralization rates of 4-n-NP were much greater (ca. 30%), suggesting higher microbial activity compared to medium-grown plants or roots (Table IX).

Table IX. Fractional distribution of radioactivity in plant/soil systems incubated with 4-n-nonylphenol for 21 days (n = 3)

Species	Experiment	Shoot	Root	Soil	CO_2	Recovery
				(%)		
Lycopersicon esculentum	5 ppm[a] 4-NP	0.15	0.10	65	31	96
	10 ppm 4-NP	0.25	0.12	58	44	102
	control, without plant[b]	–	–	62	30	92
Atriplex hortensis	5 ppm 4-NP	0.49	0.33	49	11	60
	10 ppm 4-NP	0.69	0.55	63	17	81
	control, without plant[b]	–	–	49	12	61

[a] mg/kg soil.
[b] 5 ppm 4-*n*-NP.

Due to the low amounts of radioactivity in plants, respective materials from three experiments were combined and mixed to obtain reliable measurements; standard deviations were <12%. The two plant species (*L. esculentum* and *A. hortensis*) took up 4-*n*-NP at both test concentration levels. However, over a period of 21 days, the amounts of radioactivity did not exceed 1.5% of that applied. Although only small proportions were taken up, it is noteworthy that higher concentration led to higher uptake with both species, not only in absolute, but also in relative (%), amounts, and that radioactivity was transported into the shoot. Due to the experimental technique used, refixation of released $^{14}CO_2$ can be excluded. This confirms findings with aseptically grown plants, that 4-*n*-NP or its metabolites are transported within the plant. Uptake rates strongly differed between species, which has been shown also for cell cultures with 4-*n*-NP and other chemicals [15,16]. About 60% of the applied compound remained in the soil. The soil used in experiments with *A. hortensis* was extracted with methanol/dichloromethane/water (according to the extraction procedure for the plant material). 11.5% (10 ppm) or 20 to 25% (5 ppm) of the radioactivity in the soil was found to be soluble. The major part of the radioactivity was associated with the bound residue in the soil. The extractable amount of 4-*n*-NP in soil was within the range determined by Kirchmann et al. [17], although in that study soil-bound residues were not determined. For predictions of bioavailability of 4-*n*-NP from soils, more detailed studies would be necessary to elucidate complex interactions between soil microorganisms and soil physics and chemistry [18].

5. NON-EXTRACTABLE (BOUND) RESIDUES

After application of radiolabelled compounds to cell cultures, and especially to intact plants, large amounts of the radioactivity were found associated with insoluble plant components. These residues are referred to as “bound” or “non-extractable residues,” which typically cannot be released from the plant matrix by extraction with solvents. The patterns of binding of various xenobiotics depended on the plant species, and on the physical and chemical properties of the compound. Bound residues were assigned to defined cell-wall fractions using a sequential fractionation procedure. Table X shows the distribution of radioactivity in the various cell wall components of wheat cultures after treatment with various ^{14}C-labelled chemicals.

The lignin contained some radioactivity with all the compounds tested. However, in the cell-wall components, nearly 50% of the applied 4-chloroaniline was located in the pectin fraction and 27% in the lignin, whereas this distribution pattern was reversed for the 3,4-dichloro analogue. With pentachlorophenol, most radioactivity was in the hemicellulose fraction, although some was associated with the lignin and pectin fractions. Pentachloronitrobenzene, however, was predominantly associated with lignin and only a lesser portion was in the pectin.

Table X. Radioactivity released from cell-wall fractions of plants treated with various ^{14}C-labelled xenobiotics

Reagent	Material liberated	Radioactivity (%) released from cell-wall fractions of plants that received[a]			
		4-CA	3,4-DCA	PCP	PCNB
α-Amylase	starch	2.3	3.4	2.8	4.3
Pronase E	protein	5.6	3.4	23	20
EGTA[b]	pectin	48	21	6.9	14
Dioxane/HCl (2 *M*)	lignin	27	52	22	43
KOH (24%)	hemicellulose	4.8	12	36	6.0
H_2SO_4 (72%)	cellulose	1.6	0.2	1.9	1.8
	residue	2.8	0.3	2.8	2.8

Table XI. Distribution of radioactivity in bound-residue fractions of cell cultures incubated with 4-n-nonylphenol

Species	Radioactivity in bound residue	Relative distribution in fractions					
		Starch	Protein	Pectin	Lignin	Hemi-cellulose	Cellulose
	(%)						
Glycine max	32[a]	16	25	13	35	5.3	5.5
Lupinus polyphyllus	26	0.0	40	14	26	19	1.4
Lupinus hartwegii	12	17	4.3	4.9	31	27	15
Daucus carota	22	4.6	9.4	18	46	16	6.1
Lactuca sativa	38	8.1	13	26	44	7.8	1.4
L. esculentum	6.6	28	4	11	11	4.6	1.9
Atriplex hortensis	4.8	2.4	6.7	6.7	12	44	28
Chenopodium rubrum	3.8	0.0	27	18	17	15	24
B. vulgaris esculenta	12	2.2	11	18	47	1.3	2.8
Hordeum vulgare	6.5	0.0	43	8.8	40	7.3	1.7
Pennisetum americanum	10	2.0	23	13	51	2.8	9.0
Triticum aestivum	16	12	5.2	14	31	28	9.2

[a] All values are means from five combined parallel experiments.

The data for phytotoxicity and the amount of non-extractable residues of the respective cell cultures suggest that the formation of nonextractable residues may be associated with higher tolerance to 4-*n*-NP. This confirms the hypothesis that the bound-residue fraction (e.g. plant-cell wall) is important for detoxification [19]. From the data presented in Table XI, it is obvious that the species with the highest tolerance had high proportions of radioactivity in bound residues. The most sensitive species adsorbed only low proportions of radioactivity to the bound-residue fraction. A similar phenomenon was observed when ^{14}C-metribuzin was applied to cultivars of soybean; the more tolerant cultivar had higher amounts of bound residues than the more susceptible [20]. However, for species exhibiting EC_{50} values between 50 and 500×10^{-3} mM 4-n-NP, such a relationship was less obvious. Thus, there may be additional processes influencing sensitivity or tolerance. Phytotoxicity and bound-residue formation seem to be characteristic of plant families. Among the species tested, the members of the *Fabaceae* exhibited high capacity for residue formation and were at the same time quite tolerant to 4-*n*-NP, whereas the tested *Chenopodiaceae* species formed only limited amounts of bound residues and were found to be relatively sensitive.

The use of plants for animal or human food raises the question of bioavailability, which is generally accepted for the soluble fraction. For non-extractable residues, bioavailability is still a matter of discussion and has rarely been tested (e.g. [21]). The binding type and binding site of a compound to residue fractions might be important factors [22]. Non-extractable residues of twelve cell-suspension cultures were characterized with respect to the main binding sites of radioactivity derived from 4-NP. All cell cultures exhibited a specific distribution of radioactivity in fractions of bound residues (Table XI). In most of the cultures (seven of twelve), lignin was the fraction with which the major part of radio-activity was associated, but in some of the cultures, protein *(Hordeum vulgare, Chenopodium rubrum* and *Lycopersicon esculentum*) and hemicellulose (*Atriplex hortensis*) were prevalent in fractions containing radioactivity. The distribution of radioactivity in cell-wall fractions seems to be species-specific.

The pattern of binding of a given chemical and its metabolites to the various cell-wall components indicates that these persistent bound residues also differ in their bioavailability. Knowledge of the cell-wall components with which xenobiotics are associated would enable an estimate of the ecotoxicological risks of these chemicals.

7. CONCLUSIONS

In addition to several advantages that accrue from the use of sewage sludge and biowastes for crop nutrition, certain limitations should be taken into consideration. Undoubtedly the utilization of municipal sewage sludge and biowastes on agricultural land is an economic and environmentally acceptable method of disposal. On the other hand, these sludges and biowastes contain varied amounts of organic contaminants and heavy metals that have implications for soil fertility and quality of crops for human and animal consumption. Even very low concentrations of some chemicals may represent serious health and environmental hazards. Because of these potential toxicological properties the public expects and demands more legislative control of environmental contamination problems. Consequently, reliable data on the concentrations of organic contaminants and heavy metals in sludge and biowastes are required to assess risks associated with land use.

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UPTAKE OF MACRONUTRIENTS AND HEAVY METALS BY MAIZE/GREEN-GRAM CROPS FROM AN INCEPTISOL AMENDED WITH NORMAL AND IRRADIATED SEWAGE SLUDGE

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Abstract

The high organic matter content and nutritional value of sewage sludge make it a potentially useful soil amendment for integrated nutrient management in agriculture. Four successive crops in a maize/green-gram rotation were grown on an inceptisol at Trombay to assess the benefits of application of normal (NSS) and irradiated (ISS) sewage sludge. At 1 to 8 t ha^{-1} crop yields and N, P, K concentrations in plants were maintained. Heavy-metal contents in plants showed no signs showed no cumulative effects due to repeated addition of sludges. The results are discussed in the context of efficacy of NSS and ISS for sustainable crop production.

1. INTRODUCTION

Complementary use of organic manures along with chemical fertilizers is an essential component of integrated crop-nutrient management in agriculture for sustainable productivity. Among available organic and biological sources, sewage sludge has long been used as an amendment to improve soil fertility. Rich in organic matter content and moderate in levels of macro- and micronutrients, sewage sludge, a by-product of municipal wastewater treatment, has been used for many years to improve soil fertility [1].

However, since sewage sludges are of disparate origin—municipal, household, and industrial—they differ widely in chemical and biological composition, with respect to heavy metals and pathogenic organisms, that limit their usage in agriculture. They generally contain high concentrations of parasites, and pathogenic bacteria and viruses. Elimination of these organisms is effected by disinfection processes, such as changing pH (lime treatment), heat treatment (pasteurization) and irradiation (gamma or electron beam) [2]. The current status of radiation treatment of sludge and wastewater was reviewed by Pikaev [3]. Application of sewage sludge also results in accumulation of heavy metals in soil, leading to phytotoxicity and entry to the human food chain via food and feed crops [4,5]. Plants can accumulate high levels of heavy metals not toxic to plants but deleterious to animals and humans [6].

A survey of sewage sludges from municipal treatment plants in cities in India indicated wide ranges in concentrations of micronutrients and heavy metals [7]. Although there is insufficient sewage sludge available to make an impact on the national fertilizer needs of India, low cost, availability, and renewable supply make it an attractive alternative to synthetic fertilizers in areas near sewage-treatment plants.

Pot-culture studies on the effects of sewage sludge and city compost from Mumbai on heavy-metal pollution of soil and plants have already been reported [8]. Moderate doses of gamma rays (3–5 kGy) are known to completely inactivate disease-causing organisms in sewage sludge [9].

The Department of Atomic Energy, India, has set up a pilot plant for irradiation of sewage sludge at Baroda, Gujarat. In an ongoing program on the use of irradiated sludge to improve soil fertility and crop yields, and to preserve the environment, we report field studies on the effects of sewage sludge, untreated and irradiated, on crop growth and heavy-metal uptake.

2. MATERIALS AND METHODS

2.1. Field

Microplot field experiments were conducted to evaluate irradiated sewage sludge in comparison to normal sewage sludge, with respect to soil fertility, crop yields, and to preservation of the environment. The physicochemical properties of the soil, an inceptisol, at the field station of the Bhabha Atomic Research Centre, Trombay, are shown in Table I; it was near neutral in pH, had high CEC, adequate organic C content, and was inherently fertile as it had been out of cultivation for at least 15 years. After levelling, the field was divided into microplots in a randomized block design; each gross plot in the quadruplicate trial measured 2.8 m^2 while each net plot measured 1.5 m^2. All of the microplots received a uniform basal dose of 20:40:30 kg ha^{-1} as N:P:K in the form of ammonium sulphate, superphosphate, and potassium chloride, respectively.

2.2. Sewage sludges

The normal (non-irradiated) sewage sludge (NSS) for these experiments was collected from the drying beds of the conventional municipal sewage-sludge treatment plant at Baroda. The irradiated sewage sludge (ISS) was obtained from a sludge hygienization research irradiator (SHRI) facility located adjacent to the treatment plant. The irradiation process involves collection of sludge from the digestor of a conventional treatment plant in the reservoir tank of the SHRI facility. It is then pumped into the metering silo, which measures the sludge quantity to 3 m^3. The sludge is then drained under gravity into the irradiation vessel containing a maximum load capacity of 68 kCi of ^{60}Co. The sludge is constantly recirculated in the irradiation vessel for a predetermined time (4 to 6 h) to obtain the homogeneous dose of 300 to 500 krad, equivalent to 3 to 5 kGy. The ISS was dried separately and powdered to pass through a 5-mm sieve. The properties of both the NSS and ISS are shown in Table II; there were no appreciable differences. In general, they were rich in organic C, were near neutral in pH, had considerable amounts of heavy metals, and the C:N ratio was in the range 2.1 to 4.2 for NSS and 2.9 to 3.8 for ISS.

Table I. Characteristics of the inceptisol[a]

Characteristic		Value
pH in $CaCl_2$(1:5)		6.8
Organic C (%)		0.67
Water-holding capacity (%)		67
CEC (mEq 100 g^{-1})		48
Total N (%)		0.14
Available P (mg kg^{-1})		50
Available K_2O (mg kg^{-1})		88
C:N ratio		4.8
Metals (mg kg^{-1})	Aqua-regia extract	DTPA[b] extract
Cu	169	11.0
Zn	117	2.3
Mn	1,350	51
Cd	2	0.1
Co	67	0.5
Ni	246	8.0
Pb	24	0.6
Cr	249	ND

[a] Sand, 27%; silt, 27%; clay, 46%; texture: clayey; predominant clay mineral: 2:1 (montmorillonite).
[b] Diethylenetriaminepentaacetic acid.

Table II. Analysis of normal (NSS) and irradiated (ISS) sludge

Property	NSS	ISS
pH	6.2–7.0	6.5–7.1
Organic C (%)	6.1–10.3	7.8–11.1
Water-holding capacity (%)	127–150	102–125
CEC (mEq 100 g^{-1})	22.0–25.6	24.4–40.0
Total N (%)	2.2–3.0	2.7–3.0
Available P (mg kg^{-1})	200–262	205–325
Total K (mg kg^{-1})	2050–2500	1600–2450
C:N ratio	2.1–4.2	2.9–3.8
Metals (mg kg^{-1})[a]		
Cu	277–328	383–478
Zn	53–73	95–133
Mn	204–233	276–319
Cd	4–5	6–7
Co	11–12	12–16
Ni	77–82	97–129
Pb	143–146	201–207
Cr	181–260	186–283
Faecal coliform (MPN g^{-1})	565–1,600	11–116

[a] Aqua-regia extract.

2.3. Experimental

The treatments for the first two crops were: T_0, no sewage sludge control; T_1, NSS 1 t ha^{-1}; T_2, NSS 2 t ha^{-1}; T_3, NSS 3 t ha^{-1}; T_4, NSS 4 t ha^{-1}; T_5, ISS 1 t ha^{-1}; T_6, ISS 2 t ha^{-1}; T_7, ISS 3 t ha^{-1}; T_8, ISS 4 t ha^{-1}. These sludge rates corresponded to 30, 60, 90 and 120 kg N ha^{-1}, respectively. Maize (*Zea mays* L. cv. Ganga) was grown to physiological maturity (14 weeks) as the first and third crops, divided into cobs and shoots and sun dried (38±2ºC). Suitable samples were oven-dried (95±5ºC), powdered, and used for analysis.

After harvest of the maize crops (first and third), the microplots were kept fallow for a period of 1 to 2 weeks. Sludge treatments were reapplied and green gram (*Phaseolus aureus* Roxb. cv. ML 5) was grown to physiological maturity (8 weeks) as the second and fourth crops. The crop was harvested by separating pods and shoots, processed as described above and used for analysis.

The third and fourth crops of maize and green gram respectively were grown after keeping the microplots fallow for five months of the rainy season. For the third and fourth crops, the sludge doses (Treatments 1 to 8) were augmented to double (i.e. from 2 to 8 t ha $^{-1}$) as compared to the doses described above for the first and second crops. A severe attack of yellow mosaic virus on the fourth crop of green gram affected plant growth.

2.4. Analysis

Soil and plant analyses were performed by standard recommended methods [10,11]. Heavy metals in soil samples were analysed by aqua-regia extraction. Two-gram aliquots of finely ground plant material were wet-ashed with 30 mL of 5:1 HNO_3:$HClO_4$ acid mixture. The concentration of heavy metals in the clear acid extracts of soil and plant samples were determined using a Perkin-Elmer Model 380 atomic absorption spectrophotometer equipped with a D_2-arc background compensator.

3. RESULTS AND DISCUSSION

3.1. Dry-matter yields

Data on the effects of NSS and ISS at application rates of 0 to 4 t ha^{-1} on the dry-matter yields (DMYs) of the first two crops (Table III) show that there were no significant differences for shoot or grain of the two crops. The DMYs of shoot and grain of maize varied from 4.0 to 5.1 t ha^{-1} and from 2.9 to 4.1 t ha^{-1}, respectively. The DMYs of shoot and grain of green gram ranged from 1.9 to 2.5 t ha^{-1} and from 1.1 to 1.4 t ha^{-1}, respectively. The DMYs from the controls were similar to those from the sludge-treated soil. The DMY values of green gram were between 30 and 50% of those of maize mainly due to differences in species, biomass, and duration of plant growth.

Data on the influence of NSS and ISS, after doubling the rates of application up to 8 t ha^{-1}, on the DMY of third and fourth crops are shown in Table IV. No significant differences were observed for shoot or grain of either the maize or green gram among the sludge treatments. The overall range of DMYs of shoot and grain of maize were between 4.3 to 5.2 t ha^{-1} and between 2.0 to 3.9 t ha^{-1}, respectively.

The results in Tables III and IV together indicate that the DMYs of the first and third crops were similar. The DMYs of shoot and grain of green gram as the fourth crop ranged from 0.35 to 0.6 and 0.18 to 0.37 t ha^{-1}, respectively. Although sludge application, in general, increased green gram yield as compared to no sludge (control), no significant differences were seen in the DMYs of shoot or grain as affected by NSS and ISS at the various dosage levels. However, the DMYs of the fourth crop (Table IV) were about 75% lower compared to the second crop of green gram (Table III) primarily due to the yellow mosaic virus infection.

Table III. Effect of normal (NSS) and irradiated (ISS) sewage sludge at various levels on dry matter yields of maize (first) and green-gram (second) crops

Treatment	Maize			Green gram		
	Shoot	Grain	Total	Shoot	Grain	Total
	(t ha^{-1})					
T0[a]	5.1	3.7	8.8	2.5	1.3	3.8
T1[b]	5.1	4.1	9.2	2.1	1.1	3.2
T2	4.4	3.9	8.3	2.2	1.3	3.5
T3	4.4	3.1	7.5	2.2	1.1	3.3
T4	4.3	3.4	7.7	2.1	1.2	3.3
T5	4.0	2.9	6.9	2.1	1.2	3.3
T6	4.5	3.3	7.9	2.2	1.3	3.5
T7	4.3	3.4	7.7	2.0	1.4	3.8
T8	4.5	3.4	7.9	1.9	1.1	3.0
CD[c] source	NS[d]	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS
CD interaction	NS	NS	NS	NS	NS	NS

[a]Control, no sludge. [b]T1–T4: NSS at 1–4 t ha^{-1}; T5–T8: ISS at 1–4 t ha^{-1}. [c]At $P = 0.05$; [d]Not significant.

Table IV. Effect of normal (NSS) and irradiated (ISS) sewage sludge at various levels on the dry matter yields of maize (third) and green-gram (fourth) crops

Treatment	Maize			Green gram		
	Shoot	Grain	Total	Shoot	Grain	Total
	(t ha^{-1})					
T0[a]	4.8	3.4	8.2	0.35	0.18	0.53
T1[b]	5.2	3.2	8.4	0.46	0.29	0.75
T2	4.6	3.2	7.8	0.43	0.27	0.70
T3	4.8	2.5	7.3	0.45	0.27	0.72
T4	4.9	2.8	7.7	0.52	0.33	0.85
T5	4.4	2.8	7.2	0.49	0.26	0.75
T6	4.9	3.9	8.8	0.52	0.29	0.81
T7	4.7	3.7	8.4	0.60	0.37	0.97
T8	4.3	2.0	6.3	0.43	0.30	0.73
CD[c] source	NS[d]	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS
CD interaction	NS	NS	NS	NS	NS	NS

[a]Control, no sludge. [b]T1–T4: NSS at 2–8 t ha^{-1}; T5 – T8: ISS at 2 to 8 t ha^{-1}. [c]At $P = 0.05$. [d]Not significant.

The results in Tables III and IV together reveal that DMYs of maize and green gram crops with NSS and ISS were similar to results obtained in pot-culture studies on the sewage-sludge addition to an ultisol [8] and to field studies conducted in Germany [12].

3.2. Plant analysis

3.2.1. Nitrogen, phosphorus and potassium uptake

Data on N, P and K contents in the first and second crops are presented in Table V. These data indicate no differences in the percent N, P and K contents either in the shoot or in the grain of maize and green gram at the various rates of NSS and ISS.

Similarly, data in Table VI reveal no effect of different rates of NSS and ISS application on N, P and K contents in the third (maize) or fourth (green gram) crops, in spite of increasing the application rates as compared to the first two crops.

Nitrogen and P contents in the grain were higher than in the shoots of all the four crops, thus maintaining a consistent trend. In contrast, K content was higher in the shoot than in the grain of all the four crops, attributable to the role of K in giving strength to the shoot to support the grain. In addition, the data on %N, P and K contents in the shoot and grain of the first and third maize crops were at directly comparable levels and seem to be stabilized even after application rates were doubled.

Although N and P contents in the maize crops (first and third) were, in general, lower than in green gram (second and fourth crops), K contents were similar. However, when compared to K content of green gram as the second crop (Table V), that of the fourth crop was higher (Table VI). This effect was probably due to poor growth of the second crop due to yellow mosaic virus.

Table V. Contents of N, P and K in shoots and grain of maize (first) and green-gram (second) crops

Treatment	Maize						Green gram					
	N		P		K		N		P		K	
	S[a]	G[b]	S	G	S	G	S	G	S	G	S	G
	(%)											
T0[c]	0.75	1.58	0.19	0.40	1.46	0.45	2.3	3.9	0.24	0.38	1.36	0.87
T1[d]	0.73	1.57	0.20	0.42	1.84	0.38	2.3	3.9	0.23	0.38	1.23	0.83
T2	0.79	1.52	0.19	0.51	1.51	0.39	2.2	3.8	0.24	0.31	1.58	0.92
T3	0.81	1.46	0.20	0.44	1.54	0.62	2.2	3.7	0.21	0.36	1.15	0.88
T4	0.82	1.45	0.18	0.37	1.44	0.39	2.3	3.9	0.24	0.34	1.55	0.79
T5	0.84	1.62	0.19	0.43	1.35	0.44	2.1	3.9	0.22	0.35	1.88	0.82
T6	0.82	1.46	0.19	0.42	1.36	0.46	2.3	3.9	0.22	0.33	1.24	0.84
T7	0.75	1.52	0.21	0.40	1.44	0.59	2.4	3.7	0.28	0.35	1.37	0.83
T8	0.67	1.53	0.20	0.45	1.35	0.50	2.2	3.7	0.25	0.31	1.28	0.87
CD[e] source	NS[f]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Shoot. [b] Grain. [c] Control, no sludge. [d] T1–T4: NSS at 1–4 t ha^{-1}; T5–T8: ISS at 1–4 t ha^{-1}. [e] At *P*=0.05. [f] Not significant.

Table VI. Contents of N, P, and K in shoots and grain of maize (third) and green-gram (fourth) crops

Treatment	Maize						Green gram					
	N		P		K		N		P		K	
	S[a]	G[b]	S	G	S	G	S	G	S	G	S	G
	(%)											
T0[c]	0.92	1.50	0.23	0.40	1.28	0.47	2.06	3.42	0.26	0.32	1.62	1.28
T1[d]	0.71	1.42	0.25	0.46	1.33	0.46	2.14	3.37	0.25	0.49	1.48	1.28
T2	0.78	1.48	0.28	0.47	1.32	0.45	2.11	3.50	0.25	0.49	1.62	1.25
T3	0.80	1.58	0.33	0.50	1.07	0.55	2.13	3.57	0.25	0.52	1.51	1.30
T4	0.69	1.59	0.23	0.47	1.47	0.41	2.03	3.49	0.28	0.50	1.80	1.34
T5	0.74	1.59	0.28	0.48	1.48	0.46	2.15	3.42	0.28	0.46	1.99	1.46
T6	0.76	1.53	0.23	0.54	1.27	0.51	2.18	3.44	0.28	0.51	1.76	1.30
T7	0.82	1.62	0.23	0.48	1.37	0.43	2.11	3.44	0.26	0.50	1.71	1.36
T8	0.77	1.43	0.26	0.47	1.25	0.58	1.92	3.47	0.24	0.50	1.87	1.35
CD[e] source	NS[f]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Shoot. [b] Grain. [c] Control, no sludge. [d] T1–T4: NSS at 1–4 t ha^{-1}; T5–T8: ISS at 1–4 t ha^{-1}. [e] At *P*=0.05. [f] Not significant.

Table VII. Heavy metals in the first crop, maize

Treatment	Shoot[a]							Grain[b]				
	Cu	Zn	Mn	Co	Ni	Pb	Cr	Cu	Zn	Mn	Ni	Cr
	(mg kg^{-1})											
T0[c]	10	60	30	3.1	4.2	6.8	5.5	3.2	31	6.8	1.7	0.5
T1[d]	8	59	26	3.1	4.0	6.1	4.3	3.1	31	6.9	1.7	0.8
T2	9	56	26	2.4	4.6	6.8	4.5	3.3	32	6.5	1.4	0.7
T3	10	65	28	2.5	4.8	8.3	5.7	3.4	33	6.3	1.5	0.9
T4	9	52	29	2.6	4.4	7.6	5.1	3.3	31	6.4	1.3	0.5
T5	10	59	27	2.7	4.4	6.9	5.3	3.3	32	6.6	1.8	0.5
T6	9	64	26	2.6	3.3	6.1	4.5	3.2	31	6.8	1.5	0.5
T7	10	56	28	2.6	4.3	6.0	4.2	3.2	30	6.7	1.5	0.5
T8	8	61	26	2.8	3.7	7.4	4.1	3.4	34	6.8	1.4	0.8
CD source[e]	NS[f]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD interaction	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Cd was not detectable. [b]Cd, Pb, and Co were not detectable. [c]Control, no sludge. [d]T1–T4: NSS at 1–4 t ha^{-1}; T5–T8: ISS at 1–4 t ha^{-1}. [e]At $P = 0.05$. [f]Not significant.

3.2.2. Heavy metals

The heavy-metal (Cu, Zn, Mn, Co, Ni, Pb, and Cr) contents of maize shoot and grain (first crop) are shown in Table VII. Cadmium was not detected in the shoot or grain, neither were Co nor Pb found in the grain. The data, in general, indicated no significant differences in the heavy-metal contents of shoot or grain in response to the treatments. Metals were higher in the shoot than in the grain in the order: Zn > Mn > Cu >Pb > Cr > Ni > Co.

Similarly, results for concentrations of heavy metals in green gram (second crop) shoot and grain (Table VIII) revealed no significant differences in the contents of these elements as a result of different rates of NSS or ISS application. Cadmium and Pb were not detected in grain. Comparisons between shoot and grain contents indicated higher contents in the former, similar to the trend in the first maize crop. In green gram, shoot Mn concentration was highest followed by Zn, Cu, Ni, Pb, Cr, and Co, and Cd was the least. Further, in general, all the heavy-metal concentrations except for Zn were higher than in maize both in shoot and grain.

Data on the heavy-metal contents in the shoot and grain of maize (third crop) presented in Table IX again indicate no differences in the contents of heavy metals due to NSS and ISS application. However, the contents of these metals were higher in the shoot than in the grain. Cadmium could not be detected in the grain. Manganese and Zn contents were highest in the shoot and grain, respectively, followed by Cu, Pb, Ni, Cr, and Co in both tissues. Further, the heavy-metal contents of the third crop were similar to those of first maize crop (Table VII) indicating thereby no adverse effects even after increasing the rate of application.

Data on the heavy-metal concentrations in the shoot and grain of green gram (fourth) crop are in Table X. Although Co and Cr were detected in the second crop (Table VIII), they fell below detectable levels in the fourth crop, in spite of doubling the rates of sludge application, whereas the reverse was true for Pb content.

Table VIII. Heavy metals in the second crop, green gram

	Shoot								Grain[a]					
Treatment	Cu	Zn	Mn	Cd	Co	Ni	Pb	Cr	Cu	Zn	Mn	Co	Ni	Cr
	(mg kg^{-1})													
T0[b]	18	36	80	1.1	5.0	10.6	10.4	7.5	16	32	12	0.8	7.3	1.7
T1[c]	17	35	74	1.2	5.4	10.1	10.1	6.9	16	31	11	0.9	7.2	1.9
T2	17	35	73	1.2	4.9	9.5	9.6	6.1	16	31	12	0.9	7.1	1.5
T3	17	35	68	1.1	4.7	9.8	9.8	6.8	16	32	12	0.9	7.1	1.7
T4	17	38	73	1.1	4.8	9.6	10.5	5.8	17	31	12	0.6	7.2	1.7
T5	17	33	68	1.2	5.1	9.4	9.4	6.5	16	32	12	0.7	6.8	1.7
T6	17	32	66	1.1	5.0	9.5	9.7	6.3	16	31	11	0.9	7.2	1.9
T7	17	35	70	1.1	5.1	9.4	9.3	6.0	16	31	11	0.7	7.5	1.7
T8	19	36	71	1.1	5.6	11.6	9.8	9.3	16	31	11	0.8	7.2	2.1
CD source[d]	NS[e]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD int.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Cd and Pb were not detectable. [b]Control, no sludge. [c]T1–T4: NSS at 1–4 t ha^{-1}; T5–T8: ISS at 1–4 t ha^{-1}. [d]At P=0.05. [e]Not significant.

Results for heavy metals in Table X showed no significant differences as a result of various rates of application of NSS compared to ISS application. As seen in the maize (third crop), among the heavy metals, Mn and Zn contents of fourth crop (green gram) also were highest in the shoot and grain, respectively followed by Cu, Ni, and Pb. Further, the contents of these heavy metals were higher in the shoot than in the grain and this trend was common to all the four crops. In addition, the heavy metal contents in the fourth crop were at levels comparable with those of the second crop (green gram Table VIII) except for Cu which was lower in the fourth crop grain and Ni in shoots of second crop.

3.3. Soil analysis

Soil samples were taken after the harvest of each crop and analysed for the various parameters under study. Data on these properties are not presented since they did not show appreciable changes.

Data on soil properties after the harvest of green gram (fourth crop) are presented in Table XI. In comparison to NSS, application of ISS significantly increased total N in soil at all rates of application. Furthermore, in general, significant differences are observed between the doses within both the NSS and ISS treatments and retention of residual N in soil increased with the dose of sewage sludge. However, organic C of soil in ISS treatments was significantly lower than with NSS. Results in Table XI showed no variation in pH, CEC, WHC, P or K contents of the soil as a result of either NSS or ISS application at any application rate. When these soil properties are compared to those before starting the experiment (Table I) a definite trend is seen. Soil pH increased from 6.7 to 7.4; CEC shifted from 48 to about 53 mEq 100 g^{-1}; available P increased from 50 to 54 mg kg^{-1}; available K increased from 88 to around 95 mg kg^{-1}; water holding capacity increased from 67 to about 70%; organic C increased from 0.67 to about 0.80% and C:N ratio increased from 4.8 to about 5.3. Thus, application of sewage sludge to soil revealed a slow changes in these parameters attributable to the mineralization of N and conversion of organic C into humic acid fraction [12,13].

Table IX. Heavy metals in the third crop, maize

Treatment	Shoot								Grain[a]						
	Cu	Zn	Mn	Cd	Co	Ni	Pb	Cr	Cu	Zn	Mn	Co	Ni	Pb	Cr
	(mg kg^{-1})														
T0[b]	14	34	53	0.7	1.8	3.4	6.4	5.6	2.7	29	7.0	0.2	1.0	1.2	0.6
T1[c]	8	35	38	0.4	1.0	2.4	5.7	4.7	3.0	32	6.5	0.1	1.1	1.4	0.3
T2	10	37	36	0.6	1.4	2.4	4.9	5.0	2.9	28	6.6	0.2	0.4	1.1	0.1
T3	10	39	33	0.7	1.4	2.8	5.9	4.5	4.4	32	6.8	0.4	1.0	1.2	0.1
T4	9	32	34	0.6	1.1	1.1	4.6	4.3	2.9	29	5.7	0.4	0.6	1.4	0.6
T5	9	37	32	0.4	1.5	1.5	5.6	4.8	4.1	28	6.3	0.2	0.8	1.1	0.1
T6	10	32	38	0.6	1.7	1.7	6.4	5.4	3.6	33	5.7	0.2	0.1	1.1	0.1
T7	11	31	42	0.4	1.2	1.2	6.2	5.1	3.2	32	7.0	0.4	0.5	1.1	0.2
T8	9	39	35	0.5	1.4	1.4	6.5	5.5	3.7	31	6.0	0.2	0.7	0.8	ND
CD source[d]	NS[e]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD inter'n	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Cd was not detectable. [b]Control, no sludge. [c]T1–T4: NSS at 2–8 t ha^{-1}; T5–T8: ISS at 2–8 t ha^{-1}. [d]At P=0.05. [e]Not significant.

Table X. Heavy metals in the fourth crop, green gram

Treatment	Shoot								Grain[a]				
	Cu	Zn	Mn	Cd	Co	Ni	Pb	Cr	Cu	Zn	Mn	Ni	Pb
	(mg kg^{-1})												
T0[b]	18	42	81	1.7	5.4	16	16	7.5	14	32	12	7.2	1.7
T1[c]	22	44	76	1.6	5.7	16	18	10.3	13	32	12	7.6	1.1
T2	17	39	74	1.4	5.2	14	17	7.4	13	33	12	7.4	0.6
T3	19	40	65	1.4	4.9	12	15	7.1	13	34	12	6.1	0.7
T4	18	43	65	1.4	5.7	14	13	6.8	13	34	12	6.6	1.0
T5	17	41	64	1.7	5.6	14	13	6.8	13	33	12	9.0	0.9
T6	16	43	66	1.4	5.3	13	16	7.9	13	33	12	7.5	1.6
T7	19	46	71	1.4	6.0	17	15	7.1	13	32	12	6.9	0.7
T8	17	43	63	1.4	5.7	14	14	8.3	13	33	12	5.6	1.2
CD[d] source	NS[e]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CD intn.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a]Cd ,Cr and Co were not detectable. [b]Control, no sludge. [c]T1–T4: NSS at 2–8 t ha^{-1}; T5–T8: ISS at 2–8 t ha^{-1}. [d]At P=0.05. [e]Not significant.

Table XI. Soil analysis after harvest of the fourth crop, green gram

Treatment	pH	Org. C (%)	WHC (%)	CEC (mEq $100g^{-1}$)	Total N (%)	Av. P (mg kg^{-1})	Av. K_2O (mg kg^{-1})	C:N ratio
T0[a]	7.5	0.748	69	52	0.154	57	101	5.0
T1[b]	7.5	0.773	73	54	0.161	53	101	4.8
T2	7.5	0.932	70	54	0.133	54	99	7.1
T3	7.4	0.990	72	52	0.161	49	89	6.2
T4	7.3	1.023	73	52	0.182	53	104	5.7
T5	7.4	0.870	69	52	0.161	53	95	5.4
T6	7.4	0.850	69	52	0.168	57	88	5.0
T7	7.4	0.830	67	52	0.182	59	95	4.6
T8	7.5	0.780	69	52	0.196	55	98	3.9
CD[c] source	NS[d]	0.042	NS	NS	0.009	NS	NS	NS
CD level	NS	0.026	NS	NS	0.014	NS	NS	NS
CD inter'n	NS	NS	NS	NS	NS	NS	NS	NS

[a]Control, no sludge. [b]T1–T4: NSS at 2–8 t ha^{-1}; T5–T8: ISS at 2–8 t ha^{-1}. [c]At *P*= 0.05. [d]Not significant.

Table XII. Heavy metals in aqua-regia extracts of soil

Treatment	Cu	Zn	Mn	Pb	Co	Ni	Cr
	(mg kg^{-1})						
T0[a]	253	173	1,838	37	98	731	404
T1[b]	255	180	1,955	39	100	728	402
T2	258	185	1,769	39	100	742	405
T3	239	171	1,666	40	97	746	402
T4	262	188	1,813	40	97	745	408
T5	256	184	1,852	40	103	746	405
T6	251	188	1,650	38	99	732	396
T7	252	181	1,673	38	96	739	416
T8	251	187	1,798	40	97	703	387
CD[c] source	NS[d]	NS	NS	NS	NS	NS	NS
CD level	NS	NS	NS	NS	NS	NS	NS
CD interaction	NS	NS	NS	NS	NS	NS	NS

[a]Control, no sludge. [b]T1–T4: NSS at 2–8 t ha^{-1}; T5–T8: ISS at 2–8 t ha^{-1}. [c]At *P* = 0.05; [d]Not sig.
(Cd = 2 mg kg^{-1} in all the treatments.)

Data on the heavy-metal contents (aqua-regia extractable) in soil (Table XII) showed no appreciable changes in values due to applications of either NSS or ISS; Cd content was 2 mg kg^{-1} soil in all the treatments. Furthermore, the heavy-metal contents were in the following decreasing order: Mn > Ni > Cr > Cu > Zn > Co > Pb > Cd. The data in Table XII, when compared to the basal values (Table I) reveal accumulation of all of the heavy metals except Cd as a result of repeated applications of both types of sludges. The mean (%) increases over initial values were: Ni, 200; Pb and Cr, 62; Zn, 55; Cu, 49; Co, 47; and Mn, 31. Although these values show a cumulative trend, they do not constitute cause for concern [14]. It is important to note that, of these heavy metals, Zn, Cu, Mn and Co are essential micronutrients for plants; thus, sewage sludges may serve a useful source of same. The order of heavy-metal concentrations in soil does not necessarily reflect the order of concentrations in plants, because soil-plant relationships with respect to metals are complex and governed by diverse processes [15]. The other noteworthy factor is that since plants are highly selective, the heavy-metal concentrations in the four crops remained fairly constant in spite of repeated additions of sludge. However, the results in Table XII indicate that monitoring of levels in soil and plants is an essential component of sewage sludge application, particularly when it contains industrial waste.

4. CONCLUSIONS

The microplot field trial with repeated applications of normal (non-irradiated) and irradiated sewage sludge to an inceptisol with four crops of a maize/green-gram rotation demonstrated that both sludges were similar with respect to plant productivity. Since limited data are available on the evaluation of sewage sludge for fertilizing effects and heavy-metal contribution in Indian soils, the present investigations indicate that sludge is a good source of macro- and micronutrients for crop growth and irradiated sludge was as good as its untreated counterpart. Incremental applications of sludge did not result in increased crop yields probably due to inherent soil fertility. Uptake of N, P and K in the four crops was unaffected by type of sludge. The heavy-metal concentrations in plants remained low, stable and unaffected by type of sludge or level of application. Although repeated applications of sludge led to accumulation of heavy metals in soil, no deleterious effects were observed in the crops. Therefore, further work is needed to establish guidelines for safe application of sludge to different Indian soils taking into account heavy-metal levels, crop requirements and soil type. More long-term studies are needed to understand improvements in biological and physical properties of these soils as a result of sludge application.

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DOMESTIC SEWAGE-SLUDGE APPLICATION TO FIVE CYCLES OF CORN: EFFECTS ON N-UPTAKE AND HEAVY-METAL ACCUMULATION

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Abstract

In a field experiment, the effects on corn of the locally recommended rate of inorganic N (ammonium sulphate, 140 kg N/ha) were compared to those of irradiated (IR) and non-irradiated (NIR) sludge at 150, 300, 450, and 600% of the recommended fertilizer-N rate. No sludge was applied to a second corn. During the third, fourth and fifth corn cycles, the treatments were similar to the first cycle with the exception that the rates were 100, 200, 300, and 400% of the recommended fertilizer-N rate. For all of the cycles, there were no significant differences in yields with the various rates of, or between, the IR and NIR sludges, due to high variability in dry-matter yields. Overall, 200% N-equivalent of sludge was sufficient to give optimum corn dry-matter yields. There were no significant differences in concentrations of Cu and Zn in the corn grain between IR and NIR sludge treatments for the five cycles. However, Cu and Zn accumulated in the soil.

1. INTRODUCTION

Malaysia produces about 5 million m^3/year (wet-weight basis) of domestic sludge, and this is expected to increase to 7 million m^3 by 2020 [1]—a huge amount of waste that has to be disposed of. Therefore, pressures exist to devise beneficial methods of utilization. Although the sludge is of domestic origin, such wastes can have high concentrations of Cu and Zn [2]. In addition to heavy metals, pathogens, such as feacal colliform, hepatitis virus, and *Ascaris*, can be found in sludge. Treatment such as irradiation can help to sanitize sludge. Research is needed on utilization of domestic sewage sludge on agricultural land as a means of recycling, in order to minimize costs of safe and efficient disposal.

Sludge contains nutrients required for plant growth, including N. Therefore, the N contribution from sludge was investigated in this study as well as the quality of crops in terms of concentrations of Cu and Zn.

2. MATERIALS AND METHODS

For the first and third cycles of corn crops, the sludge was collected from the Indah Water Consortium treatment plant in Lembah Pantai, Kuala Lumpur. It can be classified as anaerobically digested septic-tank sludge that had been drying beds for about 6 months. Due to unforeseen circumstances, sludge collected at the Taman Tun Dr. Ismail plant had to be used for the fourth and fifth cycles. This sludge is mechanically treated using an extended aeration process.

A field experiment using a block design was set up. Soil at the site is a Bungor, clayey kaolinitic, isohyperthermic Typic Paleudults. The total number of plots was forty, 4×6 m, and the planting distance was 0.75×0.25 m. Distance between plots was 1.5 m. The treatments for the first, third, fourth, and fifth corn cycles are shown in Table I. No sludge was applied during the second cycle.

3. RESULTS AND DISCUSSION

The characteristics of the sludges are shown in Table II. They were acidic and the C/N ratio favoured mineralization. The sludge used in cycle 1 had high concentrations of Cu and Zn. According to the Commission of the European Community, 1986, the maximum permitted concentrations of Cu and Zn in sewage sludge for agricultural purposes are 1,000 to 1,750 and 2,500 to 4,000 mg/kg, respectively [3].

Table I. Treatments for the corn field studies

Treatment	Cycle 1[a]	Cycles 3[b], 4 and 5
T1	140 kg N/ha NH_4SO_4	140 kg N/ha NH_4SO_4
T2	0 kg/ha sludge	0 kg/ha sludge
T3	150% N eq.[c] IR[d]	100% N eq. IR
T4	300% N eq. IR	200% N eq. IR
T5	450% N eq. IR	300% N eq. IR
T6	600% N eq. IR	400% N eq. IR
T7	150% N eq. NIR[e]	100% N eq. NIR
T8	300% N eq. NIR	200% N eq. NIR
T9	450% N eq. NIR	300% N eq. NIR
T10	600% N eq. NIR	400% N eq. NIR

[a]Maximum amount of sludge 42 t/ha. [b]Maximum amount of sludge 28 t/ha. [c]Equivalent. [d]Irradiated sludge. [e]Non-irradiated sludge.

Table II. Characteristics of the sewage sludges

Component	Cycle 1	Cycle 3	Cycle 4	Cycle 5
Moisture content (%)	7.29	29.4		42.5
pH	5.87	6.09	4.9	
C (%)	44.9			
N (%)	2.56	2.76	0.93	2.30
C/N ratio	17.5			
P (%)	0.63	0.37	0.82	0.50
Al (%)	1.14		0.93	1.08
K (mg/kg)		336	373	188
Ca (%)		0.47	0.41	0.27
Mg (mg/kg)		1,071	4,139	300
Fe (%)	3.13	3.84	1.85	1.76
Cd (mg/kg)	16	4.4	10.2	2.2
Cu (mg/kg)	259	92	85	93
Mn (mg/kg)	112	88	85	74
Pb (mg/kg)	147	155	93	59
Zn (mg/kg)	2,766	526	1,062	1,205
Ni (mg/kg)		39	44	18

3.1. Nitrogen uptake

The N yields for the first, second, third, fourth and fifth cycles are in Fig. 1 a–e. The data indicate a soil-fertility benefit from sewage sludge. For the first cycle, a corn-yield calibration curve was obtained for the various rates of sludge application. More N was taken up with higher sludge application rates (up to 450% of the recommended fertilizer-N rate). This was probably because, initially, the higher amounts of mineralized N in the sludge were not vulnerable to immobilization. There were no significant differences in the yields between different rates or between the IR and NIR sludges due to high variability, probably due to spatial heterogeneity of the soil.

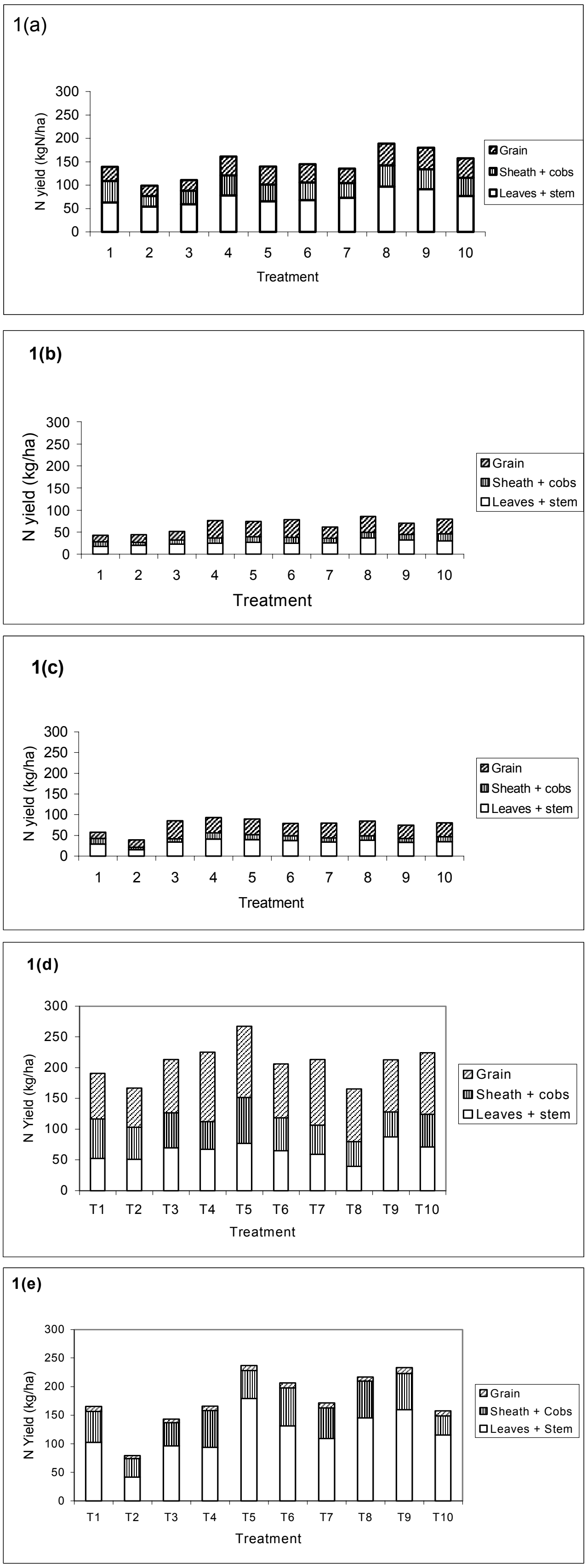

FIG. 1. Nitrogen yields of corn components for the first (a), second (b), third (c), fourth (d) and fifth cycles (e).

Table III. Nutrient status of the soil before planting and liming

Nutrient	Block 1	Block 2	Block 3	Block 4	Mean ± SD
%N	0.05	0.06	0.04	0.08	0.06 ± 0.01
Avail. P (mg/kg)	4.00	2.60	2.45	3.41	3.12 ± 0.63
Exch. K (mg/kg)	33.2	27.5	28.1	33.1	30.5 ± 2.68
Exch. Ca (mg/kg)	22.6	51.3	55.1	78.9	52.1 ± 20.2
Exch. Mg (mg/kg)	8.06	11.1	12.7	36.8	17.3 ± 11.4

In N-fertility experiments, it is not unusual that treatment effects are not significant during the first year if the soil has adequate amounts of major nutrients to support crop growth. This soil was inherently low in N, but contained substantial amounts of other essential major plant nutrients (Table III). For the first cycle of corn, on a relative basis, dry matter yields of all sludge treatments (except T3) were 30 to 60% greater than the no-fertilizer and the chemical-fertilizer controls.

The N yields for the second cycle were about half those of the first cycle (Fig. 1). However, residual fertility benefits of sewage sludge were pronounced. Without applying additional fertilizer, the dry matter yields of all sludge treatments (again except T3) were 25 to >100% higher than those of the controls.

During the third cycle, dry-matter yields of corn were generally low. This reduction (compared to the previous year) was not attributable to the application of sewage sludge, but due to low photosynthetic rate as a result of the haze episode of 1997. Nevertheless the relative yields with the sludge treatments (including T3 in this case) were 30 to 50% greater than those with the chemical-fertilizer and non-fertilizer controls. For the third cycle, there were slight increases in N yield up to 200% of the recommended N rate. Further increases in sludge rate did not increase available N for crop uptake, which indicated an inhibitory effect of high sludge rates on mineralization. Also, less total N was taken up in the control plot, indicating decreasing availability of soil N.

During the fourth cycle, the dry matter yields of corn were comparable to those for the first cycle, but the N yields were higher. The N yields were about 30% higher than those of the controls with the exception of T8. In this cycle, the sludge N content was lower compared to the earlier cycles, which means that at the same N-equivalent rate, the organic matter applied was commensurately higher. The trend of dry-matter yields for the fifth cycle was similar as for the earlier cycles. However, grain yields were lower as a result of spoilage due to delay in processing at the sample-preparation step.

3.2. Heavy metals

For crop quality, the maximum permitted concentration (MPC) as stated in the Malaysian Food Act 1983 and Food Regulation 1985 [4] will be used as a reference. For vegetables and grains, the MPCs for Cu and Zn were 30 and 40 mg/kg, respectively. The concentration is on an "as-consumed" basis (personal communication from the Ministry of Health), therefore, for sweet corn, the concentration will be based on fresh weight (60–70% moisture). The ranges of concentration of Cu and Zn in the grain for the five cycles are shown in Figs. 2 and 3. They were well below the MPC levels.

These were no significant differences in the concentrations of Cu and Zn in corn grain between IR and NIR sludge treatment for the five cycles (Figs. 2 and 3). Irradiation per se did not significantly affect the bioavailability of these metals. Variability in the concentration of trace elements in the grain of the corn is common in field studies. More data are needed for trends to be seen. There were, however, increases in Cu and Zn in the soil (Figs. 4 and 5).

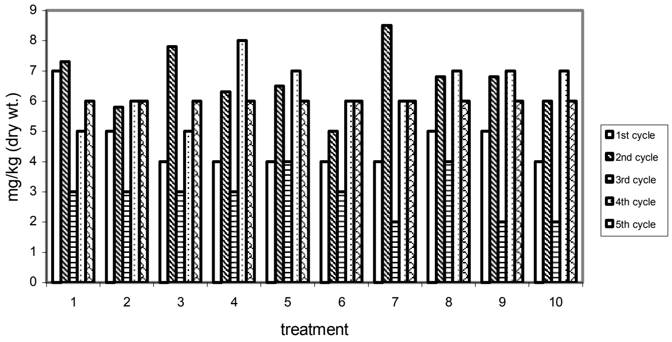

Fig. 2 :Cu concentration in corn grain

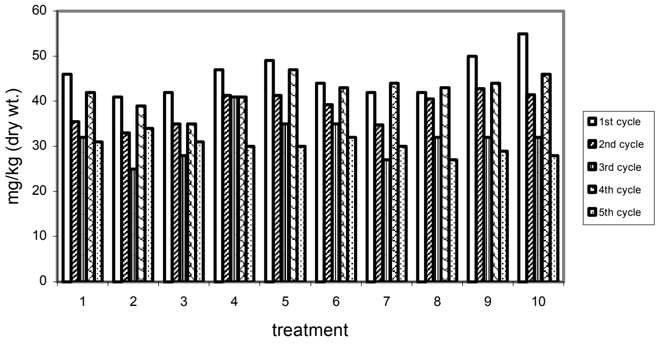

Fig. 3 : Zn concentration in corn grain

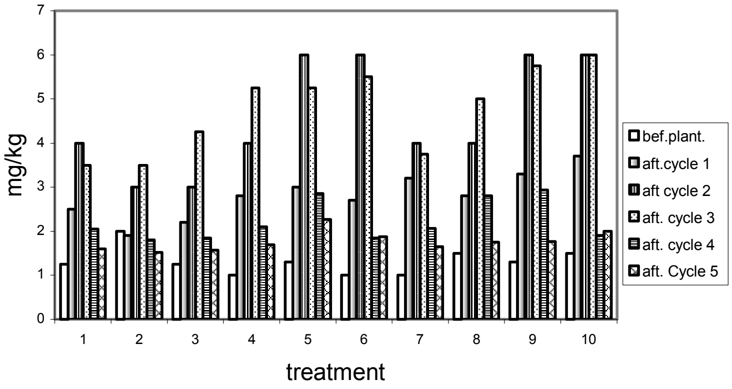

Fig. 4 : Mean concentration of Cu in soil

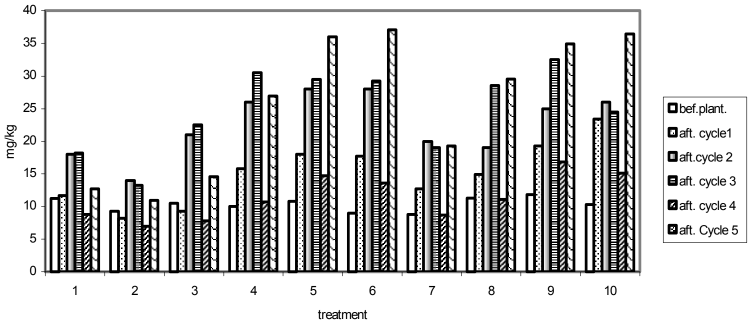

Fig. 5 : Mean concentration of Zn in soil

4. CONCLUSION

The maximum yield for the first cycles was at the rate 450% N equivalent of chemical fertilizer, but for the latter four cycles 200% N-equivalent was sufficient to give optimum yield. Thus the recommended rate of sludge application for optimum yield would be about 200% N-equivalent of the chemical fertilizer rate. Cu and Zn accumulation in the grain for the first three corn cycles was below the maximum permissible concentration levels of the Malaysian Food Act 1983 and Food Regulations 1985 [4].

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UTILIZATION OF SEWAGE SLUDGE FOR ENHANCING AGRICULTURAL PRODUCTIVITY – I. RESPONSES OF WHEAT TO FERTILIZER N AND IRRADIATED SEWAGE SLUDGE

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Abstract

A microplot field experiment was conducted to study the effects of γ-irradiated and non-irradiated sewage sludge on dry-matter yield and N uptake of wheat. Irradiation of sewage sludge at 5 kGy showed almost complete kill of coliform bacteria. Sludge was applied at rates equivalent to 120, 180, and 240 kg N ha^{-1}, either with or without ^{15}N-labelled $(NH_4)_2SO_4$ at 20 kg N ha^{-1}. In addition, one control (no treatment) and a treatment receiving 120 kg N ha^{-1} as ^{15}N-labelled $(NH_4)_2SO_4$ were also included in the experiment. Wheat was grown to maturity and dry-matter yields and N uptake obtained. A highly positive effect of sewage sludge, whether irradiated or not, was observed on dry-matter yield and N uptake. Sewage sludge not only served as an additional source of plant-available N but also helped conserve fertilizer N leading to its increased uptake by plants. The beneficial effect of sludge was more pronounced in the presence of fertilizer N and the effect increased with the rate of application. However, physico-chemical and biological properties of the soil after harvest indicated that probably the applied sewage sludge decomposed quite rapidly and thus did not add much to the soil organic matter content and other properties. Nevertheless, N content of the soil showed some improvement although not necessarily consistent with the rate of application.

1. INTRODUCTION

Increasing production of sewage sludge worldwide has led to consideration of its application to deserts, forests, and agricultural lands as a means of disposal, nutrient cycling, and enhancing ecosystem productivity [1–5]. Available information suggests highly positive effects of sewage sludge on ecosystem functioning [6–8]. However, possible negative effects are possible due to contamination of sewage sludge with pathogens, heavy metals, and organic pollutants [6,9].

The problem of pathogens in sewage sludge may be overcome by digestion and/or radiation [10]. Although this approach is less than practicable in countries lacking facilities for mass-scale radiation, it is worthy of consideration. Aside from pathogens, heavy-metal content of most sewage sludges, particularly in situations where industrial wastes are mixed with domestic, may pose serious problems following accumulation after repeated applications and entry into plant and animal food chains [8,11,12]. However, once the metals are in the soil, there is usually little removal by plants or movement down the profile, especially in heavy soils high in organic matter [13]. Persist elevated concentrations of heavy metals in the plough layer following repeated applications of sludge have been reported [14]. It would appear that soils in Pakistan, which are generally very low in organic matter, may retain relatively more-mobile heavy metals following sludge application. However, the positive effects of sewage sludge through supplemental nutrient supply and improvement in physico-chemical and biological properties of soil may more than balance the negative effects of heavy metals. However, the positive effects of sludge may be fairly short-lived because of rapid decomposition.

Soils in Pakistan generally have less than 1% organic matter because prevailing climatic conditions are conducive to rapid loss of C [15]. Sewage sludge may prove to be beneficial for such soils. Traditionally, application of sewage sludge to farm lands is not common in Pakistan, and there is no systematic collection for use in agriculture. However, with increasing environmental concerns over unorganized dumping of sewage sludge and the realization that sludges may help increase agricultural productivity, the situation may change in the near future.

The objectives of the experiment reported here were to study the effect of irradiated and non-irradiated sewage sludge on: i) growth of wheat, ii) uptake of N from ^{15}N-labelled fertilizer and from soil/sewage sludge.

2. MATERIALS AND METHODS

The experiment was conducted at the experimental farm of the Nuclear Institute for Agriculture and Biology, Faisalabad. The soil at the study site belongs to the Hafizabad series (Haplic Yermosol; FAO, 1966) and is a deep, well drained sandy-clay loam. The region has a semiarid subtropical climate with mean annual rainfall of 340 mm, most of which falls in July and August. Air-dried and sieved (<2 mm) soil, sampled to a depth of 20 cm, had the following characteristics: organic C, 0.56%; total N, 0.06%; pH (saturation paste), 7.7; water-holding capacity, 28%; sand, 52%; silt 28%; clay, 20%.

The sludge, obtained from a water-treatment plant in Islamabad, had the following characteristics: pH, 7.05; organic matter, 23%; total N, 1.5%; P, 0.55%; K, 0.2%; Zn, 63.5 ppm; Fe, 5,596 ppm; Mn, 288 ppm; Co, 18.7 ppm; Cu, 113 ppm. A portion of the sludge was radiated at Pakistan Radiation Services (PARAS) Lahore at a dose of 5 kGy in cuboid packets. Densities of coliform bacteria in irradiated and non-irradiated sludge samples were 12 and 900 (MPN index per 100 mL) respectively.

Forty-eight plots measuring 1.4×2.0 m with a plot-to-plot distance of 1 m were established and were treated in quadruplicate in a completely randomized block design as follows: T1, control (no treatment); T2, 120 kg FN; T3, 20 kg FN at; T4, 20 kg FN + 120 N as RSS; T5, 20 FN + 180 kg N as RSS; T6, 20 FN + 240 kg N as RSS; T7, 20 kg FN + 120 kg N as SS; T8, 20 kg FN + 180 kg N as SS; T9, 20 kg FN + 240 kg N as SS; T10, 120 kg N as SS; T11, 180 kg N as SS; and T12, 240 kg N as SS, where FN is fertilizer N applied as $(^{15}NH_4)_2SO_4$, RSS is irradiated sewage sludge and SS is non-irradiated sludge. Atom % ^{15}N excess of $(^{15}NH_4)_2SO_4$ in T2 was 10, whereas in all other treatments (T3–T9) it was 1.0. The recommended rate of N for wheat in Pakistan is 120 kg ha^{-1} and, accordingly, the doses of sewage sludge were adjusted to obtain N equivalents of 120, 180 and 240 kg ha^{-1}.

Sewage sludge was uniformly spread on the soil surface and N was applied in solution as a spray. Superphosphate and muriate of potash were applied to obtain P and K rates of 50 kg ha^{-1} each. The sludge was then worked well into the top 15-cm soil layer and seeds of wheat (*Triticum aestivum* var. Inqilab) were sown in rows (six rows, seven plants per row) such that each experimental ^{15}N area was 1×1.5 m with twenty plants; twenty-two plants on the boundary were considered non-experimental. Plants were harvested at maturity (last week of April) and data on grain and straw yields were expressed as kg ha^{-1}. Aliquots of oven-dried (70°C) and finely powdered straw and grain were analysed in triplicate for Kjeldahl N [15]. The distillates thus obtained were processed for ^{15}N-isotope-ratio analysis on an IRGA mass spectrometer.

Triplicate soil samples (0–15 cm) collected from each experimental plot after harvesting wheat were pooled, wet-sieved and stored at 4°C before analysis. Total N, mineral N, organic C, WHC, pH, and microbial biomass N of the moist samples was determined. Microbial biomass C was determined by the chloroform-fumigation-incubation method [16].

3. RESULTS

Irradiation of sewage sludge at 5 kGy significantly reduced the population of coliform bacteria. Results of the presumptive phase showed MPNs of 2 and 240 in irradiated and non-irradiated sludge, respectively after 24 h of incubation, and MPNs of 300 and 1,600 after 48 h. In general, production of both acid and gas was noticed for non-irradiated samples, while production of acid was the predominant feature for irradiated samples. Confirmation tests on brilliant-green lactose broth showed MPNs of 12 and 900 in irradiated and non-irradiated samples, respectively.

Table I. Dry-matter yield and harvest index (HI) as affected by fertilizer N and/or irradiated (RSS) or non-irradiated (SS) sewage sludge

Treatment[a]	Dry-matter yield			HI
	Straw	Grain	Total	
	($\mathrm{kg\ ha^{-1}}$)			
Control	171f[b]	190d	361f	0.53b
120 kg N	559a	318a	876a	0.36f
20 kg N	168f	239c	406e	0.59a
20 kg N + RSS1	280e	223cd	503de	0.44cde
20 kg N + RSS2	369bc	277b	646b	0.43de
20 kg N + RSS3	376b	276b	652b	0.42ef
20 kg N + SS1	338bcd	268b	606bc	0.44cdc
20 kg N + SS2	368bc	269b	637bc	0.42de
20 kg N + SS3	344bc	321a	665b	0.48bc
SS1	262e	206cd	467e	0.44cd
SS2	295de	212cd	507de	0.42cde
SS3	333cd	237e	570cd	0.42cde

[a] $(^{15}NH_4)_2SO_4$ at 120 and 20 kg N $\mathrm{ha^{-1}}$ had atom % ^{15}N excess values of 1 and 10, respectively; 1, 2, 3 represent N-equivalents of 120, 240 and 360 kg $\mathrm{ha^{-1}}$, respectively. [b] Figures with the same letter are not significantly different at $P=0.05$ according to DMRT.

Maximum dry-matter yields of shoots (grain + straw), obtained with fertilizer applied alone at 120 kg N $\mathrm{ha^{-1}}$, were significantly higher (143%) than the control and other soil treatments (Table I). Application of sewage sludge, whether irradiated or non-irradiated, caused substantial increases (17–69%) in total dry matter yield, and the effect increased with the rate of application. No significant differences were observed between irradiated and non-irradiated sewage sludge as far as dry-matter production was concerned. Application of fertilizer at 20 kg N $\mathrm{ha^{-1}}$ along with the sewage sludge caused a significant increase in dry-matter yield compared to sewage sludge applied alone. The effects of the treatments on straw and grain yields were similar to those observed for total dry-matter yield. Sewage sludge caused a significant decrease in harvest index, but the decrease was substantially less than that for fertilizer applied at 120 kg N $\mathrm{ha^{-1}}$. The form and amount of sewage sludge had no significant effect on the harvest index. In general, ca. 60% of the dry matter was allocated to straw and 40% to grains.

Trends in N content (or N yield) were similar to those observed for dry-matter yield (Table II); the two parameters showed a significant positive correlation. Sewage-sludge treatment led to a significant decrease in N-harvest index compared to the control, while values similar to those for fertilizer treatment were observed. The grain portion contained 78 to 91% of the total plant N, while allocation to straw was 9 to 22%.

Table II. Nitrogen yield and N harvest index (NHI) as affected by fertilizer N and/or irradiated (RSS) or non-irradiated (SS) sewage sludge

Treatment[a]	Nitrogen yield			NHI
	Straw	Grain	Total	
	(g plot^{-1})			
Control	421g[a]	3,758fgh	4,179fg	0.90a
120 kg N	1554a	6,122a	7,656a	0.80d
20 kg N	454g	4,389d	4,843de	0.91b
20 kg N + RSS1	772ef	3,884efg	4,656cde	0.83bcd
20 kg N + RSS2	847de	5,5523b	6,400abc	0.87abc
20 kg N + RSS3	1,339b	4,758c	6,097d	0.78d
20 kg N + SS1	1,092c	4,146def	5,239cd	0.79d
20 kg N + SS2	1,106c	4,246de	5,352c	0.79d
20 kg N + SS3	1046c	4,971c	6,018b	0.83bcd
SS1	733f	3,133I	3,867g	0.81bcd
SS2	781ef	3,310hi	4,090g	0.81cd
SS3	916d	3,690gh	4,606ef	0.80d

[a]See footnotes for Table I.

Table III. Plant N derived from fertilizer (Ndff) and from soil + sewage sludge (Ndfss) and percent fertilizer N in plant tops (%FNP)

Treatment[a]	Ndfss	Ndff	%Ndff	%FNP
	(mg plot^{-1})			
Control	4,179de[a]	n.a.	n.a.	n.a.
120 kg N	5,792a	1,863a	24.3a	9.5e
20 kg N	4,574bcd	270e	5.6f	9.0e
20 kg N + RSS1	4,231cde	426d	9.1cd	14.2c
20 kg N + RSS2	5,872a	528b	8.3dc	17.6a
20 kg N + RSS3	5,619a	478c	7.8e	16.0b
20 kg N + SS1	4,703b	536b	10.2b	17.9a
20 kg N + SS2	4,855b	497bc	9.3c	16.6b
20 kg N + SS3	5,637a	381d	6.3f	12.7d
SS1	3,867e	NA	NA	NA
SS2	4,090e	NA	NA	NA
SS3	4,606bc	NA	NA	NA

[a]See footnotes for Table I.

In the various treatments, 5.6 to 24% of plant N was derived from inorganic fertilizer; the maximum value was recorded for fertilizer N added at 120 kg ha^{-1} (Table III). Contribution to total plant N of fertilizer N when applied alone at 20 kg N ha^{-1} was 5.6%. However, when applied along with sewage sludge, the contribution increased significantly. With the increase in rate of application, both irradiated and non-irradiated sludge caused reductions in the contribution of fertilizer N to total plant N. However, when the values for different rates are averaged, the sludge had no significant bearing on contribution of fertilizer N.

Net amounts of fertilizer N in plants also increased, from 270 to 477 and 471 mg plot^{-1} (averages of three application rates) due to irradiated and non-irradiated sewage sludge, respectively. Likewise, plant utilization of fertilizer N increased from 9.0% to averages of 16% and 16% in the presence of irradiated and non-irradiated sewage sludge, respectively. Interestingly, only 9% of the fertilizer N applied at 120 kg ha^{-1} was taken up by the plants—an uptake efficiency lower than generally obtained. In spite of this low uptake efficiency, however, dry matter yields were substantially improved (Table I).

Application of fertilizer N at 120 kg ha^{-1} significantly improved (27%) the uptake of N from unlabelled sources (possibly including N from soil organic matter and from N_2 fixation in the rhizosphere), an increase of 12% was observed for fertilizer N applied at 20 kg ha^{-1}(Table III) Application of irradiated or non-irradiated sewage sludge with 20 kg N ha^{-1} had a positive effect on the uptake of unlabelled N; the effect increased with the amount of sewage sludge. It would appear that fertilizer N had a positive bearing on availability of N from unlabelled sources (soil, sewage sludge, etc.) due to the so-called “added nitrogen interaction,” sewage sludge having an additive effect. However, when applied alone, sewage sludge had a negligible effect on uptake of unlabelled N. Apparently, sewage sludge did not make a measurable contribution to plant-available N, probably due to its low N concentration (1.5%). Indeed, plants grown in soil with different sludge treatments had consistently higher ^{15}N-enrichment values (data not shown) suggesting that no dilution was caused by N from sewage sludge. Use of sewage sludge with a high N concentration could have helped determine its role in N nutrition of plants using isotopic-dilution equations.

Soil samples obtained after harvesting wheat were analyzed for microbial biomass N, total N, mineral N, organic C, pH, and WHC (Table IV). Comparison of different treatments indicated a substantial increase in total N content, while pH decreased to some extent. Total organic C was almost unaffected, suggesting a rapid decomposition of the applied sludge. However, there was some improvement in WHC, while mineral-N content was fairly low.

Microbial biomass was determined using the chloroform-fumigation-incubation method. The amount of NH_4-N in soil from differently treated plots increased significantly due to the fumigation, whereas the amount of NO_3-N decreased during incubation of fumigated soils, suggesting its immobilization (data not shown). The flush of NH_4-N (difference between fumigated and untreated soils) was used to calculate biomass N employing a k_N factor of 0.3. Biomass N was not greatly affected by different soil treatments and ranged between 41 and 69 µg g^{-1} soil. On average, irradiated and non-irradiated sludge and sludge without fertilizer did not show any consistent differences in amounts of biomass N.

4. DISCUSSION

Organic amendments, including sewage sludge, have been reported to increase crop yields as a result of improvements in physico-chemical and biological characteristics of the soil and increased availability of plant nutrients, especially N [1–8]. In the present study, application of sewage sludge, whether irradiated or not, had significant beneficial effects on yields of wheat (Table I). The increases were more pronounced when small quantities (20 kg ha^{-1}) of fertilizer N were also applied. However, this benefit could not be attributed to the N-supplying potential of the sludge as plant N did not increase (Table II) concomitant with the dry-matter yield. Hence, any increase in crop yield is attributable to improvement in physico-chemical and biological characteristics of the soil. Such effects of organic amendments on fertility have frequently been reported [17].

Table IV. Soil analyses after harvesting wheat

Treatment[a]	Biomass N	Total N	Mineral N	Organic C	pH	WHC
		(μg g^{-1})		(%)		(%)
Control	64.1[a]	542	2.3	0.28	7.12	25.1
120 kg N	68.7	573	3.1	0.29	6.98	24.5
20 kg N	59.0	575	5.6	0.22	6.95	25.4
20 kg N + RSS1	59.3	546	9.9	0.25	6.75	26.3
20 kg N + RSS2	56.7	573	9.1	0.21	6.66	26.4
20 kg N + RSS3	57.3	603	5.8	0.23	6.87	25.2
20 kg N + SS1	55.3	543	3.5	0.23	6.81	26.0
20 kg N + SS2	55.3	645	4.1	0.21	6.96	28.8
20 kg N + SS3	64.3	579	5.2	0.23	7.12	26.8
SS1	65.0	653	3.3	0.23	7.11	25.6
SS2	45.7	608	4.5	0.21	7.16	27.1
SS3	41.3	667	4.3	0.23	6.71	27.0
$LSD_{0.05}$	4.5	29	0.29	0.03	0.33	1.6

[a] See footnote for Table I.

Nitrogen benefit from organic amendments result mainly from net release of N from decomposing organic matter with a high N concentration and narrow C/N ratio [18,19]. However, studies carried out with ^{15}N have shown that only a small proportion of the organic N applied as plant residues is available to plants [20,21]. Sewage sludge used in this study had a low N concentration of 1.5% and thus a C/N ratio wider than that considered desirable for net mineralization. Consistent ^{15}N enrichment instead of dilution of plant N (data not presented) in different treatments also suggested that sewage sludge did not make a net contribution to plant-available N. Use of sewage sludge with an N concentration of >3% would have helped to study its N supplying potential using the dilution equation:

$$\%Ndfss = \left[1 - \frac{atom\%\,excess_{with\ sludge}}{atom\%\,excess_{without\ sludge}}\right] \times 100$$

Several studies have indicated that organic amendments can have an indirect rather than a direct effect on N availability to plants. Nitrogen-15-labelled plant residues with high N concentration, and thus a narrow C/N ratio, caused an increase in the accumulation in soil and availability to plants of unlabelled N [20]. This extra unlabelled N was thought to result from enhanced mineralization of native organic matter [20,22]. Such an effect (the so-called “added nitrogen interaction”) was not observed for sewage sludge in the present study (Table III). However, there was a significant increase in uptake of unlabelled N by plants grown in soil treated with fertilizer N at 120 kg ha^{-1}, which is in line with results from several other studies and is attributable to pool substitution, enhanced mineralization of native soil N, as well as increased root proliferation and thus exploration of a greater soil volume for nutrient uptake [20, 23–26].

Increase in plant uptake of unlabelled N was also observed with 20 kg N ha^{-1} of ^{15}N-labelled fertilizer although less than that observed for 120 kg N ha^{-1}. The added-N interaction has been reported to increase with the rate of fertilizer N applied [27,28]. Uptake of unlabelled N caused by 20 kg ha^{-1} of fertilizer N was significantly increased in the presence of sewage sludge, more so at the higher rate of sludge application. It would appear that small amounts of fertilizer N accelerated the mineralization of sewage sludge N, an effect similar to that observed for the native soil N. Favourable effects of inorganic N on mineralization of organic N from sources like green manures have been reported [29].

Application of fertilizer N with sewage sludge not only enhanced the uptake of unlabelled N by plants, but its own availability was also improved (Table III). The atom % ^{15}N excess of plants grown in sludge-treated soils always remained higher than that of plants grown without sludge (data not shown). The extra amount of unlabelled N was not enough to offset this effect so as to lead to a substantial dilution of plant ^{15}N. These observations demand further study, particularly on the rate of N uptake from fertilizer and other sources at various stages of plant growth. In the present study, sewage sludge enhanced the availability of added ^{15}N. Not only did the plants treated with sewage sludge derive a higher percentage of their N from applied ^{15}N-labelled fertilizer, but the absolute amounts of fertilizer N taken up were also substantially higher compared to the non-sludge treatments. This may mean that sludge conserved fertilizer N (by providing additional C for microbial immobilization) which the plants were able to make use of over an extended period of time. In view of being a labile source of C but not of N [5], sewage sludge could be expected to cause an immobilization of fertilizer N with negligible release of N over the short term.

Although sewage sludge had beneficial effects on wheat yields, long-term improvements in physico-chemical and biological properties of the soil were not so obvious (Table IV). Some improvement was observed in water-holding capacity and N content after harvest. Such effects have frequently been reported following organic amendment [17].

The results of this study suggest a positive effect of sewage sludge on physico-chemical and biological properties of the soil as well as substantial beneficial effects on crop yield and nutrient (N) uptake. The potential of sewage sludge to enhance agricultural productivity could substantially be improved by nutrient (e.g. N) supplementation. However, the material used in this study seemed to be fairly readily decomposable, resulting in negligible addition to the total organic matter content of the soil. Results of coliform enumeration indicated almost complete eradication at 5 kGy. However, irradiation had little effect in terms of crop yield and uptake of nutrients.

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UTILIZATION OF SEWAGE SLUDGE FOR ENHANCING AGRICULTURAL PRODUCTIVITY – II. RESPONSES OF RICE TO FERTILIZER N AND IRRADIATED SEWAGE SLUDGE

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Abstract

A greenhouse experiment was conducted to study the effects of γ-irradiated sewage sludge, applied alone or with ^{15}N-labelled ammonium sulphate (1.0 atom % ^{15}N excess), on rice yield and N uptake. Six-kg portions of a clay loam were amended with sewage sludge to obtain N addition rates of 30, 60, 90 and 120 mg kg^{-1} soil. In other treatments, N was applied at 120 mg kg^{-1} as ^{15}N-labelled ammonium sulphate or 120 mg kg^{-1} as $^{15}NH_4$-N plus sludge-N in the ratio of 1:3, 1:1, or 3:1. All of the treatments were made before transplanting the rice. Three healthy seedlings (4 weeks old) of rice (*Oryza sativa* L., var. Bas-Pak) were transplanted per pot, and the plants were harvested at maturity. Applications of sewage sludge caused significant improvements in rice yield (grain yield increased by 188% with sludge-N at 120 mg N kg^{-1}, whereas the yield benefit at a similar rate of fertilizer N was 304%); increases were greater at higher rates of application. Increase in rice yield was dependent on uptake of N, and sewage sludge significantly improved the plant availability of N. The additional plant N in sludge-treated soil was partially attributable to enhanced mineralization of soil N and N_2 fixation by free-living micro-organisms. Application of inorganic N led to a significant increase in the plant availability of N from soil organic matter and sewage sludge. Data from combined applications suggest that substantial savings of fertilizer N can be made by disposing of sewage sludge on rice fields.

1. INTRODUCTION

Organic manuring is a widely recognized method of improving soil fertility. In rice-production systems, animal wastes, crop residues, green manures, and urban wastes are applied as manures [1]. Of these, leguminous green manures have traditionally been used as a source of plant-available N and as a means of improving soil productivity [2–4]; they are reported to provide a substantial portion of the N required by rice [3,5]. However, some other reports show that N-supplying potential of green manures is limited, with rice utilizing 10 to 50% of the N applied [2,3,5–8]. Indirectly, however, leguminous crop residues with high N content may lead to increased plant-available N from soil organic matter [6,7,9,10]. The low and variable N-supplying potential of leguminous residues can be attributed to differences in N concentration and the resultant C/N ratios—the factors that play an important role in net N mineralization [7,11,12].

Application of inorganic N simultaneously with plant residues of low-N concentration may lead to enhanced N availability from the latter and improved crop yields [13]. However, in spite of the reported benefits of leguminous plant residues in terms of nutrient supply (especially of N), increased rice yields are attributed mainly to overall improvement of soil conditions as a result of organic matter addition. It has been suggested that the role of organic matter as a source of N and other nutrients is less important than improvements in physical, chemical, and biological properties [14].

Like plant residues, sewage sludge may serve as a good source of organic matter and nutrients for soil and crops, respectively. However, the release of N from sewage sludge may be restricted because of a relatively wide C/N ratio. Supplementing sewage sludge with fertilizer N may help overcome this problem. Our objective was to study N uptake and growth of flooded rice in soil treated with sewage sludge and inorganic fertilizer, applied separately and in combination.

2. MATERIAL AND METHODS

The soil was collected from experimental fields at the Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan. Air-dried and sieved (<2 mm) soil, sampled to a depth of 20 cm, had the following characteristics: organic C, 0.56%; total N, 0.06%; pH (saturation paste), 7.7; water-holding capacity, 28%; sand, 52%; silt, 28%; clay, 20%.

Six-kg portions of the soil, placed in plastic pots, were amended with: i) γ-irradiated sewage sludge to obtain N-addition rates of 30, 60, 90, and 120 mg kg^{-1} soil, ii) ^{15}N-labelled ammonium sulphate at 120 mg N kg^{-1} soil, and iii) 120 mg N kg^{-1} soil as sewage sludge and ammonium sulphate in the ratios of 1:3, 1:1or 3:1 on the basis of N. All the treatments were given to triplicate pots before transplanting rice. Three healthy seedlings (4 weeks old) of rice (*Oryza sativa* L., var. Bas-Pak) were transplanted per pot. A second dose of fertilizer N (80 mg kg^{-1}) was given 6 weeks after transplanting as signs of N deficiency appeared. Harvested plants were separated into root, straw, and grain portions, and were dried, weighed, and finely powdered. Portions were analysed for total N [15] and the resulting distillates processed for ^{15}N-isotope-ratio analysis on a mass spectrometer.

3. RESULTS

Table I presents dry-matter yields and harvest indices. The root portion showed no response to sewage sludge when applied alone; however, a significant improvement was recorded when fertilizer N was applied alone or along with sewage sludge. Apparently, the positive effect was due mainly to fertilizer N, since it increased with the amount applied. The straw portion exhibited significant increases due to both sewage sludge and fertilizer N. The increase was greater when both sewage sludge and fertilizer N were applied together. It appeared, however, that sewage sludge decreased the benefit of fertilizer N since straw yield decreased with the increase in the proportion of sewage sludge. Similar results were observed for grain yield, but the benefit from sewage sludge was more pronounced.

Table I. Dry-matter yield, its distribution and harvest index as affected by application of sewage sludge (SS) and ammonium sulphate (AS) separately or in ratios of 1:3, 1:1 and 3:1

Treatment	Dry-matter yield				Harvest index
	Roots	Straw	Grain	Total	
	(g pot^{-1})				
Nil (control)	8.0c[a]	40.2e	9.5f	57.7g	0.19c
SS-N30[b]	8.0c	41.3e	9.5f	58.9g	0.19c
SS-N60	7.2d	41.3e	16.8e	65.3ef	0.29ab
SS-N90	7.6cd	43.4e	19.9d	70.8e	0.31a
SS-N120	7.6cd	49.8d	24.6c	82.0d	0.33a
AS-N120	14.5a	80.1a	31.2b	125a	0.28b
SS+AS, 1:3	13.2a	72.9b	34.9a	121a	0.32a
SS+AS, 1:1	13.3a	66.5bc	29.6b	109b	0.31a
SS+AS, 3:1	12.3b	63.2c	25.1c	101c	0.28b

[a] Figures in a column followed by the same letter are not significantly different at P=0.05 by DMRT.
[b] 30–120 = amount of N (mg kg^{-1}) applied.

Table II. Nitrogen yield, its distribution and harvest index as affected by application of sewage sludge (SS) and ammonium sulphate (AS) separately or in ratios of 1:3, 1:1 and 3:1

Treatment[a]	N yield				N-harvest index
	Roots	Straw	Grain	Total	
	$(g\ pot^{-1})$				
Nil (control)	48.3c[a]	183ef	103f	334h	0.36d
SS-N30	49.6c	185ef	10f	336h	0.36d
SS-N60	45.5d	178f	188e	411g	0.51c
SS-N90	46.4cd	194de	211d	451fg	0.52c
SS-N120	45.9d	216d	294c	557e	0.58a
AS-N120	94.5a	377a	413ab	884a	0.58a
SS+AS, 1:3	83.2b	315b	428a	827bc	0.58a
SS+AS, 1:1	82.2b	296bc	386b	764c	0.57b
SS+AS, 3:1	77.7b	282c	303c	662d	0.52c

[a]See Table I for details.

Table III. Percentage increase or decrease (–) in dry matter and N content of plant components in response to application of sewage sludge and N fertilizer

Treatment[a]	Dry matter yield			N yield		
	Root	Straw	Grain	Root	Straw	Grain
	(%)					
SS-N30	1.1f[a]	2.6g	0g	2.7e	0.6g	0f
SS-N60	–9.6d	2.8g	76.4f	–5.8d	–3.0f	83.6e
SS-N90	–5.0e	7.8f	109e	–3.9d	5.6e	106d
SS-N120	–4.2e	23.8e	158d	–5.0d	17.9d	188c
AS-N120	81.8a	99.0a	227bc	95.7a	105a	304a
SS+AS, 1:3	66.0b	81.2b	267a	72.3b	71.8b	319a
SS+AS, 1:1	67.3b	65.4c	211c	70.1b	61.7b	278b
SS+AS, 3:1	54.1c	57.1d	163d	60.8c	53.6c	197c

[a]See Table I for details.

Combined application of sewage sludge and ammonium sulphate (3:1) gave a grain yield almost equivalent to that obtained with sewage sludge alone applied at 120 mg N kg^{-1} soil (Table I). However, total dry matter yield was better when 25% of the applied N was in the form of ammonium sulphate. Soil treatment caused significant increases in harvest index (in contrast with wheat).

Trends in N yield and N-harvest index were similar to those recorded for dry-matter yield (Table II) and a highly significant relationship was obtained between the two parameters (r = 91, when all values of Tables I and II were computed). However, compared to dry matter, partitioning of N to grain was more positively affected by treatment, particularly with fertilizer N alone or in combination with sludge.

Table III compares treatments in terms of percent changes in dry matter and N yield of plant components. The dry matter and N content of roots showed decreases of 4.2 to 9.6% and 3.9 to 5.8%, respectively. However, fertilizer N applied alone or with sewage sludge stimulated increases of 54 to 82% in root dry matter and 61 to 96% in root N content; the lowest values were recorded for the highest proportion of sludge-N, and the highest values were recorded for the fertilizer N alone. The increase in straw dry matter was 24% at the highest level of sludge. Application of sludge along with fertilizer N in different ratios produced increases of 57 to 99% in straw dry mater and 61 to 96% in N content. Much higher increases in grain yield and grain N content (76–266.5% and 84–319%, respectively) were obtained due to combined effects of sewage sludge and fertilizer N.

Percent N contents of root and straw portions were not affected significantly by treatment (Table IV). However, an increase in grain-N concentration was observed at the highest level of sewage sludge when applied alone and at all levels when applied with fertilizer N.

Table V shows the contributions of ^{15}N-labelled fertilizer to the N content of the whole plant and its components. As the amount of applied fertilizer N decreased, its contribution to the total plant N (and to its components) also decreased, as expected. (Similar observations were recorded for wheat.) Among the three plant components, most fertilizer N was found in the grain portion. The efficiency of fertilizer-N uptake remained fairly low and the plants contained only 22% of the fertilizer N when applied alone. Although percentage of fertilizer N in whole plant decreased to some extent with increasing proportion of sewage sludge, the decrease was most pronounced for the grain portion. However, the role of sewage sludge in affecting the fate of fertilizer N could not be established in this study where the main objective was to study the combined effect of two N sources. It would be worthwhile to study the effects of different levels of sewage sludge on the fate of fertilizer N applied at a constant rate.

Table IV. N concentration of components of rice in response to sewage sludge and N fertilizer

Treatment[a]	%N content		
	Root	Straw	Grain
Nil (cont.)	0.61a[a]	0.46ab	1.08c
SS-N30	0.62a	0.45ab	1.07c
SS-N60	0.63a	0.43b	1.12c
SS-N90	0.61a	0.45ab	1.06c
SS-N120	0.60a	0.43b	1.20b
AS-N120	0.65a	0.47a	1.32a
SS+AS, 1:3	0.63a	0.43b	1.23ab
SS+AS, 1:1	0.62a	0.45ab	1.30a
SS+AS, 3:1	0.63a	0.45ab	1.21ab

[a]See Table I for details.

Table V. Contribution of fertilizer N when applied alone or with sewage sludge

Treatment[a]	Root	Straw	Grain	Whole plant
	%N derived from fertilizer (%Ndff)			
AS-N120	21.3a[a]	16.8a	18.8a	18.2a
SS+AS, 1:3	11.2b	13.5b	15.4b	14.3b
SS+AS, 1:1	11.3b	7.5c	6.6c	7.5c
SS+AS, 3:1	7.6c	4.5d	4.8d	5.0d
	% fertilizer N taken up			
AS-N120	2.8b	8.8a	10.8b	22.4a
SS+AS, 1:3	1.7c	7.9b	12.3a	21.8b
SS+AS, 1:1	2.6b	6.2d	7.1c	15.8d
SS+AS, 3:1	3.3a	7.0c	8.1c	18.3c

[a] See Table I for details; statistics applies independently to each of the two data sets of four values each.

In this study, up to 70% of the applied fertilizer N (i.e. ^{15}N-labelled fertilizer applied at the start of experiment, excluding the second dose of unlabelled N applied during plant growth) was lost from the soil-plant system. The amount of sewage sludge did not have a strong effect on fertilizer-N loss. Also, application of sewage sludge in the absence of ^{15}N-labelled fertilizer led to a dilution of plant ^{15}N that was most pronounced at higher rates of application (data not presented). This dilution could be attributed either to sewage sludge N (which had lower ^{15}N-abundance as compared to native soil N taken up by plants) and/or to enhanced N_2 fixation. We do not yet have ^{15}N data for sewage sludge to ascertain its role in ^{15}N dilution.

Table VI shows N uptake from sewage sludge and/or soil as affected by treatment. Application of sewage sludge alone had negligible effects, while application of fertilizer N led to significant increases in unlabelled N of roots. Similar trends were observed for straw, with which fertilizer N alone had the maximum positive effect on unlabelled-N content. The grain portion contained significantly higher amounts of unlabelled N at the two higher doses of sludge. Again, the application of fertilizer N had a significant, positive effect on unlabelled-N content of the grain portion; the effect was greater in the presence of sludge. On a whole-plant basis, the amount of unlabelled N almost doubled due to combined application of fertilizer N and sewage sludge. Application of sewage sludge alone at the two higher rates also caused significant increases in unlabelled N. In all treatments, a significant proportion of the unlabelled N could, however, have been obtained from unlabelled fertilizer that was applied at a later stage of plant growth following appearance of N-deficiency symptoms.

4. DISCUSSION

Application of sewage sludge had a significant, positive effects on rice yield (increases of 3–8% and up to 158% in straw and grain yields, respectively) and harvest index, observations consistent with other reports in which different manures were used [3,16,17]. Yield responses of 22 to 133% have been reported in some studies [17]; sewage sludge has been found to compensate for inorganic fertilizer to a significant extent in terms of grain production. Combinations of green manure and inorganic fertilizer are reported to have positive effects on rice yield [6,18]. The results of this study show that a substantial savings in fertilizer N could accrue, simultaneously with sewage-sludge disposal on rice fields.

Table VI. Treatment effects on the content of unlabelled soil N in the whole plant and its components

Treatment[a]	N derived from soil			
	Root	Straw	Grain	Whole plant
	(mg pot^{-1})			
Nil (Cont)	48.3b[a]	183de	103g	334d
SS-N30	49.6b	185de	102g	336d
SS-N60	45.5b	178e	188f	411c
SS-N90	46.4b	194d	211e	451c
SS-N120	45.9b	216c	294cd	557b
AS-N120	74.4a	313a	335b	723a
SS+AS, 1:3	73.9a	273b	362a	709a
SS+AS, 1:1	72.9a	274b	360a	708a
SS+AS, 3:1	71.8a	269b	289d	629b

[a]See Table I for details.

Application of sewage sludge also caused significant increases in plant N. The effect of different soil treatments were fairly dependent on N uptake, as revealed by a correlation (r = 0.91) between dry-matter yield and N content of the plants; dependence of crop yields on net N uptake is quite well established. Benefits from organic amendments result mainly from net release of N from decomposing organic matter high N in concentration and narrow in C/N ratio [11,19]. However, the increases in yield observed in the present study seem to be more than would be expected from sewage sludge with a wide C/N ratio. Sewage sludge had more of a positive than a negative effect on N availability; plant N increased by 0.6% to 67% at the lowest and highest rates of addition, respectively. Since N mineralized from wide-C/N-ratio residues (like the sewage sludge in this study) tends to be re-immobilized by the soil microflora [20], a significant proportion of additional plant N in sludge-treated soil could have resulted from N_2 fixation by free-living microbes. Indeed, a significant dilution of plant ^{15}N was observed (data not presented) as a result of soil amendment with sewage sludge in the absence of ^{15}N-labelled fertilizer. Stimulation of N_2-fixation by the addition of organic matter has been reported [21]. In addition, the sewage sludge may have enhanced the mineralization of native soil N in a way similar to that reported for other organic materials [6,22].

The influence of inorganic ^{15}N on plant availability of unlabelled N was significantly greater than that observed for similar amounts of N added as sewage sludge. Enhanced uptake of unlabelled N by plants and accumulation of unlabelled mineral N in soil following application of ^{15}N-labelled NH_4 and NO_3 has been reported [23–26]. As a result, contribution of unlabelled N to the total plant would decrease as observed in this study (Table V). However, since different ratios of inorganic and organic N (sewage sludge) were used in this study, it was not possible to clearly demonstrate the effect of either N source on their respective plant availability.

In summary, the application of sewage sludge had significant positive effects on rice yield and N uptake. Substantial savings in chemical fertilizers seem possible while disposing of sewage sludge on rice fields. More studies are, however, needed to determine i) the influence of inorganic fertilizer on the release of N from sewage sludge, and ii) the effect of sewage sludge on plant availability and fate of inorganic N.

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THE USE OF SEWAGE SLUDGE AS A FERTILIZER IN PASTURES

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Abstract

In a 4-year field experiment, we tested irradiated sewage sludge as a fertilizer for a clover-based pasture (*Trifolium subterraneum* and *Lolium multiflorum*). A calcaric cambisol (pH 7.2) and anaerobically digested sludge, at 5, 10, 20 and 50 t ha^{-1} (S1–S4), were used. Two controls were included: one without sludge amendment but with the addition of a standard mineral fertilization (P and K) and a second with 5 t ha^{-1} of non-irradiated sludge (control). Irradiation drastically reduced the number of the coliforms and eliminated fungi. After the first growth cycle, coliforms were not detected in the soil. The beneficial effects of the sludge application were meaningful, especially for the non-legume, increasing dry-matter production with increasing rates of sludge. The %N derived from fixation (NdfFix) and amount of N fixed decreased when rates of sludge increased, probably due to its N content. With mineral fertilizers the %NdfFix (80) was significantly higher than for S2 (72%), S3 (63%) or S4 (51%). No harmful effects were detected on rhizobial numbers or intrinsic soil quality, even with the highest rate of sludge (50 t ha^{-1}).

1. INTRODUCTION

The application of sewage sludge to agricultural fields is gaining acceptance as an economical means of disposal. Sewage sludge usually has high organic matter and essential plant nutrients, in particular N and P. Its beneficial effects include those on soil physical characteristics [1–3]. However, the presence of potentially harmful contaminants, such as heavy metals, salts, and toxic organics, can limit their application to land due to risks to the environment and to human health through the food chain

Adverse effects of heavy metals on legume nodulation, N_2 fixation, and plant growth have been reported [4,5]. Generally, the application of low-metal sludges has beneficial effects on clover nodules, dry weight of shoots [6], microbial biomass, and on soil microbial activity, whereas higher heavy metal contamination of soils can substantially decrease soil microbial biomass [7].

At present, information is limited on the availability of nutrients from sewage sludge to crops, its benefits as an organic amendment, and the harmful effects of heavy metals on crop growth. Isotope and radiation techniques provide potentially valuable tools for obtaining fuller understanding of these issues.

2. MATERIALS AND METHODS

Data from all four years of field experiments are presented and discussed in general terms. Using the same soil, a pot experiment was also carried out. Some results for which data are not presented will be discussed.

2.1. Soil and sludge

The field experiment was carried out near Estação Agronómica Nacional on a calcaric cambisol (Table I). Before the experiment, the numbers of *Rhizobium leguminosarum* bv. *trifolii* in the soil were determined to be in the range 10^4 to 10^5 cells g^{-1}.

An irradiated (average of absorbed dose 6.17 kGy) urban sewage sludge was collected from a drying bed in Lisbon (Table II).

2.2. Climate

Total rainfall measurements for the four growing seasons were (mm), 1995/96, 1,283; 1996/97, 782; 1997/98, 877; and 1998/99 378. Such irregularity is common in this Mediterranean climate.

Table I. Physical and chemical characteristics of the soil

pH ($CaCl_2$)	7.2	K_2O available (mg kg^{-1})	111
OM (g kg^{-1})	16.6	Cd (mg kg^{-1})[a]	0.20
Total N (g kg^{-1})	1.13	Cr (mg kg^{-1})	39.0
Organic C (g kg^{-1})	9.60	Cu (mg kg^{-1})	11.0
WHC (%)	39	Fe (g kg^{-1})	24.7
Exch.Ca ($cmol_{(c)}$ kg^{-1})	12.1	K (g kg^{-1})	2.20
Exch.Mg ($cmol_{(c)}$ kg^{-1})	0.37	Zn (mg kg^{-1})	59.5
Exch.K ($cmol_{(c)}$ kg^{-1})	0.24	Mn (mg kg^{-1})	150
Exch.Na ($cmol_{(c)}$ kg^{-1})	0.25	Pb (mg kg^{-1})	25.5
P_2O_5 available (mg kg^{-1})	385	Ni (mg kg^{-1})	17.5

[a] All metals from aqua-regia digests.

Table II. Physical and chemical characteristics of the sewage sludge

	Sludge sample	EC limits [8]	Portuguese limits [9]
pH (H_2O)	6.25		
OM (%)	47.9		
Total N (%)	2.67		
Total P (%)	3.55		
K (g kg^{-1})[a]	1.01		
Na (g kg^{-1})	2.0		
Ca (g kg^{-1})	80.0		
Mg (g kg^{-1})	6.6		
Al (g kg^{-1})	13.5		
Fe (g kg^{-1})	107		
Co (mg kg^{-1})	13.0		
Mn (mg kg^{-1})	110		
Zn (mg kg^{-1})	1,780	2,500–4,000	2,500
Cr (mg kg^{-1})	64.0	1,500–2,500	1,000
Ni (mg kg^{-1})	36.0	300–400	300
Pb (mg kg^{-1})	132	750–1,200	750–1,200
Cd (mg kg^{-1})	1.8	20–40	20–40
Cu (mg kg^{-1})	302	1,000–1,750	1,000–1,750

[a] All metals extracted with aqua regia.

2.3. Procedures and experimental design

The experiment had a completely randomized block design with four replicates, using the following rates of sludge (t ha^{-1}): S1, 5; S2, 10; S3, 20; and S4, 50. These were applied only once, at the beginning of the experiment. Two controls were included: one without sludge amendment but with the addition of a standard mineral fertilization (PK) equivalent to 300 kg ha^{-1} of superphosphate (18% P_2O_5) and 60 kg ha^{-1} of KCl (60% K_2O,) and a second one using 5 t ha^{-1} of non-irradiated sludge (control) for monitoring pathogens in the soil.

In the first year, a mixture of *Lolium multiflorum* cv. Prima (20 kg ha^{-1}) and *Trifolium subterraneum* cv. Clare (30 kg ha^{-1}) inoculated with a selected strain of *Rhizobium leguminosarum* bv. *trifolii* (10^6 bacteria per seed) were sown in each plot (1×1m). In the subsequent three years, the plots were resown without addition of sewage sludge or fertilizer; the legume seeds were not inoculated and the grass was sown at 10 kg ha^{-1}.

2.4. Sample analysis and monitoring pathogens

Soil and sludge samples were air-dried and ground (2 mm). Their heavy-metal contents were determined by aqua-regia extraction with reflux and measured by atomic absorption [10].

Pathogens in the irradiated and non-irradiated sludge were evaluated using methods employed in the local public-health laboratory.

2.5. Plant growth

The shoots were washed free of soil, dried at 70°C for 24 h in a forced-draught oven, and then weighed and ground for chemical analysis

2.6. Nitrogen-15 treatment and N_2 fixation

Ammonium nitrate (NH_4NO_3) containing 5% ^{15}N atom excess was applied as a uniform spray, at the rate of 2 kg N ha^{-1}, one month after sowing (clover had three trifoliolates) or immediately after harvesting.

The ^{15}N-dilution technique was used to estimate the amount and the fraction of N derived from biological N_2 fixation [11].

2.7. Numbers and effectiveness of the rhizobial population

Populations of *Rhizobium leguminosarum* bv. *trifolii* were estimated by the most probable number (MPN) method [12], using a ten-fold dilution series with *Trifolium subterraneum* L. cv. Clare as the test plant.

The effectiveness of the rhizobial population was evaluated using the acetylene reduction assay in the tubes with the plants used to estimate MPNs. Rate of acetylene reduction to ethylene (determined by gas chromatography) is an indirect measure of nitrogenase activity [13].

3. RESULTS AND DISCUSSION

3.1. Detection and enumeration of pathogens

Pathogens were evaluated in the irradiated and non-irradiated sludge, before addition to soil, and also in the soil at the end of the first growth cycle.

3.1.1. Sludge

3.1.1.1. Coliforms

After 24 h incubation, 10^6 bacteria g^{-1} were detected in non-irradiated sludge and none were detected in irradiated sludge. After 36 h of incubation there were 10^6 bacteria g^{-1} in non-irradiated sludge and 2×10^3 bacteria g^{-1} in irradiated sludge.

3.1.1.2. Fungi

After 72 h of incubation, fungi were present in non-irradiated sludge but were not detected in irradiated sludge.

3.1.1.3. Other microorganisms

Salmonella, ova, cysts or parasites were not detected in non-irradiated or in irradiated sludge..

3.1.2. Soils

At eight months after application of the sewage sludge, no coliforms were detected in the soil. *Escherichia coli*, one of the pathogenic bacteria of importance in sewage sludge, which was present in large numbers even in the irradiated sludge (2×10^3 bacteria g^{-1}), disappeared from the soil during the first growth cycle. These results agree with those referred to by Smith [14]: faecal coliforms do not survive in soil.

3.2. Plant growth

The results for dry-matter production (g $plot^{-1}$) are illustrated in Figs. 1, 2, and 3, with averages (AVG) included.

Dry-matter production of the non-legumes and total dry matter, increased while that of legume decreased with increasing rates of sludge applied. Non-legume dry-matter production was generally superior to that of the legume.

In an overall analysis, averages of four growth cycles, for legume plants (Fig. 1) the yields in treatments PK (79 g $plot^{-1}$), S1 (81 g $plot^{-1}$), and the control (88 g $plot^{-1}$), were significantly higher than in S3 (38 g $plot^{-1}$) and S4 (40 g $plot^{-1}$).

For non-legumes (Fig. 2), yield in the S4 treatment (384 g $plot^{-1}$) was significantly higher than with all the other soil treatments, and those in S3 (251 g $plot^{-1}$) and S2 (220 g $plot^{-1}$) were significantly higher than in the PK treatment (133 g $plot^{-1}$).

The total dry-matter production of legumes and non-legumes (Fig. 3) of treatment S4 (421 g $plot^{-1}$) was significantly superior to all the other soil treatments, with control (273 g $plot^{-1}$), S2 (283 g $plot^{-1}$) and S3 (291 g $plot^{-1}$) significantly higher than PK (212 g $plot^{-1}$). The irregular weather conditions, especially the precipitation, during all 4 years significantly affected dry-matter production.

The introduced sludge seems to have characteristics appropriate for use as fertilizer. Even at the highest rate, only small amounts of heavy metals were introduced to the soil. Only Cu and Zn concentrations were high, but without problems of toxicity to the plants. Chemical analyses showed that the highest values were obtained with treatment S4 (during the 1st, 2nd and 3rd years), 83 and 13 mg kg^{-1}, for Zn and Cu respectively in clover. For non-legume, 48 and 10 mg kg^{-1}, were obtained for Zn and Cu, respectively. No toxic effects of heavy metals were found, and the values were adequate for these plants [15] and well below critical phytotoxic concentrations of 500 mg Zn kg^{-1} and 25 mg Cu kg^{-1} for legumes [16] and 221 mg Zn kg^{-1} and 21 mg Cu kg^{-1} for non-legume plants [17].

Our results showed beneficial effects of the sewage sludge application to clover-based pastures, increasing the total dry-matter production without problems of toxicity. When applied at agricultural rates (5–10 t ha^{-1}), the sludge can be used as a fertilizer in pastures without affecting the legume/non-legume equilibrium. Legume dry matter decreased with increasing rates of sludge, which can be explained by the increased growth of the non-legume.

3.3. Numbers and effectiveness of the rhizobial population

During the period of the experiment, the population size of *Rhizobium leguminosarum* bv. *trifolii* was not significantly affected by the different rates of sludge (Fig. 4). At the beginning of the second rowing season (October 1996), the rhizobial population varied between 4.1×10^5 (S2) and 2.0×10^6 (control) and between 2.2×10^6 (PK) and 1.5×10^7 (S1) at the beginning of the fourth season (October 1998).

No significant differences in the nitrogenase activity of nodules induced by the rhizobial populations, were detected in October 1996 or October 1998 (Fig. 5).

Rhizobia are soil bacteria that have the ability to nodulate leguminous plants and to fix atmospheric N_2. Presence of heavy metals in the soil is known to affect their survival [18] and their N_2-fixing capability [4]. Heavy-metal concentration in this soil, even with the highest application rate of sludge (50 t ha^{-1}), remained far lower than the amounts allowed under current EC guidelines [9]. This sludge did not have a detrimental effect on soil rhizobial numbers (Fig. 4.), neither did it decrease nitrogenase activity (Fig. 5).

3.4. Biological N_2 fixation

The results for fractional contribution of biological N_2 fixation (%NdfFix) and amount fixed (kg N ha^{-1} year^{-1}) are illustrated in Figs. 6 and 7.

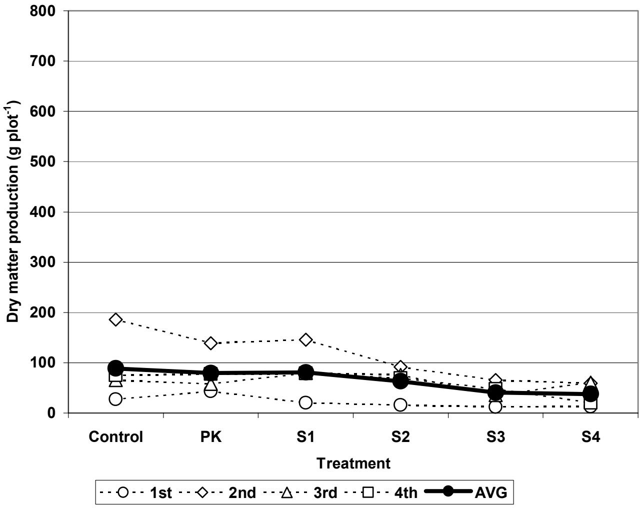

FIG. 1. Dry-matter production of the legume, in each of the four years.

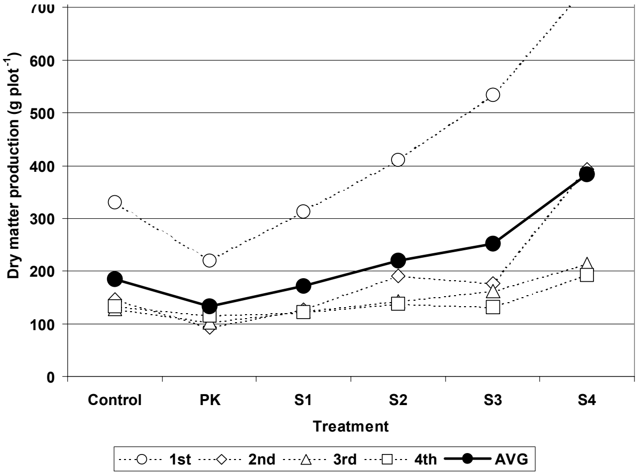

FIG. 2. Dry-matter production of the non-legume, in each of the four years.

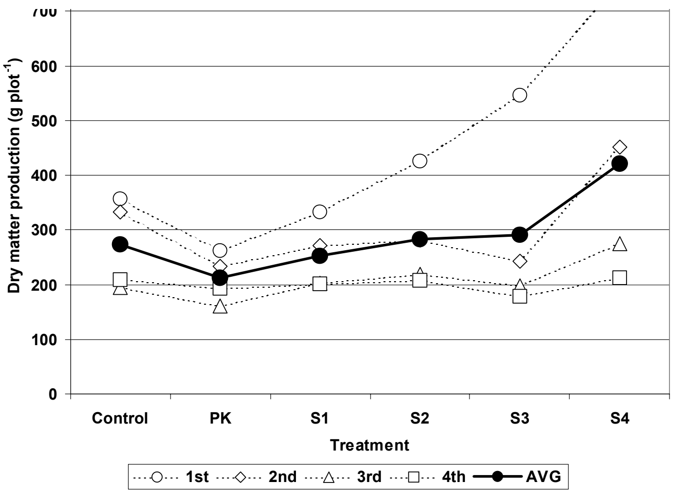

FIG. 3. *Total dry-matter production of the pasture, in each of the four years.*

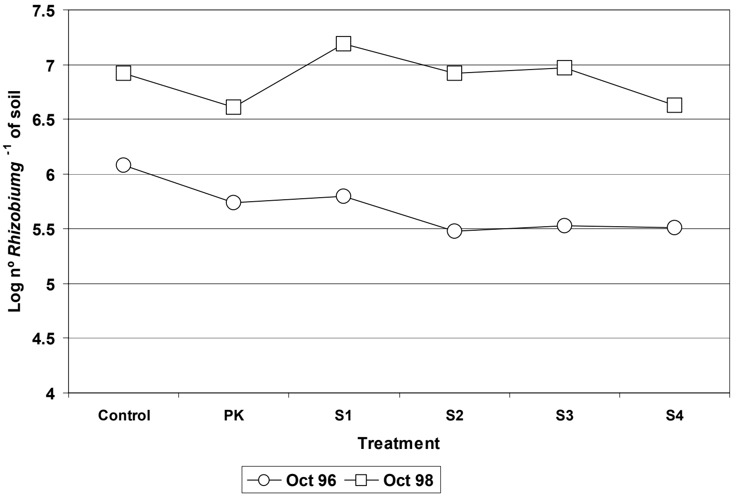

FIG. 4. *Number (MPN) of rhizobia per gram of soil at the beginning of the second and fourth growing seasons (October 1996 and October 1998).*

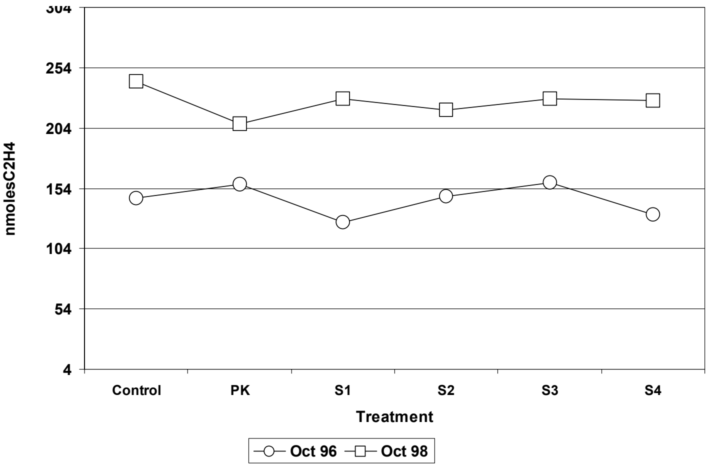

FIG. 5. *Nitrogenase activity (nmoles/C_2H_4/three plants) at the beginning of the second and fourth growing seasons (October 1996 and October 1998).*

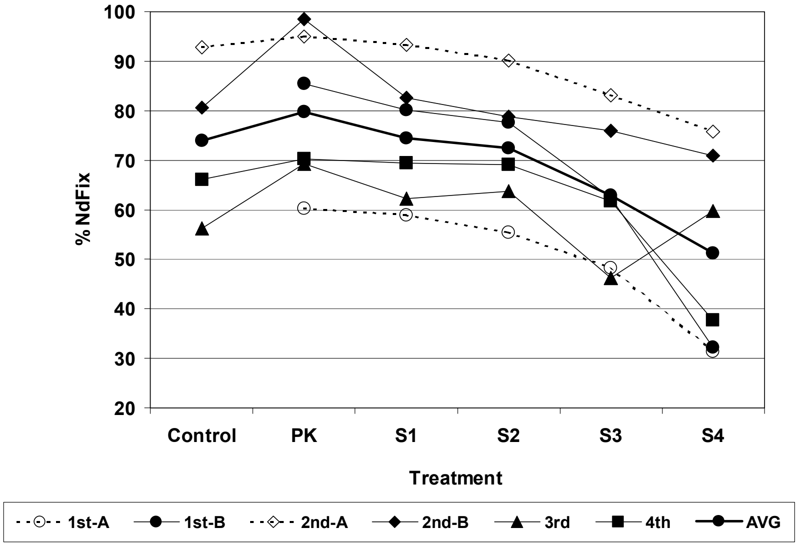

FIG. 6. %NdfFix during the four growth cycles. Six cuts: first year (cut A and cut B), second year (cut A and cut B), third year, fourth year and average of all cuts (AVG).

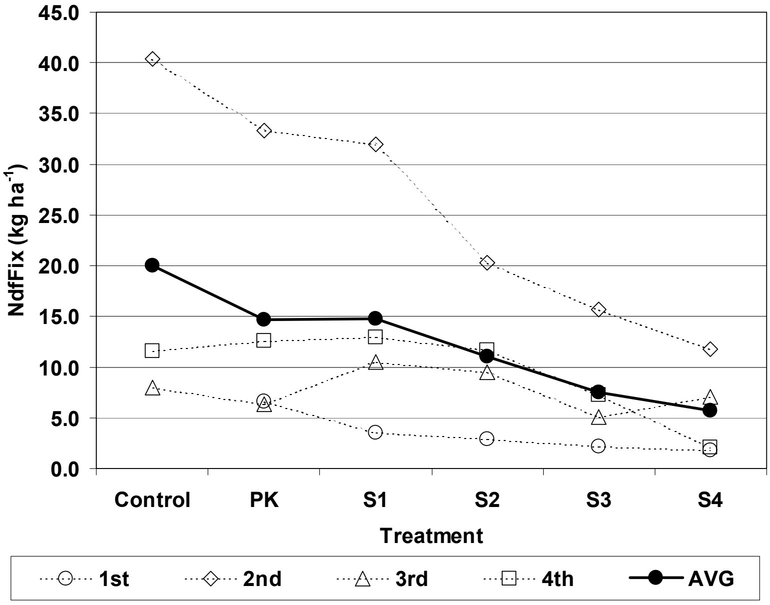

FIG. 7. Amounts of N fixed during the four years of experiments and the overall average (AVG).

In general, the % NdfFix (Fig. 6), and amount of N fixed (Fig. 7), decreased as the rate of sludge increased.

With mineral fertilization (PK) the %NdfFix (80%) was significantly higher than for S2 (72%), S3 (63%) and S4 (51%).

The amounts of N fixed in the control (20 kg ha^{-1}), PK (15 kg ha^{-1}), and S1 (15 kg ha^{-1}) were significantly greater that with S3 (8 kg ha^{-1}) and S4 (6 kg ha^{-1}).

Our results for %NdfFix for the lowest rates of sludge (5 and 10 t ha^{-1}) and for PK, agree with those of Hogh-Jensen and Schjoerring [19], where the values ranged from 50 to 96%, and with those of Bolger et al. [20], where the values ranged from 65 to 95%.

However, the average amounts of fixed N (three years) varied between 20 and 6 kg ha^{-1}, markedly less than those obtained by Hogh-Jensen and Schjoerring [19] (31–72 kg N ha^{-1}) and by Bolger et al. [20] (50–125 kg ha^{-1}). Our results can, at least partially, be due to the irregular climatic conditions. In the second year (a normal year) we obtained a similar amount (40 kg N ha^{-1}) for the control.

Clearly, when applied at high rates (>20 t ha^{-1}) sludge can negatively affect %NdfFix and the amount of N fixed.

The %N derived from sludge (data not shown) increased with rates of sludge for the legume and non-legume alike, ranging between 5 and 27% for the former and between 17 and 49% for the latter, for S1 and S4, respectively.

Data obtained in the pot experiment suggested that even sludge applied at the rate of 80 t ha^{-1} did not supply the N requirements of the grass, but only supplement it; however, biological N_2 fixation did supply the N requirements of the legume.

4. CONCLUDING REMARKS

Dry-matter production of the non-legume increased while that of the legume decreased with increasing rates of sludge applied. Sewage sludge can be used as a fertilizer in clover-based pastures to increase the total dry-matter production without problems of toxic effects on the plants or the soil, even at the highest rate of application (50 t ha^{-1}).

This sludge, of low metal content, had no detrimental effects on soil rhizobial numbers or on root-nodule nitrogenase activity.

When applied at agricultural rates (5–10 t ha^{-1}), the sludge can be used as a fertilizer in pastures, increasing soil fertility without causing problems either in terms of the equilibrium of the pasture (relative numbers of legume and non-legume plants), on the % NdfFix, or on amount of N fixed.

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USE OF NUCLEAR TECHNIQUES FOR EVALUATING AGRICULTURAL USE OF SEWAGE SLUDGE

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Abstract

In a field experiment, we examined the effects of irradiated and non-irradiated sewage sludge on oats and maize. After 3 years of the maximum rate of 400 kg N/ha-equivalent of sewage sludge, significant changes were observed in physical, chemical and biological soil properties and in content of P, K, Ca, Cu, Zn, Pb, Co, Ni, Cr, and Cd in oat grain and straw as well as in maize stalks, leaves, husks, cobs, and kernels. Treatments with sewage sludge, at doses equivalent to 200 kg N/ha for oats and 300 kg N/ha for maize, gave the same yields as those with 100 kg N/ha as ammonium sulphate. The fraction of N derived from sewage sludge (%Ndfss) had values between 12 and 32% for non-irradiated sludge, and 8.7 to 22% for irradiated sludge. Recoveries of sewage-sludge N were 10 to 13.5% for non-irradiated and 9.5 to 17% for irradiated sludge applied to oats, and 9.3 to 20% for non-irradiated and 11 to 19% for irradiated sludge applied to maize. The data support the possibility of using irradiated sewage sludge as fertilizer, with only minor negative effects on environmental quality.

1. INTRODUCTION

In Romania, moderately and severely acidified soils cover 3,352,000 ha. Moreover, soils on 7,178,000 ha have low and very low humus content, 6,246,000 ha have low and very low available P, 4,812,000 ha are low in N, 694,000 ha have low available K, 1,500,000 ha are deficient in Zn, 900,000 ha are affected by pollution, and 33,000 ha are otherwise disturbed and covered with solid wastes. Fertilizer application to cultivated land decreased from 102 kg/ha active ingredient in 1989 to 28 kg/ha in 1997, and organic-fertilizer usage decreased from 3.64 to 1.2 t/ha. In the same period, large quantities of sewage sludge from municipal wastewater treatment plants were applied to non-arable land, polluting the environment. Researchers have suggested that the least expensive means of sludge disposal is application to agricultural land, thus its organic matter and nutrients would be efficiently recycled.

To be applied on agricultural lands, sewage sludge should meet the following criteria:

- It is not a source of pathogenic organisms (in Romania sludge is decontaminated by mesophilic anaerobic digestion and lime stabilization of liquid sludge; in other countries, many other methods are used, e.g. pasteurization, incineration, composting, pyrolysis, wet oxidation, thermophilic aerobic digestion, irradiation, and lime treatment to high pH [1–3]).
- It does not contain heavy metals or synthetic organic compounds (polychlorinated biphenyls, etc.) over maximum allowable limits [4,5].
- It is applied according to prescribed technologies (suitable land, rates, time and means of application, adequate crop structure and rotation, intervals, etc.) [6–8].

Implementation of a system to monitor environmental quality is necessary [9,10].

Research previously carried out by IAEA demonstrated the effectiveness of ionizing radiation in removal or inactivation of pathogenic micro-organisms and protozoon parasites, and in degrading atrazine, 2-ethylhexylphthalate, hexachlorobenzene, and parathion. Maximum degradation was achieved when the compounds were dissolved in water. Atrazine and parathion were easily degraded, even at low doses of accelerated electrons, 5 kGy [11,12]. Although the problem of decontamination is solved by using irradiation, research is still needed to establish guidelines for land application of sewage sludge without adversely affecting the environment and to elucidate its influence on soil fertility, agricultural production, and yield quality.

2. MATERIALS AND METHODS

A field experiment with irradiated and non-irradiated sewage sludge, on a Haplic Phaeozem at Teleorman Agricultural Research Station, Drăgăneşti Vlaşca, using oats and maize as test crops, was organized. This soil has a pH (H_2O) of 6.1 and ($CaCl_2$) 5.4, humus content 3.6%, total N 0.26%, available P 34 mg/kg, available K 155 mg/kg, CEC 28.2 mEq/100 g, base saturation 84%, clay (<2 μm) 46%, silt (20–2 μm) 33%, sand (2,000–20 μm) 21%, Mitscherlich hygroscopic coefficient 11.5%, total porosity 49%, and bulk density 1.37 Mg/m^3.

The experiments had a completely randomized block design with ten treatments (T_1: 100 kg N/ha from 1% a.e. ^{15}N-labelled fertilizer; T_2: 20 kg N/ha from 10% a.e. ^{15}N-labelled fertilizer; T_3–T_6 non-irradiated and T_7–T_{10} irradiated sewage sludge equivalent to 100, 200, 300, and 400 kg N/ha + 20 kg N/ha of 10% a.e. ^{15}N labelled fertilizer). Ammonium sulphate with 10% ^{15}N abundance and unenriched ammonium sulphate were used as N sources for isotope dilution. These doses of sewage sludge were applied in 1997 and 1998. In the first year (1996) they were applied at half of these levels.

The sewage sludges (Table I) had the following average chemical characteristics: 2.09% N, 0.52% P, 0.38% Na, 0.70% Ca, 0.31% Mg, 63% water content, pH 6.98, 107 mg/kg Cu, 767 mg/kg Zn, 150 mg/kg Pb, 16 mg/kg Co, 35 mg/kg Ni, 391 mg/kg Mn, 396 mg/kg Cr, 10 mg/kg Cd, and 63% organic matter.

By comparing the heavy-metal contents with the maximum admissible limits (alert values) for agricultural use of sewage sludge in Romania (10 mg/kg Cd, 50 mg/kg Co, 500 mg/kg Cr, 1,200 mg/kg Mn, 100 mg/kg Ni, 300 mg/kg Pb and 2,000 mg/kg Zn) or with those of the European Community Directive (20–40 mg/kg Cd, 1,000–1,750 mg/kg Cu, 750–1,200 mg/kg Pb, 300–400 mg/kg Ni, and 2,500–4,000 mg/kg Zn), clearly this sludge could be used as a fertilizer.

In the second and third years, maize was sown instead of oats and vice-versa. After harvesting the two crops, 80 kg/ha P_2O_5 were uniformly spread and the plots were ploughed to a depth of 25 to 28 cm. The seeding density for oats (cv. Solidor) was 500/m^2 and for maize (hybrid Olt) 50,000/ha. In spring, the sewage sludge was applied and the seed bed prepared by disking and harrowing.

The irradiation of sewage sludge from the Piteşti wastewater-treatment plant was done in the High Activity Gamma Irradiation Station (SIGMA), which uses a ^{60}Co source. The sludge was irradiated after packaging in airtight polyethylene bags. All samples were irradiated under the same conditions:

— an absorbed dose of 4.95 kGy,
— a dose rate of 0.90 kGy/unit time,
— a dose uniformity ratio of 1.14,
— environment, air.

The plants were harvested at physiological maturity. Grain yields were determined, as well as total above-ground biomass, on a dry-weight basis.

Table I. Characteristics of the sewage sludge

Characteristic		1996		1997		1998	
		Range	Average	Range	Average	Range	Average
pH (1:2.5 H_2O)		7.12–8.03	7.44	5.84–7.90	6.80	5.90–7.27	6.85
Moisture	(%)	39–82	60	61–75	70	43–80	58
Organic matter				73–89	82	37–50	44
N		1.51–2.74	2.17	1.80–2.70	2.39	1.60–2.20	1.81
P		0.24–0.96	0.69	0.21–0.41	0.34	0.52–0.75	0.67
K		0.30–0.75	0.48	0.29–0.41	0.35	0.54–0.86	0.72
Na		0.15–0.29	0.23	0.34–0.54	0.48	0.30–0.38	0.34
Ca		0.10–0.19	0.14	0.57–1.88	1.19	0.09–0.83	0.38
Mg		0.05–0.07	0.06	0.31–0.40	0.35	0.35–0.38	0.36
Cu	(mg/kg)	36–93	69	95–165	141	65–129	94
Zn		387–603	548	726–1,070	979	534–1,099	700
Pb		83–145	125	150–217	177	96–261	141
Co		7–10	8	12–17	14	10–24	20
Ni		22–29	26	32–47	41	19–49	34
Mn		315–529	396	262–443	321	229–535	439
Cr		160–370	280	330–534	462	156–348	286
Cd		5–11	9	7–14	11	6–15	9

Soil samples were analyzed for:

- pH in water suspension (1:2.5) and in 0.01 *M* $CaCl_2$ (1:5),
- organic C, by the Turin method,
- total N, by the Kjeldahl method,
- mobile P and K, by extraction in 0.1 *M* ammonium lactate – 0.4 *M* acetic acid solution at pH 3.7 (Egner Riehm Domingo method, 1960) followed by colorimetric determination of P as phosphomolybdenum blue and flame-photometric determination of K,
- total Cd, Co, Cu, Pb, Mn, Ni, and Zn by aqua regia (4:1 HCl:HNO_3 v:v) digestion followed by atomic absorption spectrometric determination,
- soluble Cd, Co, Cu, Pb, Mn, Ni, and Zn by extraction in 1 *M* NH_4NO_3 followed by atomic absorption spectrometry.

Plant samples were analysed for:

- total N, by digestion in sulphuric acid (Kjeldahl-Rittenberg oxidation method) followed by titration,
- ^{15}N, by mass spectrometry, after the above-mentioned digestion,
- heavy-metal content by digestion with a mixture of acids: HNO_3-$HClO_4$ (85:15) followed by atomic absorption spectrometry.

According to the meteorological data, the mean annual air temperature is 10.8°C and the annual rainfall averages 538 mm, with a significant decrease during the summer and non-uniform distribution in time, causing periods of severe drought. Two irrigations were applied to oats and three for maize, in order to ensure normal growth and development.

3. RESULTS AND DISCUSSIONS

3.1. Yields

Data showing the effects of application of irradiated and non-irradiated sewage sludge on oat and maize crops are in Tables II and III. In the first year, there was a tendency of increasing oat yield in response to fertilization with sewage sludge, but the only significant increases (compared with treatment with 100 kg N/ha) occurred with the maximum dose of sludge (equivalent to 200 kg N/ha). In the second and the third years, fertilization with the higher doses of sewage sludge significantly influenced yields of oat grain and stover. The highest oat yields were obtained with the maximum sludge treatment (400 kg N/ha equivalent). As compared with the control (T_2), oat yields increased by 28% for irradiated sewage sludge and by 33% for non-irradiated sewage sludge; as compared with 100 kg N/ha treatment, the yields were increased by 8% and 12%, respectively. After 3 years of yearly application of sewage sludge equivalent to 200 kg N/ha, the oat yields were the same as in the treatment receiving ^{15}N labelled fertilizer as 100 kg N/ha at 1% a.e.

Data presented in Table III show that, with sewage sludge in doses equivalent to 300 and 400 kg N/ha, maize yields were similar to those obtained with 100 kg N/ha fertilization. In comparison with non-treated plots, applying sludge equivalent to 400 kg N/ha increased maize yield by 17% as non-irradiated sewage sludge and by 15% as irradiated.

In another experiment, on a Haplic Phaeozem phreatic phase from Timişoara, fertilization with sewage sludge gave strong increases in maize-grain yields. Taking as reference the 3-year averaged control yield (5,284 kg/ha) the increases were: 9.8%, 26%, 33%, 36%, and 39% at 10, 25, 50, 75, and 100 t/ha sewage sludge, respectively. The $N_{120}P_{80}$ mineral fertilization assured an average yield increase of 39%, almost equal to that with 100 t/ha sewage sludge [13].

As a matter of fact, all of our field, pot and greenhouse experiments so far have indicated that sludge rates of less than 30 t/ha rarely give significant yield increases in the first year of application [e.g. 14,15].

Table II. Effects of sewage sludge on oat yields (1996–1998)

Year	T_1	T_2[a]	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
					Grain (t/ha)					
1996	4.39ab[b]	3.97 a	4.31ab	4.30ab	4.47ab	4.56b	4.47ab	4.53ab	4.37ab	4.57b
1997	2.30bcd	1.95abc	1.72a	1.79a	2.38d	2.34cd	1.84a	1.91ab	1.80a	2.00abcd
1998	2.72ab	2.06a	3.22bc	3.31bc	3.59c	3.70c	2.99bc	3.18bc	3.27bc	3.64c
Mean	3.14	2.66	3.08	3.13	3.48	3.53	3.10	3.21	3.15	3.40
					Straw (t/ha)					
1996	6.98bc	5.90a	6.73abc	6.59abc	7.00bc	7.40	6.28ab	6.38ab	6.14ab	6.87bc
1997	6.00 ab	5.46a	6.50bcd	7.20d	6.92cd	7.10d	6.19abc	6.15abc	6.70bcd	7.25d
1998	6.58ab	6.25a	7.38bc	7.97cde	8.35de	8.51e	7.22bc	7.42c	7.67cd	8.56e
Mean	6.52	5.87	6.87	7.25	7.42	7.67	6.56	6.65	6.84	7.56

[a] Reference for treatments T_3–T_{10}. [b] Numbers within a row followed by the same letter are not significantly different ($P<0.05$) using Tukey's HSD procedure.

Table III. Effects of sewage sludge on maize grain yields

Year	T_1	T_2[a]	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
	(t/ha)									
1996	10.9ab[b]	9.2 a	10.4ab	10.2ab	11.2b	10.4ab	10.2 ab	10ab	9.8ab	9.9ab
1997	12.7c	10.4a	10.8ab	11.9abc	12.0abc	12.2bc	11.1abc	11.6abc	12.4bc	12.0abc
1998	11.6bc	9.9a	10.6ab	11.1bc	11.8c	12.0c	10.6ab	10.7ab	11.2bc	11.4bc
Mean	11.7bc	9.8a	10.6ab	11.1bc	11.7c	11.5c	10.6ab	10.8ab	11.1bc	11.1bc

[a]Reference for treatments T_3–T_{10}. [b]Numbers within a row followed by the same letter are not significantly different ($P<0.05$) using Tukey's HSD procedure.

Table IV. Contributions of soil, mineral fertilizer and sewage sludge to N taken up by oats

Characteristic	T_1	T_2[a]	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
	1996									
N yield (kg/ha)	135	117	123	115	133	135	121	130	127	134
Ndfs[b] (%)	61	96	96	90	95	90	96	96	93	91
Ndff[c]	39	4.0	4.3	3.7	4.1	3.7	4.2	4.1	3.8	3.8
Ndfss[d]			0.0	6.4	1.4	6.7	0.2	0.0	2.9	4.8
Sewage-sludge N yield (kg/ha)			0.0	7.4	1.9	9.1	0.2	0.0	3.7	6.4
Recovery of sewage-sludge N (%)			0.0	7.4	1.3	4.6	0.4	0.0	2.5	3.2
	1997									
N yield (kg/ha)	111	98.4	111	126	136	151	111	122	127	142
Ndfs (%)	70	94	84	79	81	73	80	79	75	68
Ndff	30	5.6	4.9	4.6	4.8	4.4	4.7	4.6	4.5	4.1
Ndfss			11	17	14	23	15	16	21	28
Sewage sludge N yield (kg/ha)			12.3	21.2	18.8	34.0	17.1	19.6	26.3	39.4
Recovery of sewage-sludge N (%)			12.3	10.6	6.3	8.5	17.1	9.8	8.8	9.9
	1998									
N yield (kg/ha)	97.7	87.5	106	120	134	168	109	122	149	166
Ndfs (%)	66	93	81	76	72	63	85	77	70	55
Ndff	34	7.3	6.3	5.9	5.7	4.8	6.5	6.1	5.4	4.3
Ndfss			12	18	22	32	8.7	17	25	41
Sewage sludge N yield (kg/ha)			13.2	21.2	30.1	54.1	9.5	20.3	37.0	67.2
Recovery of sewage-sludge N (%)			13.2	10.6	10.0	13.5	9.5	10.2	12.3	16.8

[a]Reference for treatments T_3–T_{10}. [b]N derived from soil. [c]N derived from fertilizer. [d]N derived from sewage sludge.

3.2. Contributions of sludge to N nutrition

In the first year of the experiment, the N contents of oat grain and straw showed no significant effects of the application of sewage sludge. The fractions of N derived from fertilizer (Ndff), either with irradiated or non-irradiated sludge were low (3.7–4.3%) (Table IV).

Percent N derived from sewage sludge (%Ndfss) had values between 0 and 6.7. The sludge had passed through a digestion procedure, and thus was stabilized, explaining the low indices of N release in the first year. Recovery of sewage-sludge N was very low (0–7.4%).

In the second and third years, values of %Ndff remained low (4.1–6.5), but Ndfss increased with the rate of sludge, and had values between 12 and 32% for non-irradiated and 8.7 to 41% for irradiated. Nitrogen yields increased with dose of sewage sludge although recoveries of sludge N remained low (6.3–17%) as compared with other reported data, e.g. between 40% and 50% during the first year, and 15 to 50% during the second and third years [6]. In the second and third years, fractions of N derived from sludge were higher with irradiated sludge than with the corresponding treatments with the non-irradiated counterpart. This could be due to a stimulatory effect of irradiation on degradation of organic compounds, e.g. atrazine and parathion [1].

During the experiments with maize, in all treatments N yield increased from the first year to the third, and every year increased with sludge doses (Table V). In all years, %Ndff had similar low values (>2), for all either irradiated or non-irradiated sludge applied at maize. The N derived from non-irradiated sewage sludge had values between 13 and 23% in the first year, 4.6 to 23% in the second and 4.0 to 27% in the third. Nitrogen derived from irradiated sewage sludge had the following values: 1.8 to 19%, 2.9 to 17%, and 7.4 to 22%, respectively. In the third year, recovery of sewage sludge N increased with quantity of sewage sludge applied and had values between 9.4 and 20% for non-irradiated and 11 and 19% for irradiated sludge.

The N content of sewage sludge was between 1.5 and 2.7% with an average of 2%. The content in NH_4-N was 173 to 480 mg/kg (average 300 mg/kg) and NO_3-N was 56 to 198 mg/kg (average 87 mg/kg); less than 2% was in soluble form, the majority being organically bound.

Nitrogen availability from raw sludge relative to fertilizer in a clay loam soil (similar to that in our experiment) was 27 to 41%. Decomposition, and hence N release to the crop, occurs more slowly for anaerobically digested sludges; data in the literature [16] provide values between 10% and 20% in first year of application. Our data were close to this. In the third year, for the 400 kg N/ha treatment (around 20 t/ha), sewage sludge N yields were 60 to 80 kg/ha, which means 2- to 3-fold more than the average doses of mineral fertilizers used in Romania in recent years.

3.3. Effects on soil

To assess effects of non-irradiated and irradiated sewage sludge on the physical state of the soil's surface layer (0–10 cm), the following were determined: bulk density, resistance to penetration, degree of compaction, total and air porosity, saturated hydraulic conductivity, water retention in the range 0.1 to 10 kPa, water-aggregate stability and particle-size distribution. All of the measurements were made during vegetative growth of maize, after harvest of oats.

There were no significant changes in physical properties, which may be explained by the "quality" of the Haplic Phaeozem, i.e. good structure resiliency and low vulnerability or sensitivity to structural modifications. Therefore, sewage sludge can be applied at agronomic rates if other restrictions do not apply. There is evidence of bulk-density decreases in soils treated with sludge; significant linear correlations between the increasing content of soil organic matter and bulk density were reported in a sandy and a loamy soil [17].

Table V. Contributions of soil, mineral fertilizer and sewage sludge to N taken up by maize

Characteristic	T_1	T_2[a]	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
					1996					
N yield (kg/ha)	179	169	177	186	189	213	198	198	197	202
Ndfs[b] (%)	88.5	97.8	85.3	84.4	83.9	75.4	79.0	96.1	91.1	92.9
Ndff[c]	11.5	2.2	1.9	1.9	1.9	1.7	1.8	2.1	2.0	2.1
Ndfss[d]			12.8	13.7	14.2	22.9	19.2	1.8	6.9	5.0
Sewage sludge N yield (kg/ha)			22.7	25.5	26.9	48.8	38.0	3.6	13.6	10.1
Recovery of sewage-sludge N (%)			45.4	25.5	17.9	24.4	76.0	3.6	9.1	5.1
					1997					
N yield (kg/ha)	198	181	187	198	218	216	176	206	216	210
Ndfs (%)	92.9	98.5	94.0	84.3	84.3	75.6	95.6	86.2	87.4	82.1
Ndff	7.1	1.5	1.4	1.3	1.3	1.1	1.5	1.3	1.3	1.2
Ndfss			4.6	14.4	14.4	23.3	2.9	12.5	11.3	16.7
Sewage sludge N yield (kg/ha)			8.6	28.4	31.4	50.3	5.1	25.8	24.4	35.1
Recovery of sewage sludge N (%)			8.6	14.2	10.5	12.6	5.1	12.9	8.1	8.8
					1998					
N yield (kg/ha)	243	231	234	259	296	301	252	260	276	284
Ndfs (%)	82.2	98.1	94.1	85.7	78.4	72.0	90.8	90.0	85.2	76.8
Ndff	17.8	1.9	1.9	1.7	1.5	1.4	1.8	1.8	1.7	1.5
Ndfss			4.0	12.6	20.1	26.6	7.4	8.2	13.1	21.7
Sewage sludge N yield (kg/ha)			9.4	32.6	59.4	79.9	18.6	21.3	36.2	61.6
Recovery of sewage sludge N (%)			9.4	16.3	19.8	20.0	18.6	10.7	12.1	15.4

[a]Reference for treatments T_3–T_{10}. [b]N derived from soil. [c]N derived from fertilizer. [d]N derived from sewage sludge.

Changes in structural (decrease in density, increase in porosity and aggregate stability) and hydraulic (increase in water-holding capacity and conductivity) properties have important implications for agriculture on both heavy- and light-textured soils, for improving moisture-retention characteristics for example.

In our experiment, the microbiological activity (number of bacteria, number of microscopic fungi and dehydrogenase activity) of the Haplic Phaeozem showed no significant changes after 3 years of application of non-irradiated and irradiated sewage sludge (12–50 t/ha). Usually, increases in microbiological activity occur after fertilization with sewage sludge—if contents of heavy metals [18,19] and other pollutants are low—with decreased microbial biomass and changes in community structure [20–24].

During the 3-year period, 12.5 to 50 t/ha of irradiated or non-irradiated sewage sludge were applied. These low doses had significant effects on chemical properties of soil: base saturation, CEC, pH, humus content, total N, available P and K (Table VI), or total and soluble contents in Cu, Zn, Pb, Cd (Table VII). Only tendencies in increasing humus content, and available P, Cu and Zn were observed.

In Romania, the maximum permissible concentrations (mg/kg) of heavy metals in soils are: 100 Cu, 300 Zn, 100 Pb, 50 Co, 50 Ni, 1500 Mn, 100 Cr, and 3 Cd. These limits correspond to those recommended by the European Union (1–3 Cd, 100–150 Cr, 50–140 Cu, 50–300 Pb, 30–75 Ni and 150–300 Zn) [22]. In comparison with these limits, it can be seen that no significant changes occurred for any of the analysed elements (Cu, Zn, Pb, Co, Ni, Cd, Cr) in soil to which 20 t/ha sewage sludge was applied three years in a row. Among soluble heavy metals, only Pb had significantly increased values after treatment with sludge.

The total allowable amount of municipal sewage sludge for application over 30 years on agricultural lands with medium-textured soils and pH above 6.5 can be established by using the formula:

$$D_{ss30} = \frac{0.8 \times C_{max} - C_{soil}}{C_{ss}} \times K$$

where

D_{ss30} is the amount of sludge applied during 30 years per hectare (dry tonnes/ha),
C_{max} is the alert value (mg/kg),
C_{soil} is the heavy-metal concentration in soil (mg/kg),
C_{ss} is the heavy-metal concentration in sewage sludge (mg/kg),
K is the mass of ploughed layer for 1 ha considering the layer thickness and bulk density (t/ha),
and 0.8 is the constant used to limit the loading of soil with heavy metals from sludge to the 80% of alert value level.

A separate calculation is necessary for every heavy metal with potentially adverse environmental effects. In order to establish the dose of sludge to be applied, the lowest value resulting from the calculation is chosen. If we want to increase yields, according to our results, every year at least 20 t/ha irradiated sewage sludge (equivalent to 400 kg N/ha) may be applied.

In samples collected from a greenhouse experiment, the amounts of six polychlorinated biphenyls (PCBs) were determined: PCB 28, PCB 52, PCB 101, PCB 138, PCB 153 and PCB 180. Data are presented in Table VIII. Only PCB 101, PCB 138, PCB 153 and PCB 180 were present both in sludge and in soil. The amounts of PCB 138, PCB 153, PCB 180 found in soil exceeded by far the maximum permissible limit (0.0004 mg/kg) for Romania.

The contents of PCB 180, the most persistent, were very close to the alert threshold (0.01 mg/kg) considered for sensitive uses of land [25]. The PCB contents of soil were high because pots were treated with sludge 3 years in a row.

Table VI. Effects of sewage sludge on oats (1998)

Characteristic	T_1	T_2[a]	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
pH ($CaCl_2$)	5.27	5.33	5.43	5.38	5.47	5.41	5.48	5.38	5.40	5.41
pH (H_2O)	5.82	6.22	6.07	6.10	6.08	6.11	6.14	6.03	6.09	5.94
Humus (%)	3.69a[b]	3.46a	3.64a	3.70a	3.79a	3.79a	3.62a	3.62a	3.72a	3.77a
Total N (%)	0.28a	0.26a	0.26a	0.27a	0.28a	0.29a	0.26a	0.27a	0.27a	0.28a
Av. P (mg/kg)	28a	40a	30a	36a	46a	48a	34a	40a	40a	42a
Av. K (mg/kg)	147a	145a	163a	157a	158a	173a	155a	158a	163a	175a
CEC (mEq/100g)	28.2a	27.8a	27.0a	28.1a	27.2a	27.5a	27.9a	28.6a	28.5a	28.9a
Base sat'n (%)	80.1a	82.5ab	82.7ab	82.0ab	82.4b	83.4b	83.1b	83.3b	82.0ab	81.3ab

[a] Reference for treatments T_3–T_{10}. [b] Numbers within a row followed by the same letter are not significantly different ($P<0.05$) using Tukey's HSD procedure.

Table VII. Effects of sewage sludge application on Cu, Zn, Pb and Cd in soil and in oats (1998)

Characteristic	T_1	T_2[a]	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
Cu										
Grain (mg/kg)	5.5a[b]	5.2a	5.2a	5.2a	5.4a	6.1a	4.8a	5.1a	4.8a	4.6a
Straw	4.2a	5.4abcd	4.6ab	5.3abcd	5.5abcd	5.7abcd	4.9abc	6.0bcd	6.5cd	6.7d
Soil, soluble (µg/kg)	19a	19a	19a	16a	15a	16a	15a	20a	16a	20a
Soil total (mg/kg)	18a	18a	18a	21a	19a	18a	19a	21a	23a	21a
Zn										
Grain (mg/kg)	35a	39a	30a	38a	34a	37a	31a	33a	37a	43a
Straw	19ab	24b	17ab	17ab	21ab	20ab	19ab	17ab	19ab	15a
Soil, soluble (µg/kg)	58a	75a	79a	81a	81a	88a	54a	70a	80a	91a
Soil, total (mg/kg)	47a	51a	50a	51a	51a	51a	51a	52a	56a	56a
Pb										
Grain (µg/kg)	490a	509a	563a	575a	631a	662a	575a	600a	670a	683a
Straw	180a	181a	203a	210a	228a	255a	186a	226a	279a	266a
Soil, soluble (µg/kg)	236a	254ab	312abc	412bcd	447cd	557de	440cd	659e	833f	830f
Soil, total (mg/kg)	34a	32a	32a	34a	32a	32a	32a	36a	33a	34a
Cd										
Grain (µg/kg)	211a	184a	268a	266a	235a	223a	210a	222a	275a	236a
Straw	153a	146a	194ab	245ab	258ab	298b	204ab	208ab	224ab	227ab
Soil, soluble (µg/kg)	2.8a	2.8a	3.1a	3.0a	3.5a	4.0a	2.9a	2.9a	3.1a	2.5a
Soil, total (mg/kg)	1.0a	1.1a	1.1a	1.2a	1.2a	1.2a	1.2a	1.3a	1.2a	1.1a

[a] Reference for treatments T_3–T_{10}. [b] Numbers within a row followed by the same letter are not significantly different ($P<0.05$) using Tukey's HSD procedure.

3.4. Influence on plant chemical composition

The low doses of sludge applied (maximum 20 t/ha/yr) did not effect significant changes in P, K, Ca, Pb, Co, Ni, Cr, Zn, or Cd contents of oats (grain and straw) or of maize (stalk, leaf and grain).The contents were within normal limits. Data in the literature indicate that normal levels (mg/kg) are below 0.5 for Cd, 8 for Cu, 2 for Ni, 3 for Pb, and 40 for Zn, although plant genotype is an important factor that may affect content, and phytotoxic levels (mg/kg) are 8 for Cd, 20 for Cu, 35 for Pb, 200 for Zn and zootoxic levels (mg/kg): 1 for Cd, 30 for Cu, 5 for Pb and 500 for Zn [26].

Polyelectrolytes, as coagulating agents for sludge treatment, can change the form of a metal in sludge and significantly affect its availability to plants, and they can influence extraction from sludge in the laboratory. Therefore, decisions concerning allowable levels of metal addition to soil should be based on total quantity [27]. Only tendencies were observed to increase contents of Cu and Cd in oat straw and of Zn in maize leaves.

Metal concentration in grains were rarely sufficient to cause usage interdictions for animal food [28].

Research carried out for 5 years showed that doses of sludge up to 70 t/ha applied annually did not induce statistically significant changes in the agrophysical properties of a Reddish Brown soil; a significant pH increase occurred, as well as increases in organic matter, nutrient and heavy-metal contents. However, the heavy-metal contents remained well below the alert values and had no negative effects on maize, sugar beet, soybean, or wheat crops, quantitatively or qualitatively [29].

Not only should sludge application to agricultural land sbe considered in terms of its effects on crop yields., but also in terms of improving soil physical characteristics, of productive use of its organic matter content and its fertilizing potential. Moreover, it helps to increase the degree of utilization of nutrients from mineral fertilizers as well as alleviating micronutrient deficiencies of some soils. By freeing up sludge-storage areas, pollution of ground water in those areas is minimized. The aim is not to replace mineral fertilizers with sewage sludge, but to use both in combination.

4. CONCLUSIONS

The sewage sludge resulting from the Piteşti wastewater-treatment plant has relatively rich in organic matter and nutrients. The heavy-metal contents were below the maximum admissible limits for agricultural use of sludge in Romania. Therefore, it is acceptable as a fertilizer.

There were no significant effects on physical, biological, or chemical characteristics of the soil as a result of application of low doses of sludge (up to 400% of the recommended N rate, i.e., about 20 t/ha) over a 3-year period.

Fertilization with the higher doses of sewage sludge significantly influenced the yield of oats and maize. The highest yields were obtained at maximum doses of sewage sludge irrespective of whether it had been irradiated.

Treatments with sewage sludge equivalent to 200 kg N/ha (for oat) and 300 kg N/ha (for maize) provided the same yields as those produced as a result of 100 kg N/ha fertilization with ammonium sulphate.

Table VIII. Polychlorinated biphenyls in municipal sewage sludge and in sludge-treated soil

Sample	PCB 28[a]	PCB 52	PCB 101	PCB 138	PCB 153	PCB 180	Total PCB
	(µg/kg)						
Sewage sludge	ND[b]	ND	4.5	9.5	19.6	18.5	52.1
Soil treated with:							
150 t sludge/ha	ND	ND	0.4	1.8	5.1	8.6	15.9
180 t sludge/ha	ND	ND	0.4	3.3	5.8	9.9	19.4
210 t sludge/ha	ND	ND	0.5	2.1	5.5	8.7	16.8

[a] IUPAC numbers.

[b] Not detected.

For oats, %Ndfss increased with time, and had values between 12 and 32% for non-irradiated and 8.7-41% for irradiated sludge. For maize, in the last year of experimentation, Ndfss had values of 4.0 to 27% for non-irradiated and 7.4 to 22% for irradiated sewage sludge.

Recovery values for sewage sludge N were low: 10 to 14% for non-irradiated and 9.5 to 17% for irradiated sludge applied to oats and 9.3 to 20% for non-irradiated and 11 to 19% for irradiated sewage sludge applied to maize.

In terms of efficiency of use as fertilizer, the effects of irradiated and non-irradiated sewage sludge were the same. But the irradiated sludge was pathogen-free, and non-irradiated sludge represents a risk to human and animal health. For this reason, irradiated sewage sludge is recommended for use in agriculture.

After 3 years of treatment with 20 t/ha irradiated and non-irradiated sludges, there were no significant changes in physical, biological and chemical characteristics of the Haplic Phaeozem or in terms of chemical composition of oat (straw and grain) and of maize (stalk, leaf, husk, cobs, and grain).

Our data confirm the utility of irradiated sewage sludge as a fertilizer, with minimal negative effects on environmental quality.

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THE EFFECTS OF LONG-TERM APPLICATION OF SEWAGE SLUDGE ON SOIL PROPERTIES

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Abstract

Long-term addition of sewage sludge to a fine-textured soil (36.5% clay, 41% silt, 22.5% sand) resulted in a significant increase of soil organic C, which improved soil structure, as indicated by an increase in total porosity from 44 to 63 vol% including both macro- and microporosity. In terms of changes in absolute pore volume, macropores were of main importance. However, in relative terms, the increase in microporosity was comparable to that of macroporosity (75% and 90%, respectively). Micropores with diameters in the range of 1 to 30 μm were highly significantly correlated with the soil C content. A C-balance sheet of the sewage-treated plots showed that the stable portion of added sludge after 35 years of continuous input was 55%.

1. INTRODUCTION

The recommended rate for sewage-sludge application in Sweden is of 5 tons per 5 years per hectare (Swedish Sewage Sludge Regulation, 1960). This means that about 150 to 200 kg of total N, 125 kg of total P, and 50 kg of total S are applied with a one-time addition. Swedish sewage sludges consist of about 3 to 4% total N, 0.4 % ammonium-N, 2.5% total P, and 1% total S in dry matter [1,2].

Three types of sludge are produced in Swedish sewage-treatment plants: mechanical sludge through sedimentation of raw wastewater, biological sludge through aeration of wastewater, and chemical sludge through precipitation of P using aluminium sulphate or iron (III) chloride. After mixing the three sludges, the material is anaerobically digested for methane production, and thereafter dewatered. Recently a fourth cleaning step, removal of ammonium-N dissolved in cleaned sewage water has been introduced in large sewage works. Sewage water is alternately nitrified and denitrified whereby ammonium is released as dinitrogen gas to the atmosphere.

When considering reactions and decomposition of sewage sludge in soil, it is useful to be aware of the fact that Swedish sewage sludge has been treated both aerobically and anaerobically. Most of the easily decomposable C compounds have been used by the microflora during treatment and the sludge consists of recalcitrant organic matter. This is reflected in a high ash-content of about 44% in Swedish sludge [2].

In this paper are described some biological and physical changes in soil in a long-term field experiment in which regular applications of sewage sludge were made.

2. THE LONG-TERM SOIL ORGANIC MATTER EXPERIMENT

The field experiment is located at Uppsala in central Sweden, on a clay loam consisting of 370 g clay kg^{-1}, 410 g silt (0.002–0.06 mm) kg^{-1}, 220 g sand (0.06–2 mm) kg^{-1} and classified as a Typic Eutrochrep or Eutric Cambisol. When the experimental plots were established in 1956, C content was15 mg g^{-1}, N content was 1.7 mg g^{-1}, S content was 0.23 mg g^{-1}, and pH was 6.6.

There are fourteen treatments, laid out in a randomized block design with four replicates per treatment. The individual plots, 2×2 me, were separated by pressure-treated wooden frames. Since 1956, tillage, sowing, fertilization, organic-matter addition, and harvesting have been performed by hand.

Sewage sludge was compared to five other treatments: no-N fallow, no-N cropped, $Ca(NO_3)_2$, green manure (grass), and farmyard manure (well decomposed). Organic amendments, all analysed before use, were made in 1956, 1960, and 1963, and thereafter every second year based on equivalents of organic C (2,000 kg

C ha^{-1} yr^{-1}). Nitrogen fertilizers were applied at 80 kg N ha^{-1} yr^{-1}. To all treatments, K was added at a rate of 35 kg ha^{-1} yr^{-1} as potassium chloride, and superphosphate was added at 20 kg P ha^{-1} yr^{-1}. Barley, oats, winter wheat, spring wheat, and fodder rape were rotated. At harvest, the above-ground portions of the crop were completely removed, weighed, analysed, and nutrient removal calculated.

Average chemical composition of the organic amendments are shown in Table I.

3. BULK DENSITY, POROSITY AND PORE-SIZE DISTRIBUTION

Long-term amendments of organic matter to soil resulted in significant changes in bulk density, ranging from 0.96 to 1.19 kg dm^{-3} of soil (Table II). Also, the non-amended treatments had lower bulk densities than at the start, because the plots were tilled by hand with no compaction from farm vehicles. The highest value for soil porosity was obtained on the sewage-sludge treated plots, 63 vol%.

The organic amendments caused significant changes ($P<0.05$) of the proportions of micro-, meso-, and macropores (Table III). The proportion of coarse to fine macropores (>600 µm) was highest in the calcium-nitrate treatment (13 vol%). This treatment also had the highest pH value (7.0). However, the increase of pore volume was largest in the very fine macropore fraction (60–600 µm). The volume of these pores differed between 11% in the fallow to 2 vol% in the sludge treatment, which means a relative difference of 90%. The volume of the mesopore fraction (30–60 µm) was much smaller (1.2–2.1 vol%) with similar differences between treatments as for the very fine macropores.

Table I. Average chemical composition of organic materials added to the experimental soils

Material	Organic matter	C	N	P	K	S	Ash
	(% of dry matter)						
Sewage sludge	56	28	3.2	2.6	0.27	1.05	4
Animal manure	81	41	2.0	0.76	2.24	0.39	19
Green manure	89	45	2.5	0.30	2.40	0.26	11

Table II. Physical properties of the Ultuna topsoil (0–20 cm)

Treatment	Bulk density				Porosity			
	1956	1975	1991	1997	1956	1975	1991	1997
	(Mg m^{-3})				(Vol%)			
No-N, fallow	1.44	1.38	1.36	1.28	45	47	48	52
No-N	1.44	1.41	1.43	1.25	45	46	45	53
$Ca(NO_3)_2$	1.44	1.38	1.34	1.14	45	47	49	57
Green manure	1.44	1.35	1.36	1.19	45	48	48	55
Animal manure	1.44	1.32	1.30	1.16	45	50	50	56
Sewage sludge	1.44	1.22	1.11	0.96	45	53	58	63

Table III. Equivalent pore-diameter[a] fractions in the Ultuna topsoil (0–10 cm) derived from moisture-retention characteristics

Treatment	Macropores		Mesopores	Micropores	Ultramicropores	
	> 600 µm	60–600 µm	30–60 µm	5–30 µm	1–5 µm	<1 µm
			(Vol%)			
Fallow	12.6ab	10.9cd	1.2c	3.3c	3.8e	20.2bc
No-N	8.3b	14.7bc	1.2c	4.2b	4.4de	20.4bc
$Ca(NO_3)_2$	13.4a	13.9bcd	1.3bc	3.9bc	4.7cde	19.9bc
Green manure	10.1ab	12.1cd	1.3c	4.5ab	5.3bcd	21.3ab
Animal manure	9.3ab	11.6cd	1.3bc	4.6ab	6.2ab	22.8a
Sewage sludge	9.3ab	20.6a	2.1a	4.9a	6.7a	19.7c

[a] Soil water suction and equivalent pore diameter: 0.05 m = 600 µm; 0.5 m = 60 µm; 1.0 m = 30 µm; 6.0 m = 5 µm; 30 m =1 µm.

Table IV. Organic matter of the Ultuna topsoil (0–20 cm) expressed in 1997 after 41 years (initial values: pH, 6.5; 15 g C kg^{-1}, 1.7 g N kg^{-1} [2,4]

Treatment	Organic C	Total N	C/N	pH (H_2O)
	(g kg^{-1}soil)			
Fallow	10.0±0.2g	1.00±0.0f	10.0	6.2+0.18d
No-N	12.1±0.4gf	1.17±0.0e	10.3	6.5±0.04c
$Ca(NO_3)_2$	14.0±0.3ef	1.37±0.0d	10.2	7.0±0.04a
Green manure	17.2±0.4cd	1.65±0.0c	10.4	6.2±0.05d
Animal manure	21.0±0.3b	2.00±0.0b	10.7	6.7±0.04b
Sewage sludge	29.3±1.1a	2.92±0.1a	10.0	5.4±0.08e

The volume of the micropore fraction (5–30 µm) was larger (3.3–4.9 vol%) than of mesopores, but the largest relative difference due to treatments was only around 50%. Again, the most micropores were present in the sewage-sludge-treated soil and the least in the permanently fallowed soil. The volume of the ultramicropore fraction (1–5 µm) ranged from 3.8 to 6.7 vol%, which means a relative difference of 76%. Concerning the volume fraction of pores less than 1 µm, small absolute and relative differences were found.

There was a significant ($P<0.05$) positive correlation between soil organic C and the total volume of pores. However, increases in soil pore volume and in soil C concentration were not uniform. Correlations increased with smaller pore sizes and the highest significant correlation ($P<0.001$; $R^2 = 0.918$) was found between micropores of 1 to 5 µm diameter and soil C concentrations [3]. However, the smallest pores, ultramicropores, were not correlated with soil C concentration.

4. TURNOVER OF C

The organic C and N contents of the topsoil changed significantly over the 41 years [4]. Contents decreased in the fallow and no-N treatment, and increased in the animal-manure and sewage-sludge treatments. In the green-manure treatment, soil organic matter remained relatively constant. The largest changes with respect to C, N and pH took place in the sewage-sludge treatment (Table IV). The soil pH decreased below 6 in the sludge treatment after 41 years. An estimate of the role of acidifying processes in sludge-treated soil showed that N transformation, but mainly S-related processes such as S-mineralization and S leaching, are proton-producing [5].

The C balance (Table V) showed that total C losses were about six-fold higher in plots amended with organic materials than in the fallow plots. No equilibrium between input and output of C had been reached [4]. The C balance was used to estimate the humification of the organic materials added, calculation of which is described elsewhere [2,4]. The portions of C present in soil after 35 years were 19% from green manure, 34% from animal manure, and 55% from sewage sludge. As these values are averaged over a period of decades, they do not reflect differences in the decomposition velocity of C in manures added.

5. DISCUSSION

In a long-term (13 years) field experiment, in which 2 and 5 t ha^{-1} of organic matter in the form of sewage sludge were applied annually, changes in soil C content, but no differences in soil aggregate stability, were found [6]. Higher application rates, 30 to 500 t ha^{-1} yr^{-1}, as used in American studies [7,8], however, caused significant effects on soil physical properties. Bulk density decreased linearly with sludge application, and there were increases in porosity, aggregate stability, and mean diameter of aggregates. The presence of olestra in sewage sludge, a fat replacer in the human diet, did not affect soil physical properties [9].

The effects of sewage sludges on soil physical properties depend on their contributions of organic C and also of inorganic precipitants used for P removal from sewage water. Organic C and precipitates such as aluminium sulphate or iron chloride are cementing materials binding smaller soil particles, such as clay, into larger particles, and in this way aggregation is favoured. Mixing of biologically and chemically produced sludge and treating it anaerobically before application to land, which is common in Sweden, increases the recalcitrant C fraction of sewage sludge. When evaluating the effects of sewage sludge on soil properties, the ways sewage sludge is produced and treated need to be considered.

Table V. Carbon balance[a]: cumulative inputs and losses for the period 1956–91

Treatment	Cumulative C input			Change in soil C	Cumulative C losses			
	Organic addition	Roots	Total		Total	Native humus	Roots	Organic addition
	(kg C m^{-2})							
Fallow	0	0	0	–1.31	1.31	1.31	0	0
No-N	0	0.94	0.94	–0.80	1.74	1.31	0.44	0
$Ca(NO_3)_2$	0	1.70	1.70	–0.23	1.93	1.31	0.80	0
Green manure	6.71	1.71	8.42	+0.83	7.59	1.31	0.81	5.47
Animal manure	6.61	1.56	8.17	+1.75	6.42	1.31	0.74	4.37
Sewage sludge	6.48	2.17	8.65	+3.24	5.25	1.31	1.03	2.91

[a] Loss of initial soil humus was assumed to be the same in all treatments (no priming effect); loss of root C was assumed to be 47.5% (mean of no-N and inorganic fertilizer treatments).

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THE USE OF IRRADIATED WASTEWATER SLUDGE CAKE TO INCREASE SOIL FERTILITY AND CROP YIELDS, AND TO PRESERVE THE ENVIRONMENT

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Abstract

A 4-year field experiment was conducted to compare the effects of ammonium sulphate and irradiated wastewater sludge cake on growth, yields and uptake of N by maize (*Zea mays* L.). Six crops were grown. Heavy metals were determined in soil and plant. Total coliforms and some soil properties were determined after harvest. Results of experiments showed that wastewater sludge cake from Sripraya wastewater-treatment plant can be used for agricultural application. In the third application of sludge cake (fourth year of the experiment) dry weight and N uptake at the higher rate of sludge-cake application gave better results than did chemical fertilizer. Nitrogen was residual from the sludge cake, but not from chemical fertilizer. Nitrogen derived from sludge cake (Ndfss) was calculated for the whole experiment; %Ndfss values were higher when the frequency of application increased and with higher application rates of sludge cake. Plots receiving higher rates of sludge cake gave higher percent fertilizer-use efficiency (%FUE) values. Comparison of irradiated sludge cake and non-irradiated sludge indicated that the latter gave higher values in terms of maize yields, N uptake, %Ndfss and %FUE. Zinc, Mn, and Ni were detected in maize in the order Zn > Mn > Ni. Copper, Pb, Cr, and Cd were not detected. The concentrations did not increase with higher application of sludge cake. Micro-organisms, yeasts and molds, and coliforms, including *Escherichia coli* were reduced in number by irradiation. Some pathogens, such as *Salmonella, Staphylococcus aureus, Ascaris* ova were not found in the wastewater sludge cake. Sludge-cake applications had no influence on soil properties, consistent with lack of treatment effects, in the first 2 years of the experiment.

1. INTRODUCTION

The disposal of sewage is a serious problem in many countries including Thailand, because of urbanization and population expansion. Appropriate utilization of wastewater has long been studied, but few practical applications have resulted. Wastewater and sewage sludge have potential as agricultural fertilizers, because they contain essential plant nutrients, e.g. N, P, K, and Zn, Fe, Cu, Mn and other trace elements. At the same time, pathogenic organisms, heavy metals and other toxic materials in these waste materials can cause health problems and have negative residual effects in soil. Research has indicated that radiation is an effective means of reducing pathogen numbers in sewage sludge, suggesting the possibility of its utility on a commercial scale.

The present work, conducted over 4 years, examined the effects of irradiated wastewater sludge cake on the growth of maize. In addition, pathogen numbers, heavy-metal content and some soil properties were examined.

2. MATERIALS AND METHODS

2.1. Wastewater sludge cake

Wastewater sludge cake from Sripraya wastewater treatment plant, moisture content 35%, was mixed and divided into two parts, one of which was irradiated at 2 kGy (200 krad) at the Thai Irradiation Center, Office of Atomic Energy for Peace, Patumthani Province, which has a carrier type gamma irradiator, model JS 8900, designed by Nordion International Incorporation. A ^{60}Cb source, with initial load of 450 kCi has been used for food irradiation and other products since 1989. Operation and monitoring, including the safety system, are designed to meet national regulations and international standards.

Sripraya wastewater treatment plant is part of the centralized wastewater treatment project of the Metropolitan Administration of Bangkok. It is capable of treating about 30,000 m^3 of domestic wastewater per day to an effluent standard level of 20 mg/L BOD. The plant's catchment area is 2.7 km^2, serving a population of about 100,000 covering the canal area of Bangkok, comprising the Semphan Thavong and Pomerab districts. The plant has been operating since January 1994 and remains under the maintenance period guaranteed by the contractor. Due to the restricted site area, the plant is three-storey building with treatment-process units on each floor, i.e. the contact stabilizing activated sludge process, comprising a primary physical-treatment unit, a secondary biological-treatment unit, and an excess-sludge-treatment unit.

The wastewater from the collection system flows to the equalizing tank and is then pumped up to the treatment units on each floor. The wastewater goes to the inlet channel through screens and a chamber in order to remove sand and grit, then flows into the contact tank to mix with return sludge from the stabilization tank before flowing into the clarifier tank. The effluent from the clarifier tank flows to a disinfection tank before discharging to the river. The sludge in the clarifier tank is pumped to the stabilization tank; part of the sludge is returned to the contact process and the rest is sent to the sludge-treatment unit.

2.2. Experiment site and soil

A field experiment was conducted at Praputtabart Crop Experiment Station, Saraburee Province. The soil is classified as in the Pakchong series, a reddish brown lateritic great group (Paleustult). Maize (hybrid DK 888) was grown. A randomized complete block design with four replications and ten treatments was employed. Descriptions of treatments are given in Table I. Sub-plot size was 4.5×5.0 m; microplots for ^{15}N were 1.5×1.5 m, within each sub-plot. Plant rows were arranged along the longer sides of sub-plots. Plant spacing was 75 cm between rows and 25 cm between hills. Treatment AS, ammonium sulphate, was applied by in-row banding. In treatments designated SS and IS, sludge cake was applied by broadcasting and incorporating into the soil over each plot 1 month before planting. For the microplots of treatments C, SS and IS, ^{15}N-labelled ammonium sulphate at 20 kg N/ha 10% a.e. was dissolved in water and applied with a plastic shower bottle over the soil surface. Triple superphosphate at 60 kg P_2O_5/ha and potassium chloride at 30 kg K_2O/ha were applied as basal fertilizers by in-row banding before planting. Supplemental irrigation was made with sprinklers. Maize was harvested at 120 days.

2.3. Temperature, relative humidity, and rainfall

Temperature, percent relative humidity, and rainfall (mm) were determined every day for the duration of the experiment (Fig. 1).

3. RESULTS AND DISCUSSION

3.1. Yields of maize

With the third application of sludge cake (in the fourth year of the experiment) dry weight and N uptake of maize grain was higher with sludge cake than with chemical fertilizer (Fig. 2). Süss [1] found that residual beneficial effects of sewage sludge, seen in the third year of a crop rotation, but not in the second year, varied depending on the location and the amount applied.

In the second year, there was N residual from sludge cake, but none from chemical fertilizer. In the third year, after 2 years of sludge cake applications, N derived from sludge (%Ndfss) cake, calculated using the N-balance method, showed that N was derived from sludge cake with 200% of the recommended rate of fertilizer. Percent N derived from sludge cake for the whole experiment, calculated using the N-balance method, increased when the times of application increased and with the higher rate of sludge cake applied (Fig. 3).

Calculation of %Ndfss failed using the ^{15}N-dilution method. The values of atom % ^{15}N from the third-year crop showed that the plots with higher rates of sludge cake had higher %FUE values (Fig. 4). Suss [1] showed that application of sludge also improved biological and physical properties of soils.

Comparison of the effects of irradiated and non-irradiated sludge indicated that irradiation indicated greater yields of maize, and more N uptake, %Ndfss, and %FUE. Mitrosuhardjo and Harsojo [2] found that irradiated sludge at 5 t/ha increased dry-matter production and P uptake. Pandya et al. [3] suggested that gamma radiation induced inactivation of toxic substance(s) in sludge, and that sludges from the digesters of conventional domestic sewage treatment plant are inhibitory to several growth parameters and irradiation may remove this inhibition.

3.2. Heavy metals

The wastewater sludge cake from the Sripraya treatment plant was analysed for heavy metals before and after irradiation (Fig. 5).

Table I. Treatments

Treatment	First year	Second year	Third year	Fourth year
AS	$(NH_4)_2SO_4$ 62.5 kg N/ha	$(NH_4)_2SO_4$ 62.5 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 60 kg N/ha, 1% a.e.	$(NH_4)_2SO_4$ 62.5 kg N/ha
C	No fertilizer	No fertilizer	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	No fertilizer,
SS 1	Sludge cake 31.3 kg N/ha	Sludge cake 62.5 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	Sludge cake 62.5 kg N/ha
SS 2	Sludge cake 62.5 kg N/ha	Sludge cake 125 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	Sludge cake 125 kg N/ha
SS 3	Sludge cake 93.8 kg N/ha	Sludge cake 187.5 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	Sludge cake 187.5 kg N/ha
SS 4	Sludge cake 125 kg N/ha	Sludge cake 250 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	Sludge cake 250 kg N/ha
IS 1	ISC[a] 31.3 kg N/ha	ISC 62.5 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	ISC 62.5 kg N/ha
IS 2	ISC 62.5 kg N/ha	ISC 125 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	ISC 125 kg N/ha
IS 3	ISC 93.8 kg N/ha	ISC 187.5 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha,10% a.e.	ISC 187.5 kg N/ha
IS 4	ISC 125 kg N/ha	ISC 250 kg N/ha	^{15}N labelled $(NH_4)_2SO_4$ 20 kg N/ha, 10% a.e.	ISC 250 kg N/ha

[a] Irradiated sludge cake.

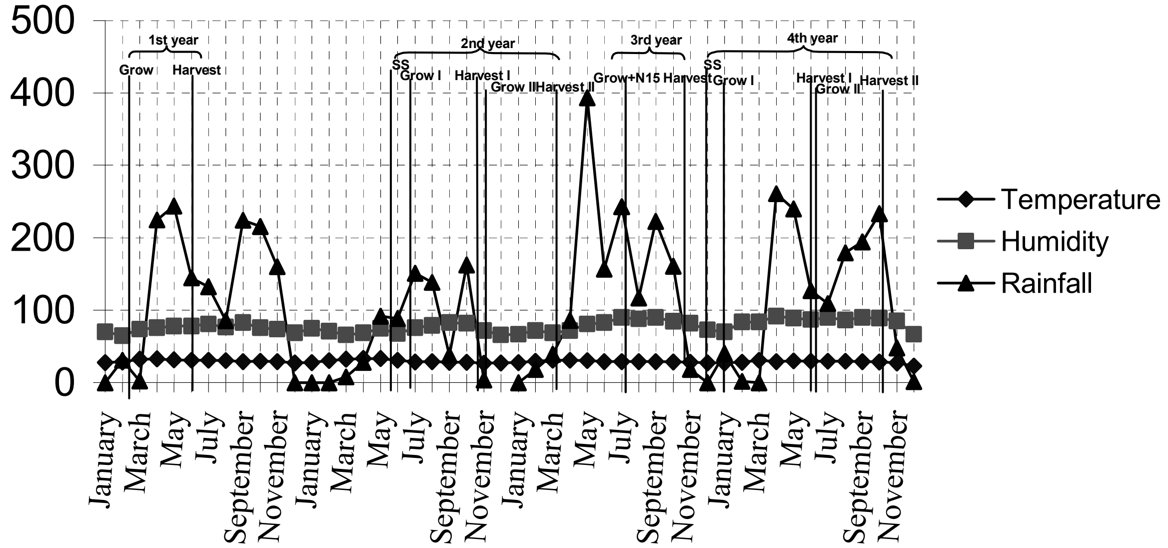
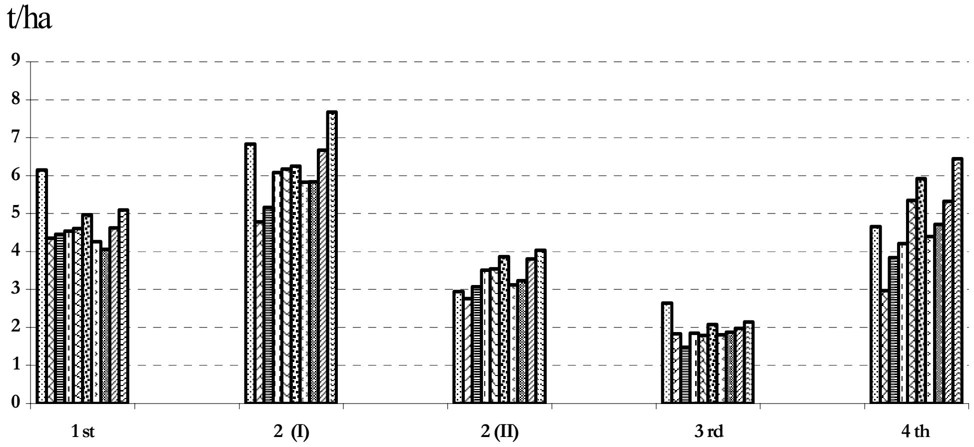


FIG. 1. Temperature, percent relative humidity, and rainfall.



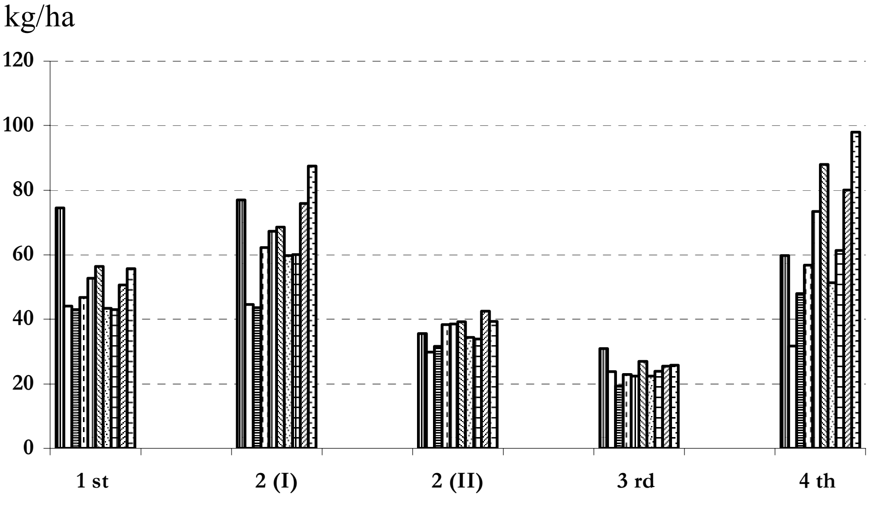


FIG. 2. Dry weight (upper) and N-uptake (lower) of maize grain, years 1 to 4.

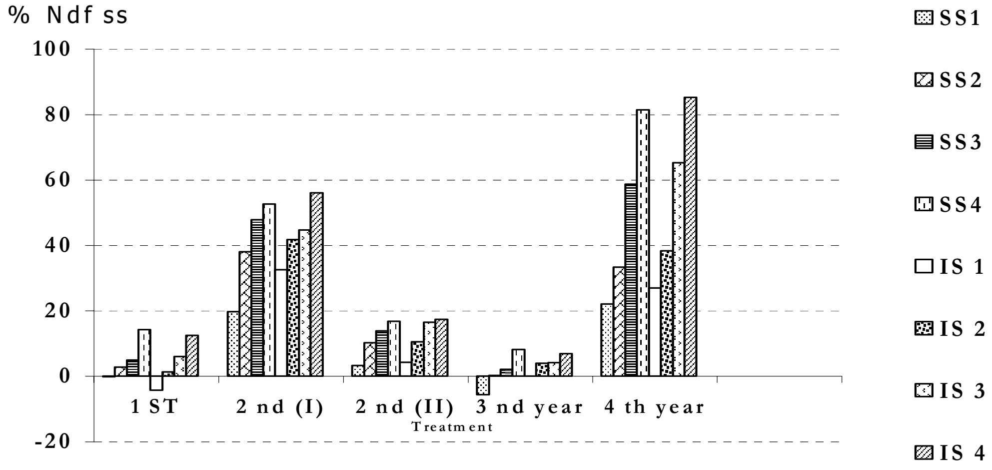


FIG. 3. Percent N derived from sewage sludge in maize, calculated by using the N-balance method, years 1 to 4.

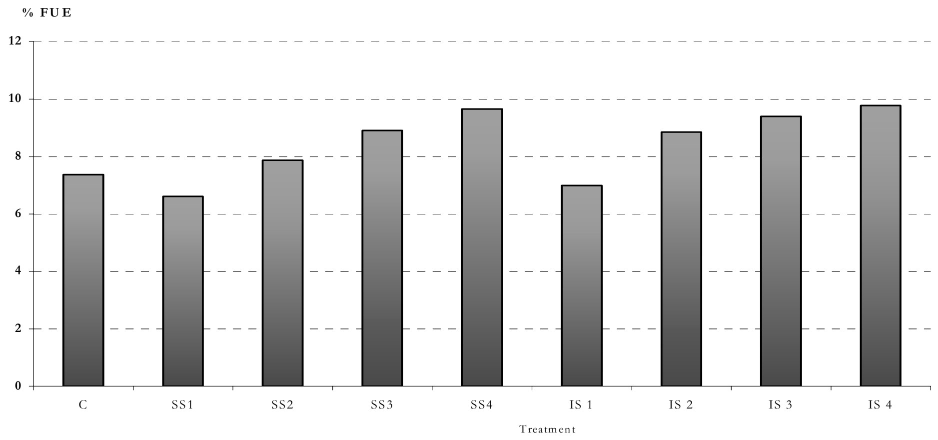


FIG. 4. Percent fertilizer use efficiency by maize on plots to which sludge cake was applied for two years.

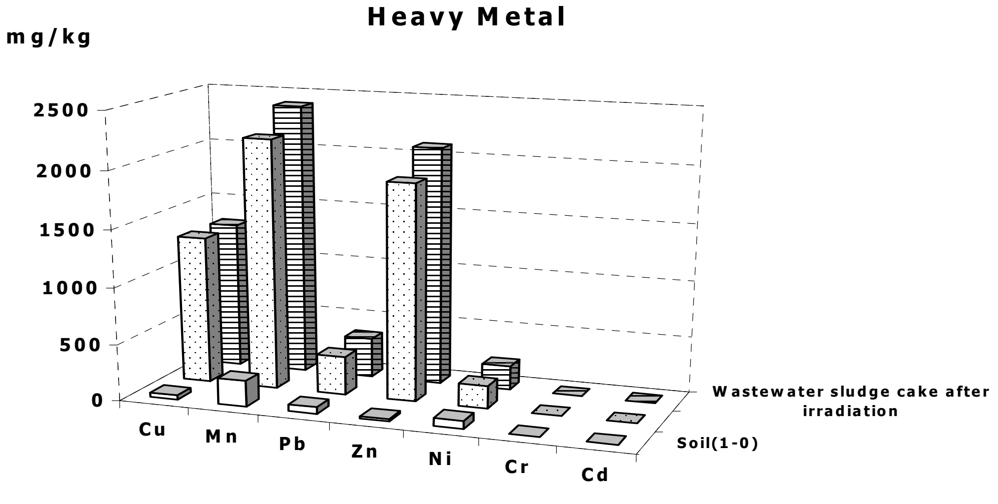


FIG. 5. Heavy metal concentrations in soil (1–10 cm) and in sludge cake before and after irradiation.

Table II. Annual loading rates for heavy metals in wastewater sludge cake

Metal	First year	Second year	Fourth year
	(kg/ha/year)		
Cu	5.98	12.0	12.0
Mn	10.1	20.2	20.2
Pb	1.56	3.12	3.12
Zn	8.74	17.5	17.5
Ni	0.92	1.84	1.84
Cu	ND[a]	ND	ND
Cd	ND	ND	ND

[a] Not detectable.

The results of the analysis were compared with the standards of developed nations for suitability for agricultural application. Australian guidelines would not permit the unrestricted use of Sripraya wastewater sludge cake, especially with respect to heavy metals: Zn, Cu, Pb, and Ni, the concentrations of which were well above recommended limits. However, concentrations were well below the Class B agricultural category. The sludge was considered safe for agricultural application.

The concentrations of all the heavy metals in wastewater sludge cake, except Cu and Ni, were within permissible limits for Germany, although restrictions to agricultural application may be required in the long term.

Compared with the code for the United Kingdom [4], the wastewater sludge cake would be considered safe for agricultural application.

Regulations of Environmental Protection Agency (EPA) of the United States [5] permit the recycling of significantly more sludge compared with the more precautionary approach adopted by some European countries. The heavy-metal loading rates (Table II) were well below the annual agricultural-land loading rates of the EPA. Accordingly we regard the wastewater sludge cake from the Sripraya treatment plant to be safe for agricultural application.

After applying sludge cake and after the harvest, the concentrations of Cu, Mn, Pb, Zn, and Ni were not significantly different from the values determined in the original soil (namely 40, 230, 60, 20, and 70 mg/kg); Ni and Zn were slightly higher in all treatments, probably from the environment or contamination. Neither Cr nor Cd could be detected.

Cichos and Singliar [6] showed that inputs of Ni and Sn to the sewer are mainly from industry. Zinc and Cu are found mainly in domestic wastewaters, whereas Pb comes from surface run-off, and Ar and Cd are from mining.

Only Zn, Mn, and Ni were detected in maize, with the order of accumulation Zn > Mn > Ni. Copper, Pb, Cr, Cd were not detectable.

Manganese concentrations were in the order stover > grain > cob (10–80, ND–60, and ND–30 mg/kg dry weight, respectively); the concentrations did not increase with the application of sludge cake. The irrigation water contained high concentrations of Mn, which was deposited on the leaf tissue. The tolerable limit for Mn in agricultural crops is 300 mg/kg dry matter [4].

Zinc concentrations in maize were in the order cob > stover > grain (50–90, 50–70, and 30–50 mg/kg dry matter, respectively), all higher than in the soil (1–10 cm) after applying sludge cake (20–30 mg/kg) and after harvest (20–30 mg/kg). The higher accumulation of Zn in plant tissue was due to its mobility and bio-availability. Mature leaves accumulated Zn in excess of 400 mg/kg dry matter, which is considered to be toxic [7]. Zinc concentrations in the non-sludge treatments (AS, C) were also higher than in soil, possibly from the water supply.

Nickel was not detected in first- and second-year maize. In the third year, it was detected in stover and cob (40–60 and ND–60 mg/kg dry matter, respectively), but not in grain. Nickel concentrations up to 50 mg/kg dry matter are regarded as tolerable in agriculture crops [4].

Manganese and Zn are expected to be present in plant tissues due to the fact that they are micro-nutrients essential for growth and development.

Diez [8] reported that although the heavy metal contents greatly exceeded the limiting values in Germany, there were no depressions of yield. He suggested that the uptake of Cd, Zn, and Ni could be reduced considerably by applying lime with sewage sludge. Heavy-metal uptake by crops depends on the species of plant and on the metal.

3.3. Pathogens

Counts of microorganisms in sludge cake before and after irradiation demonstrated the utility of the treatment (Table III). It is noteworthy that *Salmonella, Staphylococcus aureus,* and *Ascaris* ova were not found in the wastewater sludge cake.

Total-coliform counts in soil from yield sub-plots before the experiment showed high variation in numbers. Machi and Hashimota [9] reported that irradiation of 15 kGy reduced total numbers of bacteria by six to seven orders of magnitude. Irradiation of 2 kGy can almost completely exterminate coliforms, which are highly sensitive to radiations.

After the crop in the second year no coliforms were found in the soil in any of the treatments (Table IV). At the harvest of the second crop, the weather was very hot and dry. Reddy et al. [10] found that temperature is important in the survival of bacteria and viruses in soil; inactivation and die-off are more rapid at higher ambient temperatures. In the case of bacteria, and probably viruses, the die-off rate is approximately doubled with each 10°C rise between 5 and 30°C. Dunigan and Dick [11] reported that faecal coliform (FC) indicator bacteria counts in surface run-off waters from sewage-treated plots were very high within the first two to three weeks. Counts of FCs were as high as 55,000/mL. However, numbers decreased rapidly as the soil dried.

Table III. Pathogen counts in non-irradiated and irradiated sludge cake

Pathogen	Non-irridiated	Irradiated
Microorganisms/g	4.4×10^6	7.6×10^4
Yeasts and molds/g	3.1×10^4	2.7×10^3
Coliforms (MPN/g)	1,500	240
E. coli/g	20	<3
Salmonella/25 g	ND	ND
Staphylococcus aureus/0.1 g	ND	ND
Ascaris ova	ND	ND

Table IV. Total coliform counts in the soil collected from yield sub-plots before and after the first- and second-year crops

Treatment	First year		Second year	
	After applying SC	After harvest	After applying SC	After harvest
	(MPN/g)			
AS	0–240	15–1,100	0–14.7	0
C	0–40	460–1,100	3–462	0
SS 1	0–7	7–460	3.57–240	0
SS 2	0–43	4–460	23–462	0
SS 3	0–93	21–93	42.7–1,100	0
SS 4	4–150	75–240	9.18–2,400	0
IS 1	4–93	7–240	0–93.3	0
IS 2	0–43	9–460	0–93.3	0
IS 3	0–210	25–240	0–23.1	0
IS 4	0–93	9–460	9.18–74.9	0

Table V. Soil characteristics, pH, percent organic matter, cation-exchange capacity, percent organic C, and water-holding capacity after the first- and second-year crops

Treatment	1st a	2nd a	1st a	2nd a	1st a	2nd a	1st a	2nd a	1st a	2nd a
	pH		%OM		CEC		%OC		WHC	
AS	6.63	7.6	1.34	1.45	6.0	7.33	0.61	0.84	33.4	31.5
C	6.75	7.6	1.38	1.33	5.7	7.23	0.62	0.78	33.1	31.1
SS 1	6.73	7.6	1.39	1.46	5.8	7.00	0.62	0.85	32.7	30.0
SS 2	6.70	7.6	1.45	1.35	5.9	7.00	0.65	0.79	33.0	30.1
SS 3	6.78	7.7	1.40	1.43	5.9	7.10	0.63	0.83	32.8	30.6
SS 4	6.65	7.6	1.35	1.39	6.0	7.20	0.61	0.81	33.9	31.6
IS 1	6.80	7.6	1.36	1.34	5.50	7.10	0.61	0.78	32.0	30.1
IS 2	6.80	7.6	1.44	1.40	5.70	7.05	0.64	0.82	33.8	31.4
IS 3	6.88	7.6	1.41	1.31	5.7	7.13	0.63	0.77	33.8	31.3
IS 4	6.93	7.6	1.43	1.40	6.2	7.13	0.64	0.81	33.1	30.4

3.4. Soil properties

Soil properties after harvest of the first- and second-year crops showed no differences among treatments, but, between years, there were trends of increasing pH, CEC, and %organic C and decreasing water-holding capacity (Table V).

Adsorption to clay minerals and organic matter is increased with a raise in soil pH [4]. Zinc, Ni, and Cd tend to be influenced strongly by soil pH, whereas Cu, Pb and Cr are not. Cation-exchange capacity increases with increasing soil pH, organic matter, and clay content [4]; it is considered to be important in controlling the retention and, thus, the toxicity of metals in sludge-amended soils. Smith [4] showed that applying organic matter reduced the mobility of potentially toxic elements in soil and thus lowered availability to plants.

4. ON-GOING WORK

- We have harvested the fourth-year crop.
- We are determining the concentrations of heavy metals in plants and soil after the fourth-year crop.
- We are making faecal-coliform counts and assessing soil properties after the fourth-year crop.

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BIOAVAILABILITY OF HEAVY METALS FROM SEWAGE SLUDGE AND SOME LONG-TERM EFFECTS ON SOIL MICROBES IN AGRICULTURAL ECOSYSTEMS

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Abstract

Metals may affect the growth of plants and soil microbial activity, and soil fertility in the long term. Less is known of their adverse long-term effects on soil micro-organisms than on crop yields and metal uptake. This is not surprising, since their effects when added to soils in sewage sludge are difficult to assess, and few long-term experiments exist. This paper reviews evidence from long-term field experiments with sewage sludges in the United Kingdom, Sweden, Germany, and the United States. Changes in the extractability and uptake by crops of sludge metals in the Woburn experiment (UK), started in 1942, were measured to assess whether Zn and Cd are fixed by the sludge/soil constituents or are released as the sludge organic matter decomposes. Extractability of Zn by 0.1 *M* $CaCl_2$ as a proportion of the total ranged from 0.5 to 3% and that of Cd from 4 to 18%, and were higher in sludge-amended than in farmyard-manure- or fertilizer-amended soils. Over a 33-year period after 1961, when sludge was last applied, the extractability of both metals fluctuated, but neither decreased nor increased consistently. The relationships between soil- and crop-metal concentrations were linear, with no evidence of a plateau across the concentrations achieved. The slopes of the soil-plant relationships depended on the type of crop or crop part examined, but was generally in the order red beet > sugar beet > carrot > barley. However, there were large seasonal differences in metal concentrations in the crops. It is concluded that up to 33 years after sludge applications ceased, Zn and Cd extractability and bioavailability do not decrease. Adverse effects on microbial activity and populations of cyanobacteria, *Rhizobium leguminosarum* bv. *trifolii*, mycorrhiza, and the total microbial biomass have been detected, in some cases below the European Community's maximum allowable concentration limits for metals in sludge-treated soils. For example, N_2-fixation by free-living heterotrophic bacteria was found to be inhibited at metal concentrations (mg kg^{-1}) of 127 Zn, 37 Cu, 21 Ni, 3.4 Cd, 52 Cr, and 71 Pb. Fixation by free-living cyanobacteria was reduced by 50% at metal concentrations (mg kg^{-1}) of 114 Zn, 33 Cu, 17 Ni, 2.9 Cd, 80 Cr, and 40 Pb. Numbers of *R. leguminosarum* bv. *trifolii* decreased by several orders of magnitude at metal concentrations (mg kg^{-1}) of 130 to 200 Zn, 27 to 48 Cu, 11 to 15 Ni, and 0.8 to 1.0 Cd. Important factors influencing the severity of toxicity were soil texture and pH. Higher pH, clay and organic-C contents reduced metal toxicity considerably. The evidence presented in this review of long-term field experiments suggests that adverse effects on microbial parameters were generally found at modest concentrations of metals in soils. It is concluded that prevention of adverse effects on soil microbial processes, and ultimately soil fertility, should be a factor that influences soil-protection legislation.

1. INTRODUCTION

Land application of sewage sludge is projected to increase in European countries because of the ban on sea disposal starting in 1999 and increases in wastewater treatment lasting into the new millennium, resulting from the EU Wastewater Treatment Directive [1]. This is also likely to happen in countries elsewhere as a result of international agreements to cease sea disposal of sludge.

Sewage sludge can be a valuable source of plant nutrients, such as N, P and S, and its organic matter content can help improve soil physical conditions. One of the major environmental problems associated with the land use of sludge is the addition of potentially toxic heavy metals. Heavy metals in sewage sludges often originate from industrial sources, but domestic waste can contain significant amounts of Zn and Cu. Repeated applications of heavy-metal-contaminated sewage sludge can result in accumulation of toxic metals in the soil. Once accumulated, heavy metals are highly persistent [2–4], and can cause problems such as phytotoxicity [5], injury to soil microorganisms [6], or elevated transfer to the food chain [7–9]. Because of the persistence of heavy metals in soils, it is important to understand how their bioavailability changes over the long term.

Table I. Maximum concentrations of metals allowed in agricultural soils treated with sewage sludge

		Zn	Cu	Ni	Cd	Cr	Pb	Hg
		(mg kg^{-1} soil)						
EU	1986	150–300	50–140	30–75	1–3	100–150[a]	50–300	1–1.5
US[b]	1993	1,400	750	210	20	1,500	150	8

[a]Now withdrawn. [b]Calculated [10] from maximum cumulative pollutant-loading limits, assuming incorporation to 15 cm depth and average soil bulk density of 1.33 g cm^{-3}, but not including the background concentrations of these elements in soils.

The European Community and some countries have set maximum permissible values for metal concentrations in agricultural soils through the application of sewage sludge (Table I). These values resulted from work on metal uptake by crops, animals, and humans, but take little or no account of possible effects of metals on soil-microbial populations [11–15]). However, concern has been expressed over the effects of relatively small concentrations of metals on soil micro-organisms and soil-microbial activity [16–21].

In this paper, results are presented on long-term changes in the bioavailability of sludge-borne heavy metals after the termination of sludge applications. Long-term effects of metals added to soils in sewage sludge are very difficult to assess, because they are hidden and there are few such experiments and, consequently, a lack of good long-term data. Appropriate field experiments exist in the United Kingdom, Sweden, Germany, and the United States. This paper also reviews evidence from these field experiments on the impact of metals on soil-microbial activity and on long-term soil fertility.

2. MATERIALS AND METHODS

2.1. Field experiments

2.1.1. Woburn, England

A long-term experiment at Woburn received twenty-five applications of sewage sludge or a compost made from the same sewage sludge plus straw, both at two rates, from 1942 to 1961. The sludges were always from the same sewage works in west London and the soil was a sandy loam with 9% clay. The original aims of the experiment were to compare their agronomic value with either locally made farmyard manure (FYM) or inorganic fertilizers (IF). The rates of addition of all the organic manures were constant on a fresh-weight basis, but they had different dry matters (DM). Sewage sludge was added at 15 or 30 t ha^{-1} DM per application and its mean composition [22] was (mg metal kg^{-1} DM): 2,780 Zn, 1138 Cu, 188 Ni, 99 Cd, 900 Pb, and 919 Cr. Soil and crop samples were taken at irregular intervals, and stored in the Rothamsted archive. These samples were retrieved, subjected to chemical analyses using modern digestion or extraction methods (aqua regia for total metals, 0.05 *M* EDTA at pH 7, and 0.1 *M* $CaCl_2$), and analysed by ICP or GF-AAS.

A range of mainly vegetable crops were grown in the experiment, of which carrot (*Daucus carota)* and red beet (*Beta vulgaris*) were the most numerous, and samples were available of the same varieties grown some 20 years apart. These were chosen for further study and regressions of the metal concentration in the plant tissue against either total or extractable metal concentrations in soil were made. Soil pH (H_2O) was maintained at 6.5 throughout.

2.1.2. Gleadthorpe, England

The soil at this site is a sandy loam with 9% clay and 1 to 2% organic C (control soil). This experiment was started in 1982 and the sewage sludges used were artificially contaminated by adding metal salts to raw sewage and then dewatering. One application of either Zn- or Cu- or Ni-contaminated sludge was made to all plots with a further application to some, but not all, 5 years later. Apart from these single metal treatments, mixed metal treatments of Zn plus Cu and Zn plus Ni were also applied. There were also treatments with "uncontaminated" sewage sludge at approximately 100 t dry solids (DS) ha^{-1} in 1982, or inorganic fertilizers.

2.1.3. Braunschweig, Germany

Two experiments were begun in 1980 on the same field and both received the same treatments consisting of inorganic fertilizers or "moderately" contaminated or metal-amended liquid sludge added at rates of 100 or 300 m^3 ha^{-1} a^{-1} for 10 years. These were equivalent to 5.5 or 16.5 t DS ha^{-1} a^{-1} from 1980 to 1990. The moderately contaminated sewage sludges were obtained from a local sewage works and were naturally contaminated. However, the contaminated sludges were from a different works in 1980, then from 1981 to 1990 this treatment was made from the same moderately contaminated sewage-works sludge, artificially contaminated with metal salts and anaerobically incubated for 6 weeks before use. One experimental site (Braunschweig 1) was on an old arable soil with plot pH values ranging from 6.0 to 7.0 and 0.8 to 1.5% organic C; the other experimental site (Braunschweig 2) was on an ex-woodland soil with plot pH values ranging from 5.3 to 5.7 and 1.6 to 2.6% organic C. Both soils are silty loams with 50% silt, 45% sand and 5% clay.

2.1.4. Lee Valley and Luddington, England

The soil at Lee Valley is a heavy silt loam with 21% clay, 4% organic C, and a pH of 5.6 to 5.9. That at Luddington is a sandy loam with 15% clay, 3% organic C, and a pH of 6.5. Both experiments received the same treatments, starting in 1968. Sewage sludges from various wastewater-treatment works were used and the different treatments received either a single large dose of 125 t DS ha^{-1} of four sewage sludges contaminated predominately with Zn (16,000 mg kg^{-1}), or Cu (8,000 mg kg^{-1}), or Ni (4,000 mg kg^{-1}), or Cr (8,000 mg kg^{-1}), or 31 t ha^{-1} a^{-1} of the same sludges for 4 years from 1968. In addition, there was a relatively uncontaminated sludge and a control treatment with inorganic fertilizers only.

2.1.5. Ultuna, Sweden

Starting in 1956, sewage sludge, naturally contaminated with metals, from wastewater-treatment plants in Uppsala and FYM were both added at 14 t and 10 t ha^{-1} every 2 years, respectively, from 1956 to 1988. Inorganic fertilizers were also added as treatments over the same period. The soil is post-glacial, with 35% clay, 35% silt, and 21% fine sand, and pH values of 6.2 and 6.6 in the unfertilized and FYM-treated plots, respectively, and 5.3 in the sludge-treated plots. The organic C content in the sludge-treated soil was 2.7%, whereas the unfertilized and FYM-treated plots contained 1.2 and 1.9% organic C respectively.

2.1.6. Fairland and Beltsville, Maryland, USA

Two field experiments were established at Fairland and Beltsville, MD, in 1975 and 1976 respectively. The soil at Fairland is a sandy loam with pH values in the plots ranging from 6.4 to 6.9. Anaerobically digested sludge was applied at 112 t ha^{-1}. The soil at Beltsville is a fine sandy loam with two types of sludge applications. Some plots received heat-treated sludge from Annapolis at 224 t ha^{-1} and these had pH values ranging from 5.1 to 6.0; other plots received Chicago "Nu-Earth" sludge at 100 t ha^{-1} and had plot pH values ranging from 5.7 to 6.6.

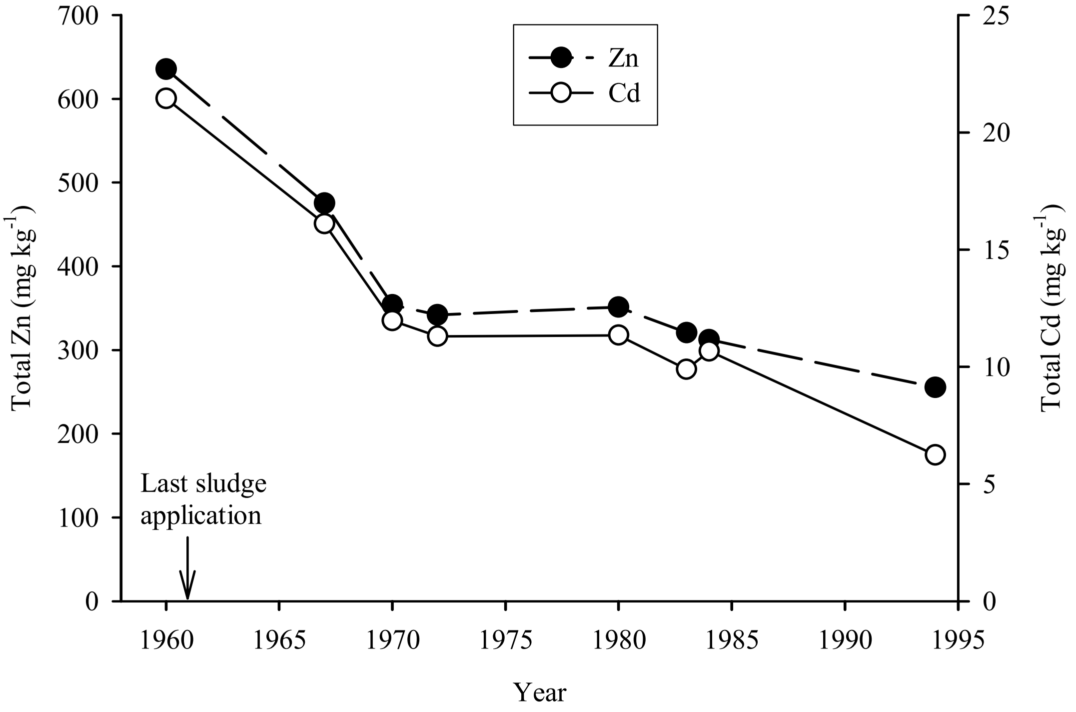

FIG. 1. Changes in concentration of total Zn and Cd in the topsoil after the termination of sludge application at Woburn. (Data are means of the highest sludge treatment.)

3. RESULTS AND DISCUSSION

3.1. Long-term changes in the bioavailability of heavy metals after termination of sludge applications at Woburn

Total Zn and Cd concentrations decreased substantially in the sludge-amended plots after termination of sewage-sludge applications (Fig. 1). McGrath and Lane [4] showed that these changes were caused largely by lateral movement of soil due to cultivation. Using a two-dimensional model to account for the dispersion of metals from the treated plots to the adjacent path and neighbouring plots, they estimated that about 80% of the metal load applied between 1942 and 1961 remained in the top soil in 1985. Examination of the soil profiles showed little evidence of downward movement in this situation, although the possibility of metal leaching with organic matter through macro-pores cannot be ruled out [23].

The percentages of the organic C added in sewage sludge or FYM that were unaccounted for were calculated for the years 1960 and 1984 (Table II). During the first 19 years, when manures were being applied annually, 60 to 75% of the added organic C was lost (unaccounted for within the 23-cm topsoil of the treated plot) at the end of this period, with FYM disappearing slightly faster than sludge or sludge compost. By 1984, when the applications of sludge and compost had been terminated for 23 years, and of FYM for 17 years, 77 to 88% of the added organic C had been lost, with little difference being observed for different manure treatments. The Rothamsted C model was also used to predict decomposition of organic matter in the FYM plots, using parameters that have been validated by fitting organic-C data from several long-term (>150 years) experiments at Rothamsted [24]. The model predicted that 73% of the added C from FYM would be lost from the topsoil in 1960, and 87% by 1984.

Table II. The amounts of organic C added and the percentages of the added C lost in different treatments

	FYM1	FYM2	S1	S2	SC1	SC2
Total C added 1942–60 (t ha^{-1})[a]	59	118	89	177	64	128
% of added C lost by 1960	76	71	65	66	59	65
Total C added 1942–61 or 67[b] (t ha^{-1})	79	158	95	191	69	138
% of added C lost by 1984	88	87	83	85	77	88

[a] Using a mean C concentration of 58% in the organic matter from FYM and sewage sludge.

[b] Organic matter was added 1942–61 for the sludge-amendment and 1942–67 for the FYM treatments.

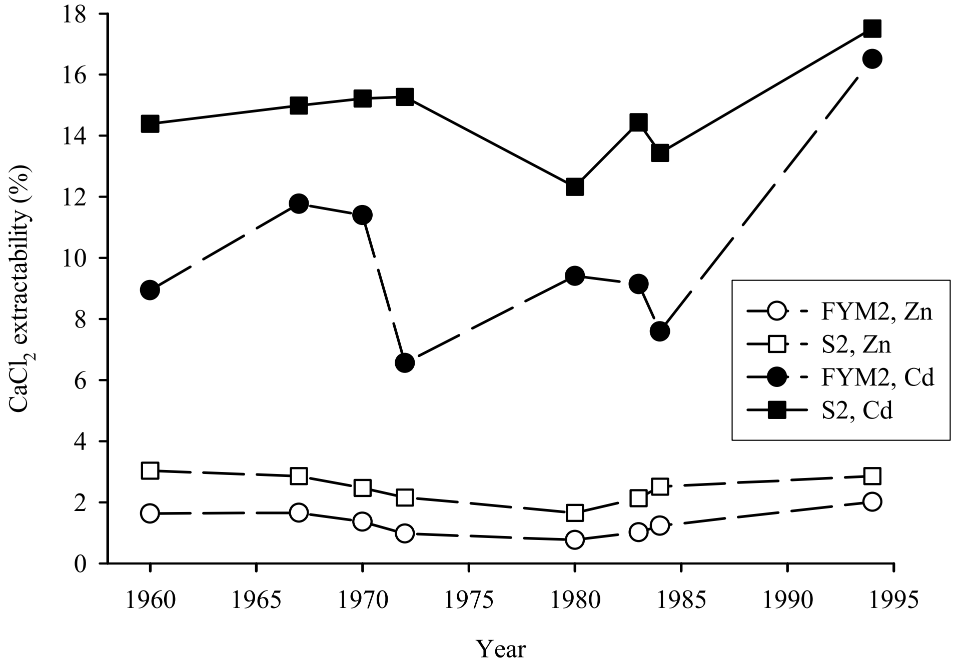

FIG. 2. Changes in the extractability of Zn and Cd by CaCl$_2$ in plots amended with sludge (S2) and farmyard manure (FYM).

Judging from the data in Table II, the rate of decomposition of sludge organic matter is likely to be slightly slower than this. Assuming an arable-farming scenario, the model predicted that 6% of the organic matter added as FYM would remain undecomposed even 100 years after the termination of application. This is equivalent to a total organic-C content in the FYM2 topsoil that is 9 t ha^{-1} higher than in the control soil, or an organic-C concentration 0.3% higher than that in the control soil. Overall, these results indicate that, although the easily decomposable fraction of sludge organic matter undergoes rapid decomposition during the initial years, the recalcitrant fraction will remain for a long time, taking hundreds of years before the soil organic matter concentration reverts to the background level at the experimental site. One may argue that the time-bomb hypothesis [25] cannot be fully tested until soil organic matter in the sludge-amended plots reverts to the background level, because the recalcitrant fraction of sludge-derived organic matter may be more important for metal adsorption than easily decomposable organic matter.

Table III. Ranges of plant Zn and Cd concentrations (mg kg^{-1}) and linear-regression parameters between plant and soil-metal concentrations in the Woburn experiment

Year	Species	Tissue	Zn				Cd			
			Range	Inter-cept	Slope	R^2_{adj}	Range	Inter-cept	Slope	R^2_{adj}
1963	Carrot	Top	28–71	27.0	0.07	0.80	0.4–3.1	0.73	0.09	0.50
		Root	22–49	21.1	0.04	0.77	0.2–1.7	0.39	0.06	0.60
1984	Carrot	Top	21–60	16.0	0.09	0.68	0.2–2.1	0.19	0.18	0.78
		Root	20–41	17.9	0.05	0.62	0.3–1.3	0.23	0.10	0.75
1985	Carrot	Top	26–83	16.9	0.17	0.84	0.5–4.3	–0.08	0.35	0.88
		Root	21–50	15.9	0.09	0.82	0.3–3.2	0.002	0.23	0.79
1970	Sugar beet	Top	49–256	22.7	0.58	0.79	0.4–5.6	0.008	0.44	0.83
		Root	31–85	23.0	0.15	0.91	0.1–1.1	–0.02	0.08	0.95
1971	Barley	Grain	31–64	28.6	0.09	0.93	0.03–0.2	0.004	0.015	0.94
		Straw	9–65	–2.3	0.14	0.84	0.2–4.6	–0.42	0.28	0.77
1983	Red beet	Top	25–172	7.8	0.36	0.78	0.5–6.4	–0.13	0.56	0.84
		Root	30–75	19.2	0.13	0.80	0.1–1.3	–0.08	0.09	0.88
1984	Red beet	Top	56–337	–10.3	0.78	0.78	0.6–7.5	–1.32	0.74	0.91
		Root	29–95	14.6	0.19	0.75	0.1–1.0	–0.18	0.09	0.92
1985	Red beet	Top	73–547	–54.0	1.54	0.91	0.8–11.3	–1.93	1.07	0.94
		Root	34–146	7.8	0.34	0.89	0.1–1.6	–0.32	0.15	0.93

Calcium chloride extracts mainly soluble and exchangeable metals from soils. The $CaCl_2$-extractable fraction correlates closely with plant uptake [26–28]. The percentages of total Zn and Cd in soils that were extractable with $CaCl_2$ varied between 0.5 and 3.2% for Zn, and from 4 to 18% for Cd (Fig. 2). The extractability for Cd was higher than that for Zn, probably because Cl can form soluble complexes with Cd. It is clear that the sludge-amended soils had considerably higher percentages of $CaCl_2$-extractable Zn and Cd than did the FYM soils. This is consistent with the results of several previous studies [27,29], and indicates that higher proportions of sludge-borne Zn and Cd than soil-native Zn and Cd were in soluble or exchangeable forms, and hence were of potentially higher bioavailability. Although the percentages of $CaCl_2$-extractable Zn and Cd fluctuated over time, there was no clear evidence of either an increasing or a decreasing trend. Similarly, Hyun et al. [30] reported that soluble Cd concentrations did not decrease over a 10-year period after sludge applications were terminated.

3.2. Relationships between soil- and plant-metal concentrations at Woburn

Samples of eight crops grown between 1963 and 1985 in the Woburn experiment were retrieved from the Rothamsted archive, and analysed for concentrations of Zn and Cd. The relationships between crop and soil concentrations were largely linear. Figure 3 shows examples of carrot and red beet grown in 1985. Table III shows the concentration ranges in plants and the parameters of linear regressions for Zn and Cd. In the majority of cases, the percentages of variation accounted for by the linear regression ($R^2_{adj.}$) were greater than 70% for both Zn and Cd. The slope of regression indicates the transfer efficiency of metals from soil to plant. Several general points can be observed.

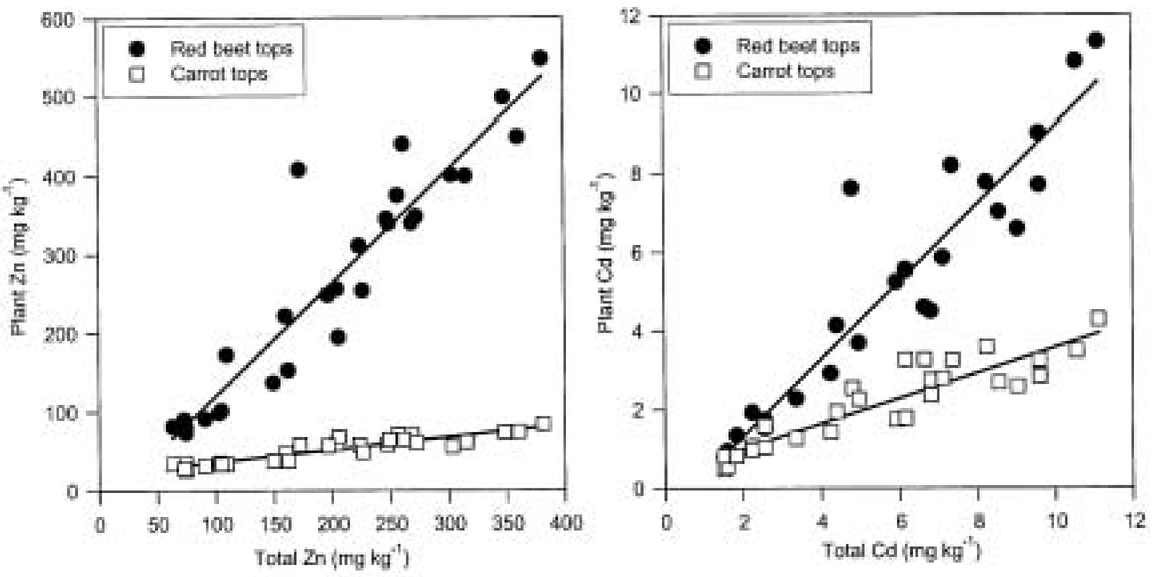

FIG. 3. Relationship between plant Zn and Cd concentrations and soil total Zn and Cd.

First, the slopes for Zn and Cd fall broadly into similar ranges, indicating a similarity between the two metals. Second, vegetative tissues had greater slopes than storage tissues (grain or beet roots), indicating a physiological barrier in the transfer of Zn and Cd from primary roots or vegetative tissues to storage organs. Third, crop species differed markedly in the slopes. Compared to carrot and barley, sugar beet and red beet had considerably higher concentrations of Zn both in roots and in tops, and responded more steeply to the increasing Zn concentration in the soils. Sugar beet and red beet also took up more Cd in their tops than did carrot, but had slightly lower concentrations of Cd in their tuber roots than did carrot. For Zn, the slopes for red-beet roots and tops were approximately four and nine times, respectively, greater than those for carrot. For Cd, the slopes for red-beet tops were three to four times higher than those for carrot tops, whereas the slopes for red-beet roots were only 65 to 90% of those for carrot roots. The order of Cd accumulation in different species was consistent with previous reports [7,31].

Transfer of Cd to food crops is of major concern [31]. In Germany, the current guideline limit for Cd in cereal grain is 0.12 mg kg^{-1} DM [32], and adoption of the same limit is being discussed in the European Union. The linear regression obtained with barley (Fig. 4) indicates that this limit would be breached when soil total Cd exceeds 6.4 mg kg^{-1}. This is greater than the maximum concentration (3 mg kg^{-1}) allowed under EU legislation [13]. Under the United States EPA-503 regulation, the maximum is limited by loading rather than soil concentration [33], but the loadings that gave rise to average grain Cd levels of more than 0.12 mg kg^{-1} included all of the sludge-amended treatments in the Woburn experiment. These begin at a loading of 20 kg Cd ha^{-1} in the SC1 treatment, which is roughly half the EPA-503 maximum cumulative loading (39 kg Cd ha^{-1}). Most of the sludges applied at Woburn would not meet the “clean” criterion for under EPA-503. However, a maximum cumulative loading of 39 kg ha^{-1} means that a soil concentration of 20 mg kg^{-1} would be allowed, but would take longer to build up in the soil. Whether applying “clean” sludge with <39 mg Cd kg^{-1} for a longer time would avoid grain with >0.12 mg Cd kg^{-1} has not been tested anywhere to our knowledge, and must, therefore, be in question. Other studies not using “clean” sludge [3,34] have shown that, depending on the soil, the concentration of Cd in barley grain could exceed 0.12 mg kg^{-1} with a total Cd concentration in the sludge-amended soil well below the value obtained in this experiment.

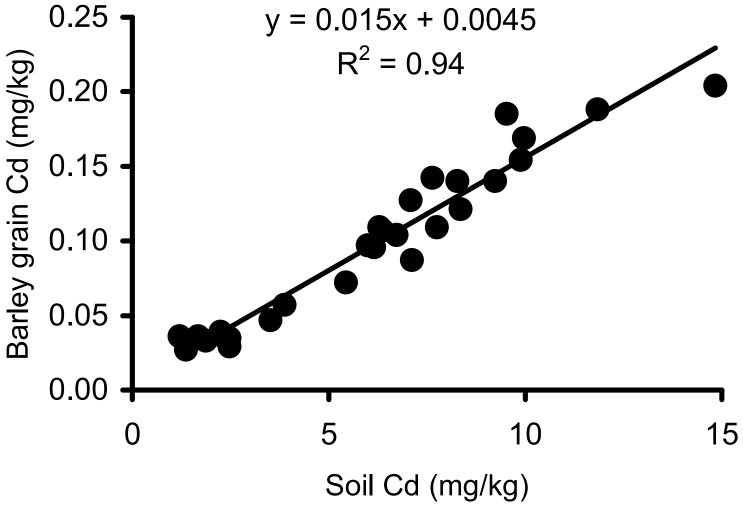

FIG. 4. Relationship between barley grain Cd and soil total Cd.

3.3. Effects of heavy metals from sewage sludge on biological nitrogen fixation

Atmospheric nitrogen (N_2) is fixed only by micro-organisms that have the enzyme nitrogenase [35]. It occurs in prokaryotes, including free-living heterotrophic bacteria, phototrophic cyanobacteria (blue-green algae) and bacteria that have symbiotic associations with plants, the most important of which are rhizobia that form nodules on the roots of legumes.

3.3.1. Free-living heterotrophic bacteria

Free-living N_2-fixing bacteria are ubiquitous in soil, and include species that live under aerobic, microaerobic, and anaerobic conditions. Significant decreases in acetylene reduction activity (ARA, an indirect indication of nitrogenase activity) by aerobic and microaerophilic N_2-fixers were reported in metal-contaminated soils from Woburn compared to FYM-treated soils [36]. These decreases occurred at metal concentrations close to the EU upper limits for Zn and Cu, and at three to four times the limit for Cd (Table I). Mårtensson and Witter [37] found heterotrophic N_2-fixation to be strongly decreased in metal-contaminated soil compared to FYM-treated soil at Ultuna, central Sweden. Nitrogen-fixing activity by aerobic diazotrophs in the metal-contaminated soil was decreased to 2% of that measured in the FYM-treated soil. Metal concentrations in the FYM-treated soil were at background levels, and those in the sludge-treated soil are given in Table IV. However, the pH of the sludge-treated plot was low (pH 5.3) and this may also have affected both numbers of, and N_2-fixation by, free-living heterotrophic bacteria at this site in combination with the metals.

Fliessbach and Reber [38] confirmed the great sensitivity of N_2-fixation by free-living heterotrophic bacteria to metals in the old arable soil at Braunschweig in Germany. The metal-concentration ranges (mg kg^{-1}) were: 157 to 381 Zn, 42 to 102 Cu, 12 to 25 Ni, 0.7 to 2.5 Cd, 41 to 90 Cr, and 61 to 88 Pb. However, no N_2-fixation by free-living heterotrophic bacteria could be measured in the ex-woodland soil (Braunschweig 2) in the same field. The pH of the soil in these plots ranged from 5.3 to 5.7 and the metal concentrations were similar to those in the old arable experiment (Braunschweig 1).

Lorenz et al. [39] tested the possibility of using N_2-fixation by free-living heterotrophic bacteria as a sensitive biological indicator of metal pollution on sludge-treated soils from the Woburn, Luddington, Lee Valley, and Gleadthorpe experiments. The Woburn soils gave the same results as those of Brookes et al. [36], but no activity could be found in either the Luddington or Lee Valley soils.

There was variable activity in Gleadthorpe soils that did not correlate with metal concentration. In a further experiment in which metal-contaminated soil and FYM-treated soil, from Woburn, were mixed in various proportions to give soils of increasing metal concentrations, free-living heterotrophic N_2-fixation was significantly inhibited at metal concentrations (mg kg^{-1}) of: 127 Zn, 37 Cu, 21 Ni, 3.4 Cd, 52 Cr, and 71 Pb. However Lorenz et al. [39], concluded that free-living heterotrophic bacteria were not ubiquitous or active enough to be used as indicator organisms for detecting metal pollution of soil.

3.3.2. Effects of metals on free-living cyanobacteria

These autotrophs grow on the soil surface and use light to fix CO_2 in photosynthesis. Brookes et al. [40] showed that 25 years after the treatments ceased, cyanobacterial N_2-fixing activity reduced by 30% on metal-contaminated soil from Woburn compared to FYM-treated soil. Also, the growth of cyanobacteria was much inhibited on the metal-contaminated soil. They also sampled a gradient of increasing metal concentrations in soil between plots previously treated with either FYM or sewage sludge, and a smooth decrease in nitrogenase activity with increasing metal-concentration was found. Nitrogen fixation was reduced by 50% at metal concentrations given in Table IV. Even stronger inhibition of cyanobacteria has been reported [37] on the metal-contaminated soil at Ultuna compared to FYM-treated control soil (Table IV).

Fliessbach and Reber [38] reported large decreases in counts of cyanobacteria in plots of the two field experiments at Braunschweig, to which increasing amounts of "moderately" contaminated unamended or metal-contaminated sludge had been added, compared to the control NPK or moderately contaminated unamended low-sludge-treated plots. Using ARA to assess nitrogenase activity in cyanobacteria, they found that, after 30 days incubation, AR and hence N_2-fixation, was suppressed by 30 and 70% in the low and high sludge-treated plots, respectively, at the old arable site (Braunschweig 1, pH 6.1-6.8). In the ex-woodland site (Braunschweig 2, pH 5.3-5.7) the corresponding suppression was about 25 and 100% respectively (Table IV). Interestingly, annual additions of the moderately contaminated unamended sludge from a normal sewage works also decreased the abundance of cyanobacteria, compared with the NPK treatment. The large amount of available N and C may suppress N_2-fixation in cyanobacteria and also may favour competition by heterotrophic organisms [38].

Table IV. Metal concentrations in soils at which negative effects on N_2-fixation by cyanobacteria were observed

Experimental site		Zn	Cu	Ni	Cd	Cr	Pb
		(mg kg^{-1} soil)					
Woburn, UK[a]		114	33	17	2.9	80	40
Ultuna, Sweden[b]		230	125	35	0.7	85	40
Braunschweig[a], Germany[c]	L	42–93	–	1.75–4.5	0.36–0.81	–	–
	H	132–305	–	5.4–17	0.58–2.38	–	–
Braunschweig[b,d]	L	26–81	–	1.5–4.6	0.4–0.88	–	–
	H	86–240	–	3.7–16	0.7–2.15	–	–

[a]50% reduction in N_2-fixation. [b]>50% reduction in N_2-fixation. [c]Braunschweig 1: 30 and 70% reductions in N_2 fixation at the low (L) and high (H) rates of sludge application respectively. [d]Braunschweig 2: 25 and 100% reductions in N_2 fixation at L and H respectively.

3.3.3. Symbiotic N_2 fixation

The most important agricultural associations involving rhizobia include lucerne (alfalfa), pulses such as peas and beans, and clover in grass-clover leys. For example, N_2-fixation by white clover (*Trifolium repens* L.) in agroecosystems can amount to more than 200 kg N ha^{-1} a^{-1} [41]. This is, therefore, an important source of N in low-input agroecosystems such as grass-clover leys to which very little or no inorganic N fertilizers are applied.

3.3.4. Effects of metals on clover growth and N_2 fixation

Significant decreases in white-clover yields in a mixed grass/clover sward were reported by Vaidyanathan [42] in plots of the Lee Valley experiment contaminated predominantly by Zn 7 years previously. Copper also decreased the yield of clover, but to a lesser extent, whereas Ni and Cr had no effect. When grown in monoculture at Woburn, white-clover yields were decreased by 60% on metal-contaminated sludge-treated plots compared to FYM-treated plots more than 20 years after the sludge had been applied [43].

Red clover failed almost completely in all three harvests taken in 1985 from some of the plots at Luddington that were most contaminated with metals, particularly those with 455 and 511 mg Zn kg^{-1} soil [44]. In the "low"-Zn treatments, with 238 mg Zn kg^{-1} soil, there was no decrease in yield. Other treatments, with 118 and 91 mg Ni kg^{-1}, soil also gave poor yields, but only at the first harvest. However, these plots also contained 128 and 104 mg Zn kg^{-1} soil, respectively, and it is likely that the combination of Zn and Ni caused the initial poor establishment. Similarly, at Gleadthorpe, yields of white clover were decreased in Zn and Cu-contaminated soils. Zinc and Cu together at concentrations (Zn:Cu mg kg^{-1}) of 172:47, 173:107, and 209:94 decreased the clover yields by 20, 31 and 67%, respectively, compared to the controls [45]. Nickel alone had no effect on yields at the largest concentration of 31 mg kg^{-1} soil, nor were there any yield decreases in any of the combined Zn and Ni treatments. This may be because, in all the Ni treatments, the Zn concentrations were smaller than the concentrations at which yield reductions occurred.

From the above studies, it is not possible to determine whether the adverse effects reported on clover yield were due to phytotoxicity of the metals or due to an effect on N_2 fixation or the rhizobia in these soils. At Lee Valley, Luddington, or Gleadthorpe, no data were collected at the time on the effects of these metals on N_2 fixation and/or on rhizobial populations. However, McGrath et al. [17] showed that yield decreases on the metal-contaminated soil were not due to phytotoxicity at Woburn, as addition of fertilizer N restored the clover yields to those in the control FYM-treated soil. The reductions in clover yields were due to an effect on N_2 fixation which was reduced in a pot experiment by more than 50% in soils containing more than (mg kg^{-1}): 334 Zn, 99 Cu, 27 Ni, and 10 Cd. It seems likely that the metal effects on clover yields at Lee Valley, Luddington, and Gleadthorpe were also due to effects on N_2 fixation rather than to phytotoxicity, since the metal concentrations were similar to those found in the metal-contaminated soil at Woburn.

3.3.4.1. Rhizobia

Rhizobia are well adapted to life as free-living soil bacteria and can survive for long periods in the absence of the host [46]. They have the ability to nodulate leguminous plants, but due to a deficiency in either the host or micro-symbiont, some nodules can be ineffective and fix no N_2. These ineffective nodules are usually relatively small and white, whereas effective nodules are larger and pink due to the presence of leghaemoglobin.

The decrease in clover yield reported by McGrath et al. [43] in metal-contaminated soil at Woburn was due to a lack of N_2 fixation as a result of ineffective nodules [17], whereas plants grown in FYM-treated control soil had effective nodules. *Rhizobium leguminosarum* bv. *trifolii*, isolated from nodules on clover plants grown in the metal-contaminated soil, were found to be ineffective in N_2

fixation in plant-infection tests in the absence of metals [18]. No effective rhizobia were present in the metal-contaminated plots, whereas the FYM-treated control plots, some 2 m away, had effective rhizobia. In a further experiment in which increasing numbers of effective *R. leguminosarum* bv. *trifolii* were added to metal-contaminated soil from Woburn, and the soils were incubated for 2 months in a moist condition in the laboratory, no effective nodulation was obtained on clover plants where 10^4 cells g^{-1} soil or fewer were added. Only with the additions of extremely large numbers of effective rhizobia >10^4 cells g^{-1} soil) did sufficient survive to give effective nodules on clover after 2 months. It was concluded that effective clover rhizobia were unable to survive in the free-living state outside the protected root-nodule environment in the metal-contaminated soil at Woburn, and that Cd, Zn and Cu were likely to be the most toxic to rhizobia.

Smith et al. [47] enumerated the population of indigenous *R. leguminosarum* bv. *trifolii* in soils from plots of the Luddington experiment. Even though all the plots had clover growing on them when the soil samples were taken, the numbers of rhizobia were an order of magnitude smaller in the high-Zn and -Cu treatments compared to the control soils. Rhizobial numbers also decreased in the high-Cu soil, but to a lesser extent, whereas Cr and Ni had no effect. Smith et al. [47] concluded from their Luddington data that the order of toxicity to the rhizobial population was Zn > Cu > Ni > Cr. The metal concentrations (mg kg^{-1}) reported by these authors in this soil were: 542 Zn, 34 Cu, 48 Ni, and 160 Cr (no data were given for Cd). However, there is some dispute as to the metal concentrations in the high-Zn plots since Chander and Brookes [48], using soils from the same plots for microbial biomass work, reported metal concentrations (mg kg^{-1}) of: 281 Zn, 30 Cu, 41 Ni, and 1.5 Cd. The Zn concentration was approximately half that reported by Smith et al. (1990)[47].

In the field experiment at Ultuna, *R. leguminosarum* bv. *trifolii* numbers were also found to be an order of magnitude smaller in soil previously treated with metal-contaminated sludge (e.g. 2,500 cell g^{-1} soil) compared to the unfertilized soil (e.g. 19,000 cells g^{-1} soil) and FYM-treated soil (e.g. 18,000 cell g^{-1} soil) [37]. No legumes had been grown at this site for 30 years. Rhizobia isolated from clover plants grown in the metal-contaminated soil showed a distinct delay in nodulation in plant-infection tests compared to isolates from plants both in the unfertilized and in the FYM-treated soils. The unfertilized and FYM-treated plots contained background metal concentrations, whereas those in the sludge-treated plot were the same as those given in Table IV. The large contents of clay and organic C in the sludge-treated soil may have reduced metal toxicity to rhizobia at this site, even though the pH was low.

Giller et al. [19] constructed a gradient of increasing metal concentrations by mixing metal-contaminated and FYM-treated soils, from Woburn, in various proportions. To these they added equal numbers of *R. leguminosarum* bv. *trifolii* and monitored their survival over a 171-day period. At 53 and 171 days, the numbers of rhizobia in the 2/3 sludge soil had decreased to <10,000 cells g^{-1} soil, whereas those in the control FYM soil remained at >10^6 cells g^{-1} soil. A portion of the survivors in the soils containing the largest amounts of sludge (i.e. >1/2 sludged soils), at 171 days, may have been ineffective metal-tolerant rhizobia. In earlier studies, these ineffective rhizobia were found to number 100 cells g^{-1} soil in freshly sampled rhizosphere soil from the metal-contaminated plot at Woburn [18]. The metal concentrations in the 2/3 sludge soil, at which significant reductions in the numbers of rhizobia occurred after 53 days, were (mg kg^{-1}): 228 Zn, 67 Cu, 31 Ni, 6.9 Cd, 81 Cr, and 84 Pb.

Chaudri et al. [49] sampled all the plots of two field experiments at Braunschweig and found that numbers of *R. leguminosarum* bv. *trifolii* had decreased by several orders of magnitude, compared to the control plots, at metal concentrations well below the current CEC limits (Fig. 5).

Although the concentrations of several metals increased simultaneously in both field experiment, it was suggested that Zn had a strong effect on the numbers of rhizobia [49]. Also, metal toxicity to rhizobia occurred at slightly smaller concentrations in the ex-woodland site (Braunschweig 2) due to the lower pH compared to the old arable site (Braunschweig 1).

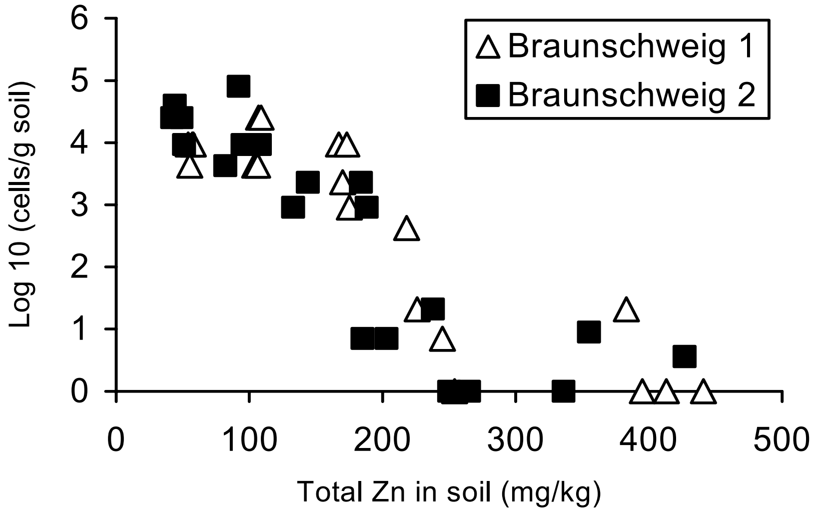

FIG. 5. Numbers of indigenous effective R. leguminosarum *bv.* trifolii *in soils from the old arable experiment (Braunschweig 1) and ex-woodland experiment (Braunschweig 2) at Braunschweig.*

3.3.5. Effects of metals on other species of legumes/rhizobia

Spring beans (*Vicia faba*) grown in the metal-contaminated plot at Woburn during 1968 and 1969 (more than 7 years after sludge-applications ceased) showed no difference in yields compared to the control FYM-treated plot [50]. In fact, the yields from these plots were identical in both years, and both were greater than the yield from the plot treated with NPK fertilizer. The spring beans were not inoculated with rhizobia nor was N fertilizer added and, therefore, must have been infected by indigenous effective *R. leguminosarum* bv. *viciae*. The yield data for the 2 years suggest that *R. leguminosarum* bv. *viciae* is less sensitive to heavy metals than is *R. leguminosarum* bv. *trifolii*.

Work on the effects of heavy metals, through the application of sludge, on yields and N_2 fixation in soyabean was carried out in the field experiments at Beltsville and Fairland in Maryland [51–54]. In pot experiments using soils to which heat-treated sludge had been applied, Heckman et al. [51] found increasing yields and N_2 fixation by soyabean with increasing sludge-application rates. In 1983 and 1984 non-nodulating and nodulating isolines of soyabean were used to determine toxic effects of metals on N_2 fixation. No metal toxicity occurred at Beltsville, but at Fairland there were decreases of 12 to 55% in the %N derived from fixation in soyabean in the metal-contaminated plots, suggesting toxicity [53]. Kinkle et al. [54] found that numbers of *Bradyrhizobium japonicum* increased with increasing rates of sludge application, but number of serotypes and their metal sensitivities remained the same regardless of the treatment. Neither of these studies reported metal concentrations in the sludge-treated soils at the two sites. But it is now known that all of the metal concentrations at these two sites were below those in most of the European experiments, even in the most heavily contaminated plots [55]. It is, therefore, possible that the metals were not present in concentrations high enough to cause adverse effects on yields, N_2 fixation or on free-living *B. japonicum* in these two experiments. The increases in numbers of *B. japonicum* with increasing applications of sludge presumably were due to the additional substrate from the organic matter. It is important to distinguish between short-term positive effects due to substrate and longer-term negative effects due to metal toxicity.

Differences in sensitivity of different species were shown by Giller et al. [19], e.g. *R. loti* was present only in soil from the FYM-treated plot, at Woburn, and not in the metal-contaminated plot. No *R. meliloti* was present in either plot, which is not surprising as alfalfa (*Medicago sativa*) often requires inoculation with *R. meliloti* when grown in the UK. In a further experiment, they added equal numbers of *R. loti* and *R. meliloti* to gradients of increasing metal concentrations prepared by mixing metal-contaminated soil with FYM-treated soil from Woburn. After 50 days exposure, the numbers of *R. meliloti* in soil mixtures containing 5/6 or more sludge soil were an order of magnitude smaller compared to the control FYM-treated soil, but were above 100,000 cells g^{-1} for all soils. In contrast, *R. loti* numbers decreased by several orders of magnitude (i.e. 100 cells g^{-1} soil) in soil mixtures containing 1/2 or more sludge soil, compared to the FYM soil, which contained 100,000 cells g^{-1} soil. These authors concluded that *R. meliloti* was less sensitive to heavy metals than *R. loti*, which was similar in sensitivity to *R. leguminosarum* bv. *trifolii*, but, because these results were for single strains of each species, concluded that confirmation was required with a broader range of strains.

3.4. Effects of metals on the soil-microbial biomass

In uncontaminated soils, there is normally a positive linear relationship between the organic-C content of and biomass-C content [56]. The soil microbial biomass is the most labile fraction of the organic matter and, therefore, can be a useful indicator of changes in soil conditions due to alterations in management practices.

Brookes and McGrath [16] measured the microbial biomass in soils from the Woburn experiment some 20 years after the last treatments were applied. They found that in the high-metal soils (sludge-treated) the microbial biomass was approximately half that in the low-metal soils (FYM-treated) (Table V). They also showed that in the low-metal soils the usual linear relationship between organic C and biomass C existed, but no relationship was seen with the high-metal soils. Also, the rate of respiration per unit weight of biomass (specific respiration) was much higher in the high-metal soils than in the low-metal soils, whereas the adenylate energy charge (thought to indicate the level of metabolic activity [57]) was high in both soils [58].

Further work by Chander [59] and Chander and Brookes [60] on soils from Woburn confirmed the higher specific respiration rate of the microbial biomass in the metal-contaminated soils first reported by Brookes and McGrath [58].

Table V. Minimum concentrations of metals in soils that negatively affected soil microbial biomass

Experimental site	Zn	Cd	Cu	Ni	Pb	Cr
			(mg kg^{-1} soil)			
Woburn, UK	180	6.0	70	22	100	105
Luddington, UK	281	–	150	–	–	–
Lee Valley, UK	857	–	384	–	–	–
Ultuna, Sweden	230	0.7	125	35	40	85
Braunschweig 1, Germany	360	2.8	102	23	101	95
Braunschweig 2, Germany	386	2.9	111	24	114	105

When glucose and maize-straw substrates were added to the Woburn soil, proportionately more was respired as CO_2 from contaminated soils compared to soils from uncontaminated plots [60]. Consequently, less new microbial biomass was formed from the added substrate and it was concluded that the lower efficiency of conversion of C into biomass is one of the explanations for the lower biomass in the metal-contaminated soils. The fact that specific respiration was greater in the metal-contaminated soils means that measurements of soil respiration alone cannot give a good indication of effects of metals on the size of the microbial biomass. In fact, increased respiration could be interpreted as an indication of increased stress and/or an increased death rate of microbes.

Carbon and N in the soil microbial biomass were found to be reduced by about 60% in soil from the metal-contaminated sewage-sludge-treated plot at Ultuna, Sweden, compared to soil from the control FYM-treated plot (Table V) [61].

Both at the low and high rates of sludge application in both field experiments at Braunschweig, microbial biomass increased with increasing additions of "moderately" contaminated unamended sludge each year. In contrast, increases in biomass were less pronounced or even absent in the inorganic fertilizer treatments and metal-contaminated sludge treatments, especially at the largest rates of addition. These effects on biomass were apparent after only 7 years of sludge addition [21], at the concentrations shown in Table V.

3.4.1. Effects of metals on mycorrhizas

The effects of metal-contaminated soil and FYM-treated control soil, from Woburn, on both native and an introduced species of vesicular-arbuscular mycorrhiza (VAM) (*Glomus mosseae*) were examined by Koomen et al. [20] in pot experiments. In the control soil, 60% of the white-clover roots were infected with native VAM compared to only 1% for the metal-contaminated soil. In the experiment where VAM was introduced, the control soil had 46% and 21% mycorrhizal infection in the inoculated and non-inoculated treatments respectively. In contrast, none of the plant roots were infected with mycorrhizas in the metal-contaminated soil from either treatment. Clover roots were also sampled in the field at Woburn, to see if this trend was repeated. When clover roots were first sampled, no mycorrhizal infection was present. But after 6 months, 52% of the root length sampled from the FYM-treated plots and 69% of the root length from the sludge-treated plots were infected. Hence, mycorrhizal infection was delayed in the FYM- and sludge-treated plots.

Koomen et al. [20] suggested that the apparent contradiction between their pot and field experiments could be explained by the greater time that had elapsed during field samplings, which may have allowed the infection by a small inoculum-density of mycorrhizas present in the sludge-treated plots to gradually increase to the levels observed on the FYM-treated plots. They further suggested that metals may delay the development of mycorrhizal infection rather than completely suppress it, and that the infection that developed in the metal-contaminated soil after some time was likely due to indigenous metal-tolerant mycorrhizas present in the soil. Gildon and Tinker [62] found an isolate of *G. mosseae* that was tolerant of large concentrations of Zn and Cd in heavily polluted soils, and which was as effective in enhancing the growth of clover as a "normal" isolate of *G. mosseae* with comparable infection levels after 6 weeks. Reductions in VAM infections of maize with increasing metal-contaminated sludge applications have also been reported at Braunschweig, with a relative shift of mycorrhizal infection to the uncontaminated subsoil [63] possibly to avoid the toxic effects of metals in the topsoil.

However, caution should be exercised in interpreting these results, as large amounts of available-P is a general property of long-term sludge-treated soils which could have inhibited infection. Also, in some of the work discussed [63] the reductions in VAM infections occurred in plots where the pHs were low, which may have affected both the infection of the roots and their growth. Even after taking these considerations into account, these studies suggest that there is a strong negative effect of metals on VAM infection and development.

4. CONCLUSIONS

In the Woburn long-term sewage-sludge experiment, the $CaCl_2$ extractability of soil Zn and Cd was higher in sludge-amended than in control plots, and did not show a clear decreasing trend over the 33 years since termination of sludge applications. The concentrations of Zn and Cd, both in vegetative and in storage tissues of eight crops, correlated linearly with soil total-metal concentrations. Plant species differed markedly in the transfer efficiency of Zn and Cd from soil to plants. There were large differences between years in metal-transfer efficiency. Overall, the results indicate that the bioavailability of sludge-borne Zn and Cd did not decrease with time after the termination of sludge applications.

The reported adverse effects on soil-microbial parameters were generally found at surprisingly modest concentrations of metals in soils. Indeed, they were smaller than the concentrations reported by Chang et al. [64] to be likely to decrease growth of sensitive crop species. However, one should be cautious in interpreting the results summarized in Tables IV and V. This is because, as with any sewage sludge, the metals were present simultaneously and their effects were, therefore, confounded. Thus, in Table IV, in which Cu appeared to have an adverse effect in three experiments at well below the upper CEC limit, it should be remembered that Zn and Cd were also present. Copper was near background in some of those soils in which effects were observed, and so was Cd; it is, therefore, likely that Zn, at concentrations below the CEC limit, was toxic to the clover-rhizobia system. In other studies, Chaudri et al. [65] showed that Zn was toxic to the survival of rhizobia as a free heterotroph in soil, and Cd needed to be present at much larger concentrations than those at Braunschweig. Similar arguments hold for the observed effects on microbial biomass, for which Cu appeared to approach toxic concentration.

It is concluded that prevention of adverse effects on soil-microbial processes and ultimately soil fertility, should be an aim of soil-protection legislation.

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ASSESSING BIO-AVAILABILITY OF METALS IN BIOSOLID-AMENDED SOILS: ROOT EXUDATES AND THEIR EFFECTS ON SOLUBILITY OF METALS

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Abstract

The existence of root exudates has been known to research scientists for a long time. Their role and function in plant nutrition and soil chemistry have only recently begun to be understood. The primary constituents of root exudates are low molecular weight organic acids that play an essential role in making the sparingly soluble soil Fe, P, and Zn available to plants. While root exudates are reasonably well characterized, the influence of environmental factors on their chemical composition and volume require further investigation. This study was initiated to investigate the role of root exudates on the solubilization and bioavailability of soil-borne heavy metals in biosolid-treated soils. Corn, wheat, canola, Sudan grass, chickpea, and Swiss chard were grown on standard and biosolid-treated sand media to characterize root exudates and evaluate the plants' metal-uptake patterns. Recent results indicate that: (i) the same organic acids were present over a 16-week growing season. However, the primary constituents of exudates from corn grown on standard sand media and biosolid-treated media were different. (ii) Metal concentrations in plant roots were considerably higher than those present in respective plant shoots, and plants grown in the biosolid-treated rooting medium had significantly higher concentrations of metals than those of the standard sand media at all stages of the growth. (iii) Based on mass present in the growth media and absorbed by corn, the phyto-availability of biosolid-borne metals were in the order: Cd > Ni = Zn > Cu = Pb > Cr. The availability of Mo was comparable to that of Cr. (iv) In the biosolid-treated growth medium, the uptake rates of Cd, Pb, and Zn by corn shoots (measured as mg of metal absorbed per g of biomass increment per unit time) were relatively constant over the active growing phase of the plants and were proportional to respective mass input of the metals. Work is in progress to define the role of root exudates on solubility and bioavailability of biosolid-borne metals in soils

1. INTRODUCTION

When applied to crops, sewage sludges are potentially harmful because of the pathogens and/or chemical pollutants they contain. In the soil, pathogens rapidly die off and do not present a long-lasting detrimental environmental effect. But chemical pollutants in such biosolids, especially metals and trace elements, may persist in soils and be absorbed by plants in sufficient quantities to adversely affect the health of consumers and/or plants for long periods of time. In 1972, the Congress of the United States directed the Environmental Protection Agency (EPA), through Section 405(a) of the Federal Water Pollution Control Act, to develop guidelines for the use and disposal of biosolids, including cropland application. The Code of Federal Regulations, Title 40, Parts 257, 403, and 503 Standards for the Use or Disposal of Sewage Sludge were finalized on February 19, 1993 [1].

The EPA's goal was to encourage beneficial use of biosolids while protecting public health and the environment from any "reasonably anticipated adverse effect" of pollutants found in biosolids. These regulations define the domain within which biosolids may be safely disposed of or beneficially used. They have far-reaching implications on how biosolids generated by the more than 15,000 publicly owned treatment works (POTW) nationwide are managed, and provide the POTWs with guidelines to develop long-range, cost-effective biosolid-management plans. With these regulations in place, cropland application has become a viable option for the management of biosolids. It has been estimated that 55% of the annual production of biosolids in the United States (6.85×10^6 tons yr^{-1}) is now applied to land [2].

Are EPA regulations protective with respect to accumulation of toxic pollutants from cropland application of biosolids? To address this question, the long-term bioavailability of metals in biosolid-amended soils must be objectively evaluated.

Plants and animals absorb metals from those dissolved in solutions. As potentially toxic metals exist in the natural environment primarily as sparingly soluble solids; their reactivity and bioavailability in terrestrial and aquatic environments are dependent on the chemical equilibria between those in solution and those associated with solid phases as well as by the kinetics of these reactions [3–5]. Activities of the free metals or their ions in the soil solution determine their availability to plants [6–7]).

In land application of biosolids, metals are added to the soil in the solid phase, in which case, plant absorption may take place through root extraction from those present in bulk soil solution via the solid-solution phase equilibration process or at the rhizosphere and soil interface where root exudates mobilize the solid-phase metals. Considering that the metal concentrations in soil solution are always low and dissolution kinetics in soil are also low, plant absorption from the bulk solution probably is not the most important process by which metals are taken up. Eaton [8] reported that plants grown in sand culture obtained Fe and P "from water insoluble minerals by absorption across the particle-root interfaces rather than by absorption from culture or soil solution." Jenny and Overstreet [9] postulated that mineral elements are taken up through contact and ion interchange between plant roots and soil colloids. Mench and Martin [10] found that soil Cd, Cu, Fe, Mn, Ni, and Zn were solubilized by root exudates of *Nicotiana tabacum* L., *N. rustica* L., and *Zea mays* L. Chemical compositions of these exudates differed for the three species and the extent of metal extraction by root exudates was similar to the order of metal bioavailability when these species were grown on the same soils. Krishnamurti et al. [11] demonstrated that soil Cd might be mobilized by low-molecular-weight organic acids such as acetic, citric, oxalic, fumaric, and succinic, which are often found in root exudates. At the same concentration, Cd release followed the order: fumaric > citric > oxalic > acetic > succinic. The extent of Cd solubilized from each soil was proportional to the kinetics of Cd release.

The bioavailability of metals in soil is customarily defined by the amounts absorbed by growing plants. Plants typically absorb <1% of the metals present, therefore, it would take a long time to exhaust biosolid-borne metals that are available for plant absorption. We hypothesize that total amounts of bioavailable metals may be determined by those solubilized by root exudates. Uptake by plants, which determines the plant-tissue concentration and phytotoxicity of a metal, will be determined by the kinetics of metal released to the root-exudate solution. In this manner, the bioavailability of biosolid-borne metals may be defined in terms of a capacity factor (metals solubilized by root exudates), which describes how long (and how much) metals in biosolid-treated soil will be available, and a rate factor (the rate at which metals may be solubilized by root exudates), which indicates the amounts taken up.

The overall purpose of this investigation was to define the role of root exudates in plant uptake of metals from, and to evaluate the bioavailability of metals accumulated in, biosolid-treated soils. Specific objectives were as follows:

- Develop an experimental procedure to collect root exudates of plants grown with and without biosolids, using a non-sterile sand-culture system.
- Characterize the chemical composition of root exudates of selected species.
- Evaluate the effects of plant species, soil and biosolid properties, and the length of time metals are present in soil on the chemical composition of root exudates.
- Formulate synthetic "exudates" that can be used as surrogates for actual root exudates to study the chemistry of metals in biosolid-amended soils.
- Evaluate the ability of root exudates to solubilize metals in biosolid-amended soils by determining root-exudate-specific metal solubility and dissolution rate constants of biosolid-treated soils.
- Correlate metal concentrations of plants grown on biosolid-amended soils with root-exudate-specific metal-dissolution-rate coefficients of the soils on which the plants are grown.

2. METHODS AND PROCEDURES

In a greenhouse experiment, plants were grown in sand culture with or without biosolids, designated “biosolid treated” and “standard,” respectively. The purpose was to determine whether root-exudate composition changes with stage of growth; to examine the effect of biosolids on root-exudate composition; and to evaluate exudate effects on uptake of metals. Corn (*Zea mays* L.), wheat (*Triticum turgidum* var. *durum* L.), canola (*Brassica napus* L.), Sudan grass [*Sorghum sudanense* Piper (Stapf.)], chickpea (*Cicer arietinum* L.), and Swiss chard (*Beta vulgaris* L.) were studied.

Each plant-culture unit consisted of a reservoir for nutrient solution and four 10-L plastic pots situated immediately above the nutrient reservoir. The plastic pot had a standpipe to control the water level during irrigation and had perforations at the bottom for drainage. Each pot contained approximately 11.5 kg of silica sand. For the biosolid treatment, a compost produced in the wastewater reclamation district of greater Chicago in 1975 (Nu-earth) was used at a rate equivalent to 20 Mg ha^{-1}. The concentrations of selected metal elements in this biosolid compost are summarized in Table I. The metal concentrations of this material exceeded the federally mandated maximum contaminant level (MCL) for biosolids of exceptional quality. It represented the upper limits of what we investigated. In later experiments, we compared responses to contemporary biosolids against those to Nu-earth.

Seeds were germinated in a growth chamber and seedlings transplanted at 3 to 5 days. After irrigation, excess nutrient solution drained into the reservoir under gravity. Each reservoir contained 100 L of nutrient solution. For the biosolid treatment, Zn, Mn, Cu, and Ni along with H_2-EDTA in the nutrient solution were withheld. Thus, uptake of metals came exclusively from the biosolid. All of the pots supported by a reservoir received the same treatments (i.e. species, growing period, and biosolid).

2.1. Root-exudate extraction

For root-exudate collection, we grow the plants in septic conditions because plant-nutrient media foster microbial growth. Root exudates accumulating under septic conditions were a more realistic depiction of field conditions. To collect exudates, pots were removed from the plant-growing unit, the sand media were flushed three times with deionized water, and the plants were allowed to photosynthesize for 5 h. Root exudates were flushed out with 2 L of deionized water, collected in plastic bottles, and stored at –80°C.

2.2. Biomass and plant-tissue samples

Following the collection of exudates, plants were removed from the pots and separated into shoot and root portions, cleaned with deionized water, dried at 65°C for 5 days and weighed for biomass. The plant tissues were digested in Teflon Parr bombs by a perchloric acid microwave procedure for metal determination using ICP-OES spectroscopy [13].

Table I. Concentrations of selected metals in the biosolid (from [12])

Element	Concentration (mg kg^{-1})		
	Range	Mean	St. Dev.
As	20–25	23	1
Cd	204–250	229	12
Cr	3,100–3,800	3,480	202
Cu	1,030–1,460	1,330	92
Ni	656–771	721	31
Se	2.0–3.8	2.8	0.3
Zn	4,260–5,090	4,800	274

2.3. Concentration of root exudates

The amounts of root exudates collected were small relative to the volume of water used for flushing. For chemical analyses, 250-mL aliquots of the stored solution were freeze-dried and stored at –20°C. Organic acids in the concentrated exudate preparations were analyzed. Data in the literature indicate that organic acids are the primary constituents of root exudates and are the components that are most reactive with metals. We employed a Dionex Anion Chromatographic Instrument with an IonPac ICE-AS6 column for rapid determination of the primary organic-acid components.

3. RESULTS AND DISCUSSION

3.1. Plant growth

The rate of biomass accumulation over the growing season was significantly affected by the biosolid treatment. With corn, the differences in plant growth between those treated and those not treated with biosolids was apparent from 2 weeks after planting (Table II). Shoot and root biomass exhibited similar growth patterns.Differences in rates of growth continued throughout the growing season.

Biomass accumulation for the various species depended on treatment. Plants grown on the standard growth medium received full-strength nutrient solution and their biomass represented the typical growth pattern for each species. The biomass accumulations of wheat, Sudan grass, corn, canola, chickpea, and Swiss chard grown on biosolid-treated growth medium were comparable to or greater than that of the corresponding species grown on the standard medium (Table III).

In this experiment, a rock-phosphate (RP) treatment was included because plants were expected to respond differently to P as affected by excretion of root exudates. This treatment may be used to calibrate the plants, in terms of responses to biosolid treatments. The plants grown on RP-treated growth medium received a nutrient solution of the same formulation except that P was withheld to induce the plants obtain P from the RP (a P source only marginally available to plants) mixed into the sand.

Ability to utilize RP was species-dependent. Dicotyledonous plants (canola, chickpea, and Swiss chard) used RP effectively (Table III). Monocotyledonous plants (wheat, Sudan grass, and corn) were unable to use RP effectively. Growth was retarded and biomass accumulations over the 4-week period were considerably less than with the other treatments.

3.2. Root exudates

Composition of root-exudates of corn were determined under biosolid-treated and standard conditions for 2 to 16 weeks. Routinely found in corn exudates were: lactic, acetic, butyric, glutaric, succinic, tartaric, maleic, and oxalic acids. Propionic, pyruvic, and valeric were occasionally found. The primary components of organic acids in root exudates were essentially the same for all stages of growth (Table IV). The relative amounts of each component, however, varied from stage to stage and varied with the biosolid treatment. Two weeks following planting, butyrate, tartrate, and acetate accounted for 86% of the total recovered organic acids of corn grown on standard medium, and lactate, acetate, butyrate, and tartrate accounted for 95% of the total recovered organic acids in corn grown on media treated with biosolids. For root exudates recovered from corn grown on medium not treated with biosolids, fractions of butyrate and tartrate in the total mixture decreased over time, while the lactate and maleate fractions increased. For root exudates recovered from corn grown on medium treated with biosolids, fractions of butyrate decreased, lactate and tartrate remained essentially the same, and acetate increased over the 16 weeks.

Although biosolid treatment did not appear to affect the types of organic acid present, it stimulated organic-acid production by two to three fold.

The relative amounts of organic acids in a typical root exudate from corn grown on the standard and biosolid-treated growth media are shown in Table V.

Table II. Mean values for growth of corn in standard and biosolid-treated sand culture media

Time (weeks)	Standard nutrient			Biosolid treated		
	Shoot	Root	Total	Shoot	Root	Total
	(g dw pot^{-1})					
2	4.9	1.9	6.8	5.9	2.3	8.2
4	8.3	3.2	10.4	40.1	7.4	47.5
8	11.3	3.8	15.1	69.2	20.2	89.4
12	21.3	4.7	26.0	81.3	25.2	107
16	21.5	5.1	26.6	83.8	26.9	111

Table III. Biomass yields of plants grown on standard nutrient solution, biosolid-treated, and rock-phosphate-treated culture media

Species	Standard medium		Biosolid treated		Rock phosphate	
	Mean (g pot^{-1})	CV (%)	Mean (g pot^{-1})	CV (%)	Mean (g pot^{-1})	CV (%)
Wheat	12.0	15	13.3	15	2.57	30
Sudan grass	17.8	30	24.0	14	3.18	7
Corn	15.8	9	22.6	8	3.29	17
Canola	11.6	10	15.5	21	15.4	2
Chickpea	11.3	4	10.9	35	12.0	11
Swiss chard	9.79	12	7.36	21	8.36	24

Table IV. Primary organic acids in root exudates of corn grown in sand culture with and without biosolids

Time (weeks)	Standard medium	Biosolid-treated
2	Acetic, butyric, and tartaric	Lactic, acetic, butyric, tartaric
4	Lactic, butyric, and tartaric	Lactic, butyric, and tartaric
8	Lactic, butyric, tartaric, and maleic	Acetic, propionic, and maleic
12	Lactic, butyric, and tartaric	Lactic and acetic
16	Acetic, butyric, tartaric, maleic, and oxalic	Acetic and tartaric

Table V. Relative amounts of organic acids in a typical root exudate from corn grown on the standard and biosolid-treated growth media contained the following

Component acid	Fraction	
	Standard medium	Biosolid treated
Lactic	0.16	0.20
Acetic	0.07	0.36
Propionic	—	0.04
Butyric	0.37	0.12
Valeric	0.06	—
Glutaric	0.02	0.03
Succinic	0.03	0.01
Tartaric	0.18	0.14
Maleic	0.12	0.07
Oxalic	0.05	0.02

There were 1.46 and 1.26 moles of COO^{-} ligand in each mole of mixed organic acids in the root exudates of corn grown in standard and biosolid-treated media, respectively, to complex. The lactic, butyric, tartaric, maleic, and oxalic acids accounted for 84% of metal-complexing capacity of root exudates in standard medium. The lactic, acetic, butyric, tartaric, and maleic acids accounted for 87% of the metal-complexing capacity of the root exudates of corn grown on biosolid-treated medium. These acids were the basis for developing synthetic root exudates.

3.3. Metal concentrations in plant tissue

In general, metal concentrations in plant roots were considerably higher than those present in the respective shoots, and plants grown in biosolid-treated medium had significantly higher concentrations of metals than those in the standard sand medium at all stages of the growth. For metals that were either not readily available (such as Pb) or present in limited amounts (such as Cd in the standard medium), the amount of metals absorbed appeared to be limited by the supply in the growth media. Their concentrations in plant tissue decreased as biomass accumulated over time through mass dilution (Tables VI and VII).

For metals that were chemically available to plants and in abundant supply (such as Cd in biosolid medium or Zn in both), the metal concentrations of plant tissue continued to rise with plant growth, especially in roots (Tables VII and VIII). In shoots, accumulations of Cd, Ni, and Zn were especially notable, because their concentrations with biosolid treatment were considerably higher than those with the standard medium. It is noteworthy that metal concentrations in plant tissues decreased with the length of the growing period, indicating that availability did not keep pace with the rate of biomass accumulation.

Based on the mass of metals that are present in the growth medium and absorbed by corn (Table IX), the phyto-availability of metals were in the following order: Cd > Ni = Zn > Cu = Pb > Cr.

The rates of metal uptake were determined as micrograms of metal absorbed per gram of biomass increase per 2-week interval ($\mu g\ g^{-1}\ 2\ wk^{-1}$). For Cd in the standard sand medium where the supply was limited, there was little additional uptake after the first 2 weeks of growth (Table X). When the metal supply was not limited, the uptake rate by corn remained reasonably constant throughout the growth period (Tables X–XII). Toward the end the growing season, however, there was a tendency for the relative uptake rate to increase as biomass accumulation slowed while plant absorption of metals continued. During the active growth period (up to approximately 12 weeks), the uptake rates by shoots (7, 12, and 160 $\mu g\ g^{-1}\ 2\ wk^{-1}$ for Cd, Pb, and Zn, respectively) appeared to be proportional to their respective mass inputs (19,198, 55,608, and 386,076 $\mu g\ pot^{-1}$, respectively) (Tables IX–XII).

Table VI. Concentration of Cd in corn grown in standard and biosolid-treated media

Time (weeks)	Shoot		Root	
	Standard	Biosolid treated	Standard	Biosolid treated
	(mg kg^{-1})			
2	0.48	7.16	1.85	36.8
4	0.27	7.25	1.79	37.5
8	n.d.[a]	9.24	n.d.[+]	47.4
12	n.d.	9.57	n.d.[+]	64.5
16	n.d.[+]	9.71	n.d.[+]	70.9

[a] Below detection limit of the instrument of <10 μg L^{-1}.

Table VII. Pb contents of corn grown in standard and biosolid-treated media

Time (weeks)	Shoot		Root	
	Standard	Biosolid treated	Standard	Biosolid treated
	(mg kg^{-1})			
2	5.73	15.3	5.57	37.4
4	4.86	11.6	2.98	17.4
8	4.29	10.1	2.98	12.5
12	3.12	9.38	2.35	10.
16	2.98	9.32	1.94	10.1

Table VIII. Zn contents of corn grown in standard and biosolid-treated media

Time (weeks)	Shoot		Root	
	Standard	Biosolid treated	Standard	Biosolid treated
	(mg kg^{-1})			
2	29.8	157	57.8	235
4	29.9	164	59.3	187
8	37.7	228	92.6	165
12	38.3	309	125	158
16	42.3	348	141	150

Table IX. Metal uptake by corn grown on biosolid-treated medium

Element	Mass input	Mass output	Plant uptake
	(μg pot^{-1})		(% input)
Cd	19,198	2,722	14
Cr	237,818	263	0.11
Cu	121,829	1,980	1.6
Ni	55,608	5,434	9.8
Pb	77,167	1,052	1.4
Zn	386,076	33,196	8.6

Table X. Rate of Cd uptake by corn at various stage of growth

Time period (weeks)	Shoot		Root	
	Standard	Biosolid treated	Standard	Biosolid treated
	($\mu g\ g^{-1}\ 2\ wk^{-1}$)			
0–2	2.4	7	3.5	37
2–4	n.d.[a]	7	n.d.	38
4–8	n.d.	6	n.d.	27
8–12	n.d.	6	n.d.	87
12–16	n.d.	7	n.d.	73

[a] Not detected.

Table XI. Rate of Zn uptake by corn at various stage of growth

Time period (weeks)	Shoot		Root	
	Standard	Biosolid treated	Standard	Biosolid treated
	($\mu g\ g^{-1}\ 2\ wk^{-1}$)			
0–2	30	157	58	235
2–4	30	165	74	166
4–8	30	158	77	77
8–12	20	386	132	63
12–16	236	813	161	17

Table XII. Rate of Pb uptake by corn at various stage of growth

Time period (weeks)	Shoot		Root	
	Standard	Biosolid treated	Standard	Biosolid treated
	($\mu g\ g^{-1}\ 2\ wk^{-1}$)			
0–2	6	15	6	37
2–4	4	11	5	8
4–8	3	8	n.d.[a]	10
8–12	2	5	n.d.	2
12–16	n.d.	7	n.d.	5

[a] Not detected.

Table XIII. Accumulation of Cd, Pb, and Zn in shoots of plants grown on standard and biosolid-treated media

Species	Cd		Pb		Zn	
	Standard	Biosolid	Standard	Biosolid	Standard	Biosolid
	(μg pot^{-1} 4 wk^{-1})					
Wheat	15	264	19	120	306	2,506
Sudan grass	22	479	18	128	354	5,768
Corn	14	377	21	190	280	3,981
Canola	18	212	10	175	289	3,366
Chickpea	12	95	22	62	103	1,375
Swiss chard	12	182	20	37	167	2,071

Table XIV. Accumulation of Cd, Pb, and Zn in roots of selected plants grown on standard and biosolid-treated media

Species	Cd		Pb		Zn	
	Standard	Biosolid	Standard	Biosolid	Standard	Biosolid
	(μg pot^{-1} 4 wk^{-1})					
Wheat	7	100	10	64	158	1,984
Sudan grass	10	163	25	60	141	3,400
Corn	7	168	36	105	148	2,419
Canola	2	50	5	68	59	756
Chickpea	4	140	26	53	157	2,580
Swiss chard	2	66	8	15	51	797

The six species absorbed similar amounts of metals from the growth media when supplies were limited, such as in the cases of Cd and Pb in the standard medium (Tables XIII and XIV). When the supplies in the growth media were not limiting, plants absorbed different amounts of Cd, Pb, and Zn.

4. CONCLUSIONS

- Although the same organic acids were present over a 16-week growing season, primary components in root exudates of corn differed when biosolids were added to the rooting medium.
- Metal concentrations in plant roots were considerably higher than in shoots and plants grown in the biosolid-treated growing media had significantly higher concentrations of metals than those of the standard sand media at all stages of the growth.
- Based on the mass of metals present in the growth media and absorbed by corn, the phyto-availability of biosolid-borne metals was in the order: Cd > Ni = Zn > Cu = Pb > Cr.
- In the biosolid-treated growth medium, the uptake rates of Cd, Pb, and Zn by corn shoots were relatively constant and were proportional to their respective mass inputs.

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EFFECTS OF IRRADIATED SEWAGE SLUDGE ON SOIL AND YIELDS OF CHILE PEPPER

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Abstract

Irradiated (3.6–4.4 kGy) and non irradiated sewage sludge were applied to a chile (*Capsicum annuum* L.) crop at 6, 12, 18, or 24 t/ha. The total N content of the sludge was approximately 1%. The presence of the sewage sludge increased soil organic matter and crop N assimilation and yield. The application of even non-irradiated sewage sludge at 24 t/ha did not increase microbial pathogen counts in the soil. Of several heavy metals examined, only the micronutrient Zn was increased in the soil as a result of application of sewage sludge. There were no increases in heavy-metal content in the harvested fruit as a result of sewage-sludge application to the soil.

1. INTRODUCTION

Sewage sludge is the solid residue that is collected during the processing of wastewater. Sludge contains valuable inorganic elements such as N, P, and K, and the organic fraction can have a soil-conditioning effect. Therefore, effort is being expended in examining the utility of recycling sludge as a fertilizer and soil conditioner in agriculture [1]. However, sewage sludge usually contains pathogens, toxic compounds, and heavy metals. It should be processed to eliminate pathogenic organisms such as *Escherichia coli* and *Salmonella* [2–4], and to reduce heavy metals and toxic compounds to safe levels for public use.

Irradiation treatment can solve these problems. Watanabe and Takehisa [5] found total bacterial and coliform counts of up to 3.0×10^9 and 3.5×10^8/g, respectively, in dewatered sludge. The coliforms in dewatered sludge were eliminated with a dose of 5 kGy, whereas liquid sludge required only 3 kGy.

The objectives of this research were:

- to quantify the contribution of N from sewage sludge to chile peppers, using ^{15}N techniques,
- to assess increases in crop yields as a result of application of sewage sludge,
- to assess improvements in soil properties, particularly increases in organic matter content and water-holding capacity,
- to estimate pathogenic-organism content in non-irradiated (NISS) and irradiated sewage sludge (ISS), and in soil to which they are applied,
- to assess the extent of soil-contamination by heavy metals by the use of sewage sludge.

2. MATERIALS AND METHODS

A field experiment was carried out in an Oxisol at Jakarta, Indonesia. The physical and chemical characteristics of the soil were: clay content 69%; silt content 30%; sand content 0.7%; pH (H_2O) 5.4; pH (KCl) 4.3; cation exchange capacity 27 mEq/100 g; N content 0.15%**;** P_2O_5 9 ppm; and K_2O 11 ppm.

Irradiated and non irradiated sewage sludge (ISS and NISS, respectively, characteristics described below) were applied at four levels. The complete treatment protocol was: 1. 100 kg N/ha + 20 kg ^{15}N/ha (“+N” control); 2. 0 kg N/ha + 20 kg ^{15}N/ha (“zero N” control); 3. 6 t NISS/ha + 20 kg ^{15}N/ha; 4. 12 t NISS/ha + 20 kg ^{15}N/ha; 5. 18 ton NISS/ha + 20 kg ^{15}N/ha; 6. 24 t NISS/ha + 20 kg ^{15}N/ha; 7. 6 t ISS/ha + 20 kg ^{15}N/ha; 8. 12 ton ISS/ha + 20 kg ^{15}N/ha; 9. 18 ton ISS/ha + 20 kg ^{15}N/ha; and 10. 24 ton ISS/ha + 20 kg ^{15}N/ha. A randomized complete block design (RCBD) was used, with four replications. The N contents of the NISS and ISS were 1.03% and 1.00% respectively. Fertilizer N was in the form of urea and ^{15}N-labelled ammonium sulphate with 10.7% ^{15}N. Fertilizer P and K were given at 120 kg P_2O_5/ha and 120 kg K_2O/ha respectively. The nationally recommended rates of application are 120 kg N/ha, 120 kg P_2O_5/ha, and 120 kg K_2O/ha respectively. Chile pepper (*Capsicum annuum* L.) was used as the experimental crop.

Sewage sludge was collected from the Pulo Gebang Municipal Sewage Sludge Receiving Station, East Jakarta. The sludge, water content 59%, was irradiated at 3.6 to 4.4 kGy using a ^{60}Co source. Both ISS and NISS were applied to the field, air-dried, at transplanting (35 day-old plantlets), together with N, P, and K. Soil sampling was done before transplanting and after harvesting. The chile fruits were harvested several times. The last harvest was at 175 days after sowing, at which time shoots (stover) were also collected. Soil samples were taken for microbe analysis, and oven-dried samples fruits, stover, and soil were used for chemical analyses. Soil water-holding capacity values were determined before and after the experiment. Soil moisture was measured by with a neutron probe, and precipitation data were collected.

3. RESULTS AND DISCUSSION

3.1. Crop yield

The yields of chile fruit and stover dry-matter production are shown in Table I.

Application of sewage sludge, both irradiated (ISS) and non irradiated (NISS) increased chile yields and dry matter production. Increasing levels of application increased yield and dry matter production up to 24 t/ha.

Table I. Yields of chile pepper

Treatment	Fruit		Stover dry wt.	Plant dry wt.
	Fresh wt.	Dry wt.		
	(t/ha)			
100 kg N + 20 kg ^{15}N	10.64	1.92	1.42	3.34
0 kg N + 20 kg ^{15}N	7.55	1.60	0.77	2.37
6 t NISS[a] + 20 kg ^{15}N	7.30	1.46	0.91	2.37
12 t NISS + 20 kg ^{15}N	9.08	1.82	1.15	2.97
18 t NISS + 20 kg ^{15}N	12.52	2.50	1.36	3.86
24 t NISS + 20 kg ^{15}N	12.65	2.38	1.51	3.89
6 t ISS[b] + 20 kg ^{15}N	6.65	1.31	1.11	2.42
12 t ISS + 20 kg ^{15}N	4.19	1.02	1.22	2.24
18 t ISS + 20 kg ^{15}N	11.45	2.15	1.11	3.26
24 t ISS + 20 kg ^{15}N	11.05	2.16	1.15	3.31
C.V. (%)	33.2	27.8	28.8	22.4
$LSD_{0.05}$	4.51	0.74	ns[c]	0.98

[a]Non-irradiated sewage sludge. [b]Irradiated sewage sludge. [c]Not significant.

The yields obtained at 18 and 24 t/ha levels of both ISS and NISS were significantly higher than those obtained at 6 t/ha sewage sludge and the zero-N control, and not significantly different from the yields with the +N control. In this experiment, ISS and NISS had similar effects on chile yields. Total dry matter production at 18 and 24 t/ha of ISS and NISS was 3.3 to 3.9 t/ha, whereas with 6 t/ha and the zero-N control it was only around 2.4 t/ha

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3.2. Nitrogen uptake

Application of sewage sludge increased total N uptake (Table II). Most of the N taken up by the chile was sequestered in the fruit. Generally, the higher the rate of application the higher was the amount of N in the fruits and the plant as a whole. Application of 18 to 24 t sewage sludge/ha resulted in total-N values not significantly different from that of the +N control. Clearly, the presence of sewage sludge at 6 t/ha was inadequate to supply the N needs of the crop.

As with N as a whole, ^{15}N was accumulated mainly in the fruits (Table II). The lowest uptake was found with the +N control, and the highest uptake resulted with ISS at 18 t/ha. The ^{15}N uptake in the zero-N treatment was not significantly different from the values obtained with ISS and NISS. The unexpectedly low uptake of ^{15}N with 12 t/ha of ISS probably was caused by variability in the soil.

Nitrogen uptake from sewage sludge by the chile plants in Table II was the difference between total N uptake when treated with sewage sludge and the total N of the zero-N control. The highest uptake was 31.6 kg N/ha accumulated with 24 t NISS/ha; the next highest uptake was with 18 t NISS/ha, and little was assimilated from 6 to 12 t NISS/ha. Uptake of N from ISS showed a similar trend, although the amounts were lower.

The lowest efficiency of uptake of ^{15}N (7.6%) was found with the +N control and the highest (21%) was obtained with 18 t ISS/ha.

Table II. Total N uptake, fertilizer N uptake, sludge N uptake, and fertilizer use efficiency (FUE)

Treatment	Total N			Fertilizer N			Sludge N[a]	FUE (%)
	Fruits	Stover	Plant	Fruits	Stover	Plant		
	(kg N /ha)							
100 kg N + 20 kg ^{15}N	33.5	19.7	53.2	1.04	0.47	1.51	–	7.6
0 kg N + 20 kg ^{15}N	28.3	12.3	40.6	2.25	0.67	2.90	–	14.5
6 t NISS + 20 kg ^{15}N	25.5	13.4	38.9	2.42	0.79	3.20	nc[b]	16.1
12 t NISS + 20 kg ^{15}N	32.3	18.6	50.9	2.32	0.66	2.98	10.3	14.9
18 t NISS + 20 kg ^{15}N	46.8	22.0	68.8	2.58	0.85	3.43	28.2	17.2
24 t NISS + 20 kg ^{15}N	44.8	27.4	72.2	2.33	0.87	3.20	31.6	16.0
6 t ISS + 20 kg ^{15}N	24.2	20.5	44.7	1.63	0.80	2.43	4.1	12.2
12 t ISS + 20 kg ^{15}N	18.4	20.5	39.0	1.44	0.78	2.22	nc	11.1
18 t ISS + 20 kg ^{15}N	39.3	20.9	60.2	3.09	1.07	4.16	19.6	20.8
24 t ISS + 20 kg ^{15}N	42.0	19.8	61.7	2.92	0.82	3.74	21.1	18.7
C.V. (%)	19.6	34.4	24.6	32.9	33.5	31.4		31.4
LSD 0.05	14.4	ns	18.9	1.05	ns	1.36		6.8

[a]Calculated by difference. [b]Not clear.

3.3. **Heavy metals**

Table III shows heavy-metal contents of the non-irradiated and irradiated sludges, and of the soil prior to the experiment, and Table IV shows the heavy-metal determinations after harvest. Only Zn content of soil after harvest showed an increasing trend resulting from application of sewage sludge: from 111 to 155 ppm for the soil for 6 to 24 t sludge/ha, whereas the control values were around 88 to 95 ppm (Table IV). Clearly, application of sewage sludge increased Zn content although Zn was taken up by the crop, and was measured at 183 ppm prior to planting (Table III).

Patterns of heavy-metal uptake by the chile plants are shown in Tables V and VI. Applications of sewage sludge at 6 to 24 t/ha did not result in a trend of increasing uptake of Fe, Zn, Co, or Mn content in chile fruits (Table V) or stover (Table VI). Nickel, Cd, Pb, Cr, and Co were undetectable.

Table III. Heavy metal content of sewage sludge and soil prior to the experiment

Metal	NIS	ISS	Soil
Fe (%)	4.43	4.49	7.00
Zn (ppm)	1500	960	183
Cu	68	88	54
Ni	22	27	17
Cd	2	2	1
Pb	71	68	45
Cr	36	37	12
Co	35	33	44
Mn (%)	0.18	0.18	0.22

Table IV. Heavy-metal content of the soil after the experiment

Treatment	Fe	Zn	Cu	Ni	Cd	Pb	Cr	Co	Mn
	(%)	(ppm)							(%)
100 kg N + 20 kg ^{15}N	7.31	95	54	12	1	41	10	42	0.25
0 kg N + 20 kg ^{15}N	7.78	88	55	12	1	37	10	42	0.26
6 t NISS + 20 kg ^{15}N	7.93	113	55	12	1	34	9	42	0.28
12 t NISS + 20 kg ^{15}N	7.97	125	56	12	1	37	9	41	0.25
18 t NISS + 20 kg ^{15}N	7.71	150	55	12	1	40	10	42	0.26
24 t NISS + 20 kg ^{15}N	7.67	155	56	12	1	39	11	40	0.26
6 t ISS + 20 kg ^{15}N	7.78	111	56	12	1	39	11	41	0.25
12 t ISS + 20 kg ^{15}N	7.56	155	58	13	1	39	9	41	0.25
18 t ISS + 20 kg ^{15}N	7.66	123	56	13	1	40	9	41	0.25
24 t ISS + 20 kg ^{15}N	7.76	125	57	13	1	38	9	42	0.26
C.V. (%)	4.8	16.6	3.3	10.2	–	13.7	12.5	4.9	8.1
LSD 0.05	ns	30	ns	ns	–	ns	ns	ns	ns

Table V. Heavy-metal content of chile fruits

Treatment	Fe	Zn	Cu	Ni	Cd	Pb	Cr	Co	Mn
	(ppm)								
100 kg N + 20 kg ^{15}N	113	15	10	Ud[a]	Ud	Ud	Ud	Ud	20
0 kg N + 20 kg ^{15}N	127	18	11	Ud	Ud	Ud	Ud	Ud	20
6 t NISS + 20 kg ^{15}N	115	15	11	Ud	Ud	Ud	Ud	Ud	18
12 t NISS + 20 kg ^{15}N	103	17	11	Ud	Ud	Ud	Ud	Ud	15
18 t NISS + 20 kg ^{15}N	90	15	11	Ud	Ud	Ud	Ud	Ud	18
24 t NISS + 20 kg ^{15}N	153	15	11	Ud	Ud	Ud	Ud	Ud	15
6 t ISS + 20 kg ^{15}N	138	18	11	Ud	Ud	Ud	Ud	Ud	18
12 t ISS + 20 kg ^{15}N	148	15	11	Ud	Ud	Ud	Ud	Ud	18
18 t ISS + 20 kg ^{15}N	90	18	11	Ud	Ud	Ud	Ud	Ud	13
24 t ISS + 20 kg ^{15}N	100	15	11	Ud	Ud	Ud	Ud	Ud	18
C.V. (%)	46.2	18.6	5.4	–	–	–	–	–	21.2
$LSD_{0.05}$	ns	ns	ns	–	–	–	–	–	ns

[a] Undetectable.

Table VI. Heavy-metal content of chile stover

Treatment	Fe	Zn	Cu	Ni	Cd	Pb	Cr	Co	Mn
	(ppm)								
100 kg N + 20 kg ^{15}N	378	38	17	Ud[a]	Ud	Ud	Ud	Ud	75
0 kg N + 20 kg ^{15}N	358	40	20	Ud	Ud	Ud	Ud	Ud	88
6 t NISS + 20 kg ^{15}N	308	38	21	Ud	Ud	Ud	Ud	Ud	55
12 t NISS + 20 kg ^{15}N	358	40	20	Ud	Ud	Ud	Ud	Ud	78
18 t NISS + 20 kg ^{15}N	360	38	18	Ud	Ud	Ud	Ud	Ud	65
24 t NISS + 20 kg ^{15}N	258	40	20	Ud	Ud	Ud	Ud	Ud	63
6 t ISS + 20 kg ^{15}N	255	35	19	Ud	Ud	Ud	Ud	Ud	65
12 t ISS + 20 kg ^{15}N	350	35	21	Ud	Ud	Ud	Ud	Ud	65
18 t ISS + 20 kg ^{15}N	275	40	19	Ud	Ud	Ud	Ud	Ud	58
24 t ISS + 20 kg ^{15}N	290	38	20	Ud	Ud	Ud	Ud	Ud	68
C.V. (%)	24.5	26.5	11.2	–	–	–	–	–	23.9
$LSD_{0.05}$	ns	ns	ns	–	–	–	–	–	ns

[a] Undetectable.

3.4. Soil organic matter

Values for organic matter content of soil prior to the experiment are shown in Table VII, and for after the experiment they are in Table VIII. The latter varied from 2.64% to 3.65%, showing responses to application of sludge. The soil organic matter contents were 2.90% to 3.65% with sewage sludge, and only 2.64% to 2.85% for the control treatments.

Table VII. Soil organic matter, water-holding capacity and pathogen counts for sewage sludge and for the soil prior to the experiment

Component	NISS	ISS	Soil
Organic matter (%)	12.83	9.97	3.34
Pathogen WHC (%)	12×10^3	2.2×10^3	36×10^3
	–	–	79

Table VIII. Soil organic matter (SOM), soil water holding capacity (WHC) and pathogen counts of the soil after harvest

Treatments	SOM (%)	WHC (%)	Pathogen content
100 kg N + 20 kg ^{15}N	2.85	81	22.6×10^4
0 kg N + 20 kg ^{15}N	2.64	81	22.0×10^4
6 t NISS + 20 kg ^{15}N	3.10	80	26.5×10^4
12 t NISS + 20 kg ^{15}N	2.90	82	23.3×10^4
18 t NISS + 20 kg ^{15}N	3.29	84	31.2×10^4
24 t NISS + 20 kg ^{15}N	3.65	83	12.8×10^4
6 t ISS + 20 kg ^{15}N	3.97	81	17.3×10^4
12 t ISS + 20 kg ^{15}N	3.14	82	23.9×10^4
18 t ISS + 20 kg ^{15}N	3.21	84	35.3×10^4
24 t ISS + 20 kg ^{15}N	3.18	84	17.3×10^4
C.V. (%)	8.10	3.2	–
$LSD_{0.05}$	0.36	ns	–

These data show that organic matter, both in soil prior to the experiment and in the sewage sludge treatment (Table VII), were used by the chile crop as a nutrient source and as a soil conditioner.

3.5. Soil water-holding capacity

Soil water-holding capacity values before (Table VII) and after (Table VIII) the experiment were similar. There was no effect of application of sewage sludge, even at 24 t/ha.

3.6. Pathogen content

Pathogen counts before (Table VII) and after (Table VIII) the experiment were low indicating that the sewage sludge from the Pulo Gebang station was effectively composted.

3.7. Soil moisture

Soil-moisture and rainfall-distribution data (Table IX) indicate that the chile crop had adequate moisture throughout the growing season, i.e. from 9 December 1995 to 8 May 1996.

Table IX. Profiles of soil moisture, soil bulk density, and precipitation

Date	Depth		
	20 cm	40 cm	60 cm
	(%)		
15/01/1966	53.8	55.3	58.9
22/01/1966	53.2	54.4	56.9
24/01/1966	52.9	54.6	56.7
05/02/1966	53.5	55.0	56.7
14/02/1966	55.3	56.5	57.2
04/03/1966	53.6	55.6	58.4
18/03/1966	52.9	54.2	55.9
08/05/1966	53.8	56.5	59.4
Soil bulk density	(g/cm^3)		
	1.080	1.196	1.188
Precipitation	December 1995 = 115 mm/6 days January 1996 = 350 mm/15 days February 1996 = 478 mm/19 days March 1996 = 202 mm/9 days April 1996 = 253 mm/16 days May 1996 = 37 mm/1 days		

4. CONCLUSIONS

From the results as a whole we draw the following conclusions:

- Sewage sludge supplied N to support the growth of the crop of chile peppers.
- Application of sewage sludge at 18 to 24 t/ha significantly increased yields.
- Application of sewage sludge significantly increased soil organic matter, but not soil water-holding capacity.
- Application of even non-irradiated sewage sludge at 24 t/ha did not increase pathogen counts in the soil.
- Even at 24 t/ha sewage sludge, a significant increase in heavy metals was obtained only for Zn. Nickel, Cd, Pb, Cr, and Co were undetectable by atomic absorption spectrometry.

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