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RESEARCH ARTICLE

Heavy metal uptake by wheat from a sewage sludge-amended calcareous soil

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Abstract The objective of this 4-year study was to determine single and repetitive effects of sewage sludge applications on the accumulation of lead (Pb), cadmium (Cd), zinc (Zn) and copper (Cu) in soil and wheat (*Triticum aestivum*). A single sludge application at a rate of 100 Mg ha^{-1} (for all the metals) and at a rate of 50 Mg ha^{-1} (for Cu) significantly increased DTPA-extractable metal concentrations 4 years later. DTPA-extractable concentrations of Pb, Zn and Cu were closely correlated with the total concentrations in soil. Their relationships between metal uptake in stalks and DTPA-extractable metal concentrations in soil were approximately linear for Pb, Cd and Cu, but better described by a quadratic equation for Cd and Zn. TF for Pb, Zn and Cu, BF for all metals and BCF for Pb, Cd and Zn were lower in wheat grown on sludge-treated than control plots.

Keywords Biosolids · Sewage sludge · Wheat · Heavy metals · Availability · Uptake

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Introduction

Sewage sludge application on agricultural land as a fertilizer enables the recycling of valuable components such as organic matter and many plant nutrients such as N, P, K and micronutrients (Logan and Harrison 1995; Singh and Agrawal 2007). This can result in beneficial effects on soil fertility and plant nutrition (Casado-Vela et al. 2007; Gascó and Lobo 2007). Moreover the organic matter added by sludge can improve many physical properties of agricultural soils such as water holding capacity, aeration and porosity (Engelhart et al. 2000). However, if the quality of the sludge is not controlled, its application can also result in excessive concentrations of trace metals such as Pb, Cd, Zn, Cu and Ni in soils (Adamu et al. 1989; Al-Najar et al. 2005; Keller et al. 2001).

Some authors reported that the plant availability of heavy metals increased following soil applic ation of sewage sludge (Logan et al. 1997; McBride 1995; McGrath et al. 2000) but other researchers postulated that “aging” would gradually decrease metal availability with time after cessation of sludge application (Rundle et al. 1982). Keller et al. (2001) found metal uptake from sludge-treated soil was different among crops and various parts of the plant. In their study, although metal bioavailability was slowly decreasing with time, bioavailable metal concentrations remained high enough to make most crop production on heavily sludge treated plots unacceptable for human or animal consumption according to the standards in Switzerland.

During a 4-year field experiment, Afyuni et al. (2006) observed that after sludge application, in subsequent years with no further sludge application, EDTA-extractable metal concentrations in soil showed a decreasing trend but, even after 4 years EDTA-extractable metal concentrations were still significantly greater in plot that had received more than 45 Mg ha^{-1} sludge than in control plots. Chang et al. (1987) reported that Cd and Zn uptake by plants (Swiss chard and radish) was highest in the first 2 years after sludge application and then decreased with time to much lower rates. Chaney and Ryan (1993) postulated an "uptake plateau," meaning that the bioavailability and crop uptake of toxic metals approaches a maximum as metal loadings increase.

The majority of calcareous soils in arid regions of Iran are low in fertility, organic matter content and productivity. These soils are usually characterized by high pH due to the presence of carbonates and by low contents in organic matter. Farmers are extensively using sewage sludge as a cheap fertilizer and there is no regulations controlling sludge quality or limiting rate of sludge application. Some studies have been done on plots with low sludge application. But with increased disposal of sewage sludge on arable lands there is a need to understand effects of high level applications of sludge and its repetitive application in consecutive years on availability and plant uptake of heavy metals in Iranian agricultural soils.

There is a hypothesis that even though the total added metals are increased with heavy sludge application, at the same time the added organic mater will significantly increase the cation exchange capacity (CEC) of the soil. This, in addition to high pH and $CaCO_3$ contents of these soils, will cause the reduction of plant availability and uptake of heavy metals by plants, especially with time. To accept or reject this hypothesis, a 4-year field experiment was carried out.

The objective of this study was to evaluate single and repetitive effects of sewage sludge applications on the concentrations of Pb, Cd, Zn and Cu in soil, their availability to plant and their uptake by wheat.

Materials and methods

Experimental design

The experiment was conducted at the Isfahan University of Technology Research Station, located approximately 40 km south west of Isfahan city (32°32′ N; 51°23′ E). The average annual rainfall at this site is 140 mm and the mean temperature is 14.5°C. The soil of the experimental plots was a fine-loamy, mixed, thermic, Typic Haplargid. Corn (*Zea mays L*) was planted as a spring crop, followed by wheat (*Triticum aestivum*) as a winter crop.

The sludge used in this experiment was secondary, aerobically digested municipal sewage sludge from the city of Isfahan. Selected chemical properties are given in Table 1. Compared to the standard values for tolerable metal concentrations in sewage sludge in the U. S. (U. S. Environmental Protection Agency 1993), none of the metals exceeded the tolerance limit.

The experimental design consisted of completely randomized blocks with treatments arranged in split plots with 1 m wide alleys between the plots. The treatments were replicated three times. Starting in June 2000, several rates (25, 50 and 100 Mg ha^{-1}) of air-dried sewage sludge were applied repeatedly over 4 years to plots of 12 × 3 m size and mixed into the topsoil (20 cm depth).

Table 1 Means of selected chemical properties of the applied sludge

Parameter	Unit	Amount
pH	–	6.4
Electrical conductivity	dS m^{-1}	9.4
Organic matter	g kg^{-1}	310
Nitrogen	g kg^{-1}	19
Phosphorus	g kg^{-1}	14.3
Potassium	g kg^{-1}	6.4
Iron	g kg^{-1}	18.7
Manganese	g kg^{-1}	0.33
Lead	mg kg^{-1}	180
Cadmium	mg kg^{-1}	5
Copper	mg kg^{-1}	385
Mercury	mg kg^{-1}	10.8
Zinc	mg kg^{-1}	1885

To study not only cumulative, but also residual effects, applications were discontinued in subsequent years on part of the area to which sludge had been applied in the preceding year. Thus, in 2001, each plot was divided into two subplots (9 × 3 m and 3 × 3 m), and sludge was applied only to the larger subplot, using the same dosage as in the first year. In the third year (2002), the same sludge dosages were applied on two-thirds (6 × 3 m), and in the fourth year (2003) on one-third of the larger subplots (3 × 3 m) only, (i.e. one-fourth of the original plots).

As a result, total sludge dosages were, 25, 50 and 100 Mg ha^{-1} for single applications, 50, 100 and 200 Mg ha^{-1} for two applications, 75, 150 and 300 Mg ha^{-1} for three applications and 100, 200 and 400 Mg ha^{-1} for 4 years of sludge applications. In addition a control plot was prepared and treated in the same way as the others, except that no sludge was applied. Thus, there were 13 treatments in total, with different numbers and rates of sludge applications (Table 2). The crops were irrigated as required using well water (with pH range of 7.6–7.8 and EC range of 0.35–0.68 dS m^{-1}) from the site. Weed control was done mechanically.

Sampling and analysis

In 2004, after the wheat had been harvested, five soil cores (0–20 cm depth) were taken from each subplot and were mixed to make a composite sample. Soil samples were air-dried, sieved (2 mm), and analyzed for pH and electrical conductivity (EC) in soil saturation extracts and organic carbon (C) by standard methods (Black 1965), for CEC using the Rhoades (1982) method and for total (Sposito et al. 1982) and DTPA-extractable (Lindsay and Norvell 1978) Pb, Cd, Zn and Cu using atomic absorption spectrophotometry (AAS).

Roots, stalks and grains were sampled separately. All plant samples were carefully washed using distilled water, dried at 65°C for 48 h, digested in a solution of 70% aqua regia (HNO_3 + concentrated HCl) and 30% H_2O_2 and analyzed for total Pb, Cd, Zn and Cu by AAS (Westerman 1990).

Statistical analysis

All statistical analyses were performed using SAS version 6.10 for personal computers (SAS 1993). Means of different treatments (level of sludge application and time after the last application) were compared using LSD ($P \leq 0.05$) test.

Results

Effect of sludge on soil properties

Selected soil chemical properties after sludge applications are shown in Table 3. Sludge application did

Table 2 Scheme of treatments and sludge application doses in different years of study

Treatment (Mg ha^{-1})	2000 First year	2001 Second year	2002 Third year	2003 Fourth year	Total amount of sludge applied	Sampling: years after last application
25	25	0	0	0	25	4
25 × 2	25	25	0	0	50	3
25 × 3	25	25	25	0	75	2
25 × 4	25	25	25	25	100	1
50	50	0	0	0	50	4
50 × 2	50	50	0	0	100	3
50 × 3	50	50	50	0	150	2
50 × 4	50	50	50	50	200	1
100	100	0	0	0	100	4
100 × 2	100	100	0	0	200	3
100 × 3	100	100	100	0	300	2
100 × 4	100	100	100	100	400	1
Control	0	0	0	0	0	–

Table 3 Means of soil properties in topsoil (0–20 cm depth) at the end of the experiment in 2004

Treatment (Mg ha^{-1})	pH	Organic C (%)	EC (dS/m)	CEC (cmol c/kg)
25	8.1^{a}	0.6^{d}	1.4^{bcd}	17.5^{bcd}
25 × 2	7.8^{a}	0.7^{d}	1.1^{d}	15.2^{ef}
25 × 3	8.2^{a}	1.2^{bcd}	1.3^{bcd}	15.8^{def}
25 × 4	8.3^{a}	1.2^{bcd}	1.4^{bcd}	17.6^{bcd}
50	8.3^{a}	0.5^{d}	1.1^{d}	16.7^{cde}
50 × 2	8.3^{a}	1.0^{cd}	1.2^{bcd}	15.9^{def}
50 × 3	8.3^{a}	1.2^{bcd}	1.5^{bc}	18.5^{bc}
50 × 4	8.2^{a}	2.1^{b}	1.6^{b}	16.5^{cde}
100	8.1^{a}	1.0^{cd}	1.2^{bcd}	17.2^{bcde}
100 × 2	8.1^{a}	1.4^{bcd}	1.2^{bcd}	18.9^{b}
100 × 3	7.6^{a}	1.8^{cb}	1.5^{bc}	21.1^{a}
100 × 4	8.1^{a}	3.4^{a}	2.1^{a}	22.9^{a}
Control	8.3^{a}	0.5^{d}	1.1^{d}	14^{f}

Values with the same letters are not significantly different from each other according to Fisher's LSD at $P \leq 0.05$

not significantly affect soil pH but organic C, EC and CEC were affected by sludge application significantly (Table 3).

Effect of sludge on total metals in soil

Sludge application significantly increased the total concentrations of metals in soil (Fig. 1). Figure 1 shows that the concentrations of metals were increased still after 4 years even by a single sludge application. However, the increases were not significant for Pb at 25 Mg ha^{-1} rate, and for Zn and Cu in any of the rates.

Effect of sludge on DTPA-extractable metals

DTPA-extractable soil metals concentrations increased approximately in proportion to the dosage and number of applications (amounts of applied sludge) (Fig. 2).

Single sludge applications at a rate of 100 Mg ha^{-1} (for all of metals) and at a rate of 50 Mg ha^{-1} (for Cu) significantly increased DTPA-extractable metal concentrations. Four years after the sludge applications had ceased, in plots which had received 25 and 50 Mg ha^{-1} of sludge, DTPA–extractable Pb, Cd and Zn concentrations were still greater than control levels but not significantly greater. However, DTPA-extractable concentrations

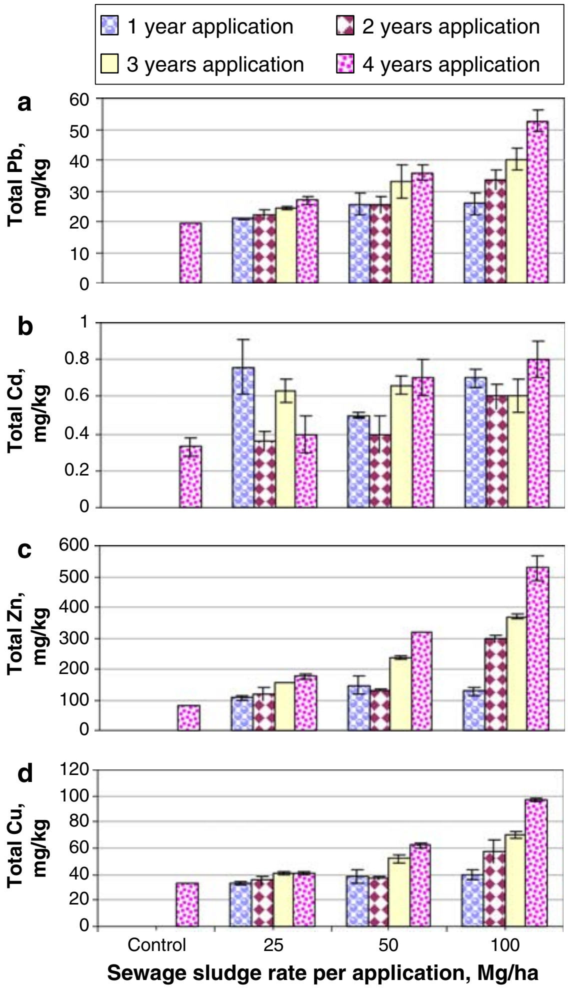

Fig. 1 Means of total metal concentrations in topsoil at the end of the experiment in 2004: (**a**) Pb; (**b**) Cd; (**c**) Zn; and (**d**) Cu. Error bars indicate standard deviation of the mean (n = 3)

of Cu were still significantly above control levels in plots that had received 50 Mg ha^{-1} or larger rates of total sludge (Fig. 2).

DTPA-extractable concentrations of Pb, Zn and Cu correlated significantly with the total concentrations of these metals in soil. The R^2-values were 0.88, 0.89 and 0.83, respectively (Fig. 3).

Effect of sludge on dry matter weight of wheat

No apparent symptoms of metal toxicity were observed in any parts of the plants. Wheat dry matter significantly increased approximately in proportion to the total amount of sludge application (Fig. 4). After a single application of 100 Mg ha^{-1}, almost 6 times

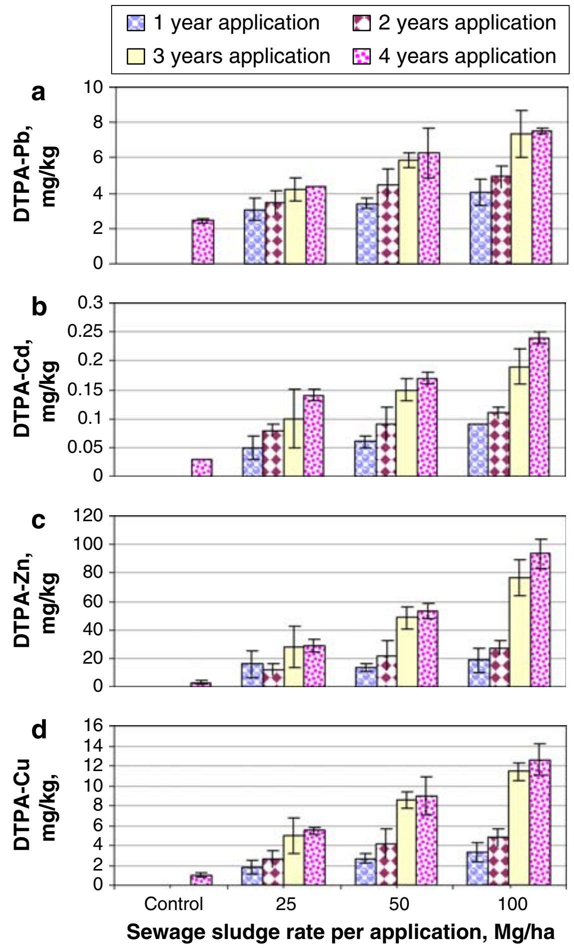

Fig. 2 Means of DTPA-extractable metal concentrations in topsoil at the end of the experiment in 2004: (**a**) Pb; (**b**) Cd; (**c**) Zn; and (**d**) Cu. Error bars indicate standard deviation of the mean ($n = 3$)

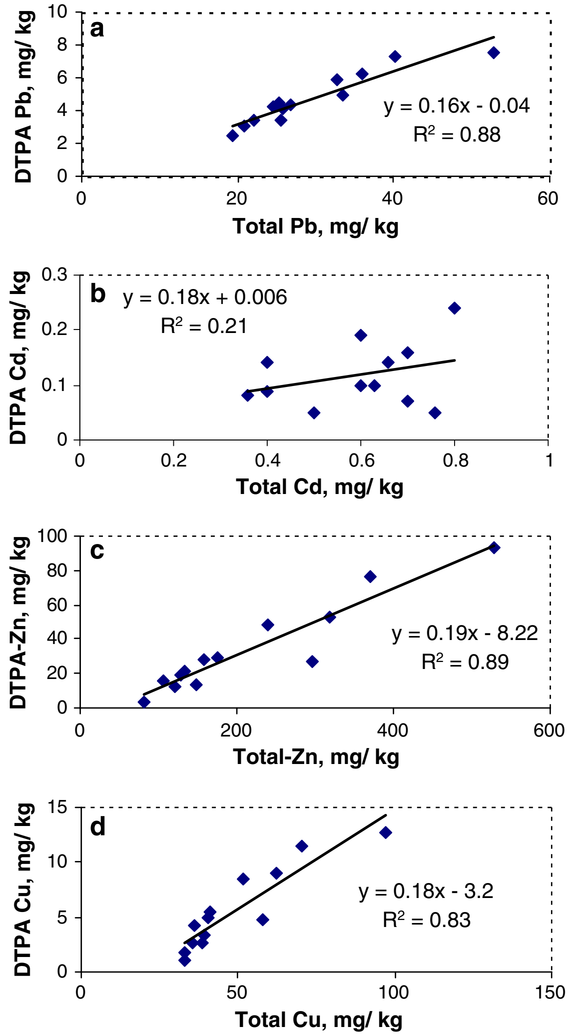

Fig. 3 Relationships between total and DTPA-extractable metal concentrations in soil: (**a**) Pb; (**b**) Cd; (**c**) Zn; and (**d**) Cu

as much dry matter was produced 4 years later as compared to that in to the control plots (Fig. 4).

Effect of sludge on plant tissue concentrations of heavy metals

Because of the great differences in yield among the treatments, "metal uptake" (product of metal concentration and plant yield) was utilized instead of "metal concentration" for comparing of metals in stalk. This matter refers to "dilution growth effect" (Han et al. 2006).

Stalk uptakes of metals were significantly affected by sludge application (Fig. 5). Metal uptakes by wheat stalks in single sludge application were still

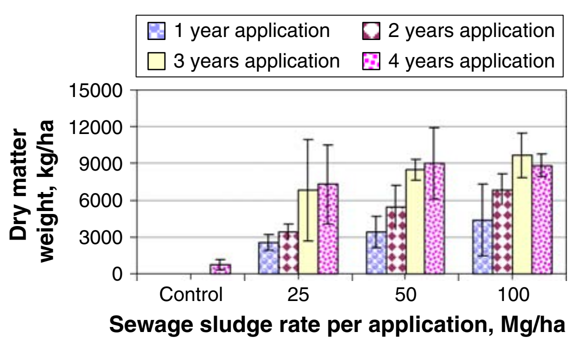

Fig. 4 Means of dry matter production by wheat in the last experimental year (2004). Error bars indicate standard deviation of the mean ($n = 3$)

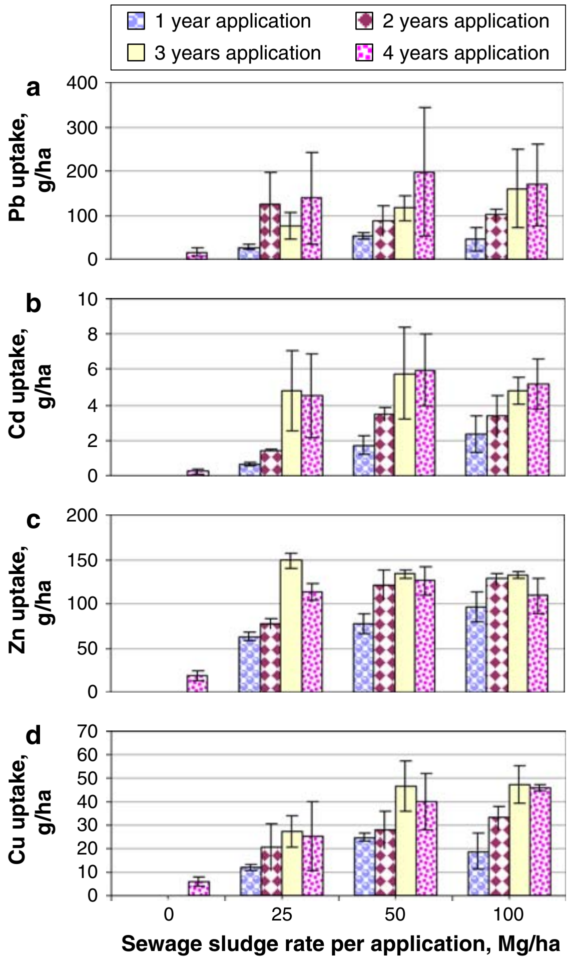

Fig. 5 Means comparisons of metals uptake by stalks at the end of the experiment in 2004: (**a**) Pb; (**b**) Cd; (**c**) Zn; and (**d**) Cu. Error bars indicate standard deviation of the mean ($n = 3$)

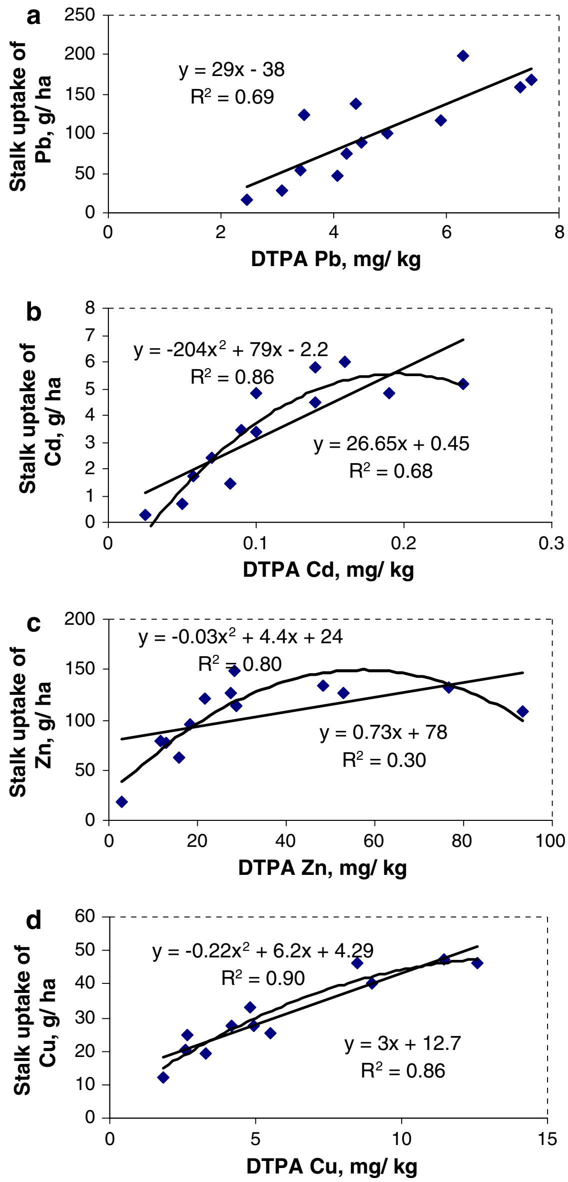

Fig. 6 Relationships between DTPA-extractable metal concentrations in soil and metal uptake by stalks: (**a**) Pb; (**b**) Cd; (**c**) Zn; and (**d**) Cu

larger than the control after 4 years and these differences are significant in some cases (Fig. 5). Metal uptakes by stalk increased as the sludge application rate increased, although Zn and Cu uptakes at first increased with increasing number of sewage sludge applications and then decreased again.

Stalk uptakes of Pb, Cd, Zn and Cu showed close and significant correlations with DTPA-extractable metals (Fig. 6) as well as to the total metals for Pb and Cu (Fig. 7). The relationship between stalk uptake and DTPA-extractable concentration was linear for Pb, but better described by quadratic equations for Cd, Zn and Cu (Fig. 6).

Stalk concentrations of Pb and Cd were not significantly different among treatments, and Zn and Cu concentrations were significantly greater in plant grown on control plots (Table 4).

No trend with sludge application rate was observed for Pb concentrations of grains. Cadmium concentrations of grains were not affected by the treatments but showed the strongest tendency of increased accumulation with increased rates of sewage sludge application among metals (Table 5).

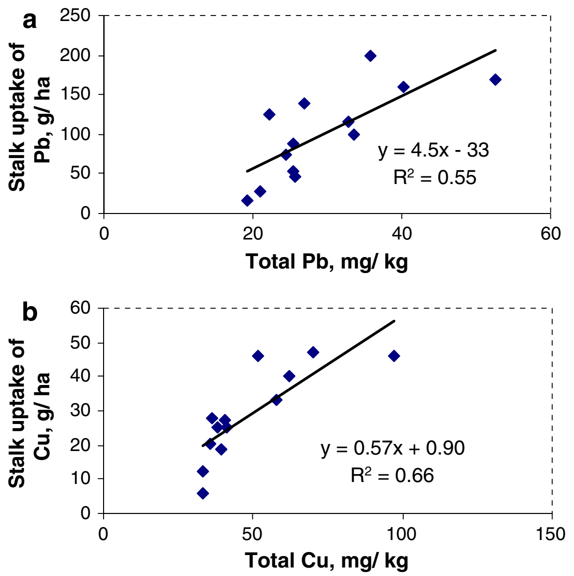

Fig. 7 Relationships between total metal concentrations in soil and metal uptake by stalks: (**a**) Pb; and (**b**) Cu

Zinc and Cu concentrations in grain were generally greater in sludge treatments than in the controls, but there was no trend with sewage sludge application rates and time of applications (Table 5).

Sludge application did not significantly affect the metal contents of the wheat roots (Data not shown). In general, the concentration of the metals in root were greater in sludge treatments than the control, the means of metal concentration of roots were 17.7, 0.7, 38.9 and 18.9 mg kg^{-1} for Pb, Cd, Zn and Cu in sludge treatments, respectively, while those concentration of roots in control were 14.0, 0.7, 31.3 and 11.1 mg kg^{-1}. In general, root metal concentrations at first increased with increasing rates of sludge application and then decreased.

Table 6 shows the values of bioconcentration factor (BCF) defined as the metal concentration ratio of plant roots to soil, bioaccumulation factor (BF) defined as the metal concentration ratio of plant shoots to soil and translocation factor (TF) defined as the metal concentration ratio of plant shoots to roots for all metals. In general, metal concentrations were greater in wheat roots than in wheat grains.

Discussion

Sludge application did not affect soil pH significantly. This mainly was because of the high buffering capacity of this calcareous soil. The increase in organic C and CEC after sludge application could be explained by the large amount of organic matter in the sludge and the large CEC of the organic matter. Also, the EC of the soil increased with sludge application because of the elevated EC of the applied sludge.

Although sludge application significantly increased the total concentrations of metals in soil (Fig. 1), the total Pb and Cd concentrations in soil remained below the respective limits of maximum acceptable concentrations (MAC) in agricultural soils for these metals in

Table 4 Means (±s.d.) of metals concentrations (mg kg^{-1}) in wheat stalks at the end of the experiment in 2004

Treatment (Mg ha^{-1})	Stalk Pb	Stalk Cd	Stalk Zn	Stalk Cu
25	$10.7^{a} \pm 0.9$	$0.26^{a} \pm 0.02$	$24.4^{a} \pm 2.5$	$4.7^{cde} \pm 1.2$
25 × 2	$36.3^{a} \pm 19.9$	$0.43^{a} \pm 0.5$	$22.9^{ab} \pm 2.0$	$5.9^{abc} \pm 0.8$
25 × 3	$11.0^{a} \pm 2.0$	$0.71^{a} \pm 0.2$	$21.7^{bc} \pm 4.4$	$4.0^{de} \pm 0.3$
25 × 4	$19.0^{a} \pm 9.5$	$0.62^{a} \pm 0.04$	$15.6^{de} \pm 2.8$	$3.4^{e} \pm 1.1$
50	$15.5^{a} \pm 1.0$	$0.50^{a} \pm 0.00$	$22.4^{ab} \pm 3.5$	$7.2^{a} \pm 2.9$
50 × 2	$16.2^{a} \pm 15.2$	$0.64^{a} \pm 0.14$	$22.3^{abc} \pm 3.5$	$5.1^{cde} \pm 0.9$
50 × 3	$13.8^{a} \pm 3.8$	$0.69^{a} \pm 0.28$	$15.8^{de} \pm 1.4$	$5.5^{bcd} \pm 1.1$
50 × 4	$22.0^{a} \pm 12.4$	$0.66^{a} \pm 0.09$	$14.0^{e} \pm 3.9$	$4.5^{cde} \pm 0.4$
100	$10.5^{a} \pm 0.6$	$0.54^{a} \pm 0.1$	$22.0^{abc} \pm 3.3$	$4.3^{de} \pm 3.2$
100 × 2	$14.6^{a} \pm 2.6$	$0.49^{a} \pm 0.2$	$18.4^{cd} \pm 1.4$	$4.8^{cde} \pm 0.3$
100 × 3	$16.5^{a} \pm 9.1$	$0.50^{a} \pm 0.1$	$13.6^{e} \pm 5.3$	$4.9^{cde} \pm 1.4$
100 × 4	$19.1^{a} \pm 11.1$	$0.60^{a} \pm 0.14$	$12.3^{e} \pm 3.4$	$5.2^{bcd} \pm 0.4$
Control	$21.5^{a} \pm 16.1$	$0.40^{a} \pm 0.05$	$25.3^{a} \pm 5.7$	$7.6^{a} \pm 6.4$

Values with the same letters are not significantly different from each other according to Fisher's LSD at $P \leq 0.05$

Table 5 Means (±s.d.) of metals concentrations (mg kg^{-1}) in wheat grains at the end of the experiment in 2004

Treatment (Mg ha^{-1})	Grain Pb	Grain Cd	Grain Zn	Grain Cu
25	$8.5^{ab} \pm 0.5$	$0.4^{a} \pm 0.1$	$35.5^{cdef} \pm 3.2$	$4.6^{bcde} \pm 0.8$
25 × 2	$8.1^{ab} \pm 0.3$	$0.5^{a} \pm 0.0$	$37.2^{bcd} \pm 0.8$	$4.2^{def} \pm 0.4$
25 × 3	$7.6^{b} \pm 1.0$	$0.5^{a} \pm 0.2$	$36.2^{bcde} \pm 0.7$	$4.7^{abcde} \pm 0.5$
25 × 4	$8.3^{ab} \pm 0.6$	$0.8^{a} \pm 0.0$	$38.5^{ab} \pm 1.7$	$4.4^{cdef} \pm 0.6$
50	$7.8^{b} \pm 0.4$	$0.3^{a} \pm 0.1$	$40.5^{a} \pm 1.5$	$4.8^{abcd} \pm 0.1$
50 × 2	$8.7^{ab} \pm 0.3$	$0.7^{a} \pm 0.2$	$37.7^{abcd} \pm 3.8$	$4.7^{abcde} \pm 0.5$
50 × 3	$9.1^{a} \pm 0.1$	$0.4^{a} \pm 0.1$	$37.3^{bcd} \pm 0.6$	$5.1^{ab} \pm 0.1$
50 × 4	$9.1^{a} \pm 0.5$	$0.5^{a} \pm 0.0$	$34.8^{defg} \pm 2.7$	$5.3^{a} \pm 0.3$
100	$8.5^{ab} \pm 0.5$	$0.4^{a} \pm 0.1$	$38.0^{abc} \pm 1.8$	$4.7^{abcde} \pm 0.5$
100 × 2	$8.7^{ab} \pm 0.4$	$0.6^{a} \pm 0.1$	$38.5^{ab} \pm 0.2$	$4.9^{abc} \pm 0.3$
100 × 3	$8.0^{ab} \pm 0.3$	$0.5^{a} \pm 0.2$	$32.3^{g} \pm 0.5$	$4.1^{ef} \pm 0.1$
100 × 4	$8.5^{ab} \pm 0.2$	$0.5^{a} \pm 0.1$	$33.7^{efg} \pm 1.2$	$4.5^{cdef} \pm 0.3$
Control	$8.3^{ab} \pm 0.2$	$0.3^{a} \pm 0.2$	$32.8^{fg} \pm 1.8$	$3.9^{f} \pm 0.2$

Values with the same letters are not significantly different from each other according to Fisher's LSD at $P \leq 0.05$

Table 6 Accumulation ratios of total heavy metals concentrations between crop parts (G, grain; St, stalk; R, root) and soil (S)

Metal	G/S		St/S = BF		R/S = BCF		St/R = TF	
	Sludge treatments	Control	Sludge treatments	Control	Sludge treatments	Control	Sludge treatments	Control
Pb	0.27*	0.37	0.56*	1.1	0.61	0.67	0.96	1.66
Cd	0.85	0.75	0.93	1.2	1.17*	2.00	0.8	0.67
Zn	0.20**	0.4	0.12**	0.3	0.21**	0.38	0.58	0.8
Cu	0.09	0.11	0.10**	0.29	0.37	0.34	0.30**	0.92

Values with an asterisk (*) or two asterisks (**) are significantly different from the control for the respective metal and ratio at $P \leq 0.05$ or $P \leq 0.01$, respectively, according to Fisher's LSD

countries such as Germany, Japan, England and Poland (Singh 1994). However, the concentrations of total Zn exceeded the MAC for Zn in England (150 mg kg^{-1}) in all treatments with three and four sludge applications and in the treatment with two sludge applications of 100 Mg ha^{-1} each year (2 × 100 Mg ha^{-1}). Similarly, the application rate of 2 × 100 Mg ha^{-1} and 3 × 50 Mg ha^{-1} sludge in consecutive years led to concentrations of Cu that exceeded the respective MAC of this metal in Germany (50 mg kg^{-1}) (Singh 1994).

In plots with a single sludge application, DTPA–extractable metals concentrations were still greater than control levels (in some cases significantly) 4 years after sludge applications were stopped. Also, repetitive sludge applications increased DTPA-extractable metals (Fig. 2). This increase, especially from 2 to 3 sludge applications, was apparent. On the other hand, the availability of metals in treatments which received sludge for only 1 or 2 years was lower than in treatments that received sludge for 3 or 4 years. This was probably due to the continued addition of microorganisms populations in the treatments where fresh sludge was applied for 3 or 4 years. There was enough time for chemical processes of metal precipitation (such as surface precipitation) in the treatments where sludge was only applied for 1 or 2 years. High temperatures and frequent irrigation may have led to substantial mineralization of the organic matter leading to a corresponding release of metals from the sludge into the soil.

The high CEC and pH of this soil (Table 3) probably were the main factors limiting the solubility of the metals. Rundle et al. (1982) suggested that bioavailability of trace metals is typically highest in the first 3–4 years following sludge application, followed by lower but generally sustained availability.

DTPA-extractable concentrations of Pb, Zn and Cu correlated significantly with the total concentrations of these metals in soil. The increases in DTPA-extractable concentrations averaged 0.16, 0.19 and 0.18 mg kg^{-1} for every mg kg^{-1} increase in total concentrations of Pb, Zn and Cu, respectively (Fig. 3). The ratios between DTPA- extractable and total metal concentrations in the control soils were 0.12, 0.03 and 0.03, respectively. This indicates that the availability of the metals introduced with the sludge remained much larger than the native metals that of in soil. In addition, the availability of micronutrient metals (Zn and Cu) increased more (from 0.03 to 0.19 for Zn and to 0.18 for Cu) than non-nutrient metal Pb (from 0.12 to 0.16) by sludge application.

This finding agrees with those of others. Naidu et al. (1997) suggested that metals added through anthropogenic activities are more available than pedogenic metals. Adamu et al. (1989) investigated the residual effect of sludge on Zn, Cu, Mn, Fe, Pb, Ni and Cd concentrations in soil and tobacco 10 years following farmland application of municipal sludge. The relationships between DTPA extractable and total soil concentrations were found to be quadratic for Pb and Ni and to be linear for all other metals studied.

Increasing production of wheat dry matter after sludge application can be attributed to the addition of macro- and micronutrients plus the improvement of soil physical properties resulting from the sludge application. Morera et al. (2002) found that the addition of sewage sludge markedly increased the average dry weight of sunflowers on soils with low fertility.

Lead and Cd uptake by stalks increased with rates of sludge application, but Zn and Cu uptake at first increased with increasing numbers of sewage sludge applications and then decreased (Fig. 5). This trend suggests that all the heavy metals in soil are not uniformly absorbed by plants and their absorption is not a concentration-dependent phenomenon for all the heavy metals (Singh and Agrawal 2007). In those plots with a single sludge application, Zn and Cu uptake by stalks was still greater than in control plots (in some cases significantly) 4 years after sludge application.

Mass balance calculations for metals showed that a large part of added metals by sludge remained within 0–20 cm depth. Crop uptake was less than 0.3% for Pb, 0.4% for Cd, 0.03% for Zn and 0.04% for Cu of total soil metals. This indicates a "sludge protection effect" which could be due to a number of constituents (such as residual organic matter, P, S and mineral residues) added to soil by sewage sludge application. Brown et al. (1998) found that lettuce (*Lactuca sativa* var. *longifolia*) uptake of Cd was generally greater from plots treated with Cd-salt treatments than similar levels of sludge-Cd, even though the soil treatments had occurred 13–15 years earlier. Logan et al. (1997) concluded that crop uptake of soil Cd would be less from soil treated with low-Cd sludge compared to a high-Cd sludge, even when actual Cd loadings were similar.

No trends with sludge application rate were observed for metal concentrations of grains. This may be due to high grain yield produced by sludge application and consequently dilution of metals in grain tissue.

In each treatment, Zn was the metal with the highest concentration in wheat root and grain (Table 5). These results are in line with the fact that Zn was also the metal with the highest concentration in the applied sewage sludge.

Table 6 shows low values ($\leq$1) of BCF and TF for metals in most cases. This means limited ability of heavy metal accumulation and translocation by the plant (Yoon et al. 2006) which indicates that significant amounts of the metals do not translocate to wheat grain. TF for Pb, Zn and Cu, BF for all metals and BCF for Pb, Cd and Zn were lower in wheat grown on sludge treated than control plots and these differences are significant in some cases (Table 6). Kidd et al. (2007) showed a reduction in the TF and BF for Cu, Mn and Zn of two wild plants (*A. serpyllifolium* and *C. ladanifer*) and maize (*Z. mays*) in sludge-amended soils compared to unamended soils. Singh and Agrawal (2007) reported that, in general, heavy metal accumulation was greater in roots than shoots with sewage sludge applications. The high accumulation of heavy metals in roots may be ascribed to complexation of heavy metals with the sulphydryl groups, resulting in less translocation of metals to shoots (Singh et al. 2004). BF values in this study confirmed that wheat did not bioaccumulate heavy metals from sludge amended soils in its aerial parts (Table 6). This may be due to high organic matter and P content in the applied sludge.

Conclusion

In summary, sewage sludge applications increased total and DTPA-extractable metal concentrations in the soil of our study. DTPA-extractable metal concentrations were closely correlated to the total metal concentrations. The increase in soil metal concentrations did not translate into corresponding increases in metal accumulation by wheat. Linear and quadratic relationships were observed between DTPA-extractable metals in this soil and metal uptake by wheat stalks. Increased metal removal from the soil was almost entirely due to increased biomass production. Zinc was the metal with the greatest uptake by wheat.

In general, despite the increase in root tissue concentrations of metals, plant uptake mechanisms clearly restricted metal transport to aerial parts. Moreover, biomass production of wheat was significantly greater in sludge-treated plots. It appears that the risk of transferring heavy metals into wheat grain could be considered less significant compared to the positive effects of sludge applications to soil. Some of these effects are increases in soil organic matter and consequent increases on CEC, as well as increases in plant availability and uptake of micronutrients such as Zn and Cu.

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