Use of sequential extraction to assess the influence of sewage sludge amendment on metal mobility in Chilean soils

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In Chile, the increasing number of plants for the treatment of wastewater has brought about an increase in the generation of sludge. One way of sludge disposal is its application on land; this, however involves some problems, some of them being heavy metal accumulation and the increase in organic matter and other components from sewage sludge which may change the distribution and mobility of heavy metals. The purpose of the present study was to determine the effect of sewage sludge application on the distribution of Cr, Ni, Cu, Zn and Pb in agricultural soils in Chile. Three different soils, two Mollisols and one Alfisol, were sampled from an agricultural area in Central Chile. The soils were treated with sewage sludge at the rates of 0 and 30 ton ha^{-1}, and were incubated at 25 °C for 45 days. Before and after incubation, the soils were sequentially extracted to obtain labile (exchangeable and sodium acetate-soluble), potentially labile (soluble in moderately reducing conditions, $K_4P_2O_7$-soluble and soluble in reducing conditions) and inert (soluble in strong acid oxidizing conditions) fractions. A two-level factored design was used to assess the effect of sludge application rate, incubation time and their interaction on the mobility of the elements under study. Among the metals determined in the sludge, zinc has the highest concentration. However, with the exception of Ni, the total content of metals was lower than the recommended limit values in sewage sludge as stated by Chilean regulations. Although 23% of zinc in sludge was in more mobile forms, the residual fraction of all metals was the predominant form in soils and sludge. The content of zinc only was significantly increased in two of the soils by sewage sludge application. On the other hand, with the exception of copper, the metals were redistributed in the first four fractions of amended soils. The effect of sludge application rate, incubation time and their interaction depended on the metal or soil type. In most cases an increase in more mobile forms of metals in soils was observed as the final effect.

Introduction

In Chile, the increasing number of plants for the treatment of wastewater has brought about an increase in sludge generation. As a matter of fact, 23% of the total wastewater generated by a population of more than 5 million in Santiago, the capital of Chile, is being treated at present. One way of sludge disposal is its application on land. This procedure, however, involves some problems that have been under study elsewhere, such as the presence of pathogens and toxic chemicals, heavy metal accumulation, and contamination of ground water.[1,2]

The use of sludge as a source of P and N affects soil properties such as pH, organic matter content, electric conductivity, available phosphorus, and trace metal content.[3,4] Mobility and bioavailability of trace metals may also vary.[5] Continued application of this kind of treatment on agricultural land may lead to accumulation of metallic elements and constitute a potential environmental hazard or, on the contrary, it might reduce availability of these elements so that they might be used to fix toxic elements, or they might change the soil physicochemical properties.[2] Total metal content in soil has been estimated to be an inadequate parameter for identifying the level of availability and toxicity.[6,7] High concentrations of metals in soil do not necessarily imply their release and, consequently, their availability to plants or ground water metal contamination.[8] Trace metals in the soil system may constitute part of its solution and solid phase. In the latter, they are distributed among the various soil components and soil association with them gives rise to the forms that determine trace metal mobility. These metal forms are the soluble, exchangeable, complexed or adsorbed in organic matter, adsorbed or occluded in oxides, associated with clay minerals and with primary minerals.[9] Knowledge of the chemical forms of metals in soils and sludge gives more information about metal behaviour and in order to determine them, sequential extraction procedures have been developed, which constitute highly utilized tools for understanding the chemistry of heavy metals in soil.[10,11] These procedures are not quite specific and overlapping could occur among metal fractions.[12–14] Despite uncertainty about extractant selectivity and possible readsorption and redistribution problems, these extraction procedures provide a semiquantitative methodology to determine trace metal forms.[15] By using these procedures, Sposito *et al.*[16] found that sludge application to soil increased the forms of Cd, Cu, Ni, Pb, and Zn bound to carbonate or to organic matter. Chang *et al.*,[17] using a similar methodology, found that the carbonate forms for Cu, Ni, and Zn prevailed in sludge-treated soils. Wang *et al.*[11] found that, in soils treated with various sludge doses, Ni occurred in the form associated with oxides, carbonates, and organic matter and that this constituted a capacity factor associated to a potential source of available Ni. Sludge application to soil involves a supply of organic matter that may modify the chemical forms of the metals present in soil. Sequential extraction is like a fingerprint of metals, giving information about their potential for leaching and availability. Thus, if the metal is in the first fractions of a soil, this exhibits a greater potential hazard when compared with other soils where the metal is mainly found in the last fractions, especially in the last, where it will be available only under extreme conditions rarely found in the environment. Studies where sludges have been applied for long periods indicate no toxic levels of accumulation of metals such as As, Cd, Cu, Pb, Hg, Mo, Ni, Se, and Zn.[18] On account of this background knowledge, it is of utmost importance to have information that permits assessment and/or prediction of the status of potentially toxic elements in these soils treated with a sewage sludge representing the treatment of most wastewaters from the city of Santiago.

The purpose of the present study was to determine the effect of sewage sludge application on the mobility of Cr, Ni, Cu, Zn and Pb in agricultural soils in Chile. A two-level factorial design was used to evaluate the effect of sludge application rate, incubation time, and interactions of both on the mobility of these elements in the soils. In addition, linear regression was used to identify the effect of macroscopic soil properties (pH, cation exchange capacity, and total organic matter) on these variables.

Materials and methods

Reagents

High purity water with a resistivity of 18 M Ω cm, obtained from a Milli-Q Plus system (Millipore Corporation, USA) was used for all solutions. Standard solutions of Cr(III), Ni(II), Cu(II), Zn(II) and Pb(II) were obtained from the respective standard solution Titrisol®, Merck. Reagents for acid digestion of soils and sludge (HNO_3, HF and H_2O_2) were Merck Suprapur® quality. The extracting solution for sequential extraction was prepared from analytical grade reagents (Merck).

Soil collection and treatment

Three soils were sampled from an agricultural land area in central Chile. Composed surface (0–10 cm) soil samples were drawn. At each site five replicates were obtained by making a 10 × 10 m plot and drawing a soil sample from each corner and the center of the plot using a stainless-steel hand auger with a plastic liner to avoid contamination by chromium or other metal. The samples were mixed, reduced and processed. All samples were air-dried and sieved through a 2 mm mesh-size polyethylene sieve. Portions of <2 mm soil fractions were ground in an agate mortar and stored in polyethylene sample bottles. As concerns soil classification, two of the soils are Mollisol (STG and MCH) and the other one belongs to the Alfisol (CQE) order. Soil pH was determined as 1 : 2.5 soil-to-water and 1 : 2.5 soil-to-0.1 M KCl solution (wt/wt).[19] Organic matter was determined by the Walkley–Black procedure.[20] Cation exchange capacity (CEC) was determined by the sodium acetate procedure at pH 7.[21] Total metal determination was done by Flame Atomic Absorption Spectrophotometry (F-AAS) after acid digestion (with a mixture of 4 mL HNO_3, 4 mL HF, and 2 mL H_2O_2) of 200 mg of soil sample, using a Milestone/mls 1200 Mega microwave oven. Metals were determined by flame AAS using a 1100 B Perkin-Elmer atomic absorption spectrometer. Characteristics of the sludge utilized for amending soils were also assessed with these methods.

Sludge incorporation and incubation method

The soils were treated with stabilized sludge from a treatment plant from Santiago at a rate of 0 and 30 ton ha^{-1} with further incubation at 25 °C for 45 days under field capacity moisture conditions. Prior and after incubation, the soils were subjected to a sequential extraction with specific reagents of differing extracting power to obtain the following operationally defined fractions for Cr, Ni, Cu, Zn, and Pb in soils: exchangeable (Step 1), sodium acetate-soluble (Step 2), soluble in moderately reducing conditions (Step 3), $K_4P_2O_7$-soluble (Step 4), soluble in reducing conditions (Step 5) and soluble in strong acid oxidizing conditions (Step 6). Metal determination in the extracts was performed by flame AAS.

Sequential extraction methods for soils and sewage sludge (SEM)

Each sample (3 g of sludge or soil) was weighed and the metals were extracted with the SEM as described by Howard and Shu.[22] At same time, 3 g of sample were dried at 105 °C to determine humidity in order to make the corresponding correction by dry weight.

Step 1: 20 mL $MgCl_2$ 1 M, shaken for 1 h at 25 °C.

Step 2: 20 mL NaOAc (sodium acetate) 1 M pH 5, shaken for 5 h at 25 °C.

Step 3: 20 mL 0.1 M $NH_2OH{\cdot}HCl$ (hydroxylamine hydrochloride) + 0.01 M HNO_3, shaken for 30 min at 25 °C.

Step 4: 20 mL 0.1 M $K_4P_2O_7$, shaken for 24 h at 25 °C.

Step 5: 20 mL 1 M $NH_2OH{\cdot}HCl$ + 25% (v/v) HOAc (acetic acid), heated with occasional stirring in a water bath; 4 h at 85 °C.

Step 6: The residue of Step 5 was dried at 30 °C, and 200 mg from it was weighed and digested the same as for total metal determination, using another portion to determine humidity in order to make the corresponding correction.

To prevent redistribution of the metals, nitrilotriacetic acid (NTA) at 200 mg L^{-1} was added to the extracting solution before they had been in contact with soils or sewage sludge samples.[23] After each individual step, supernatants were obtained by centrifugation; then they were filtered and placed in polypropylene bottles previously decontaminated with acid.

Results and discussion

Quality control

Determination of trace elements in environmental samples requires quality control of the analysis. The analytical methodologies employed for the determination of Cr, Ni, Cu, Zn and Pb in soil by F-AAS were assessed by analyzing a certified reference material (Montana Soil, Nist 2710). The values determined were not significantly different from that certified ($p < 0.05$).

Sewage sludge characteristics

Table 1 gives the total metal content of the sludge sample under analysis and some other properties such as pH, and content of organic matter, N and P in order to characterize the sludge as a fertiliser. As can be seen, the sludge contains a high content of organic matter and nitrogen, with a neutral pH, and a high content of P. As expected, conductivity and CEC values were higher than those found in soils. As regards exchangeable metals, the highest concentration is for Ca. With respect to heavy metals, Zn has the highest concentration, followed by copper, chromium, nickel and lead (Table 1). However, with the exception of Ni, the total content of the elements under study was lower than the recommended limit values in sewage sludge as stated by Chilean regulations.[24] The low heavy metal concentration is clearly accounted for by the mainly household source of the wastewater treated at the urban plant.

Sludge sequential extraction

In this study, sequential extraction focused only on Cr, Cu, Ni, Pb and Zn, because total metal concentration was high enough to allow fractionation with no analytical difficulties.

The metals in the sludge were differently distributed,

Table 1 Some properties of sewage sludge and soils under study

	Sewage sludge	STG soil	MCH soil	CQE soil
Organic matter/g kg^{-1}	440	39	42	27
pH (H_2O) 1 : 2.5	6.7	8.1	6.7	6.2
pH (KCl) 1:2.5	6.4	7.2	5.2	4.6
N/g kg^{-1}	27	1.0	1.8	1.4
P/g kg^{-1}	14	1.1	0.71	0.37
N available/mg kg^{-1}	5440	10	7.0	7.0
P available/mg kg^{-1}	370	3.8	7.3	18
C E/dS m^{-1}	16	0.90	0.6	0.30
CEC/cmol kg^{-1}	66	13	16	7.0
Ca/cmol kg^{-1}	22	24	8.8	4.8
Mg/cmol kg^{-1}	4.8	1.4	1.7	1.4
K/cmol kg^{-1}	1.4	0.30	1.1	0.32
Na/cmol kg^{-1}	2.0	0.38	0.10	0.06
	Total metal/mg kg^{-1}			
Cr	260	40	32	40
Ni	130	30	20	30
Cu	377	93	229	21
Zn	1214	158	105	72
Pb	67	33	30	21

although the residual form was predominant for all of them (step 6, Fig. 1), followed by step 4 for Cu, Cr, and Ni, step 5 for Pb, and steps 2 and 4 for Zn. Thus, of all the metals included in this study, it is estimated that zinc has the highest probability of mobility within the soil since it has the highest concentration and, moreover, about 23% of it is found in labile forms (steps 1 and 2), and about 50% in potentially labile forms (steps 3 to 5). Metals contained in the sewage sludge added to soils may change their form but persistence in soils in extractable and plant-available form can remain for many years.[25] On the other hand, soils are particularly important for attenuation of heavy metals in the environment because they contain surface-active mineral and humic constituents involved in metal retention.[26]

Soil characteristics

Some properties of the soils are shown in Table 1. The three soils have a similar content of organic matter and different pH values. As expected in relation to the CEC, both the STG and MCH soils show similar values and the CQE soil has the lowest CEC value. On the other hand, the total heavy metal concentration in the STG, CQE and MCH soils, unamended or amended with 30 ton ha^{-1} dry sludge, showed the same pattern (Fig. 2). For the STG soil, it was Zn > Cu > Cr > Pb > Ni; for CQE soil, it was Zn > Cr > Ni > Cu ≈ Pb; and for MCH soil, it was Cu > Zn > Cr > Pb > Ni.

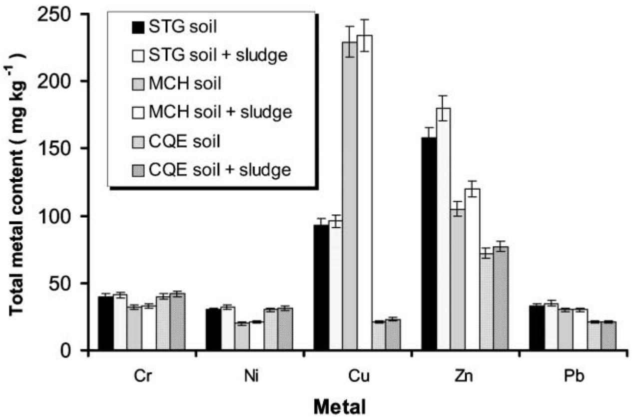

Fig. 2 Total heavy metal content in soil samples amended with sewage sludge (0 and 30 ton ha^{-1}) (results expressed as mean, $n = 3$).

Although application of sewage sludge to soils at 30 ton ha^{-1} increased the concentration of all metals in this matrix, only the content of Zn in the STG and MCH soils was significantly increased (Fig. 2). Studies have demonstrated that repeated application of sludge as a crop fertiliser may bring about accumulation of trace elements in the soil to reach phytotoxic concentrations.[27,28] On the other hand, another study has reported the opposite.[18] According to the results obtained in our work, application of sewage sludge to agricultural soils included in the study would apparently constitute no hazard.

Assessment was done of the effect of organic matter content, pH and CEC of soils on the content of metals extracted in the exchangeable fraction from the amended soils. To this end, parameters were correlated with the content of metals extracted after amending and the incubation period, which was normalized to the content extracted before incubation and without sludge application. No significant correlations were determined for any metal or parameter considered in this study (data not shown). This result indicates that the amending process, rather than the characteristics of the soils included in the study, affected metal mobility in soils.

Effect of amending on metal fractionation in soils

In order to obtain detailed information about the effect of incubation time and sludge application rate on the mobility of trace metals from amended soils, a two-level factorial design was performed with time and sludge application rate as independent variables (0–45 days, and 0–30 ton ha^{-1}, respectively), and the content of metals extracted from different

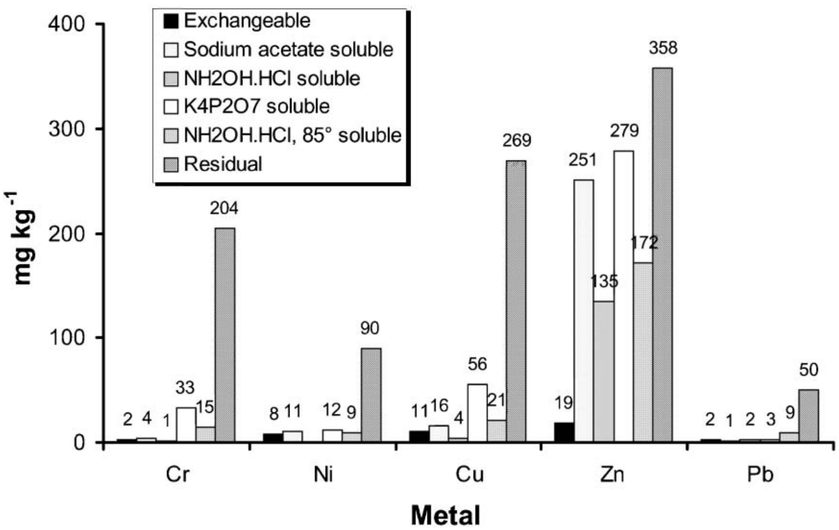

Fig. 1 Sequential extraction of the metals in sewage sludge (results expressed as mean, $n = 3$).

metal fractions (more labile, potentially labile and inert fractions) as a dependent variable. This design permits us to investigate the joint effects of both factors on the response variable (*i.e.* mean main effects and interactions). In this manner, a fitted screening linear regression model can be obtained.[29] Results are presented in Tables 2 to 6. As can be seen from the extraction before the amending process, the highest content of the elements under study was found in the residual fraction in all soils. Sludge application in almost all cases modified metal distribution in the first fractions in the soils. The changes observed vary and depend on the kind of soil and on the metal.

Chromium

Among the extraction reagents used, only the strong acids are capable of extracting chromium almost entirely from soils. This indicates that Cr is tightly bound in the matrix and would not be easily released under natural conditions. The step 5 Cr fraction follows the residual fraction in importance. Table 2 shows that the step 1 Cr fraction decreased as a result of sludge application to the STG soil, while no significant effect was observed in the other two soils. On the other hand, in the three soils, step 2 as well as the steps 3 and 4 fractions increased as a result of sludge application. Concerning time, this variable caused a decrease in Cr in most of the fractions in the STG soil, and an increase in the Cr labile fractions in the MCH and CQE soils. Upon assessing the final effect of the amending process, a significant increase in the more mobile forms of Cr was observed in the MCH and CQE soils, while the opposite was observed in the STG soil. On the other hand, a redistribution of chromium in the first four fractions in the three amended soils was observed.

Nickel

In the case of nickel (Table 3), the same as for chromium, the residual and the step 5 fractions were predominate. Time was a relevant factor in affecting the content of Ni in the various soil fractions. Thus, there was a significant increase in the exchangeable Ni form in the three soils. This, added to Ni enrichment by sludge incorporation, brought about a final net effect of significant increase in exchangeable Ni in the three soils. On the contrary, the various combined effects on the step 2 Ni fraction meant that no significant changes occurred in this fraction, or that it decreased in the MCH and CQE soils. Similar to chromium, a redistribution of nickel in the first four fractions was observed.

Copper

Considering copper fractionation, this metal is similarly distributed in both the STG and CQE soils, in the following order: step 6 > step 5 > step 2 > step 4 > step 1 > step 3. In the MCH soil, however, which also exhibits the highest total content of this element, the labile Cu forms (exchangeable and sodium acetate-soluble) become important, accounting for about 40% of the total extracted, which would indicate that Cu might be available to plants. Table 4 shows that sludge application increased almost all the Cu fractions in the CQE soil. On the contrary, Cu labile forms decreased in the other two soils. This may be related to the fact that the CQE soil has the lowest content of total Cu so that the addition is relevant, increasing the concentration of this element in most of the fractions, though not in the residual Cu fraction. Incubation time increased the labile Cu fractions in the STG and MCH soils, whereas no significant effect was observed in the CQE soil. Concerning the interaction of both factors, this variable had almost no effect on any of the fractions in the soils under study. The final effect of incubation was a significant increase of Cu associated with most of the fractions in the MCH and CQE soils, while this effect was observed only in the potentially labile Cu forms in the STG soil. Moreover, copper was the only metal that was not redistributed in any amended soil.

Zinc

About 70% of zinc is found in the residual form, followed by the step 5 fraction in all of the soils. The other forms vary in importance according to the soil. Thus, in the STG soil, the

Table 2 Two level factorial design analyses for the effect of the amending process on the sequential extraction of Cr in the STG, CQE and MCH soils. Effect of incubation time, sludge application rate and their interaction on the Cr extracted from exchangeable (F1), sodium acetate-soluble (F2), soluble in moderately reducing conditions (F3), $K_4P_2O_7$ soluble (F4), soluble in reducing conditions (F5) and soluble in strong acid oxidizing conditions (F6) fractions of the three amended soils ($n = 3$)

Soil – fraction	Extraction before amending process/mg kg^{-1}	Effect of amending process on the amount extracted of the element			
		Time	Sludge	Interaction	Extraction after amending process/mg kg^{-1}
STG-F1	0.44	−	−	+	− (nd)
STG-F2	1.7	−	+	Ns	− (0.56)
STG-F3	0.25	−	+	−	− (0.12)
STG-F4	0.33	−	+	−	+ (0.87)
STG-F5	3.5	−	Ns	−	− (1.3)
STG-F6	32	+	Ns	Ns	+ (38)
MCH-F1	0.07	+	Ns	Ns	+ (0.22)
MCH-F2	0.15	+	+	Ns	+ (0.33)
MCH-F3	0.22	+	+	Ns	+ (0.45)
MCH-F4	0.059	Ns	+	Ns	+ (0.62)
MCH-F5	1.2	Ns	+	Ns	+ (1.5)
MCH-E6	28	Ns	Ns	Ns	Ns (28)
CQE-F1	0.07	+	Ns	−	+ (0.15)
CQE-F2	0.09	+	+	Ns	+ (0.42)
CQE-F3	0.064	+	+	−	+ (0.33)
CQE-F4	0.92	+	+	+	+ (1.80)
CQE-F5	1.8	Ns	Ns	Ns	Ns (2.0)
CQE-F6	39	−	+	Ns	− (37)

+: the parameter or amending process increased significantly the amount extracted ($p < 0.05$). −: the parameter or amending process decreased significantly the amount extracted ($p < 0.05$). Ns: the parameter or amending process have a non significant effect. nd: not detected.

Table 3 Two level factorial design analyses for the effect of the amending process on the sequential extraction of Ni in the STG, CQE and MCH soils. Effect of incubation time, sludge application rate and their interaction on the Ni extracted from exchangeable (F1), sodium acetate-soluble (F2), soluble in moderately reducing conditions (F3), $K_4P_2O_7$ soluble (F4), soluble in reducing conditions (F5) and soluble in strong acid oxidizing conditions (F6) fractions of the three amended soils ($n = 3$)

Soil – fraction	Extraction before amending process/mg kg^{-1}	Effect of amending process on the amount extracted of the element			
		Time	Sludge	Interaction	Extraction after amending process/mg kg^{-1}
STG-F1	0.22	+	Ns	+	+ (0.35)
STG-F2	1.9	Ns	Ns	Ns	Ns (1.9)
STG-F3	0.086	+	Ns	Ns	+ (1.4)
STG-F4	0.43	−	+	+	Ns (0.42)
STG-F5	5.4	−	Ns	Ns	− (3.8)
STG-F6	19	Ns	+	Ns	+ (24)
MCH-F1	0.41	+	+	Ns	+ (0.78)
MCH-F2	0.77	−	+	+	− (0.48)
MCH-F3	0.55	−	−	+	− (0.39)
MCH-F4	2.4	−	Ns	+	− (0.54)
MCH-F5	1.1	+	+	Ns	+ (2.5)
MCH-E6	26	−	+	Ns	− (19)
CQE-F1	0.71	+	+	Ns	+ (1.2)
CQE-F2	0.65	−	+	−	− (0.44)
CQE-F3	0.69	−	−	+	− (0.50)
CQE-F4	0.41	+	+	Ns	+ (0.82)
CQE-F5	1.7	+	Ns	Ns	+ (2.4)
CQE-F6	22	−	Ns	+	− (18)

+: the parameter or amending process increased significantly the amount extracted ($p < 0.05$). −: the parameter or amending process decreased significantly the amount extracted ($p < 0.05$). Ns: the parameter or amending process have a non significant effect.

sodium acetate-soluble fraction ranks third, while in the CQE soil this position belongs to the exchangeable fraction. Table 5 shows that sludge application increased the presence of Zn associated with the first 4 fractions in all of the soils, not affecting the less mobile fractions. This was to be expected in view of the high content of zinc in the sludge, especially of $K_2P_2O_7$ and sodium acetate-soluble fractions. The effect of time varies, while interaction of both factors significantly favored the exchangeable form of Zn, especially in the MCH and CQE soils. On the other hand, the exchangeable Zn fraction in the MCH soil was the only fraction increased by the three parameters under consideration. The "Extraction after amending process" column shows significant increase in most of the fractions in the three soils. It may also be observed that zinc distribution changed in the three amended soils, especially in the MCH soil where the exchangeable fraction increased and became third in importance. Moreover, as previously described, almost 25% of the Zn present in sludge is in labile forms and is likely to be mobilized in soils. On the other hand, an important effect of microbial activity on Zn release from

Table 4 Two level factorial design analyses for the effect of the amending process on the sequential extraction of Cu in the STG, CQE and MCH soils. Effect of incubation time, sludge application rate and their interaction on the Cu extracted from exchangeable (F1), sodium acetate-soluble (F2), soluble in moderately reducing conditions (F3), $K_4P_2O_7$ soluble (F4), soluble in reducing conditions (F5) and soluble in strong acid oxidizing conditions (F6) fractions of the three amended soils ($n = 3$)

Soil – fraction	Extraction before amending process/mg kg^{-1}	Effect of amending process on the amount extracted of the element			
		Time	Sludge	Interaction	Extraction after amending process/mg kg^{-1}
STG-F1	2.5	+	−	Ns	Ns (2.7)
STG-F2	15	+	−	Ns	Ns (15)
STG-F3	1.9	+	+	Ns	+ (2.5)
STG-F4	6.0	Ns	+	Ns	+ (6.9)
STG-F5	24	−	Ns	Ns	− (21)
STG-F6	39	Ns	+	−	Ns (39)
MCH-F1	49	+	−	Ns	+ (52)
MCH-F2	28	+	Ns	Ns	+ (32)
MCH-F3	5.4	Ns	+	Ns	+ (5.9)
MCH-F4	22	+	+	Ns	+ (25)
MCH-F5	39	+	Ns	Ns	+ (46)
MCH-E6	62	−	Ns	Ns	− (56)
CQE-F1	1.4	Ns	+	Ns	+ (2.1)
CQE-F2	2.0	Ns	+	Ns	+ (2.6)
CQE-F3	0.36	+	+	Ns	+ (0.74)
CQE-F4	2.9	−	+	Ns	+ (3.3)
CQE-F5	2.9	Ns	+	Ns	+ (4.1)
CQE-F6	12	−	−	+	− (11)

+: the parameter or amending process increased significantly the amount extracted ($p < 0.05$). −: the parameter or amending process decreased significantly the amount extracted ($p < 0.05$). Ns : the parameter or amending process have a non significant effect.

Table 5 Two level factorial designs analyses for the effect of the amending process on the sequential extraction of Zn in the STG, CQE and MCH soils. Effect of incubation time, sludge application rate and their interaction on the Zn extracted from exchangeable (F1), sodium acetate-soluble (F2), soluble in moderately reducing conditions (F3), $K_4P_2O_7$ soluble (F4), soluble in reducing conditions (F5) and soluble in strong acid ozxidizing conditions (F6) fractions of the three amended soils ($n = 3$)

Soil – fraction	Extraction before amending process/mg kg^{-1}	Effect of amending process on the amount extracted of the element			
		Time	Sludge	Interaction	Extraction after amending process/mg kg^{-1}
STG-F1	2.0	−	+	Ns	+ (4.6)
STG-F2	5.5	+	+	+	+ (16)
STG-F3	1.7	+	+	−	+ (3.2)
STG-F4	3.2	Ns	+	Ns	+ (4.5)
STG-F5	32	+	Ns	Ns	+ (36)
STG-F6	102	−	Ns	Ns	− (85)
MCH-F1	1.4	+	+	+	+ (9.4)
MCH-F2	1.3	−	+	−	+ (3.6)
MCH-F3	0.93	−	+	Ns	Ns (0.87)
MCH-F4	1.4	+	+	+	+ (2.2)
MCH-F5	18	+	Ns	Ns	+ (23)
MCH-E6	79	−	Ns	Ns	− (73)
CQE-F1	5.1	+	+	+	+ (12)
CQE-F2	0.74	Ns	+	−	+ (3.3)
CQE-F3	0.77	−	+	+	+ (1.0)
CQE-F4	1.5	−	+	+	+ (2.4)
CQE-F5	11	+	Ns	Ns	+ (13)
CQE-F6	53	+	−	Ns	Ns (54)

+: the parameter or amending process increased significantly the amount extracted ($p < 0.05$). −: the parameter or amending process decreased significantly the amount extracted ($p < 0.05$). Ns: the parameter or amending process have a non significant effect.

sewage sludge may be also present. In fact, Qureshi *et al.*[30] have demonstrated substantial mobilization of Zn from sewage sludge when conditions for microbial activity are favored. Similar results have been described by Planquart *et al.*[31] on the availability of trace metals from soil treated with sewage sludge compost. The authors reported that, among the metals under study, only the proportion of the most labile forms of Zn was increased by compost application. Moreover, the concentration of Zn in colza leaves was higher than that of Cu or Pb and it was governed by sludge loading rates. Another report has shown a change in chemical form so that Zn and Cu are more mobile and bioavailable when the soil is amended with sewage sludge.[32]

Lead

Lead is one of the least concentrated elements both in the soils and in sludge and, the same as the other metals, it is predominantly found in the residual and step 5 fractions, the labile Pb forms being the least predominant. As seen in Table 6, Pb forms in the soils were the least affected due to the presence of sludge since, as the table shows, exchangeable Pb increased

Table 6 Two level factorial design analyses for the effect of the amending process on the sequential extraction of Pb in the STG, CQE and MCH soils. Effect of incubation time, sludge application rate and their interaction on the Pb extracted from exchangeable (F1), sodium acetate-soluble (F2), soluble in moderately reducing conditions (F3), $K_4P_2O_7$ soluble (F4), soluble in reducing conditions (F5) and soluble in strong acid oxidizing conditions (F6) fractions of the three amended soils ($n = 3$)

Soil – fraction	Extraction before amending process/mg kg^{-1}	Effect of amending process on the amount extracted of the element			
		Time	Sludge	Interaction	Extraction after amending process/mg kg^{-1}
STG-F1	0.16	+	+	−	+ (0.97)
STG-F2	1.7	+	Ns	Ns	+ (2.2)
STG-F3	2.5	−	Ns	Ns	− (1.8)
STG-F4	2.1	Ns	Ns	Ns	Ns (2.2)
STG-F5	16	−	Ns	Ns	− (12)
STG-F6	16	+	Ns	Ns	+ (22)
MCH-F1	1.1	−	Ns	Ns	− (0.81)
MCH-F2	1.0	−	+	Ns	+ (1.3)
MCH-F3	1.2	Ns	Ns	Ns	Ns (1.3)
MCH-F4	1.5	Ns	−	+	+ (1.8)
MCH-F5	10	+	Ns	Ns	+ (13)
MCH-E6	22	−	+	Ns	− (20)
CQE-F1	0.78	−	Ns	−	− (0.27)
CQE-F2	0.46	+	Ns	Ns	+ (1.1)
CQE-F3	0.30	+	Ns	−	+ (0.62)
CQE-F4	0.82	−	+	+	+ (1.3)
CQE-F5	3.1	+	Ns	Ns	+ (4.9)
CQE-F6	14	−	Ns	Ns	− (12)

+: the parameter or amending process increased significantly the amount extracted ($p < 0.05$). −: the parameter or amending process decreased significantly the amount extracted ($p < 0.05$). Ns: the parameter or amending process have a non significant effect.

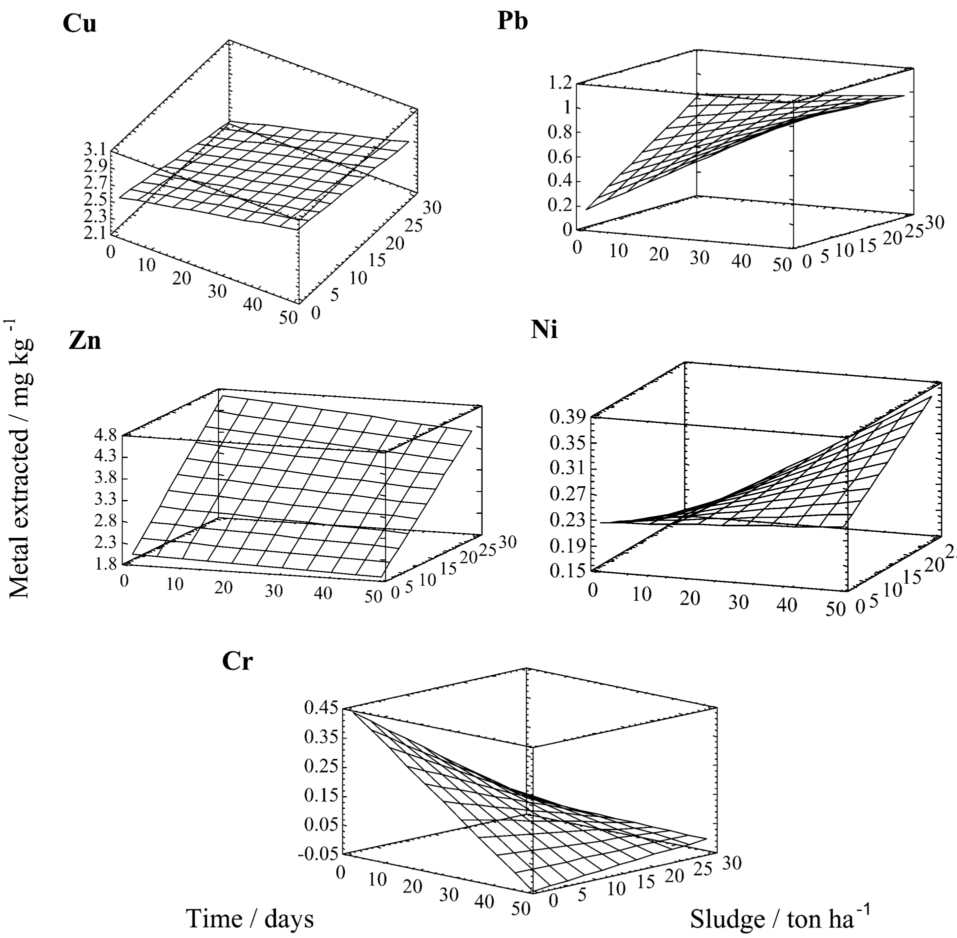

Fig. 3 Fitted models showing the relationship between the metal extracted in the first fraction from the STG amended soil, incubation time and sewage sludge application rate.

only in the STG soil. Incubation time was particularly important in the CQE soil, affecting all the Pb fractions. As for Cu, interaction was not a relevant factor concerning its effect on Pb fractions in the soils. In the extraction after the amending process, it should be pointed that in two of the three soils (CQE and MCH) the procedure caused a significant decrease in exchangeable Pb, a fact that was not observed for the other metals. It is possible that organic compounds or Fe oxides present in the sludge may be partially responsible for this reduction. Recent studies have indicated that application of high-Fe biosolids can reduce the availability of soil Pb.[33] As for the other elements, lead was redistributed in the first two or four fractions of the three amended soils. Fig. 3 shows different fitted models describing the relationship between the exchangeable form of the metals under study from the STG sludge-amended soil, incubation time, and sludge application rate. It should be pointed out that, for the same soil and fraction, behavior depends on the metal in consideration, which is also valid for the other soils in this study.

Conclusions

The results of this study indicate that sewage sludge addition led to an increase in the concentrations of almost all the metals under study, but this was significant only for Zn, which had the highest concentration in the sewage sludge applied. Except for copper, sewage sludge application modified metal distribution in the first two or four fractions in the three amended soils. Observed changes varied depending on the soil and metal type. The sequential extraction technique used in this study revealed that all the metals were mostly found in inert chemical form. The variables sludge and incubation time affected each metal and soil type differently. An increase in the labile metal forms was observed in most cases. Both variables and their interactions caused a positive final effect on almost all forms of Zn. The use of a two level factorial design as a statistical tool to assess the effect of sewage sludge application on the mobility and distribution of heavy metals in agricultural soils was satisfactorily applied.

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