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Effect of biosolid incorporation to mollisol soils on Cr, Cu, Ni, Pb, and Zn fractionation, and relationship with their bioavailability

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Abstract

Biosolid application to soil may be a supply of nutrients and micronutrients but it may also accumulate toxic compounds which would be absorbed by crops and through them be incorporated to the trophic chain.

The present study deals with the effect of biosolid application on Cr, Cu, Pb, Ni, and Zn in agricultural soils. The procedure used is sequential extraction so that the availability of those metals may be estimated and related to their bioavailability as determined through two indicator plants grown in greenhouse: ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense*). Results showed that biosolid application to soil increased total Cu and Zn content. Sequential extraction showed that the more labile Zn fractions increased after biosolid application to soil. This was confirmed when assessing the total content of this metal in shoot and root of the plants under study, since a higher content was found in plant tissues, while no significant differences were found for Cu, Cr, Ni, and Pb.

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1. Introduction

Biosolid application to soil assumes a significant supply of nutrients and micronutrients essential to plants. In addition, organic matter content, which may be above 40% dry weight, improves soil structure and porosity and increases its water retention capacity, its fertility, and activates soil microbiological life (Clapp et al., 1994; Artiola, 1996; Bitton, 1999; O'Connor et al., 2001). However, biosolid application has some inconveniences, both sanitary and environmental, due to the possible presence of pathogens, heavy metals, and toxic compounds. Wastewaters may contain contaminants (Zhou and Wong, 2001), both organic and inorganic, many of which cannot be eliminated through the various wastewater treatments, thus being transferred to sludge.

The bioavailable metal fraction in soil is constituted by ionic species present in the soil solution, which is the naturally dynamic medium where exchange, adsorption, and complexation reactions take place (Harter and Naidu, 2001). In order to determine trace metal availability and potential toxicity it is not enough to consider determining total metal content, since it gives insufficient information to estimate ecological and biological effects and to assess potential risks and toxicity in soil (Sauve et al., 2000; Pérez Cid et al., 2001). It is also necessary to know the various metal associations with the soil solid phase (Shuman et al., 1997). In order to separate the different metal associations with soil components, sequential extraction procedures are utilized (Fernández Alborés et al., 2000) through a succession of reactants with increasing solubilization strength. Thus, it is necessary to evaluate their relative lability in soil, since ideally each reactant should be specific to the component of the solid phase associated to the target trace element (Wenzel et al., 2001) and in this way to be able to predict metal distribution in the different

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fractions, which usually include exchangeable, bound to carbonates, bound to oxides, bound to organic matter, and residual (Banerjee and Shrivastava, 1998; Fernández Alborés et al., 2000; Pérez Cid et al., 2001; Lopez-Sanchez et al., 2003; Ahumada et al., 2004).

It has been reported that nickel in soil is mainly found in the residual fraction and, in biosolid-treated soils, Shuman et al. (1997) found that most Ni is retained, showing an increase in the oxide-bound fraction since this surface is highly selective for this metal (Dudka et al., 1995). On the other hand, Luo and Christie (1998) found that Cu and Zn are predominantly present in the residual fraction of soil and that in biosolid-amended soils Cu and Zn distribution is basically the same. However, such amendment caused an increase in both metals in most of the fractions. According to this information the overall purpose of this research work is to study the effect that biosolid application has on the distribution of the chemical forms, mobility and bioavailability of the trace metals Cr, Cu, Ni, Pb, and Zn in the soil or supplied by the residues.

2. Materials and methods

The study was carried out in four agricultural soils classified as mollisols. The samples were drawn from the surface layer (0–20 cm). Then they were air dried, ground and sieved through a 2-mm sieve for further storage in plastic jars at room temperature. The soils were characterized through determinations of pH, organic matter, cation exchange capacity (CEC), and total metal content. The soil samples were treated with 0 and 30 Mg ha⁻¹ of biosolid, and incubated at 25 °C for 60 days at field capacity. An anaerobically stabilized sludge obtained from the monofill at a wastewater treatment plant of the city of Santiago was used.

In order to determine the total content of Cr, Cu, Ni, Pb, and Zn, the equivalent to 200 mg of soil dried at 105 °C was weighed, with further addition of 4 ml 65% HNO₃, 4 ml HF, and 2 ml 30% H₂O₂. The samples were mineralized by digestion in a microwave oven using a 3-step program: 5 min at 250 W, 5 min at 400 W and 10 min at 500 W. Then the acids were eliminated by heating, 1 M HCl was added up to a 10 ml mark. Finally, concentration was determined by flame AAS.

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 the certified one ($p < 0.05$).

In order to fractionate the trace metals under study, the Howard and Shu methodology (1996) (Table 1) was selected, which includes the addition of the equivalent to 200 mg l⁻¹ NTA (nitrilotriacetic acid) to each extracting solution in order to prevent metal readsorption and increase selectivity. Each sample (3 g of sludge or soil) was weighed and the metals were sequentially extracted according to the following procedure:

Step 1: Addition of 20 ml 1 M MgCl₂, with 1 h-agitation at 25 °C.

Step 2: Addition of 20 ml 1 M Na OAc (sodium acetate) at pH 5, with 5 h-agitation at 25 °C.

Step 3: Addition of 20 ml 0.1 M NH₂OH·HCl (hydroxylamine hydrochloride) + 0.01 M HNO₃, with 30 min agitation at 25 °C.

Step 4: Addition of 20 ml 0.1 M K₄P₂O₇, with 24-h agitation at 25 °C.

Step 5: Addition of 20 ml 1 M NH₂OH·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.

Shoot and root tissue samples from two plants: ryegrass (*Lolium perenne* L.) and red clover (*Trifolium pratense*) plants were obtained from greenhouse assays, where pots containing the equivalent to 2 kg soil dried at 105 °C, treated with sludge in equivalent quantities to 0 and 30 Mg ha⁻¹ were used. The greenhouse assay was carried out in a completely randomized block design of 2 × 4 (sludge × soil) for both plants, considering three repetitions per treatment. The dry samples of shoot and root of both plants, separated according to kind of treatment and repe-

Table 1
Some general properties of biosolid and soils amended with 0 and 30 Mg ha⁻¹ of biosolid

Property	Method	Unit	Biosolid	Biosolid rate	Soil			
					LP	PC	EM	LL
OM	Acid digestion	g kg ⁻¹	38.6	0	2.57	3.94	4.02	2.37
				30	4.30	4.40	5.60	2.68
pH	H ₂ O 1:2.5		6.49	0	7.10	8.33	8.37	6.81
				30	7.12	8.06	8.12	7.00
CEC	NaAc 1 M pH 7	cmol kg ⁻¹	43.6	0	14.2	21.0	21.2	12.7
				30	14.4	21.6	21.6	12.8

Table 2
Total heavy metal content and sequential extraction of heavy metals in biosolid sample (result expressed as mean, $n = 3$)

THM content mg kg ⁻¹	RSD#	Exch-M		M-Carb.		M-MnO _x		M-OM		M-FeO _x		M-Res.	
		%	RSD	%	RSD	%	RSD	%	RSD	%	RSD	%	RSD
<i>Chromium</i> 189	2.1	0.40	1.9	0.70	0.8	0.3	10	20	4.7	4.5	3.5	74	7.8
<i>Copper</i> 331	5.1	3.5	0.4	4.7	0.4	1.2	2.7	21	6.1	5.8	9.4	63	8.9
<i>Nickel</i> 70	6.8	9.3	0.6	16	0.4	3.3	3.4	17	0.9	12	0.24	42	2.8
<i>Lead</i> 83	2.6	1.1	1.0	2.8	1.9	1.3	8.2	5.5	5.0	14	6.5	75	7.8
<i>Zinc</i> 1182	4.8	13	1.1	12	3.8	11	1.2	24	2.1	21	2.4	18	1.0

RSD# – relative standard deviation (%) $n = 3$; M – metal; % – percentage of total metal sequentially extracted.

tition, were mixed, ground and homogenized to obtain a compound sample.

In order to determine total Cr, Cu, Ni, Pb, and Zn content in ryegrass and red clover, about 500 mg of shoot sample and 200 mg of root sample were mineralized by microwave oven digestion with a mixture of 2 ml H₂O₂ and 4 ml HNO₃ utilizing the following 5-step program: 1 min at 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 400 W, and 5 min at 650 W, plus 1 min for ventilation. Then the resulting solution was taken to a volume of 20 ml with Milli-Q water. All of the metals were determined by inductively coupled plasma-mass spectrometry by ICP-MS Fison VG Plasma QuaD II using indium as internal standard. The analytical methodology employed for the determination of Cr, Ni, Cu, Zn and Pb in plant by ICP-MS was assessed by analyzing a certified reference material (IPE sample 722, Amaryllis Hippeastrum, leaf-Wageningen University Holland). The values determined were not significantly different from the certified one ($p < 0.05$).

Results of the effects of incubation time and sludge application on the mobility of trace metals contained in the soils were analyzed through a two-level factorial design where incubation time and biosolid application are the independent variables (0–60 days and 0–30 Mg ha⁻¹, respectively) and the content of metals extracted from the different fractions was the dependent variable. This design permits to investigate the effect of both factors on the response variable.

In order to identify the possible relationships between metal chemical forms in soils and bioavailability, a multiple linear correlation analysis was carried out between both variables, considering metal fractions as independent variables.

3. Results and discussion

Table 1 shows some of the general properties of sludge, control soil, and soils amended with 30 Mg ha⁻¹ of biosolid. In general, it may be observed that, as expected,

sludge showed the highest organic matter value, with 39%, while soil values are from 2.4% to 4.4%. Biosolid application to soil increased the content of organic matter, which would be attributed to the incorporation of organic components from the biosolid.

As to pH, the biosolid had a value of 6.5 and in soils, the values ranged from a value near neutral (6.8) to slightly

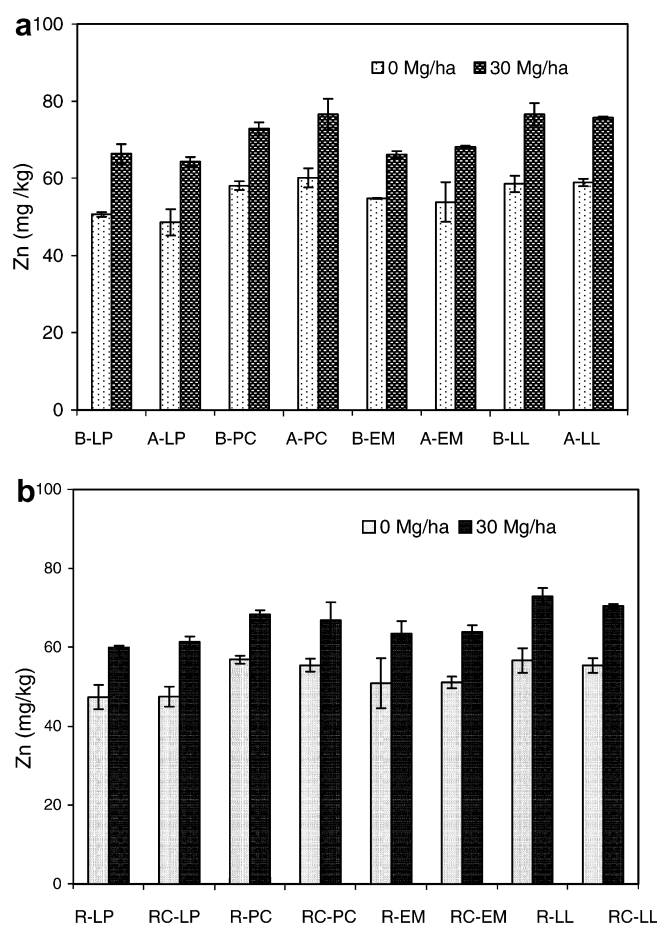


Fig. 1. Total content of Zn in control soils and soils amended with 30 Mg ha⁻¹ of biosolid: (a) before – B and after – A, incubation process and (b) after cultivation with ryegrass – R and red clover – RC.

alkaline (8.3). Respect to CEC soils PC and EM showed the highest values.

In relation to total metal concentration in the biosolid (Table 2), Zn, Cu, and Cr were in the range of 189–1182 mg kg⁻¹, while Ni and Pb were in the range of 70–83 mg kg⁻¹. The total content of the element under study was lower than the recommended limit values in sewage sludge as stated by Chilean regulation (CONAMA, 2001). The usual Zn content in biosolids is from 10 to 3000 mg kg⁻¹ (Banerjee and Shrivastava, 1998).

Sequential extraction of the biosolid under study (Table 2) shows that most Cu is in the residual form and bound to organic matter and FeO_x, while a small amount is associated to the exchangeable, carbonate, and MnO_x fractions, such as reported by McLaren and Clucas (2001). In the case of Zn, it is interesting to note the small portion represented by the residual fraction while the most labile fractions account for about 40% of the extracted metal, a result similar to the one observed by Scancar et al. (2001). Thus, it may be assumed that Zn would be found in more available forms. Most Ni extracted from the biosolid is in less mobile forms (residual, bound to oxides and to organic matter) even though the contribution of the exchangeable and bound-to-carbonate fractions is still important, about 20% of the extracted Ni. Cr and Pb are found in about 80% in their most inert form. It should be

noted that about 15% Cr is bound to organic matter, while Pb is similarly found but bound to Fe oxides.

In Fig. 1a, showing the total content of Zn, it may be observed that LL and PC soils have a higher content of Zn than EM and LP soils. Biosolid incorporation caused an increase in Zn in all of the soils, possibly due to Zn contribution from biosolid to soil. When the soils were cultivated (Fig. 1b), a decrease in total Zn concentration was found, especially in biosolid-amended soils.

This finding would coincide with the increase in the Zn available forms when the biosolid is incorporated, which is in agreement with the results obtained by Hseu (2006).

Copper is similarly distributed in the first four fractions (Table 3) for PC, EM, and LP soils prior to biosolid application, following the order F6 > F5 > F2 > F4. In biosolid-amended soils, Cu distribution is basically the same as that in the control soils, which is coincident with Luo and Christie's findings (1998), except for the bound-to-organic matter fraction, which is increased after biosolid addition. This could be accounted for by the fact that biosolid incorporation contributes Cu and offers an adequate surface for Cu interaction (Dudka et al., 1995), forming relatively strong complexes with the functional groups of the fulvic acids contained in the biosolids (Dahlgren et al., 1997). Some authors have found (Qiao et al., 2003; Vaca-Paulín et al., 2006) that biosolids amendment led to an increase

Table 3
Two level factorial design analyses for the effect of the amending process on the sequential extraction of Cu in LP, PC, EM and LL soils

Soil-fraction	Fraction before application (mg kg ⁻¹)	Time	Biosolid	Interaction	Fraction after application (mg kg ⁻¹)	Final effect
LP-F1	4.9	Ns	+	Ns	5.0	Ns
LP-F2	7.1	-	+	Ns	6.8	Ns
LP-F3	1.8	-	Ns	+	0.73	-
LP-F4	6.6	+	+	Ns	8.6	+
LP-F5	11	+	+	+	13	+
LP-F6	47	-	+	+	41	-
PC-F1	0.26	Ns	+	-	0.40	+
PC-F2	7.4	-	Ns	Ns	6.0	-
PC-F3	1.1	-	+	Ns	0.66	-
PC-F4	4.7	+	+	Ns	6.6	+
PC-F5	13	-	+	Ns	14	+
PC-E6	61	-	+	Ns	61	Ns
EM-F1	0.34	Ns	+	Ns	0.47	Ns
EM-F2	8.0	-	+	Ns	7.3	-
EM-F3	1.3	-	-	+	0.51	-
EM-F4	5.5	+	+	Ns	7.7	+
EM-F5	9.5	+	+	Ns	12	+
EM-F6	48	-	Ns	+	43	-
LL-F1	12	-	Ns	+	10	-
LL-F2	9.4	-	+	Ns	10	Ns
LL-F3	0.54	+	+	-	1.1	+
LL-F4	4.6	Ns	+	Ns	5.9	+
LL-F5	9.6	+	+	Ns	13	+
LL-F6	26	-	+	Ns	20	-

Effect of incubation time, sludge application rate and their interaction on Cu extracted from the following fractions of the four amended soils ($n = 3$): exchangeable (F1), bound to carbonate (F2), soluble in moderately reducing conditions (F3), K₄P₂O₇ soluble (F4), soluble in reducing conditions (F5) and soluble in strong acid oxidizing conditions (F6).

+: 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 is non-significant.

in Cu bound to organic matter fraction, however this tendency is observed in the present study only in EM and LL soils but to a low extent. In addition, it is observed that the labile forms (exchangeable and bound to carbonates) constitute about 12% of the total extracted, while in the case of LL soil, labile Cu accounts for about 35%.

Two level factorial design analyses for the effect of the amending process on the sequential extraction of Cu in soils (Table 3) shows that sludge application influenced positively the Cu concentration in most of the fractions of the soils under study, while incubation time has no significant effect on the labile fractions. As concerns interaction of both factors, no significant effect was observed on any of the soils under study, except for the fraction bound to Mn oxides (F3) in the case of LP and EM soils, and the exchangeable fraction (F1) of LL soil.

In the case of Zn fractionation it was found that more than 75% of the metal is in the residual form (F6), after biosolid application this percentage decreased to 60% in most of the soils.

Biosolid application increased Zn concentration in the other fractions. It could be noticed that the labile fractions (F1 and F2) increased from 5% to 20%, therefore the Zn incorporated by the biosolid should be available for the plants, the same as observed by Thompson et al. (2001). The main difference between control and amended soils is the increase of fraction F2, thus Zn contributed by biosolid

would be mainly in available and mobile forms. (Planquart et al., 1999).

On the other hand, statistical analysis of results (Table 4), shows that biosolid application affected positively and significantly the Zn fractions in all soils. On the other hand, incubation time did not have a significant effect on most Zn fractions. When evaluating the final effect of biosolid application, an increase is observed for most of the Zn fractions in all the soils, except for the residual fraction of LP and EM soils. It is known that availability of trace metals is greater in soils that have received biosolids application (Sukkariyah et al., 2005). Thus, of all the metals included in this study, it is estimated that Zn has the highest probability of mobility within the soil. Berrow and Burridge (1990) reported that metals contained in the biosolids added to soils may change their forms and their persistence in soils as labile or inert forms may last for many years. In the case of Cr, Ni, and Pb (results not shown), only the exchangeable fraction was affected significantly by incubation time.

Biosolid application increased Zn bioavailability for ryegrass and red clover. Plant Zn content is mainly distributed in the roots and the amount found in ryegrass was twice that found in red clover plants. In the case of Cu, Cr, Ni, and Pb, no significant differences were found in the contents of these metals in the selected plants. On the other hand, other authors (Bertoncini et al., 2004) have found

Table 4

Two level factorial design analyses for the effect of the amending process on the sequential extraction of Zn in LP, PC, EM and LL soils

Soil-fraction	Fraction before application (mg kg ⁻¹)	Time	Biosolid	Interaction	Fraction after application (mg kg ⁻¹)	Final effect
LP-F1	2.1	+	+	+	8.7	+
LP-F2	1.4	-	+	-	5.2	+
LP-F3	0.33	Ns	+	-	1.3	+
LP-F4	0.73	-	+	-	1.6	+
LP-F5	8.6	+	+	+	12	+
LP-F6	37	-	+	Ns	36	-
PC-F1	0.45	-	+	-	1.7	+
PC-F2	1.9	-	+	Ns	8.2	+
PC-F3	0.18	+	+	+	1.9	+
PC-F4	0.76	-	+	-	2.8	+
PC-F5	10	+	+	Ns	14	+
PC-F6	44	+	+	+	56	+
EM-F1	0.59	-	+	-	2.0	+
EM-F2	1.8	Ns	+	Ns	10	+
EM-F3	0.34	+	+	+	2.1	+
EM-F4	1.1	Ns	+	Ns	3.1	+
EM-F5	10	+	+	Ns	17	+
EM-F6	41	-	+	Ns	33	-
LL-F1	1.9	-	+	Ns	5.9	+
LL-F2	1.5	Ns	+	Ns	9.0	+
LL-F3	0.38	+	+	+	1.9	+
LL-F4	1.0	-	+	-	1.8	+
LL-F5	6.4	+	Ns	Ns	13	+
LL-F6	47	-	+	Ns	44	-

Effect of incubation time, sludge application rate and their interaction on Cu extracted from the following fractions of the four amended soils ($n = 3$): exchangeable (F1), bound to carbonate (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).

+: 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 is non-significant.

Table 5

Multiple linear regression equation between Zn content in shoots and roots of ryegrass (RS and RR) and red clover plants (RC) and different chemical fractions of these metals in soils amended with 0 and 30 Mg ha⁻¹ of biosolid

Equations of linear multiple regression	r^2
Zinc content	
<i>Control soils</i>	
ZnRS = -2.9 - 12F1 + 18F2 + 64F3 - 0.84F6	0.98
ZnRR = 176 - 10F1 - 45F3 + 5.4F4 - 3.4F5 - 0.89F6	0.99
ZnRCS = 93 - 9.3F1 + 46F3 - 24F4 - 4.6F5	0.99
ZnRCR = 14 + 8.2F2 + 9.9F3 + 13F4 - 2.8F5 - 0.16F6	0.99
<i>Soil conditioned with 30 Mg ha⁻¹</i>	
ZnRS = -117 + 17F1 + 21F2	0.97
ZnRCS = 27 - 3.1F1 - 1.9F2 - 4.1F3 + 10F4 - 1.5F5	0.99
ZnRCR = 187 - 8.2F1 - 8.8F2 + 8.1F4 - 2.2F6	0.96

Exchangeable (F1), bound to carbonates (F2), soluble in moderately reducing conditions (F3), K₄P₂O₇ soluble (F4), soluble in reducing conditions (F5) and soluble in strong acid oxidising conditions (F6).

$p < 0.05$; r : coefficient of correlation; R: roots; S: shoot; C: red clover; R: ryegrass.

that biosolid application increased the leaf concentrations of Cr, Cu Ni and Zn in clayey soils. Zinc concentration in root and shoot of ryegrass has a statistically significant correlation with most of its fractions in the control soils (Table 5). In the case of shoot, the fractions bound to carbonates and to Mn oxides have a positive contribution, while in the case of the root only the fraction bound to organic matter has a positive contribution. For red clover, Zn concentration shows a statistically significant correlation both for root and shoot, with r^2 value of 0.99. The fraction bound to Mn oxides is a positive contribution for shoot and root; in addition, the latter receives the contribution of the fractions associated to carbonates and organic matter.

In the soils treated with 30 Mg ha⁻¹, ryegrass shoot Zn content correlated with the more labile Zn fractions (F1 and F2), with $r^2 = 0.99$, indicating that those fractions in the sequential extraction should be reliable for predicting Zn bioavailability to ryegrass. Red clover, instead, shows a statistically significant correlation both for shoot ($r^2 = 0.99$), with the contribution of the Zn fraction associated to organic matter and the residual one, and for root ($r^2 = 0.96$) where the contribution comes only from the fraction bound to organic matter (F4).

4. Conclusions

Sequential extraction of the biosolid permitted to determine that the availability of the metals contained in the biosolid was the following: Zn > Ni > Cu > Pb > Cr. Thus, biosolid application to soils affected the residual metal fraction, causing an increase and a change in the concentration order of this fraction: Cr > Ni > Pb > Zn > Cu. At the same time, it was observed that all of the metals are predominantly bound to the residual fraction, with the following increasing order: Pb, Cu, Ni, Zn, and Cr, with 48%, 60%, 63%, 77%, and 80%, respectively.

A two-level (biosolid rate and incubation time) factorial design was satisfactorily utilized as a statistical tool to assess the effect of biosolid application on mobility and distribution of heavy metals in agricultural soils.

Incubation time of the control and biosolid-treated soils did not cause a significant variation in the distribution of the metal fractions. On the other hand, biosolid application caused a fourfold increase in the labile Zn fractions (F1 and F2), increasing Zn bioavailability for ryegrass and red clover. Plant Zn content is mainly distributed in root, and the amount found in ryegrass was twice that found in red clover plants. In the case of Cu, Cr, Ni, and Pb, no significant differences were found in the contents of these metals in the selected plants.

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