Australian Journal of Basic and Applied Sciences, 1(4): 354-363, 2007
ISSN 1991-8178

Cadmium, Copper, Nickel, and Lead Availability in Biosolids-amended Alkaline Soils

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Abstract: The disposal of sludge products in land fills or by incineration are feasible options currently practiced in many parts of the world, but both these strategies are expensive and can cause environmental problems. The application of stabilized sewage sludge (biosolids) to the land has therefore become an attractive option. The concentration and the availability of heavy metals is probably the main factor to bear in mind, when biosolids are used as organic amendments. The effects of different biosolids loadings on plant growth, evaluation of the availability of Cd, Cu, Ni, and Pb in a biosolids- amended alkaline soils, and determination of plant tissue metal uptake response to biosolids derived heavy metals were studied. The results of present study indicated that the concentrations of DTPA-extractable elements were significantly higher in the biosolids-amended soils than those extracted from the control treatment**.** The concentrations of heavy metals extracted with 0.05 M DTPA increased polynomialy in response to biosolids additions for all studied soils. Increases of 214, 184 and 135% in total dry matter yield were achieved as a result of applying 3% biosolids rate to sandy, calcareous and clay soils respectively. The corn plants grown in the clay soil had a higher assimilative capacity for uptake of Cd, Ni, Pb and Cu than other soils. The uptake values of heavy metals followed the following order: clay > calcareous > sandy soils. The increase in Cd, Ni, Pb and Cu concentrations peaked at the high level of biosolids application rates (3%) in all studied solis. Metal concentration in plant parts displayed a nonlinear plateau response in all cases.

Keywords: Heavy Metals, Availability, Biosolids, Soils

INTRODUCTION

In recent years, the production of sewage sludge (biosolids) has increased sharply all over the world due to the demand for better quality water and the imposition of more strict environmental laws. The accumulation of these biowastes poses a growing environmental problem. The disposal of sludge products in land fills or by incineration are feasible options currently practiced in many parts of the world, but both these strategies are expensive and can cause environmental problems. The application of stabilized sewage sludge to the land has therefore become an attractive option. This practice could solve two problems: (i) a potential source of pollution is reduced in size, and (ii) soil productivity is favoured and the need for synthetic fertilizers reduced (which has inherent economic benefits). Sewage sludge is a good source of plant nutrients such as N and P (Sims J.T., G.M. Pierzynksi, 2000. and Martinez F., *et al.,* 2003.) and it has large amounts of organic matter that can improve the physical and biological properties of the soil (Logan T.J., and B.J. Harrison, 1995.; Caravaca F., *et al.,* 2002).

The concentration and the availability of heavy metals is probably the main factor to bear in mind, when sewage-sludges are used as organic amendments. It has been suggested that applying sewage sludge to soil might provide metals in potentially toxic, labile forms (McBride M.B., 1995).

The accumulation of heavy metals and their availability to plants depend strongly on the composition of the sludge, the application rate, soil properties, and the crop species.

Previous research has shown that a number of soil properties are likely to influence heavy metal uptake by crops including pH, organic matter content and soil texture (Smith S.R., 1996). Results indicate that crops

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grown on sandy, low organic matter status soils are likely to have a greater uptake of certain heavy metals (in particular Zn and Cd) compared with crops grown on soils with higher clay and organic matter contents (Allow, B.J., 1990; Eriksson, J.E., 1988).

Sherif *et al.,* (2001) reported that increasing of sludge rates significantly increased trace elements availability in calcareous lacustrine soil and the 20 Mg fed^{-1} of sludge application rate showed a high response of tuber yield of potato. However, concerns have been raised that the availability of added trace metals may increase after termination of biosolids applications due to the decomposition of organic matter (McBride, M.B., 1995.; Beckett, P.H.T., *et al.,*1979). The risk assessment conservatively assumes that the relationship between metal added in biosolids and plant uptake will be linear despite data that suggest that uptake will attain a plateau response.

The objectives of the present study were to (i) examine the effects of different biosolids loadings on plant growth, (ii) to evaluate the availability of Cd, Cu, Ni, and Pb in biosolids- amended alkaline soils, and (iii) to determine the plant tissue metal uptake response to biosolids derived heavy metals.

MATERIALS AND METHODS

Characteristics of Soils and Biosolids:

The soils used in this study were selected and sampled (0-15 cm depth) from three different locations. The soils were: clay (Typic torrifluvents), sandy (Typic torripsamment), and calcareous (Typic calciorthids). Sub-samples of the air-dried soils were ground to pass a 2-mm sieve prior to the following chemical analyses: pH and electrical conductivity (EC) as well as soluble cations and anions were determined in soil-paste extract. [(12)] The organic matter content was determined by dichromate oxidation (Nelson, D.W. and L.E. Sommers, 1982). Cation exchange capacity (CEC) was determined by 1M Na OAC (Rhoades, J.D., 1982). Particle size analysis was determined by the hydrometer method (Day, P.R., 1965). Calcium carbonate content was determined using calcimeter (Nelson, R.E., 1982). Available P was extracted by 0.5 M $NaHCO_3$ test (Olsen, S.R., and L.E. Sommers, 1982). Available N was extracted by 2M KCl test (Keeney, D.R., and D.W. Nelson, 1982) and determined by the Kjeldahl method (Bremner, J.M., and C.S. Mulvany, 1982). Available K was extracted by 1M NH_4OAC test (Knudsen, D. *et al.,* 1982. The main characteristics of the soils are shown in Table1.

Table 1: Some chemical characteristics of the three studied soils.

Characteristics	Units	Clay	Sandy	Calcareous
EC	dSm^{-1}	2.66	3.84	2.92
pH		8.13	7.69	8.08
$CaCO_3$	g kg^{-1}	57.90	2.40	356.80
Sand	g kg^{-1}	596.4	868.2	740.00
Silt	g kg^{-1}	141.3	25.10	101.50
Clay	g kg^{-1}	262.30	106.70	158.50
Texture		S.C.L	L.S	S.L
O.M†	g kg^{-1}	8.50	1.00	4.60
CEC	$Cmol(+)kg^{-1}$	39.13	8.70	26.00
Olsen-P	mg kg^{-1}	24.75	2.89	18.70
Available-N	mg kg^{-1}	83.00	52.00	61.00
Available-K	mg kg^{-1}	350.00	70.00	301.00
DTPA-extractable elements:	mg kg^{-1}			
Cd		0.33	0.18	0.26
Ni		8.92	5.13	7.17
Pb		6.13	2.18	5.69
Cu		9.09	3.13	4.98

† O.M: organic matter

The aerobically composted biosolids were obtained from the General Organization Sanitary (GOS) of Alexandria city (station no-9). The biosolids particles were allowed to air-dry and were subsequently passed through a 1-mm sieve prior to their use (Makris, K.C, *et al.,* 2005). The applied biosolids have EC = 11.25 dsm^{-1}, pH = 6.69, organic matter = 450 g kg^{-1}, total-P = 4.60 g kg^{-1}, total-N = 32.00 g kg^{-1}, total Ni = 108 mg kg^{-1}, total Pb= 143.00 mg kg^{-1}, total Cu = 128 mg kg^{-1} and total Cd = 4.00 mg kg^{-1}. The total metals were determined by the wet digestion method (Ure, A.M., 1995).

Soils Incubation and Greenhouse Experiment:

Four biosolids rates (0, 1, 2, and 3%, ww^{-1}, on the dry weight basis) were applied to each soil and thoroughly mixed. Soil for each treatment was transferred to a large plastic bowl. Two- thirds of the water required to obtain field capacity was initially added to the soil with a water dispenser and mixed thoroughly to form a uniform soil-biosolids-water mixture. Treated soil mixture were then transferred to a plastic pot (2kg $pot^{-1)}$ and brought to field capacity. Pots were covered with perforated plastic cover and incubated at 25^0C for 60 days. Moisture content of the treated soil mixture was kept constant during incubation by periodically weighing the pots and adding deionized water to compensate for evaporative loss. After the incubation periods, corresponding soil samples were air- dried, crushed to pass a 2-mm sieve and stored for analysis. The quantity of biosolids, trace metals and total N and P applied to the soils are presented in Table 2.The changes in soil pH during the duration of the study is presented in Table 3.

Table 2: Quantity of biosolids, heavy metals, and total N and P applied at the three studied soils.

Biosolids rates	N	P	Cd	Pb	Ni	Cu
%	mg.pot^{-1}		µg.pot^{-1}			
1	640.00	92.00	80.00	2860.00	2160.00	1024.00
2	1280.00	184.00	160.00	5720.00	4320.00	2048.00
3	1920.00	276.00	240.00	8580.00	6480.00	3072.00

Table 3: Soil pH in the biosolids-amended soils.SE in parentheses.

Biosolids rates	pH		
	Clay	Sandy	Calcareous
%			
0	8.13a¶ (0.01)	7.69a (0.02)	8.08a (0.01)
1	7.96b (0.01)	7.41b (0.02)	7.98b (0.02)
2	7.65c (0.02)	7.26c (0.02)	7.75c (0.02)
3	7.29d (0.01)	7.17d (0.01)	7.48d (0.02)

¶ Values within columns followed by different letters are significant at the 0.05 probability level.

Corn seeds (*Zea mays*) were sown in pots containing 2 kg of soil (s) with biosolids rates mentioned above. The seedlings were thinned to 4 seedlings per pots and deionized water was added to bring the soil moisture to 70 % of field capacity. The experiment was arranged in completely randomized with four replicates. Plants were harvested after 105 days of growth in the tested soils.

Heavy Metals Extractability:

The diethylene triamine penta acetic acid extracting (0.005M DTPA, 0.1 TEA, and 0.01 M $CaCl_2$, adjusted to pH 7.3) solution (Lindsay, W.L., and W.A. Norvell, 1978) was employed to extract heavy metals as potential indicator of plant-available heavy metals from soils treated with and without biosolids before cultivation. The soil extracts were analyzed for Cd, Cu, Ni, and Pb with atomic absorption spectrometry (Baker, D.E. and M.C. Amacher, 1982).

Plant Analysis:

The plant shoots and roots were harvested separately, and immediately were washed thoroughly with running tap water and rinsed three times with deionized water. The plant tissues were dried in a forced air oven at 65^0C for 48 h and dry matter yield was recorded. Plant tissues were ground in a stainless steel mill. Sub-samples of ground plant material were dry-ashed in a muffle furnace at 450^0C for 6 h. Ash was dissolved in nitric acid (1:1), diluted to a constant volume with distilled water and analyzed for heavy metals (Jones, J.B., 2001).

Statistical Analysis:

Plant weight and heavy metal concentration data were evaluated by analysis of variance (ANOVA) and by the least significant difference (LSD) mean separation procedures at the 0.05 level of significance (SAS Insituate., 1994). Non linear regression was used to develop predictive equations relating available heavy metals concentration responses to biosolids application rates. Regression analysis also was employed to determine the relationships between available heavy metals concentrations in soils and heavy metals concentrations in plant tissues.

RESULTS AND DISCUSSIONS

Soil Test Extractant:

Measurements of DTPA-extractable Cd, Ni, Pb and Cu have been made before cultivation on this experiment. At the end of incubation period following biosolids application, increases of DTPA- extractable elements with increasing biosolids application was noticed. The levels of extractable Cd, Ni, Pb and Cu reached 3.66, 25.12, 15.4 and 20.29 mg kg^{-1} in clay soils respectively at the highest application rate. Whereas, in sandy soil the levels of extractable Cd, Ni, Pb and Cu were 3.15, 17.18, 12.62 and 11.19 mg kg^{-1} respectively (Table 4). The levels of these elements in calcareous soil reached 3.88, 22.08, 11.47 and 12.95 mg kg^{-1} at the highest application rate (Table 4). Anderson (Anderson, M.A., 1997) reported a linear increase in DTPA–extractable Cu (R^2 =0.992) and Zn (R^2 =0.992) concentrations with biosolids application rates in a field experiment.

Table 4: Effect of biosolids application rate on DTPA-extractable trace metals in the three studied soils.

Biosolids rate	DTPA-extractable trace metals			
	Cadmium	Nickel	Lead	Copper
%	mgkg^{-1}			
	Clay			
0	0.33d	8.92d	6.13d	9.09d
1	0.68c	15.70c	7.73c	13.83c
2	2.16b	18.92b	10.88b	16.96b
3	3.66a	25.12a	15.41a	20.29a
	Sandy			
0	0.18d	5.13d	2.18d	3.13d
1	1.61c	9.19c	4.18c	5.13c
2	2.73b	13.44b	8.95b	8.76b
3	3.15a	17.18a	12.62a	11.19a
	Calcareous			
0	0.26d	7.17d	5.69d	4.98d
1	1.18c	13.07c	7.98c	6.01c
2	2.35b	18.18b	9.65b	8.19b
3	3.58a	22.08a	11.47a	12.95a

¶ Values within columns followed by different letters are significant at the 0.05 probability level.

Table 5: Effect of biosolids application rate on dry weight of different parts of corn plants grown in the three studied soils.

Plant part	Biosolids application rate, %			
	0	1	2	3
	Clay			
Shoot	27.84d¶	43.19c	66.18b	69.94a
Root	10.46d	11.10c	16.18b	20.02a
Total	38.30d	54.29c	82.36b	89.96a
	Sandy			
Shoot	3.55d	5.13c	8.22b	10.98a
Root	1.71d	2.90c	4.48b	5.56a
Total	5.26d	8.03c	12.70b	16.54a
	Calcareous			
Shoot	6.38d	8.60c	11.19b	15.13a
Root	2.52d	4.12c	7.33b	10.15a
Total	8.90d	12.72c	18.52b	25.28a

¶ Values within rows followed by different letters are significant at the 0.05 probability level.

The concentrations of DTPA-extractable elements were significantly higher in the biosolids-amended soils than those extracted from the control treatment due to the lower soil pH (Table3). This trend in increasing extractability with increasing biosolids application rates was noticed in all studied soils. The polynomial quadratic model was used to describe the relationship between biosolids application rates and extractable elements (Fig.1). The quadratic equations were successful in describing such relationships. The concentrations of heavy metals extracted with 0.05 M DTPA increased polynomialy in response to biosolids additions (Fig.1) for all studied soils. The ability of extractant to remove metals from soils was in the following order: clay>calcareous> sandy in case of Ni, Pb and Cu, however the order was sandy >calcareous >clay in case of

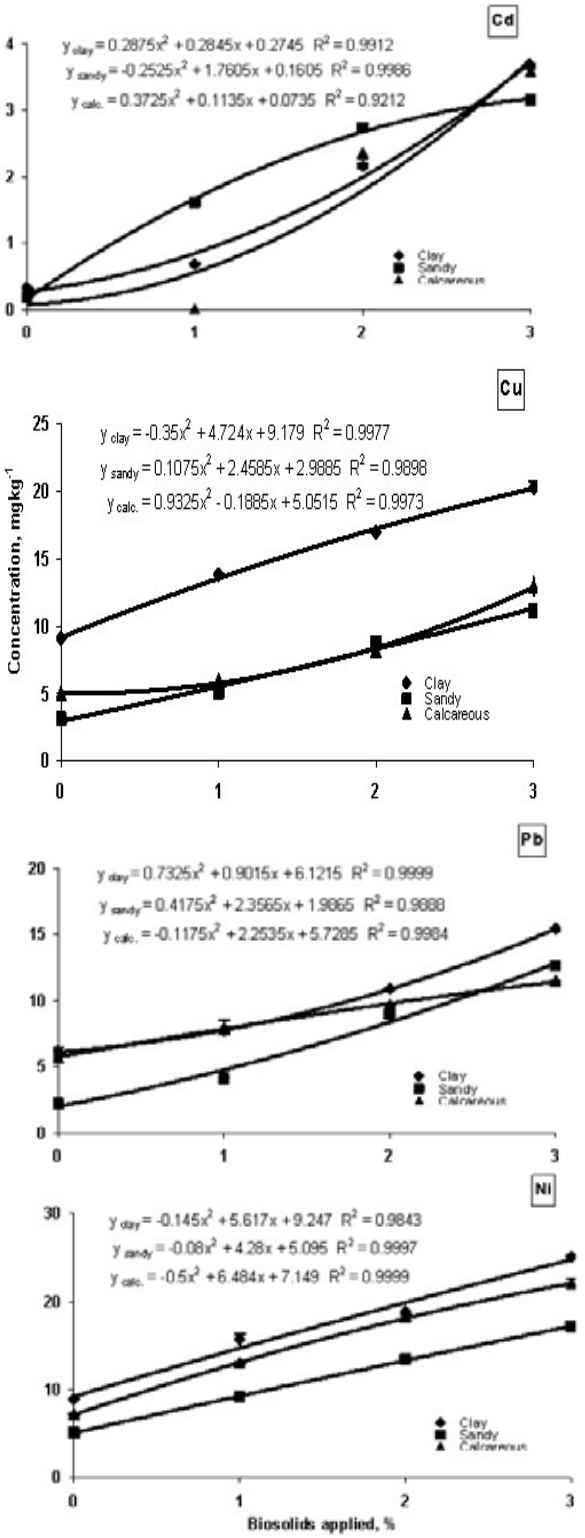

Fig. 1: Effects of biosolids application rates on the levels of DTPA-extractable Cd,Cu,Pb and Ni in the three studied soils.

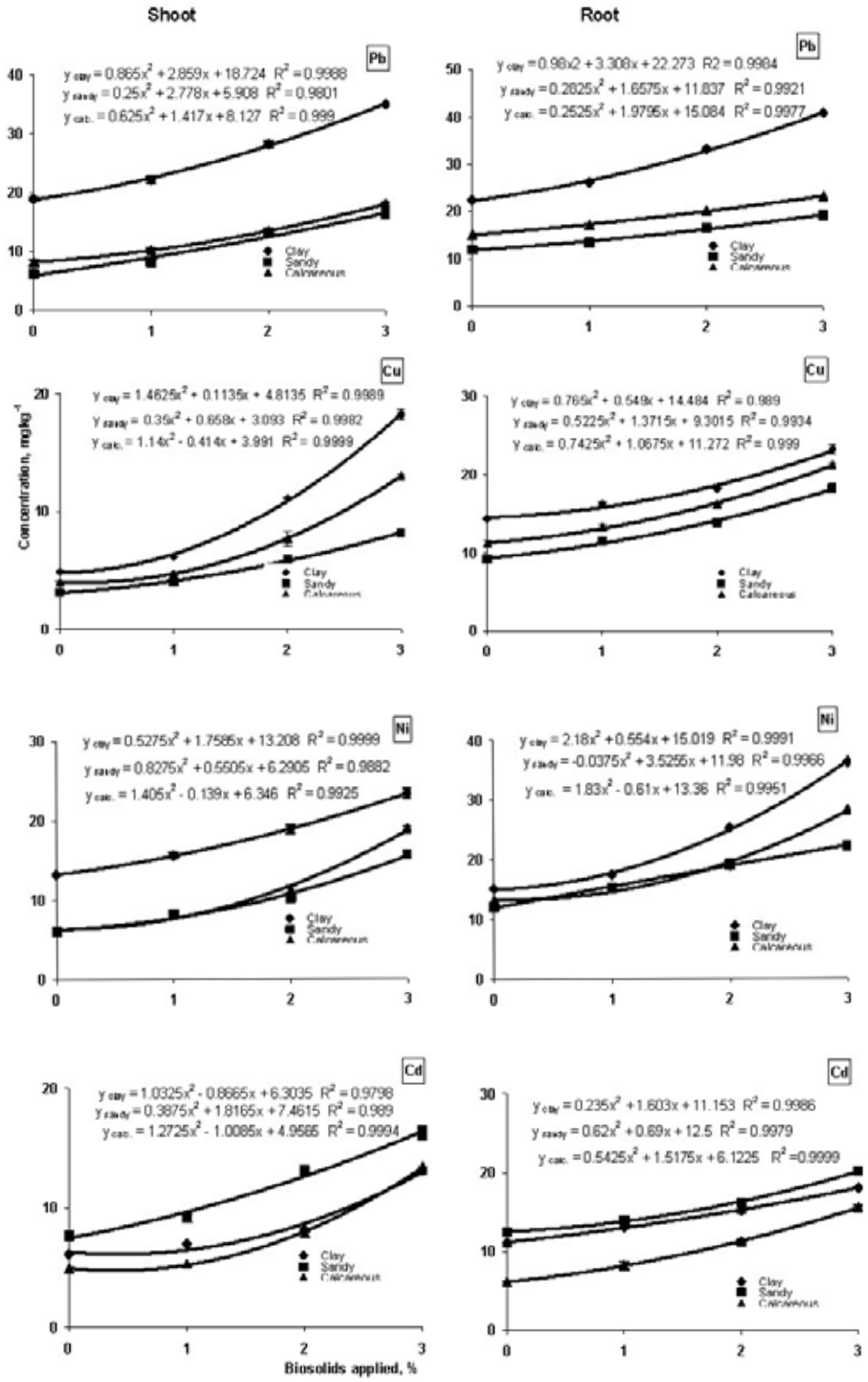

Fig. 2: Heavy metals accumulation in parts of corn plants as a function of biosolids application to the three studied soils.

Cd-across all rates. Extractable metal content of the soil, which, in addition to pH, are the most important factors in regulating the availability of Cd, Ni, Pb and Cu to several plant species (Sauerbeck, D.R., 1991).

Our results have shown a polynomial increase of Cd (clay: R^2 =0.991, sandy: R^2 =0.999 and calcareous: R^2 =0.921), Ni (clay: R^2 =0.984, sandy: R^2 =0.999 and calcareous: R^2 =0.999), Pb (clay: R^2 =0.999, sandy: R^2 =0.989 and calcareous: R^2 =0.998) and Cu (clay: R^2 =0.998, sandy: R^2 =0.990 and calcareous: R^2 =0.997) extracted with DTPA with increasing application rate.Such results clearly indicate a significant increase of metal availability with increasing of biosolids application rates in all studied soils. Sommers *et al.,* (1991) reported that the highest availability of trace metals occurred during the period immediately following biosolids application. In other studies, the availability of heavy metals has been reported to decrease with time as organic decomposition rates decreased (Walter, I. *et al.,* 2002).

Crop Yield:

Boisolids application rates significantly increased the shoots, roots and total dry matter yield of corn plants in all studied soils (Table5). There were no visual signs of toxicity or deficiency due treatments in any part of the crop, which is further evidence that plant growth was not negatively affected by the biosolids treatments. The yield of dry matter of shoots, roots and total of corn plants in the clay soil was much higher than those recorded in both sandy and calcareous soils (Table5). Increases of 214, 184 and 135% in total dry matter yield were achieved as a result of applying 3% biosolids rate to sandy, calcareous and clay soils respectively. Khalil *et al.,* (2000) and Mater (2000) reported that the addition of biosolids significantly increased the dry matter yield of corn plants as well as dwarf beans Khalil *et al.,* 2000 and Mater 2000. Also, Elsayed (1989) found that the biosolids application increased the dry weight of root, shoot and biomass of faba bean and wheat grown on the calcareous soils.

Plant Tissue Uptake Response:

The heavy metals uptake by corn plants was significantly increased at all application rates in all studied soils (Table6). The uptake values of trace metals in our study varied widely among soils. The corn plants grown in the clay soil had a higher assimilative capacity for uptake of Cd, Ni, Pb and Cu than other soils. The uptake values of heavy metals followed the following order: clay > calcareous > sandy soils.

The responses of plant tissue heavy metal concentrations to biosolids application rates are shown in Fig 2. Concentrations of the heavy metals were plant parts-specific. The metal concentrations in all plant parts were higher in the biosolids treatments than in the control for all studied soils (Table 6), but remained well within the values observed for uncontaminated soils (Kabata-Pedias, A. and H. Pendias, 1992). None of the heavy metals attained toxic concentrations. Heavy metals concentrations tend to be accumulated in parts of corn plants in the following order: roots > shoots (Table6). In general, application of biosolids significantly increased heavy metals concentration in shoots and roots of corn plants grown in all studied soils. The increase in Cd, Ni, Pb and Cu concentrations peaked at the high level of biosolids application rates (3%) in all studied soils (Table 6).Metal concentration in plant parts displayed a nonlinear plateau response in all cases (Fig 2). The nonlinear relationship indicated that an incremental decrease in the uptake rate occurred with increasing biosolids application rate (Fig 2).

Several studies (Logan, T.J., *et al.,* 1997., Brown S., *et al.,* 1998) have provided evidence that biosolids heavy metals uptake by wheat, corn and several vegetables reach a maximum with biosolids application rate. The reasons for plateau responses have been speculated by many researchers. Corey *et al.,* (1987) suggested that metal uptake would approach a maximum (Plateau) at high biosolids metal loadings because the amended soils had higher metal adsorption capacity than the unamended soils. Chaney and Rayan (1993) proposed that increasing biosolids application increases the metal adsorption capacity of soil in addition to soil metal concentration; thus, metal availability at high biosolids application declines as the specific metal adsorption capacity of the amended soil increases (Hettiarachchi, G.M., *et al.,* 2003).

Plant mechanisms such as exclusion of heavy metals, limited transport from root to shoot, and saturation of the carrier system might also contribute to the expected decrease in uptake slope at high metal loading (>3%) (McBride, M.D., 1995.; Hamon, R.E., *et al.,* 1999). Hamon *et al.,* (1999) proposed that bioavailability will plateau at some biosolids loading rate and metal uptake by plant will follow the same trend if attenuation of metal concentration is due to biosolids chemistry. Metal concentrations in plant grown in all studied soils displayed a plateau response to heavy metals from biosolids applied in all cases. Response was plant parts-dependent, metal dependent, and varied among soils. These observations show that in addition to soil reactions, plant uptake control mechanisms play an important role in regulating metal uptake.

Table 6: Heavy metals concentrations and uptake in the dry matter of corn plants grown in the three DWTR- amended soils.

Heavy metals	Concentration, mgkg-1								Uptake,mg.pot-1			
	Shoot				Root							
	Biosolids rate,%											
	0	1	2	3	0	1	2	3	0	1	2	3
	Clay											
Cd	6.13d¶	6.99c	8.18b	13.17a	11.11d	13.12c	15.17b	18.12a	0.28d	0.44c	0.78b	1.27a
Ni	13.20d	15.52c	18.81b	23.24a	15.13d	17.42c	25.18b	36.19a	0.52d	0.86c	1.71b	2.35a
Pb	18.82d	22.16c	28.19b	27.33a	22.40d	26.18c	33.19b	40.89a	0.76d	1.25c	2.40b	3.26a
Cu	4.89d	6.16c	11.12b	18.24a	14.33d	16.26c	18.18b	23.17a	0.28d	0.43c	1.00b	1.73a
	Sandy											
Cd	7.62d	9.19c	13.12b	16.24a	12.44d	13.99c	16.18b	20.21a	0.04d	0.08c	0.17b	0.28a
Ni	6.12d	8.18c	10.19b	15.56a	12.08d	15.17c	19.18b	22.12a	0.04d	0.09c	0.17b	0.29a
Pb	6.16d	8.18c	13.22b	16.24a	11.95d	13.44c	16.62b	19.24a	0.04d	0.08c	0.18b	0.29a
Cu	3.13d	3.99c	5.92b	8.18a	9.18d	11.56c	13.77b	18.24a	0.03c	0.05c	0.10b	0.18a
	Calcareous											
Cd	4.92c	5.33c	7.92b	13.42a	6.12d	8.19c	11.32b	15.56a	0.04d	0.14c	0.29b	0.35a
Ni	6.16d	8.17c	11.13b	18.76a	13.18d	15.12c	18.92b	28.18a	0.07d	0.13c	0.26b	0.57a
Pb	8.12d	10.19c	13.44b	18.01a	15.15d	17.12c	20.25b	23.23a	0.09d	0.16c	0.30b	0.51a
Cu	3.99c	4.72c	7.72b	13.01a	11.22d	13.24c	16.22b	21.21a	0.04d	0.09c	0.20b	0.40a

¶Values within rows followed by different letters are significant at the 0.05 probability level.

Conclusion:

No adverse effects on plant growth or excessive amounts of metal uptake were noted after application. Concentrations in all plants grown in the different biosolids-amended soils were well within the sufficiency range observed for agronomic crops (Kabata-Pendas A., and H. Pendias, 1992). Our results confirmed that accumulation of heavy metals differed among plant parts and soil types. Available metal concentrations were dependent on the total metal content of soil. Metal concentrations in shoot and roots of corn plants grown in the three–biosolids-amended soils displayed a plateau type response. The uptake values of heavy metals followed the following order: clay > calcareous > sandy soils.

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