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Correlation Between Heavy Metal Toxicity and Metal Fractions of Contaminated Soils in Korea

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Several metals are listed in the U.S. Environmental Protection Agency's list of 129 priority pollutants due to their abundance and toxicity in various environments. Heavy metal contamination has received much attention with regard to accumulation in soils, uptake by plants, and contamination of aquatic environments (Li and Shuman 1996). Except for very acidic soils, heavy metals are sparingly soluble in soils and occur predominantly in a sorbed state or as insoluble compounds. Because of their low solubility, movement of heavy metals in soils has generally been considered either minimal or practically nonexistent. Williams et al. (1987) found no significant movement of Cd, Pb, Cu, and Zn in soils treated with biosolids for 8 years, and demonstrated that more than 90% of applied heavy metals were found in the top 15cm of the soil. Their movement is essentially related to the physicochemical forms of the metals in soil.

It is well known that metals, reaching excessive levels, can exert serious impacts on humans, animals, and plants (Kim and Kim 1999). Metal toxicity associated with solids in the environment is of particular concern since they can be released. Nevertheless, their environmental impact is difficult to evaluate qualitatively due to the complex interactions between metals and ecological systems (Kim and Kim 1999). It is, however, well known that information on the total concentrations of toxic metals in contaminated soils is not sufficient for the understanding of their mobility and availability to the biota (Bunzl et al. 1999). For this reason, various sequential extraction procedures have been proposed to study the partitioning (or fractionation) of metals in soils or sediments (Tessier and Campbell 1988). Numerous studies have been done to determine the distribution of heavy metal species in plants and soils in heavily polluted areas (e.g., near old mines and smelters, in soils treated with sewage sludge, and in urban areas) (Keller and

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Vedy 1994; Jing and Logan 1992). Use of sequential extraction techniques to separate the soil metals into different forms can be helpful in understanding the processes of metal movement in the soil profile. Using sequential extraction, various forms of metals in a soil are frequently classified according to the following categories (Kersten and Förstner 1986): (1) easily exchangeable (readily available), (2) bound to carbonate phases, (3) bound to reducible phases (Fe-Mn oxides), (4) bound to organic matter, and (5) residual.

Metal toxicity associated with soils in the environment is of particular concern since they can be released into the water column and adversely affect the biota. Several physical, chemical, and biological factors influence the bioavailability and thus, the toxicity of metals in the environment (Newman and Jagoe 1994). These factors include mainly, pH, suspended particles, redox potential, water hardness, as well as organic and inorganic compounds. The free ionic forms of metals are generally the most toxic species to the biota while the precipitated, complexed and sorbed forms appear to be non-toxic, or at least are considerably less toxic than free metal ions.

In this study, the relationships between metal fractions and toxicity of collected samples from various contaminated soils in Korea were investigated. Distributions of five metals (Cu, Cr, Cd, Pb, and Zn) in soil were determined by a sequential chemical extraction method. Metal toxicity of soils was tested using MetPLATE^{TM}, a test kit based on inhibition of β-galactosidase activity in a mutant strain *E. coli*. This enzyme is selectively sensitive to metals but insensitive to organic toxicants (Bitton et al. 1996).

MATERIALS AND METHODS

Soil samples were collected from seventeen different sites in Korea : #1 ~ 4 near industrial areas, #5 ~ 7 near waste landfill areas, #8 ~ 14 near abandoned mine areas. Five samples from each site were collected and the mixture was used as a test soil sample. In the laboratory, the soil samples were dried at ambient temperature (22 ~ 25°C), crushed in a porcelain mortar and sieved through a 2-mm screen. The air-dried samples were stored in cloth bags for subsequent analysis.

The sequential fractionation of metals was a modified Kersten and Förstner's method (Kersten and Förstner 1986). Extraction of metal species was based on the following chemical fractions: exchangeable, carbonate bound, Fe-Mn oxide bound, organic bound, and residue. All solvents and acids were reagent grade or higher. Initially, 3 g of air-dried soil was used for extraction. Separation of the liquid from the solids after each extraction step was achieved by passing the suspension through a membrane filter following centrifugation. In brief, the following fractions were obtained: exchangeable, soil samples extracted with 1M $MgCl_2$ (pH 7); carbonate-bound, add 1M NaOAc (pH 5) to the residue from the exchangeable fraction; Fe-Mn oxides-bound, add 0.04M $NH_2OH{\cdot}HCl$ (in 20% HOAc) to the residue from carbonate fraction; organic-bound, add 0.02M HNO_3 and 30% H_2O_2 (pH 2) to the residue from Fe-Mn oxide fraction. Subsequently, add 3.2M NH_4OAc (in 20% HNO_3), and dilute with distilled water; residual, add concentrated HNO_3 to the residues from organic fraction.

Metals (Cu, Cd, Cr, Pb, and Zn) in solution were quantified by using a Flame Atomic Absorption Spectrometry (AAS, Shimadzu, Japan) or Inductively Coupled Plasma (Perkin Elmer, U.S.A.).

MetPLATE^{TM} was used to determine the toxicity of metals in soil samples. This test is based on the specific inhibition of β-galactosidase of the test bacteria (*E. coli*) by metals. The kit includes a freeze-dried *E. coli*, moderately hard water, phosphate buffer, enzyme substrate, and a 96-well microplate. In this modified test protocol, a small volume (1.0 ml) of the test bacteria was directly exposed to the soil, then β-galactosidase activity was determined. Details of the method are provided by Bitton et al. (1996).

RESULTS AND DISCUSSION

Soils consist of heterogeneous mixtures of inorganic and organic solid components as well as a variety of soluble substances (Ma and Rao 1997). Heavy metals, such as Cu, Cd, Cr, Pb, and Zn, are all potential soil pollutants, and a wide range of these elements could be found in contaminated sites.

Our results show that the total concentrations of five metals in each soil were approximately in the range of 240 ~ 1,075 mg/kg soil. The concentrations of each metal are given in Table 1. The total metal concentrations of the soils under study were in the following ranges: 11 ~ 384 mg Cu, 25 ~ 498 mg Pb, 13 ~ 78 mg Cr, 140 ~ 552 mg Zn, and 0.6 ~ 4.7 mg Cd per kg dry soil. The metal contents were in the following order: Zn > Pb > Cu > Cr >> Cd, which is in agreement with the order reported by Gimeno-Garcia et al. (1995). Metal concentrations were often higher than those obtained in studies carried out in agricultural or park soils (Gimeno-Garcia et al. 1995). As reported by other investigators, cadmium displayed the lowest (Amrhein et al. 1994; Swartzbaugh et al. 1992). As shown in Table 1, the samples from near the mining areas were the most contaminated with metals, showing average of five examined metal 722.8 mg/kg soil compared with industrial (292.0 mg/kg soil) and landfill (294.3 mg/kg soil) areas.

The average percentage distribution of all metal fractions obtained from the sequential extraction experiments is presented in Table 2. Metals were mostly distributed in the organic (38.0±11.37%) and residual fractions of the soils (41.4±12.24%). Gibbs (1973) reported that most of the Cu and Cr transported in the Amazon and the Yukon rivers was deposited in the crystalline particles (residual fraction) of the sediments. The average percentage of the other metals present in exchangeable fraction was approximately less than 2.92%, except for Pb (5.06%) and Cd (5.34%). Low metal levels in the exchangeable fraction were generally reported elsewhere (Bunzl et al. 1999). Higher percentage of Cd in some samples, especially those from the mining areas (#12 ~ #14; Table 1), was found in the exchangeable fraction, showing in the range of 14.3% ~ 29.1% (not shown in Table). However, the total concentrations of cadmium in these soils were very low (0.6 ~ 2.0 mg/kg soil), so that the corresponding absolute Cd-concentrations in exchangeable fraction were also very low.

In the carbonate-bound fraction, we generally observed higher percentages of all metals than that of the exchangeable fraction. The percentage distribution of this fraction was less than 4.24% of total content, except for Pb (10.06%) and Cd (7.63%). The carbonate forms of Cd and Pb are the elements that shows a high affinity with soil elements compared to other metals as is reported (Gimeno-Garcia et al. 1995). In the Fe-Mn oxides-bound fraction, fairly high percentages of Pb, Cr, and Zn were observed for all samples (average 5.34 ~ 19.46%). The significant percentage of Pb and Zn in Fe-Mn oxide fraction also has been observed by other investigators (Ramos et al. 1994; Ma and Rao 1997). Metal levels found in the organic fraction were rather high for all metals. As several authors recognized the affinity of Cu for the soil organic matter, Cu is also the metal that shows the greatest affinity for soil organic matter in this investigation

(Bunzl et al. 1999; Kersten and Főrstner 1995). Quite high percentages of all metals (avg. 41.4%) were observed in the residual fraction.

To estimate the bioavailability or the affinity of the contaminated heavy metals in ecosystem, values of the sequentially extracted heavy metals in each fraction have been used. In respect to the results obtained, the affinity of the metals for each fraction in investigated soil ecosystems are (based on percentage distribution) following orders : Exchangeable: Cd > Pb > Zn> Cu ≈ Cr; Precipitated as carbonates: Pb > Cd > Zn ≈ Cu > Cr; Associated with amorphous oxides: Zn > Pb ≈ Cr > Cd ≈ Cu; Bounded to the organic matter: Cu > Zn ≈ Pb ≈ Cr > Cd; Residual: Cd > Cr > Cu> Zn ≈ Pb

The average percentage of each fraction of all investigated metals is shown in Table 2. As represented, the major fraction, without considering the residual, was associated with organics. Fractions were in the following orders: Residual ≥ Organic > Fe-Mn oxides > Carbonates ≥ Exchangeable. These differences in selective distribution of heavy metals, however, may vary according to soil characteristics, such as pH, organic contents, redox potential, and heavy metal properties.

In this study, five metals in samples were measured. Fig. 1 shows the correlation between the fraction distribution of total metals and toxicity, as measured via MetPLATE^{TM}. As shown in Fig. 1, residual fractions, which are average 41.4% of total contents depending on the type of metals, show regression coefficient r^2 = 0.7054, and the rest of them were in the range of 0.0563 (organics) ~ 0.5276 (exchangeable). Such results may be due to the effects of complexity of the interactions of various metals with soil components. Some studies have shown that little correlation between total heavy metal content and sediment toxicity (Dave 1992). However, a high correlation (r^2 = 0.8552) was obtained between toxicity and total metal content in this study.

Based on our study, it is difficult to find significant correlation with metal content of each fraction and toxicity of contaminated soil samples. However, the exchangeable fraction showed a better correlation with toxicity (r^2 = 0.5276) compared with other fraction's. This may be due to the toxic effects of ionic form of metals, which can be easily exchangeable to the biota. The results of this study demonstrate the possible usage of MetPLATE^{TM} toxicity test for the assessment of the impact of ecosystems contaminated with mixture of heavy metals. More

Table 1. Total metal concentration in each collected samples

Sites		Toxicity (%)	Metal Concentration (mg/kg soil)[a]						
			Cu	Pb	Cr	Zn	Cd	Total	Avg. (std.)
Industrial areas	#1	12.6	32.2	32.2	31.2	171.6	1.6	268.9	292. (±82.06)
	#2	17.6	85.5	31.2	40.0	256.0	1.0	413.7	
	#3	5.7	41.7	31.6	23.2	147.5	1.2	245.3	
	#4	15.7	34.0	33.6	26.2	145.2	1.3	240.3	
Waste landfill areas	#5	26.9	33.3	39.9	21.0	187.4	2.1	283.4	294.3 (±29.96)
	#6	26.1	42.7	25.4	31.1	224.9	1.0	325.0	
	#7	36.2	37.5	40.2	55.0	139.9	1.9	274.5	
Mine areas	#8	34.6	76.1	53.1	27.0	454.1	2.5	612.8	722.8 (±267.79)
	#9	46.2	71.7	42.0	34.1	312.0	3.7	463.6	
	#10	72.0	149.3	54.5	35.5	429.9	4.5	673.8	
	#11	90.0	48.7	406.6	32.9	552.9	4.7	1045.8	
	#12	13.8	11.2	85.6	14.7	272.1	2.0	385.5	
	#13	61.5	74.0	497.7	12.7	218.2	0.9	803.4	
	#14	89.3	384.1	395.3	77.7	217.2	0.6	1074.9	

[a] Values show the average of triplicate. Triplicate data did not differ by more than 10%.

Table 2. Range values of the percentage distribution of heavy metals in each fraction.

Metals	% Distribution of each fraction.				
	Exchange	Carbonates	Fe-Mn oxides	Organic	Residual
Cu	0.4 ~ 6.2	2.5 ~ 6.6	0.7 ~ 12.9	10.7 ~ 70.9	21.8 ~ 72.5
Pb	2.3 ~ 8.9	2.6 ~ 17.5	4.9 ~ 17.4	2.5 ~ 56.5	19.3 ~ 74.2
Cr	0.0 ~ 2.8	0.2 ~ 12.4	6.1 ~ 52.6	18.9 ~ 46.7	11.4 ~ 64.9
Zn	0.4 ~ 13.2	1.7 ~ 5.2	7.3 ~ 29.7	2.5 ~ 58.9	16.4 ~ 71.9
Cd	0.4 ~ 29.1	2.7 ~ 17.2	0.9 ~ 17.6	10.8 ~ 36.7	8.4 ~ 79.4
Avg.	3.2	5.7	11.7	38.0	41.4

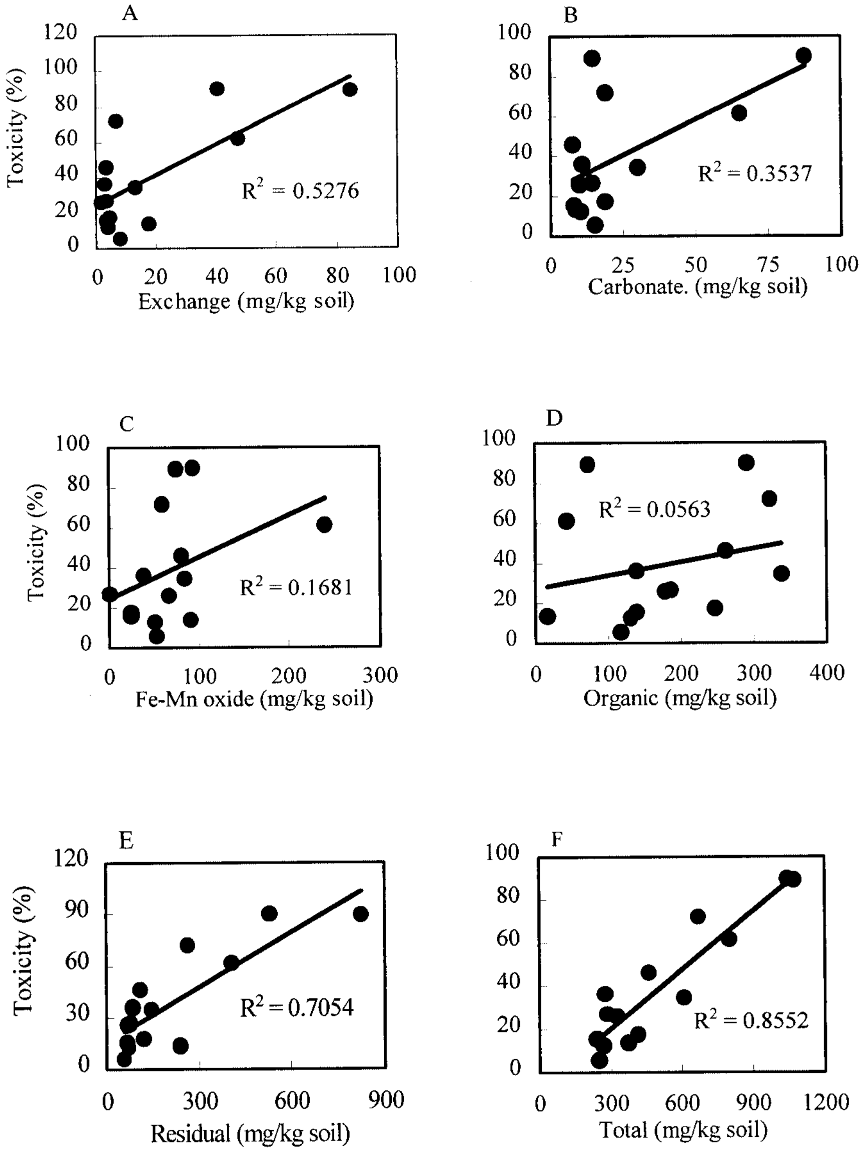

Figure 1. Correlation of soil toxicity and the percentage metal fractions in contaminated soils. A (total exchangeable form), B (total carbonates-bound), C (total Fe-Mn oxide-bound), D (total organic-bound), E (residual form), F (total metals).

effort is currently being made to test more samples from various ecosystems such as sediments, wastewater sludges or soils.

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