EVALUATION OF PLANT AVAILABILITY OF SOIL TRACE METALS BY CHEMICAL FRACTIONATION AND MULTIPLE REGRESSION ANALYSIS

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Abstract

Soil samples with a range of chemical and physical properties were collected from 10 different rural regions of China. Trace metals (Ni, Co, Cu, and Pb) in the soils were partitioned by a sequential extraction procedure into $Mg(NO_3)_2$ extractable (F1), CH_3COONa extractable (F2), $NH_2OH \cdot HCl$ extractable (F3), $HNO_3-H_2O_2$ extractable (F4), and residual (F5) fractions. Chemical fractionation showed that F1 fraction of the metals was less than 1% and residue was the dominant form for Cu and Ni in all samples, and for Co in most of the samples. Significant interrelationships of the fractions varied considerably with the different metals. Winter wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.) had been grown on the soils in a pot-culture experiment under greenhouse conditions for 40 days. Metal availability to the plants was evaluated by simple and multiple regression analysis. The $Mg(NO_3)_2$ extractable Co (F1) was significantly correlated with Co concentrations in different parts of wheat and in the whole of alfalfa. For the other metals, the independent variables of the multiple regression models, chosen by stepwise selection, were given as: F1 and F2 + F3 + F4 for Ni; F1, F2 + F3 and F4 for Cu; and F3 + F4 for Pb. The results of this study demonstrate that the sequential extraction procedure, in conjunction with multiple regression models using a combination of correlated fractions as an independent variable, may be useful for the prediction of plant absorption of trace metals in soils.

INTRODUCTION

The metal pollution in soils can be brought about by several anthropogenic activities such as sewage sludge application, mining and smelting operations, and application of commercial fertilizers (Arendt *et al.*, 1990; Nriagu, 1991). In recent years, trace metal accumulation in plants has been a serious environmental concern, primarily because their uptake by plants from contaminated soils is the principal process by which trace

metals enter the food chain. Quite a lot of work has been done to find a method that can reliably estimate bioavailability of trace metals to plants and thereby evaluate the potential health risk of the elements in contaminated soils and predict their impact on the soil ecosystem.

Metal availability in soils depends on many factors such as soil properties and plant species (Soon & Bates, 1982; Sauerbeck & Hein, 1991; Davies, 1992; Smith, 1994). These factors are not completely understood, and simple relationships are seldom found in natural soil systems between plant metal levels and total metal concentrations in soils (Iyengar *et al.*, 1981; Sims, 1986; Sims & Kline, 1991). The degree of metal association with distinct geochemical phases is strongly dependent upon the physico-chemical conditions of the soils. The different fractions of soil metals vary considerably in their chemical reactivity and bioavailability. Chemical fractionation has become a common operational approach to bridge the relationship between bioavailable fraction of a metal in soil and its content in plants (Van Valin & Morse, 1982; Lake & Lester, 1984; Shuman, 1985; Sims, 1986; Spevackova & Kucera, 1989; Sauerbeck & Hein, 1991; Mench *et al.*, 1994). Sequential extraction methods have been developed to differentiate between various soil metal fractions. It is well recognized that the sequential extraction methods suffer from nonselectivity and trace element redistribution among phases during extraction, and attempts to quantitatively predict the bioavailability and toxicity of metal species have generally failed. However, sequential extraction methods are still considered useful for evaluation of bioavailability of trace metals in soils by many research workers (Sposito *et al.*, 1982; LeClaire *et al.*, 1984; Rappaport *et al.*, 1986; Radogna, 1990; Tuin & Tels, 1990; Sims & Kline, 1991). Of the various sequential extraction methods available, the one followed most often is that proposed by Tessier *et al.* (1979).

The relationships between metal fractions and plant uptake have been estimated by simple, partial, and multiple correlation procedures. Different association forms of metals in soils and parameters such as soil pH

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were chosen as the independent variables. Reported results have differed greatly. In some of the investigations, consistent correlations between specific metal fractions and plant metal contents were found. For example, exchangeable and organically bound trace metals were found by some workers to represent plant-available forms (Iyengar *et al.*, 1981; LeClaire *et al.*, 1984; Sims, 1986). In other studies, no such correlation was observed, and therefore, multiple regression analysis between soil metal fractions, soil pH, and plant metal uptake was applied (Sims & Kline, 1991; Davies, 1992). Theoretically, in the case of multiple regression analysis, the selected variables would have no significant correlation among each other. However, significant relationships among trace metal fractions and pH were detected (Patterson, 1971; Jeng & Singh, 1993; Zhu & Alva, 1993). Correlations between soil metal fractions and their bioavailability remain an unresolved problem. Further research is therefore necessary to ascertain which relationships exist in soil-plant systems.

The present study was designed to examine further the interactions between soil metal fractions and plant metal uptake. Multiple regression analysis using step-wise selection was applied to identify independent variables and their contribution to metal availability to cultivated winter wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.), based on the fractionation data obtained by sequential extraction (Tessier *et al.*, 1979).

MATERIALS AND METHODS

Soil characterization

The soil samples used in this study were collected from 10 different rural regions of China, representing a range of chemical and physical properties. They are all from cultivated soils and the samples were taken from the surface layer (0–20 cm). The soils were air-dried, ground, and passed through a 2-mm screen to remove rocks, roots, and other large particles. Precautions were taken to avoid contamination during sampling, drying, grinding, and storage. For the laboratory analyses a representative 4-kg subsample of each soil was used for characterization, chemical fractionation, and preparation for a pot-culture experiment designed to determine the plant availability of trace metals.

Soil description is according to the American Classification System. Soil pH was measured in deionized water using a 1:1 (w:v) soil:solution ratio. After 1 h of equilibration, organic matter was determined by a Walkley–Black procedure (Nelson & Sommers, 1982). Soil texture was determined by a pipette method (Elonen, 1971), and cation exchange capacity (CEC) was determined by the method described in Rhoades (1982). Selected properties of the soils are presented in Table 1.

Metal fractionation procedure

A sequential extraction procedure (Tessier *et al.*, 1979) was employed to partition the total metals into five

Table 1. Selected chemical and physical properties of the soils used in the pot experiments and laboratory studies

Sample	Geographical origin	Order	Family	pH	CEC (me/100 g)	Organic matter (% w/w)	Texture (% w/w)		
							Sand	Silt	Clay
1	Hangzhou	Ultisol	Fine-silty, mixed	5.51	14.6	7.09	11.6	65.0	23.4
2	Mudanjiang	Mollisol	Sandy-silty, mixed	6.47	20.7	7.47	25.1	57.1	17.8
3	Changsha	Ultisol	Sandy-silty, mixed	6.78	14.2	5.16	33.5	45.9	20.6
4	Linyi	Entisol	Silty-clayed, mixed	7.59	25.8	2.06	7.3	59.2	33.5
5	Changchun	Mollisol	Sandy-silty, mixed	7.60	14.3	5.42	39.6	49.8	10.6
6	Nanjing	Alfisol	Fine-silty, mixed	7.87	10.1	4.24	11.6	63.7	24.7
7	Qinghe	Mollisol	Sandy-silty, mixed	7.91	22.7	6.45	22.9	64.8	12.3
8	Liaocheng	Alfisol	Sandy-silty, mixed	7.93	19.9	2.92	32.9	50.1	17.0
9	Bajia	Mollisol	Fine-silty, mixed	8.03	11.8	5.06	19.8	54.1	26.1
10	Miaopu	Mollisol	Fine-silty, mixed	8.11	11.7	3.04	17.9	60.3	21.8

Table 2. The sequential extraction scheme for Ni, Co, Cu, and Pb fractionation

Step ^a	Fraction	Reagent	Solute volume (ml)	Experimental conditions
1	F1	1 M Mg(NO ₃) ₂ (pH 7.0)	8	Shake 1 h, room temperature
2	F2	1 M CH ₃ COONa.3H ₂ O (pH 5.0)	8	Shake 5 h, room temperature
3	F3	0.04 M NH ₂ OH.HCl in 25% CH ₃ COOH (pH 2.0)	20	Water bath, 96°C, 6 h, occasional shaking
4	F4	0.02 M HNO ₃ 30% H ₂ O ₂ (pH 2.0) ÷ 3.2 M CH ₃ COONH ₄	3 8 ÷ 5	Water bath, 85°C, 5 h Shaking 30 min
5	F5	HNO ₃ + HF + HClO ₄	3	Under high pressure, 170°C
Total ^b		HNO ₃ + HF + HClO ₄	3	Under high pressure, 170°C

^aIn steps 1–4, 1.00 g of soil sample was used; while in step 5, 0.200 g of air-dried, finely ground residue from step 4 was used.
^bDetermination of the total metal content was performed independently on another soil sample (0.200 g dry wt).

Table 3. Total metal content in the soil samples under investigation

Metal	Concentration ($\mu\text{g/g}$ dry wt) ^a	
	Range	Mean
Ni	2.61–16.5	9.78
Co	1.04–17.2	7.40
Cu	3.93–46.9	17.2
Pb	6.57–67.5	29.4

^aMean values in triplicate determinations.

fractions (Table 2). Since the fractions are operationally defined, they are specified as: F1: $\text{Mg}(\text{NO}_3)_2$ extractable; F2: CH_3COONa extractable; F3: $\text{NH}_2\text{OH}\cdot\text{HCl}$ extractable; F4: $\text{HNO}_3\text{--H}_2\text{O}_2$ extractable; F5: residual. Extractions were performed for 1.00 g of dried soil samples in 50 ml polypropylene centrifuge tubes with mechanical shaking. All extractions were done on duplicate samples from the same site, and the average results were used for further analysis. For each step, standards were prepared in the extracting solutants to eliminate matrix differences in the subsequent analysis. Following each extraction, the mixture was centrifuged. The supernatant solutions were separated. The residues were washed with deionized water, and the obtained solutions were combined for analysis.

Total metal content in soils was determined by attacking 0.200 g of dried soil with 3 ml of $\text{HNO}_3\text{--HF--HClO}_4$ mixture followed by elemental analysis. Total contents for Ni, Co, Cu, and Pb in the investigated soils are listed in Table 3.

Pot-culture experiment

The air-dried soil samples were placed in plastic-lined pots (1000 g/pot) and planted with winter wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.) under greenhouse conditions. Triplicate samples were made at the same time. Winter wheat and alfalfa were planted at 15 and 30 seeds per pot and subsequently thinned to 12 and 20 plants per pot, respectively. The pots received supplemental fertilization with a $\text{NH}_4\text{NO}_3\text{--KH}_2\text{PO}_4$ solution supplying 10, 10, 13 mg/pot of elemental N, P

and K, respectively. The soils were initially adjusted to approximately 60% water-holding capacity, and losses from evapotranspiration were made up by daily watering with deionized water.

The plants were harvested 40 days after planting. Samples of winter wheat were divided into shoot and root by a plastic knife, while alfalfa was sampled as a whole. The harvested samples were washed with deionized water, dried at 60°C for 48 h and ground in an agate mortar and pestle to pass a 0.85 mm (20 mesh) sieve. Plant analysis for Ni, Co, Cu and Pb was accomplished by digesting a portion of dried, ground sample (0.500 g) in a 3:1 mixture of HNO_3 and HClO_4 (5 ml).

Elemental analysis

The contents of Ni, Co, Cu and Pb were determined by atomic absorption spectrophotometry (Perkin–Elmer Model 3030 equipped with a Model HGA-4000 graphite furnace) and by inductively coupled plasma–optical emission spectroscopy (Jarrell–Ash Model 1155V). Upon determination of trace metals by ICP–AES, the matrix effects were addressed by preparing standards in solutions identical to the extracting solutions. In the case of graphite furnace atomic absorption spectrometry, a palladium modifier was used for the determination of Pb (Shan & Ni, 1982). Metal content of the root ashes was checked to account for possible soil particle contamination. Accuracy of the elemental analysis was checked by the determination of trace elements in a standard reference material of soil. All reagents used were of analytical reagent grade or better.

RESULTS AND DISCUSSION

Metal fractionation in soil samples

Results from the sequential extraction procedure (Figs 1–4) reveal principally the partitioning of Ni, Co, Cu, and Pb among five fractions (F1–F5) of the soils under investigation. The duplicated analyses varied from their means by no more than 5%, except for some samples with $\text{Mg}(\text{NO}_3)_2$ extractable metal concentrations close to the lower detection limit. Similarly,

Fig. 1. The relative distribution of Ni among five fractions (F1–F5) of 10 soil samples of China.

Fig. 2. The relative distribution of Co among five fractions (F1–F5) of 10 soil samples of China.

Fig. 3. The relative distribution of Cu among five fractions (F1–F5) of 10 soil samples of China.

Fig. 4. The relative distribution of Pb among five fractions (F1–F5) of 10 soil samples of China.

total metal content and the sum of individual chemical fractions deviated from the mean value by no more than 10%. The distribution of the metals varied greatly among the samples. On average, the following orders could be arranged for these chemical fractions: Ni: F5 > F3 > F4 > F2 > > F1; Co: F5 > F2 > F3 > F4 > > F1; Cu: F5 > F4 > F3 > F2 > > F1; Pb: F3, F5 > F2 > F4 > > F1.

Among the metals studied, the $Mg(NO_3)_2$ extractable fraction (F1) was less than 1%, with no exception. Residue (F5) was the dominant fraction for Cu and Ni in all samples and for Co in most samples. With respect

to Pb, however, a more or less uniform distribution between F3 and F5 was observed. Considering that the metals under study were present mostly in the most resistant fraction, it is assumed that trace metals in these soils are to a great extent derived from geochemical background rather than anthropogenic inputs.

It should be stressed that the distribution of a given metal among the various fractions obtained by the sequential extraction procedure does not necessarily reflect metal association with discrete soil phases, but rather should be considered as defined by the methods of extraction.

Table 4. Correlation coefficients (*r*) for relationships among Ni, Co, Cu, and Pb fractions, total content and pH (** and * denote significance at 5 and 10 per cent levels)

Metal	Fraction	F1	F2	F3	F4	F5	Total
Ni	F1						
	F2	−0.062					
	F3	−0.217	−0.642*				
	F4	−0.190	−0.488	0.684*			
	F5	0.030	−0.247	0.089	0.130		
	Total	−0.070	−0.221	0.279	0.374	0.948**	
	pH	−0.678*	0.118	0.193	0.333	0.248	0.388
Co	F1						
	F2	−0.250					
	F3	−0.197	0.240				
	F4	−0.060	0.109	−0.216			
	F5	0.176	−0.891**	−0.346	0.064		
	Total	0.072	−0.108	−0.149	0.325	0.863**	
	pH	−0.887**	−0.016	0.530	0.234	0.121	0.259
Cu	F1						
	F2	0.350					
	F3	0.458	0.756**				
	F4	0.447	0.120	0.282			
	F5	−0.224	−0.306	0.228	0.016		
	Total	0.195	−0.029	0.669*	0.566	0.719*	
	pH	0.064	0.172	0.018	−0.644*	0.098	−0.303
Pb	F1						
	F2	0.345					
	F3	0.452	−0.410				
	F4	−0.548	−0.504	−0.709*			
	F5	−0.449	−0.577	−0.334	−0.109		
	Total	−0.238	−0.344	−0.131	0.219	0.330	
	pH	−0.205	0.381	−0.489	0.180	0.543	0.619

Correlations among chemical fractions, total metal content and soil pH

Single correlation analysis was performed to assess relationships among total metal content, soil pH and metal concentrations in different chemical fractions expressed on a relative scale as percentages of total contents. Correlation coefficients for these relationships are listed in Table 4.

Positive significant relationships between F5 and total content of Ni, Co and Cu were obtained ($r = 0.948^{**}$, 0.863^{**} and 0.719^* , respectively), indicating that F5 is a function of the total content of these metals in soils. However, this is not the case for Pb. It is not surprising considering that F5 was the dominant fraction for Ni, Co and Cu, whereas an even distribution between F3 and F5 existed for Pb. The percentage of Cu in F3 also was correlated with total Cu content ($r = 0.669^*$), suggesting that the proportion of $\text{NH}_2\text{OH}\cdot\text{HCl}$ extractable Cu is a function of the total Cu level in soils.

For Co, F2 was related inversely to F5 ($r = -0.891^{**}$). For the other metals, no such relation was detected. A positive correlation existed between F2 and F3 for Cu ($r = 0.756^{**}$). In comparison, an inverse relationship between the two fractions was gained for Ni ($r = -0.642^*$). The contrasting situation was also found in the correlation between F3 and F4, where a positive correlation existed for Ni ($r = 0.684^*$), whereas a negative correlation was obtained for Pb ($r = -0.709^*$).

Soil pH was not well correlated with the total metal content as well as most of the fractions, except for F1 for Ni and Co, and F4 for Cu, where negative correlations were obtained ($r = -0.678^*$, -0.887^{**} and -0.644^* , respectively). The finding suggests that soil pH is in some instance a factor that affects the distribution of these fractions in soils.

Based on the above results, it is apparent that the relationships among the chemical fractions, total metal content, and pH are greatly dependent on the discrete chemical properties of a given metal. This finding also has been reported by others (Patterson, 1971; Jeng & Singh, 1993; Zhu & Alva, 1993). To some extent, this finding means that the bioavailability of the same fraction of different metals is not identical.

Correlations between soil metal fractions and plant uptake

Results from analysis of the metals in different parts of plants (Table 5) show that Pb, Ni, and Co can be accumulated and retained by the roots of wheat so that only small portions reach the shoots. For Cu, however, no such accumulation has been found. Cu contents in roots were not much different from those in shoots. This result indicates that the high transfer of Cu to the shoots is not due to the particularly weak binding of Cu in the soils (in fact, Cu is bound strongly to soil organic matter (Stevenson, 1976)), but rather to the function of the roots.

Table 5. Range and mean metal concentrations in parts of wheat and in alfalfa grown in 10 soil samples

Elements	Metal concentrations ($\mu\text{g/g}$ dry wt) ^a					
	Wheat shoot		Wheat root		Alfalfa ^b	
	Range	Mean	Range	Mean	Range	Mean
Ni	0.14–1.03	0.42	0.48–5.32	2.19	0.65–6.13	2.13
Co	0.04–0.34	0.09	0.75–4.46	2.47	0.28–2.39	0.76
Cu	0.67–7.01	2.54	0.50–6.58	2.67	0.77–5.02	2.78
Pb	0.40–2.85	1.01	3.12–33.7	17.6	1.46–13.6	5.77

^aMean values in triplicate determinations. In general, the three values were not significantly different at the 0.10 probability level.

^bAlfalfa was put to analysis as a whole.

Table 6. Multiple and single regression equations between plant concentrations of Co, Cu, Pb, or Ni and metal concentration in soil fractions. ** and * denote significance at 5 and 10 per cent levels, respectively

Elements ^a	Regression equation	r^2
Co	$Y_a = 0.413 + 10.3F_1$	0.776**
	$Y_{sw} = 0.014 + 1.99F_1$	0.998**
	$Y_{rw} = 1.53 + 28.2F_1$	0.626**
Ni	$Y_a = -0.345 + 11.4F_1 + 0.124F_{2+3+4}$	0.778**
	$Y_{sw} = -0.114 + 2.46F_1 + 0.0268F_{2+3+4}$	0.790**
	$Y_{rw} = -0.269 + 12.7F_1 + 0.114F_{2+3+4}$	0.964**
Cu	$Y_a = 0.939 + 0.368F_1 + 0.0794F_{2+3} + 0.0655F_4$	0.791**
	$Y_{sw} = 1.054 - 2.95F_1 + 0.131F_{2+3} + 0.126F_4$	0.834**
	$Y_{rw} = 1.10 - 3.07F_1 + 0.138F_{2+3} + 0.119F_4$	0.822**
Pb	$Y_a = -5.88 + 0.181F_{3+4}$	0.472*
	$Y_{sw} = -0.816 + 0.0278F_{3+4}$	0.524*
	$Y_{rw} = -12.1 + 0.460F_{3+4}$	0.411*

^a Y_a , Y_{sw} , Y_{rw} = Concentration ($\mu\text{g/g}$ dry wt) of metal in the whole of alfalfa, shoots of wheat, roots of wheat, respectively.

Total soil metal content, either by sum of fractions or decomposition with $\text{HNO}_3 + \text{HF} + \text{HClO}_4$, was in general poorly correlated with metal concentrations in either wheat or alfalfa. This result is in agreement with the commonly observed effect that total metal level in soils is a poor indicator of bioavailability (Iyengar *et al.*, 1981; Sims, 1986; Sims & Kline, 1991).

Similarly, with the exception of Co, consistently significant correlations between plant metal concentrations and individual soil fractions were not observed. In the case of Co, however, the $\text{Mg}(\text{NO}_3)_2$ extractable fraction was significantly correlated with plant Co in shoots or roots of wheat or in the whole alfalfa ($R_{\text{sw}}^2 = 0.776^{**}$, $R_{\text{rw}}^2 = 0.998^{**}$, $R_a^2 = 0.626^{**}$). Because Co concentration in F1 seems to give the best prediction of plant Co uptake, a simple regression equation using F1 as an independent variable could be applied for predicting the bioavailability of this element (Table 6).

In order to determine the most consistently significant regression equation for estimating plant uptake of the other three metals, the data were put through a stepwise multiple regression with individual chemical fractions, combinations of the fractions, and soil pH as potential independent variables. The reason for doing so is that the most important step in dealing with multiple regression analysis is to choose proper independent variables. Theoretically, little information can be gained by multiple regressions if high multicollinearity would exist among the variables. Based on the correlation coefficients listed in Table 4, for several instances metal concentration in one chemical fraction is highly correlated with that for the same metal in another fraction. Due to this close correlation between different chemical fractions, a 'best' regression equation cannot be attained if individual chemical fractions of the metals are selected as independent variables. Nevertheless, when the fractions are combined, the regressions could account for significant percentages of variability in concentrations of Ni, Cu, or Pb in wheat and in alfalfa. Therefore combinations of the significantly correlated fractions were chosen as independent variables in multiple regressions. Each of the potential independent variables was examined for its ability to meet the criteria of entry into or remove from the regression equation. The 'probability of *F*-to-enter' at each step was 0.05, and 'the maximum *F*-to-remove' was 0.10.

The consistently significant regression equations obtained for Ni, Cu, or Pb are also listed in Table 6. The independent variables contributing to the bioavailability of these metals are found to be: F1 and F1 + F2 + F3 for Ni; F1, F2 + F3 and F4 for Cu, and F3 and F4 for Pb.

Using values of soil pH for regression failed to improve the estimation of plant contents of all the metals, so it is not selected as an independent variable. This result suggests that, among the soils investigated, pH is not a direct important factor that influences the metal bioavailability.

CONCLUSION

Based on the foregoing discussions, a significant inter-relationship between the chemical fractions was detected, and it differed greatly among the metals. Total metal content was a poor indicator of metal absorption by plants. A simple relationship existed between $\text{Mg}(\text{NO}_3)_2$ extractable Co and plant Co concentration. For Ni, Cu, or Pb, a stepwise multiple regression procedure using combinations of significantly correlated fractions as independent variables predicted metal absorption by plants. The result may indicate that the sequential extraction procedure, though operationally defined, may provide valuable data for the prediction of trace metal availability to plants, if proper independent variables in the multiple regressions are chosen.

Further study is required to develop more accurate speciation analysis methods so that well-defined species can be determined convincingly. Development of solid phase speciation techniques should be emphasized on increasing selectivity and specificity. In addition, knowledge of the complexity of the interactions between metal species and biota should be largely improved. If great progress is achieved in these aspects, the quantitative relationship between metal species and plant availability may be well understood.

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