농학석사학위논문

Effect of humic substances quality on chemical speciation and stability of heavy metals in smelter area soils

휴믹물질의 특성이 제련소 토양 내 중금속의 화학종 분포 및 안정도에 미치는 영향

2013년 8월

서울대학교 대학원

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A Thesis for the Degree of MASTER OF SCIENCE

Effect of humic substances quality on chemical speciation and stability of heavy metals in smelter area soils

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ABSTRACT

Effect of humic substances quality on chemical speciation and stability of heavy metals in smelter area soils

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Heavy metal contamination in soils such as smelter area soils has been a worldwide problem. As one of the remediation technologies, application of humic substances can decrease the availability and mobility of metals by increasing partitioning of metals into less mobile forms. Different quality of humic substances, which is the ratio of humic acid (HA) to fulvic acid (FA), is expected to influence the ability to chelate metals and the stability of humic substance-metal complexes. The ion-exchange method by Schnitzer and Skinner (1965) was employed to measure the stability constants and different metal-humic substance complexes showed variable stability constant (log K) values (As-HA: 2.64, As-FA: 3.71, Cu-HA: 7.21, Cu-FA: 3.24, Pb-HA: 8.26, Pb-FA: 8.22, respectively). As-FA complex was more stable than As-HA complex while copper and lead complexes with HA were more stable than those with FA. Different quality of humic substances (HA/FA=2, HA/FA=1, HA/FA=0.5, and control) was treated to the contaminated soil and time-variant chemical speciation of three toxic heavy metals (As, Cu, and Pb) was performed for 90 days. Sequential extraction technique (Tessier et al., 1979) was employed to fractionate metals and organo-metallic extraction method (Donisa et al., 2003; Podlešáková et al., 1992) was used to specify the treatment effect of humic substances. The addition of humic substances lessened the decrease of soil pH. Accordingly, in cases of Cu and Pb, the addition of humic substances increased less mobile chemical forms (carbonate bound, Fe-Mn oxide bound, and organic bound fractions) but decreased available forms (water soluble and exchangeable fraction) than control group. In particular, HA/FA=2 treatment was most effective among treatment groups to decrease the amount of mobile fractions and increase the stable forms. However, As did not show any significant differences between control and treatments and its chemical speciation shift to stable forms might have been attributed to the effect of soil acidification. Most metals were distributed to the fulvic fractions, which meant fulvic acids had greater chelating ability than humic acids did. However, in terms of stability constants, the log K values for metal-HA complexes were inclined to increase while the log K values for metal-FA complexes decreased. The results of the study suggested that humic substances can contribute to the immobilization and stabilization of metals in heavily polluted soils.

Key words: Heavy metals, Humic substances, Chemical speciation, Stability constant, Smelter

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Ⅰ. INTRODUCTION

Heavy metal contamination in soil has been a worldwide problem (Wei and Yang, 2010; Li et al., 2008; Huang et al., 2007; Li et al., 2001; Manz et al., 1999; Gupta et al., 1996). Heavy metals are non-degradable in nature and thus result in continuous bioaccumulation through a food chain. The toxic heavy metal contamination of soils may cause the contamination of the water systems thereby threatening ecosystems and human health. Considerable research indicates that the source of heavy metal contamination in the environment result mainly from anthropogenic activities such as mining, smelting, waste disposal, urban effluent, vehicle exhausts, sewage sludge, pesticides, fertilizers application, etc (Sud et al., 2008; Yusuf, 2007; Carrizales et al., 2006).

With increasing necessity for remediation of soils contaminated with toxic heavy metals, a number of the remediation techniques have been developed: (1) *ex-situ* (excavation) or *in-situ* (on-site) soil washing / leaching / flushing with chemical agents, (2) chemical immobilization / stabilization method to reduce the solubility of toxic metals by adding some non-toxic materials into the soils, (3) electrokinetics (electromigration), (4) covering the original polluted soil surface with clean soils, (5) dilution method (mixing polluted soils with surface and subsurface clean soils to mitigate the concentration of toxic metals), (6) phytoremediation by plants such as woody trees (Wuana et al., 2010). Of them, chemical remediation is reported as rapid, efficient technique, but the chemicals applied to the soil may destroy soil structure and cause a secondary pollution. To avoid or lessen the undesired side effects, natural organic acids and chelating agents that can substitute chemical surfactants or chelating agents are often suggested as alternatives to straight mineral acid use, since the chelating organic acids can dislodge toxic heavy metals from the exchangeable, carbonate and reducible fractions to the soluble fraction by washing procedures (Peters, 1999).

The remediation of heavy metal contamination in soils by washing with chemical agents consists of two main mechanisms: (1) dissolution of heavy metal contaminants from soil with chemical agents into the water soluble fraction and (2) separation of soil based on its particle size and reduction of the volume of contaminated soils by screening concentrated fine soil only (CL:AIRE, 2007; USEPA, 1991). It should be determined which type of soil washing is going to be applied to the metal-contaminated soil based on the forms and distribution of the contaminants in the soil. To better understand and assess the fate of toxic heavy metal contamination in the soil, knowledge of chemical forms with which the metal is associated is required.

Sequential extraction can reveal the relative distribution of toxic metals among various chemical fractions in soils. The five-step extraction method by Tessier et al. (1979) is a widely adopted extraction scheme that fractionates toxic metals into five geochemical pools (water soluble and exchangeable, carbonate bound, Fe-Mn oxide bound, organic bound, and residual fraction). Because heavy metals are partitioned into different chemical forms associated with various environmental stabilities, quantification of each chemical fraction should be assessed to understand the fate and mobility of heavy metals in the soil. In case that the metal concentrations of water soluble, exchangeable, and carbonate bound fraction are high compared to those of other fractions, it is appropriate to follow the mechanism (1): dissolution of metals with chemical agents. However, it would be suitable to follow the soil particle size separation method (mechanisms 2) if the majority of metals are associated with residual form in the soil (Moon et al., 2011).

It is well known that humic substance serves as a major reservoir of organic carbon for global carbon cycle, and is one of the major agents to transport heavy metal ions in the environment (Rashid, 1985). Because metals tend to form stable complex with organic ligands, much attention has been paid to humic substances consisting of various organic functional groups in order to understand metal retention in soils (Chen, 1996). Shuman (1999) reported that humic substances can decrease the availability of metals by increasing partitioning of metals into less mobile forms. Humic substances can be divided into three main components depending on their solubility in different media (Fig. 1). Both humic acids (HA) and fulvic acids (FA) are alkali-soluble humus fragments; humic acids are commonly extracted with diluted alkali and then precipitated with an acid, and fulvic acids are soluble in both alkali and acidic solutions. Humin represents the insoluble residue (Peña-Méndez et al., 2005).

Humic acids and fulvic acids have similar structures but show different characteristics such as molecular weights, elemental compositions, contents of functional groups (Choudhry, 1984). The elemental composition of humic acids and fulvic acids mainly consists of C, H, O, N, and S. Fulvic acids have more functional groups of an acidic characteristic, particularly -COOH. The total acidities of fulvic acids (900-1400 cmol kg^{-1}) are enormously higher than for humic acids (400-870 cmol kg^{-1}). Another important difference is that while the oxygen in fulvic acids is predominantly in known functional groups (-COOH, -OH, -C=O), with a high oxygen content, the acidity and degree of polymerization alter systematically as molecular weight increases. On the other hand, the proportion of oxygen in humic acids seems to exist as a structural component of the nucleus (Peña-Méndez et al., 2005). In this reason, fulvic acids could have greater chelating effect on metal than humic acids could.

However, even strongly bound metal ions can be released from humic substances if the interacting substructures of humic substance are degraded by microbes. Therefore, the stability of humic substance itself and the stability of humic substance-metal complexes are possibly crucial in this matter (Fan and Higashi, 2001). Generally, organic matter which has lower C:N ratio is utilized first for plants and microbes as nutrient source and because fulvic acids have lower C:N ratio than humic acids does it could be said that fulvic acids are less stable than humic acids with respect to the stability of organic matter.

Here, it was hypothesized that humic substances quality, which is the ratio of humic acid to fulvic acid, would affect metal speciation and stability of humic substance-metal complexes by time. Therefore, this hypothesis was tested by examining the effect of different quality of humic substances on the chemical partitioning of three heavy metals (As, Cu, and Pb) by the sequential extraction method (Tessier et al., 1979) and on the stability constants for metal and humic substance complexes by the ion-exchange method (Schnitzer and Skinner, 1965) through batch incubation.

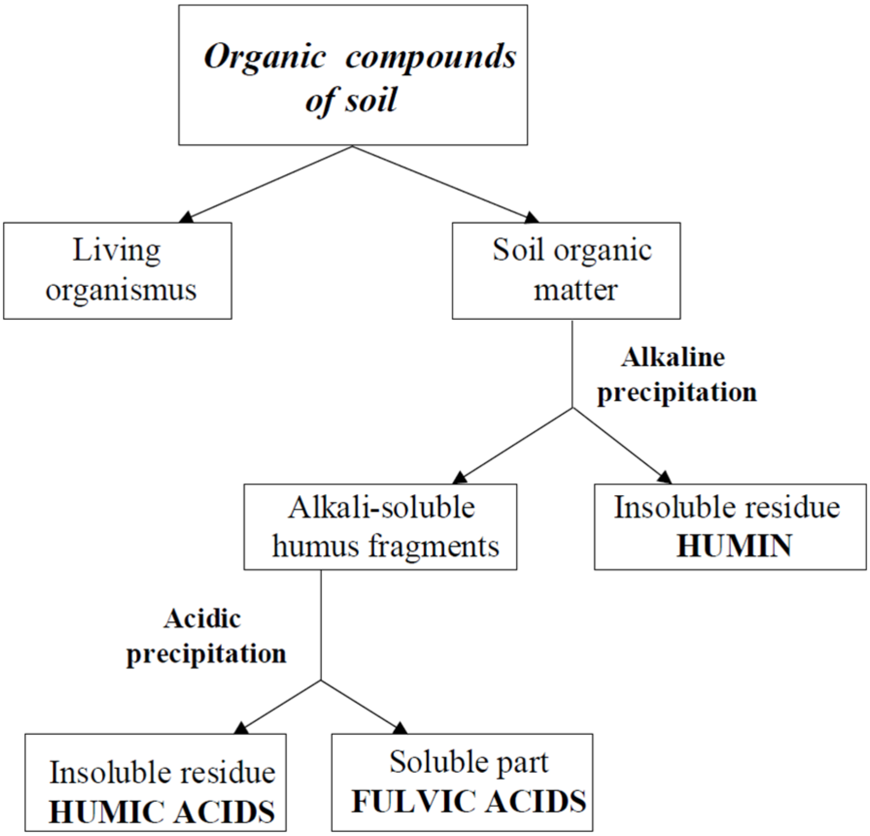

Figure 1. Classification scheme of humic substances

(adapted from Eladia M. Peña-Méndez et al., 2005).

Ⅱ. MATERIALS AND METHODS

1. Sampling site and soil / humic substances preparation

The research site (Former Janghang Smelter area) is located in Janghang-eup, Seocheon-gun, Chungcheongnam-do, Korea (Fig. 2). Due to the long smelting activities since 1936, the smelter area soils have been reported as heavily polluted environment with copper (Cu), lead (Pb), cadmium (Cd), arsenic (As), etc. The smelting activities of the Former Janghang Smelter are stopped currently, but about 2,700 people have resided in detailed investigation area only. Besides, land use near smelter area is quite high as 41 % of the total area is used for agriculture.

The sampling site is located in 417-2, Jang-am-ri, Janghang-eup, Seocheon-gun, Chungcheongnam-do, Korea (36°00'N, 126°40'E). This site is away from the chimney of smelting factory in direction of north-north-east (Fig. 3). Soil samples (0-30 cm in depth) were collected in the amount of around 20 kg in January 2013. The site was coordinated with GPS system. Right after sampling, soil samples were air-dried at room temperature, passed through 2-mm sieves, mixed thoroughly, and stored in polyethylene bags until used for incubation experiment. Soil samples were analyzed for basic soil properties such as soil texture, pH, EC (electric conductivity), CEC (cation exchange capacity), TC (total carbon content), TN (total nitrogen content), TOC (total organic carbon), organic matter, total metal contents.

Soil texture was classified based on the USDA (United States Department of Agricultures) scheme and the methods for pH and EC were followed by the official test methods for soil contamination. The values of pH in 1:5 mixtures of soil and water were measured using pH meter (Orion 3 Star, Thermo Scientific, USA). Water contents were measured with a method of KS F2306 and a method for cation exchange capacity (CEC) of soil was determined using a sodium acetate method (Chapman, 1965). TC and TN were analyzed by Elemental Analyzer (Flash EA 1112, Thermo Scientific, USA). TOC was determined by the method of Walkley and Black (1934) and organic matter was obtained by dry-wet sieving / pipette method (Day, 1965). The total contents of metals were performed by digestion with aqua regia (ISO, 1995), and analyzed using ICP (Inductively Coupled Plasma) Atomic Emission Spectrometer (ICPS-7510, Shimadzu, Japan). Exchangeable cations were determined by the ammonium acetate (pH 7) method (Sumner et al., 1996). The glassware was washed with 10 % of nitric acid (or 0.5 M hydrochloric acid) and cleaned again in a general washing process (Yusuf, 2007). Physico-chemical properties of the soil are listed in Table 1.

(http://www.aks.ac.kr)

Figure 2. The whole view of the Janghang Smelter area.

Figure 3. Sampling site at the Janghang Smelter area.

Table 1. Physico-chemical properties of soils at the Janghang Smelter area.

Parameters	Unit	Soil
Soil size distribution[*]		Silty clay loam
clay	g kg^{-1}	279.3 (0.92)[†]
silt	g kg^{-1}	605.5 (0.90)
sand	g kg^{-1}	115.2 (0.14)
Water content	%	51.7
pH (1:5)		6.3 (0.01)
EC	dS m^{-1}	0.6 (0.01)
CEC	cmol kg^{-1}	18.1(0.10)
TC	g kg^{-1}	15.9 (0.26)
TN	g kg^{-1}	1.1 (0.03)
TOC	%	1.7 (0.05)
Organic matter	%	4.3 (0.03)
Total metal contents[§]		
As	mg kg^{-1}	62.7 (0.6)
Cu	mg kg^{-1}	110.2 (3.62)
Pb	mg kg^{-1}	203.8 (5.81)
Exchangeable cations		
K	mg kg^{-1}	256.9 (11.07)
Ca	mg kg^{-1}	936.0 (11.63)
Na	mg kg^{-1}	720.2 (25.51)
Mg	mg kg^{-1}	469.2 (6.35)

[*] USDA scheme: sand (2-0.05 mm), silt (0.05-0.002 mm), clay (< 0.002 mm).

[†] The values in parentheses represent standard errors.

[§] Soil remediation concern standards (mg kg^{-1}) for As, Cu, Pb are 25, 150, 200, respectively.

Two different kinds of humic substances, humic acids and fulvic acids, were used for this research. They were purchased from an agrochemical company named Nutri-Tech Solutions (NTS, Australia). The product names are NTS Soluble Humate Granules^{TM} and NTS Soluble Fulvic Acid Powder^{TM} for humic acids and fulvic acids, respectively (Fig. 4). These humic substances were extracted from a leonardite mine located in Qitai County, Chanji City, Xinjiang Uyghur Autonomous region. NTS Soluble Humate Granules^{TM} has a minimum of 70 % potassium humates (CEC of 450 cmol kg^{-1}) and NTS Soluble Fulvic Acid Powder^{TM} is 85 % soluble potassium fulvates (CEC of 1400 cmol kg^{-1}). Their information about basic analyses was provided from the manufacturer (Table 2).

Figure 4. NTS Soluble Humate Granules™ (up) and NTS Soluble Fulvic Acid Powder™ (down).

Table 2. Basic analyses of commercial humic substances.

	Humic acid	Fulvic acid
Water soluble potassium humate / fulvate	70 %	70 %
Potassium	8.5 %	12 %
Solubility	85 %	90 %
Particle size	< 2 mm	very fine powder
Moisture	15 %	15 %
Appearance	black granules	yellow brown

2. Stability constants experiment

2-1. General theory

Stability constants (also called formation constants or binding constants) are equilibrium constants for the formation of a complex in solution. The stability constant of a complex is the numerical expression of its stability and provides a quantitative measure of affinity of the metal ion to the complexing agent; that is a measure of how much the interaction is strong between the reagents that come together to form the complex (Ram and Raman, 1984). There are two main kinds of complexes: one is compounds formed by the interaction of a metal ion with a ligand and another is supramolecular complexes, such as host-guest complexes and complexes of anions. The stability constants provide the information required to calculate the concentrations of the complexes in solution. There are several areas of application in chemistry, biology and medicine (Bjerrum and Lamm, 1972).

In the field of soil science and environmental engineering, the understanding of stability constants for different metal-soil organic matter complexes would help scientists predict the behavior of these compounds in the soil system. In this reason, numerous works have been done to determine the stability constants of the complexes formed between humic acid and / or fulvic acid with a number of metal ions (Zende and Raman, 1978; Schnitzer and Hansen, 1970; Schnitzer and Skinner, 1967, 1966; Randhawa and Broadbent, 1965). In order to determine the stability constants, it is needed to know the molar concentrations of the metal ion and of the complexing agent as well as the number of moles of complexing agent that combines with one mole of metal to form the complex. Instead of molar concentration of complexing agent, the researchers used concentration of complexing sites (Schnitzer and Skinner, 1965). In this investigation, the molecular weights of humic acid and fulvic acid were determined by elemental analysis basically, so that the concentration of complexing agent in moles per liter same as classical requirements was able to be expressed. Brief chemical properties of humic substances are listed in Table 3.

According to the Irving-Williams series, the stability of complexes of bivalent metal ions, regardless of the nature of the complexing agent involved, follows the order of Pd > Cu > Ni > Co > Zn > Cd > Fe > Mn > Mg (Irving and Williams, 1953). To figure out the purpose of this study, stability constants of complexes formed between As, Cu, Pb ions and purchased humic acid and fulvic acid were determined with the help of the Irving-Williams series. The commercial humic acid and fulvic acid were considered suiting well for these investigations because they are water-soluble and contain a comparatively large number of oxygen-containing functional groups, which make it very reactive with metals (Schintzer and Skinner, 1965).

Table 3. Chemical properties of humic substances.

Sample	Elemental composition				Atomic ratio		Molecular weight	Total metal content			pH
	C	H	O	N	H/C	O/C		As	Cu	Pb	
	------------- % (w/w) -------------						------ g mol^{-1} ------	------- mg kg^{-1} -------			
Humic acid	42.98	3.01	23.25	0.76	0.07	0.54	1297	0.74	3.89	0.47	9.94
Fulvic acid	40.58	4.08	23.77	1.57	0.10	0.59	624	0.14	1.19	1.56	5.34

2-2. Measurements of stability constants

To measure the stability constants, the ion-exchange method originally developed by Schubert (Schubert, 1948), and first applied to metal-soil organic matter complexes by Miller and Ohlrogge (Miller and Ohlrogge, 1958) and then by Randhawa and Broadbent (Randhawa and Broadbent, 1965), was employed (Schnitzer and Skinner, 1965). This method seems to be appropriate for the study of water soluble metal and organic matter complexes because (a) quite low concentrations of metals might be used, and (b) equilibriums are established quickly. According to the relationship suggested by Martell and Calvin (1952), the logarithm of the stability constant for the complex (log K) can be determined. The relation is as follows:

$$\boldsymbol{log\left(\frac{\lambda_0}{\lambda}-1\right)=logK+xlog[HA\ or\ FA]} \qquad (1)$$

where,

λ_0 = the distribution constant in absence of humic acid or fulvic acid;

λ = the distribution constant in the presence of humic acid or fulvic acid;

log K = log stability constant;

x = number of moles of humic acid or fulvic acid which combine with one mole of metal;

[HA or FA] = concentration of humic acid or fulvic acid in moles per liter – 1 mole of humic acid and fulvic acid = 1297 g and 624 g, respectively.

λ_0 was determined from the following expression:

$$\boldsymbol{\lambda_0 = \frac{\alpha_0 V}{(100-\alpha_0)g}} \qquad (2)$$

where,

α_0 = percent of total metal bound to exchange resin;

$100 - \alpha_0$ = percent of total metal remaining in solution;

V = volume of solution, which was 50 mL in all cases;

g = weight of exchange resin, which was always 1.0 g.

λ could be measured in the same manner as λ_0 but in the presence of different humic acid or fulvic acid concentrations.

x was obtained separately for each metal from the slope of the plot on log-log paper, of $\lambda_0/\lambda - 1$ vs. different humic acid or fulvic acid concentrations.

2-3. Analytical methods

Aliquots of 1 to 10 mL of humic acid and fulvic acid stock solution, containing 43.25 mg of humic acid and 20.81 mg of fulvic acid per mL (= 33.35 mM) were pipetted into 50-mL volumetric flasks and diluted to approximately 40 mL with distilled water. To each flask 5 mL of 1 N NaCl solution for ionic strength was added, followed by either 3 mL of As^{5+}, Cu^{2+}, or Pb^{2+} solutions ($Na_2HAsO_4 \cdot 7H_2O$, $CuCl_2 \cdot 2H_2O$, $PbCl_2$). The sodium arsenate dibasic heptahydrate solution and lead chloride solution were made to 16.67 mM, whereas copper chloride solution was made to 33.35 mM to simulate actual metal contamination concentrations. The pH was then adjusted to around 6.6 by the addition of a few drops of 0.1 N NaOH or 0.1 N HCl solutions. Finally, the volume of each flask was made up to 50 mL with distilled water.

Quantities (1-g) of Amberlite IR 120 Na^+ form, 100-200 mesh were weighed into 125-mL ground-glass-stoppered Erlenmeyer flasks. The solutions containing the metal, sodium chloride, and humic acid or fulvic acid were transferred to flasks and then shaken at 25±2℃, 200 rpm for one hour. It was assumed that equilibrium was reached within this period of time. The exchange resin was then removed by filtration through a quantitative filter paper (No.42, Whatman Filters) and the filtrate was analyzed using ICP (Inductively Coupled Plasma) Atomic Emission Spectrometer (ICPS-7510, Shimadzu, Japan) for arsenic, copper, and lead.

3. Batch incubation experiment

The reactions that occurred in the stability constants experiment were processed in solution state indeed. However, toxic metal contaminations happen in the soil system not only in solution. Therefore, in the realistic point of view it should be investigated how humic substances quality can influence on chemical speciation and stability of toxic metals in the soil. Soil organic matter quality can be most often assessed by the humic to fulvic acids ratio (HA/FA), and here the humic substances quality represents the ratio of humic acid to fulvic acid. The lower the value, the more polymerized and stable are the extracted organic substances

(Mládková et al., 2006).

100-g of 2-mm sieved soil samples were placed into each 250 mL polyethylene bottle and pre-incubated at 25±2℃ for 10 days. Deionized water was added to the soils in order to adjust their moisture contents to field capacity of the soils (320 g kg $^{-1}$). Four treatments were prepared in a completely randomized design with triplicate: control (X), humic substances input of HA/FA=2 (A), humic substances input of HA/FA=1 (B), humic substances input of HA/FA=0.5 (C). Different quality of humic substances was treated into the pre-incubated soil at the rate of 1.5 g kg^{-1}. A series of batch incubations was carried out in darkness in a temperature-controlled incubator at 25±2℃ for 90 days. Soils were sampled periodically (0, 5, 10, 20, 40, 60, and 90 days after treatment) and analyzed for pH, five-different fractions of toxic metals, and metal contents of organo-metallic complexes.

3-1. Sequential extraction method

The chemical forms of the metals in soil were determined by the sequential extraction method, the five-step chemical fractionation based on the work of Tessier et al. (1979) and proposed by Elliott et al. (1990). Also, Salbu and Krekling (1998) suggested a similar extraction method to Tessier’s to characterize the partitioning of toxic metals in soils. In this research, the method from Salbu and Krekling (1998) was mainly used and partially modified from the procedure of Tessier et al. (1979) and Elliott et al. (1990). Metals in soil were fractionated into five different fractions: water soluble and exchangeable (F1), carbonate bound (F2), Fe-Mn oxide bound (F3), organic bound (F4), and residual fractions (F5). The un-amended and amended soils were treated with the appropriate reagents and shaken at room temperature for the stated times to carry out the extraction (F1 to F4) (Table 4). Separation between steps was by decantation of the supernatant after centrifugation at 3,000 g for 10-30 minutes. After four sequential extractions of samples, the soil remained was dried at 65℃. The dried soil sample was digested with aqua regia until near dryness and filtered through a quantitative filter paper (No. 42, Whatman Filters). The filtrates were washed, diluted with 0.5 N HNO_3, and used for the determination of residual content of metals. The metal contents were measured using ICP (Inductively Coupled Plasma) Atomic Emission Spectrometer (ICPS-7510, Shimadzu, Japan). All the extraction procedures were performed using 50-mL sterilized polypropylene tubes. All reported values were the standard error of three replicates.

Table 4. Sequential extraction scheme for chemical speciation [a].

Fraction	Reagent conditions [b]	Shaking time (h)
Water soluble and exchangeable (F1)	20 mL 1 M NH_4OAc adjusted to pH 7.0 with HOAc (RT, 20℃)	2
Carbonate bound (F2)	20 mL 1 M NH_4OAc adjusted to pH 5.0 with HOAc (RT, 20℃)	2
Fe-Mn oxides bound (F3)	20 mL 0.04M $NH_2OH \cdot HCl$ in 25 % HOAc (water bath, 60℃)	6
Organic bound (F4)	15 mL 30 % H_2O_2 adjusted to pH 2.0 with HNO_3 (water bath, 80℃) and 5 mL 3.2 M NH_4OAc in 20 % HNO_3 after cooling	5.5 and 1
Residual (F5)	Digestion with 12 mL aqua regia	

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[a] Adapted from the procedure of Salbu and Krekling (1998), Elliott et al. (1990), and Tessier et al. (1979).

[b] Soil samples were weighed 1 g for analyses. After each successive extraction, separation was done by centrifuging at 3,000 g for 30 min. The supernatants were removed with a pipette, filtered with 0.45 ㎛ nylon membrane filters, and analyzed for metals. Before the extraction step, the residue was washed with 10 mL of deionized water followed by vigorous hand shaking, and then followed by 10 min of centrifugation at 3,000 g, and the washed solution was discarded.

3-2. Organo-metallic complexes extraction method

The aim of this research is to investigate how humic substances treatment can affect the chemical speciation and stability of metals, so it was needed to focus on the interaction between organic matter and metal specifically. Organo-metallic complexes extraction method was adapted from Podlešáková et al. (1992) and Donisa et al. (2003). In order to determine the concentration of metals bound to humic acid fraction and fulvic acid fraction, respectively, the modified procedures presented in Figure 5 were performed.

4 g of 2-mm sieved soil samples were put into 50-mL polypropylene tubes and extracted with 40 mL of 0.1 M $Na_4P_2O_7$ (pH 10.0) shaking for 2 hours at room temperature. Undissolved material was removed by centrifugation at 3,000 g for 20 minutes. After acidification of an aliquot (20 mL) of the pyrophosphate extract to pH 1.5 using 5 mL of 1 M H_2SO_4, the solution was shaken for 30 minutes and centrifuged at 3,000 g for 10 minutes. The precipitate containing the humic acid fraction was removed. The original pyrophosphate extract (20 mL) and the separated fulvic fraction were subjected to be treated with aqua regia (2 mL) for decomposition of the organic matter and conversion of pyrophosphate to orthophosphate, and then double acid hydrolysis using 4 M HCl (2 mL) and 0.5 M HCl (1 mL) was carried out for the removal of silica. The supernatants were removed with a pipette, filtered with 0.45 ㎛ nylon membrane filters, and analyzed for metals using ICP (Inductively Coupled Plasma) Atomic Emission Spectrometer (ICPS-7510, Shimadzu, Japan).

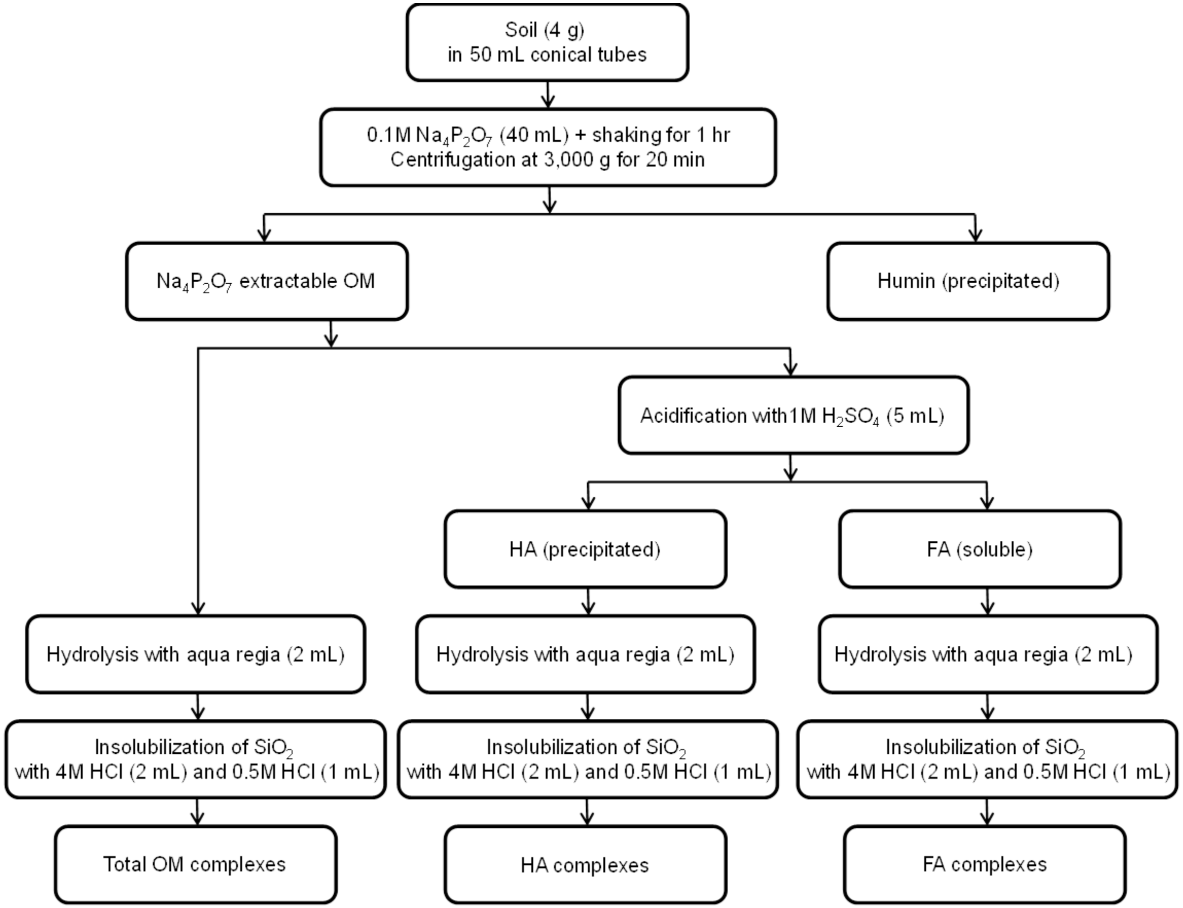

Figure 5. Flow chart for organo-metallic complexes extraction (adapted from Donisa et al., 2003; Podlešáková et al., 1992)

The metal contents in the humic acid fraction were calculated as the difference between metal contents in the total organic matter complexes and the fulvic acid fraction. However, metal content in both supernatants may include not only metal forms bound on humic substances, but also a portion of exchangeable metals (Donisa et al., 2003). Therefore, it should be allowed for these forms would increase the estimate of the metals bound in fulvic acid fraction; exchangeable metals are expected to stay in the solution during humic acid precipitation at low pH (Borůvka and Drabek, 2004).

In addition to the metal contents in the humic substances, the stability constants of each humic substance and metal complex extracted from the soil were determined by the method described above (Schnitzer and Skinner, 1965). Quantities (1-g) of Amberlite IR 120 Na^+ form, 100-200 mesh were weighed into 125-mL ground-glass-stoppered Erlenmeyer flasks. 10 mL of both supernatants were transferred to flasks and then shaken at 25±2°C, 200 rpm for one hour. The exchange resin was then removed by filtration through a quantitative filter paper (No.42, Whatman Filters) and the filtrate was analyzed using ICP (Inductively Coupled Plasma) Atomic Emission Spectrometer (ICPS-7510, Shimadzu, Japan) for arsenic, copper, and lead.

4. Statistical analysis

Data were statistically analyzed using General Linear Model procedure (SAS Institute, Version 9.3, Cary, USA). Analysis of variance (ANOVA) was used to evaluate the effect of humic substances quality on chemical speciation and stability of toxic metals in soil. The least significant difference (LSD) test at a confidence level of 95 % was used to separate means.

Ⅲ. RESULTS

1. Physico-chemical properties of soil and humic substances

To identify basic soil properties of the Former Janghang Smelter area, a representative sampling site was selected and analyzed for soil texture, pH, EC (electric conductivity), CEC (cation exchange capacity), TC (total carbon content), TN (total nitrogen content), TOC (total organic carbon), organic matter, and total metal contents. The result is listed in Table 1. The soil texture of the sampling site was silty clay loam. The pH was 6.3, which was higher than the average (pH 5.7) of paddy soils in Korea. The cation exchange capacity showed relatively low (18.1 cmol kg^{-1}). Total carbon content and total nitrogen content were 15.9 and 1.1 g kg^{-1}, respectively. The content of total organic carbon was 1.6 % and organic matter content showed 4.3 %, which is quite ideal content of organic matter in soil. In the fact that mean total contents of As, Cu, Pb in forest soils as natural background levels of metals in Korea are 6.83 (0.56-20.58) mg kg^{-1}, 15.26 (2.80-50.00) mg kg^{-1}, 18.43 (4.07-78.33) mg kg^{-1}, respectively, it can be said the site was heavily polluted with these metals (see Table S1 in the Supplemental Data).

Table 3 shows the chemical properties of humic substances. With the information from the elemental analysis, elemental compositions and molecular weights of humic substances were obtained. Based on these results, atomic ratio was calculated and humic acids have lower H/C and O/C ratios than those of fulvic acids. The molecular weights of humic acid and fulvic acid were 1297, 624 g mol^{-1}, respectively. Humic acids contained 0.74 mg kg^{-1} of As, 3.89 mg kg^{-1} of Cu, 0.47 mg kg^{-1} of Pb while fulvic acids contained 0.14 mg kg^{-1} of As, 1.19 mg kg^{-1} of Cu, 1.56 mg kg^{-1} of Pb. The pH of humic acids was 9.94 and that of fulvic acids was 5.34.

2. Stability constants experiment

2-1. Composition of complexes

The molar HA/As and FA/As ratios at pH 6.6 were 0.70 and 5.06, respectively (Fig. 6, curves a and b, and Table 5, 6). At pH 6.6, approximately one mole of humic acid complexed with one mole of As, whereas five moles of fulvic acid combined with one mole of As. The molar HA/Cu and FA/Cu ratios at pH 6.6 were 1.89 and 0.55, respectively (Fig. 6, curves c and d, and Table 7, 8). In case of Cu, nearly two moles of HA complexed with one mole of Cu at pH 6.6, while approximately one mole of FA reacted with two moles of Cu at pH 6.6. The molar HA/Pb and FA/Pb ratios at pH 6.6 were 2.01 and 1.44, respectively (Fig. 6, curves e and f, and Table 9, 10). Under the experimental conditions of this study, two moles of HA complexed with one mole of Pb, however, three moles of FA combined with two moles of Pb.

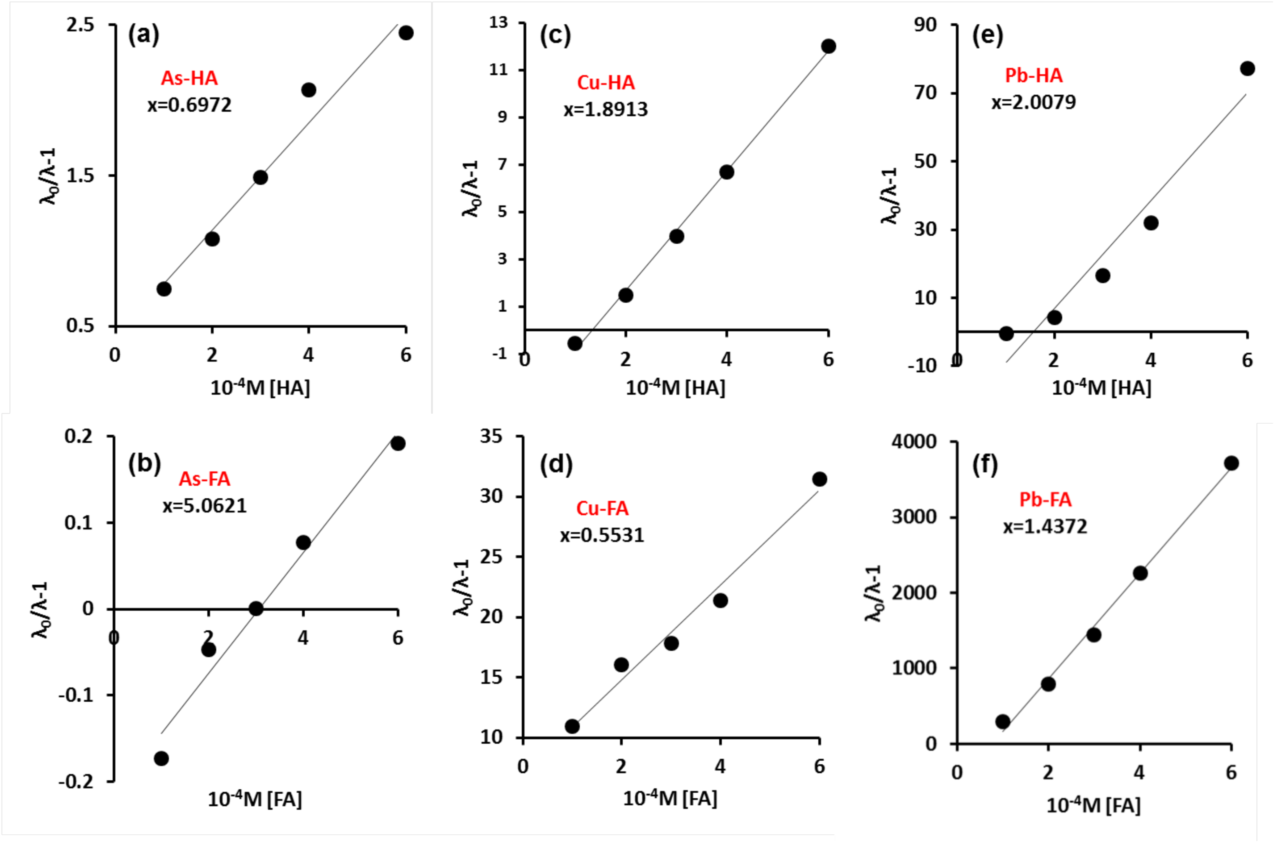

Figure 6. λ_0/λ - 1 vs. [HA] or [FA] plots of (a) As-HA at pH 6.6; (b) As-FA at pH 6.6; (c) Cu-HA at pH 6.6; (d) Cu-FA at pH 6.6; (e) Pb-HA at pH 6.6; and (f) Pb-FA at pH 6.6.

Table 5. Stability constant of As-humic acid complex at pH 6.6.

Concentration of humic acid (mol L^{-1}) x 10^{-4}	α_0	λ_0	λ	$\lambda_0/\lambda - 1$	x	Mole of HA:As	log *K*
0	5.08	2.65					
0.5	3.05		1.56	0.74			2.66
1.0	2.75		1.40	1.08			2.61
1.5	2.41		1.22	1.49			2.63
2.0	1.58		0.79	2.07			2.68
3.0	2.08		1.04	2.45	0.70	1:1	2.63
							2.64[a]

[a] Average value of conditional stability constant (log *K*).

Table 6. Stability constant of As-fulvic acid complex at pH 6.6.

Concentration of fulvic acid (mol L^{-1}) x 10^{-4}	α_0	λ_0	λ	$\lambda_0/\lambda - 1$	x	Mole of FA:As	log *K*
0	7.20	3.84					
0.5	7.74		4.14	-0.17			
1.0	7.17		3.84	-0.05			
1.5	7.51		4.03	0.00			12.40
2.0	7.01		3.74	0.08			4.51
3.0	6.31		3.36	0.19	5.06	5:1	2.92
							3.71[a]

[a] Average value of conditional stability constant (log *K*).

Table 7. Stability constant of Cu-humic acid complex at pH 6.6.

Concentration of humic acid (mol L^{-1}) x 10^{-4}	α_0	λ_0	λ	$\lambda_0/\lambda - 1$	x	Mole of HA:Cu	log *K*
0	95.61	1071.96					
0.5	97.96		2356.43	-0.541			
1.0	89.76		429.49	1.497			7.17
1.5	82.15		216.72	3.950			7.26
2.0	74.12		142.19	6.669			7.25
3.0	63.66		82.50	12.017	1.89	2:1	7.17
							7.21[a]

[a] Average value of conditional stability constant (log *K*).

Table 8. Stability constant of Cu-fulvic acid complex at pH 6.6.

Concentration of fulvic acid (mol L^{-1}) x 10^{-4}	α_0	λ_0	λ	$\lambda_0/\lambda - 1$	x	Mole of FA:Cu	log *K*
0	96.16	1228.15					
0.5	67.70		103.66	10.95			3.25
1.0	59.51		72.73	16.06			3.25
1.5	56.86		65.54	17.80			3.20
2.0	52.69		54.93	21.37			3.21
3.0	43.29		37.90	31.44	0.55	1:2	3.28
							3.24[a]

[a] Average value of conditional stability constant (log *K*).

Table 9. Stability constant of Pb-humic acid complex at pH 6.6.

Concentration of humic acid (mol L^{-1}) x 10^{-4}	α_0	λ_0	λ	$\lambda_0/\lambda - 1$	x	Mole of HA:Pb	log *K*
0	99.05	5195.32					
0.5	99.40		8341.96	-0.41			
1.0	95.14		977.23	4.33			8.06
1.5	86.31		314.05	16.56			8.29
2.0	76.99		167.50	32.01			8.33
3.0	55.32		61.44	77.26	2.01	2:1	8.36
							8.26[a]

[a] Average value of conditional stability constant (log *K*).

Table 10. Stability constant of Pb-fulvic acid complex at pH 6.6.

Concentration of fulvic acid (mol L^{-1}) x 10^{-4}	α_0	λ_0	λ	$\lambda_0/\lambda - 1$	x	Mole of FA:Pb	log *K*
0	99.99	440233					
0.5	96.83		1512.21	291.40			
1.0	91.76		553.55	794.36			8.22
1.5	85.96		304.01	1447.25			8.22
2.0	79.76		195.41	2256.80			8.24
3.0	70.37		118.71	3723.19	1.44	3:2	8.20
							8.22[a]

[a] Average value of conditional stability constant (log *K*).

2-2. Stability constants

The log K values were calculated from equation (1). The data used in these calculations are presented in Table 5 to 10. The log K value for the As-HA complex was 2.64 and that for As-FA complex was 3.71 (Table 5, 6). In case of copper, the log K value for the Cu-HA complex was 7.21 and that for Cu-FA complex was 3.24 (Table 7, 8). Even though As and Cu complexes with different humic substances showed significantly different values, on the other hand, the log K values for the Pb complexes with HA and FA were less affected by the variety of humic substances. They were 8.26 and 8.22 for Pb-HA complex and Pb-FA complex, respectively (Table 9, 10).

3. Batch incubation experiment

3-1. Changes in soil pH

The initial pH values of the soil used in this study was 6.28. Humic substances increased the pH values of the soils. Soil pH increased slightly or sharply right after the addition of humic substances. In the soils of the treatment groups (HA/FA=2, 1, and 0.5), pH increased from 6.28 to 6.87, 6.67, and 6.42, respectively (Fig. 7). However, the pH values gradually declined during the incubation. In case of control soil, the pH value dropped to 5.29 in 90 days, while the pH values of soils amended with humic substances decreased to 6.03, 5.80, and 5.61 in HA/FA=2, 1, and 0.5, respectively ($P < 0.05$).

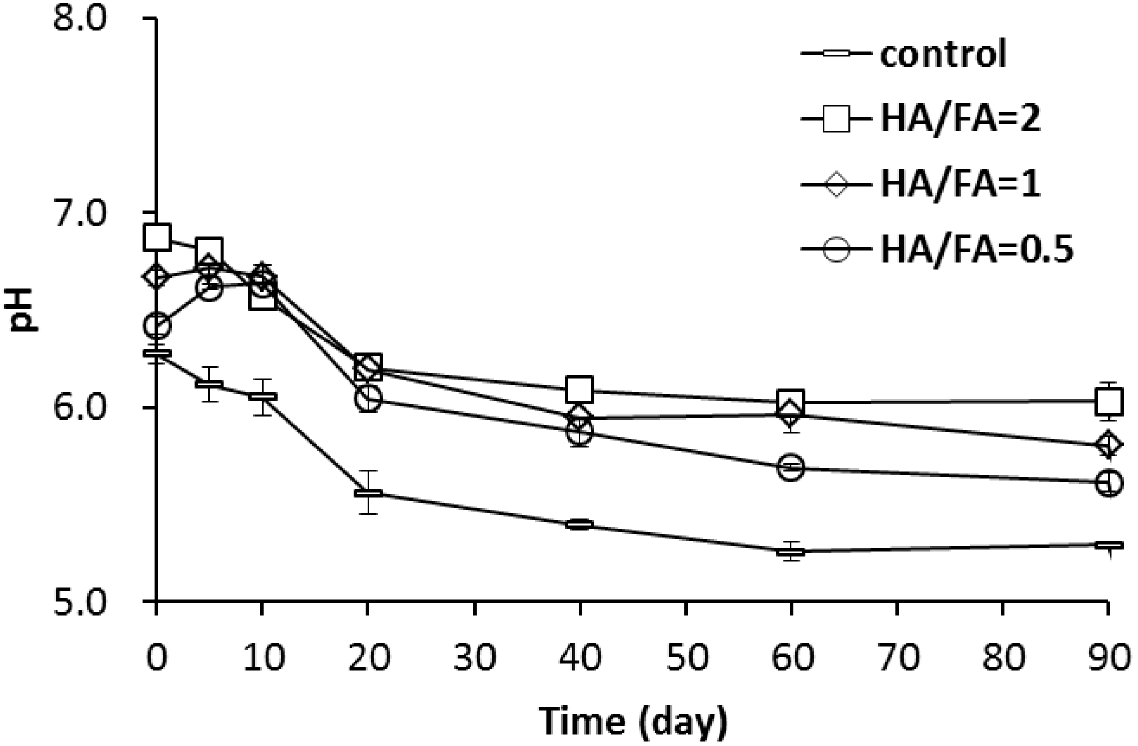

Figure 7. Changes in soil pH during 90 days. Error bars indicate standard errors of triplicates.

3-2. Chemical speciation of arsenic (As)

Humic substances hardly affected the distribution of arsenic in smelter area soils (Fig. 8, 9). The contents of water soluble and exchangeable As (F1) decreased during the incubation ($P < 0.05$) but there was no significant difference between control and treatment groups ($P > 0.05$). The contents of carbonate bound As (F2) also gradually decreased by time ($P < 0.05$); however the difference between control and treatment groups was not found either ($P > 0.05$). The contents of Fe-Mn oxide bound As (F3) declined after 20 days ($P < 0.05$) while those of organic bound As (F4) slightly increased after 40 days ($P < 0.05$). However both fractions did not show any significant differences between control and treatment groups ($P > 0.05$). In case of F5 (residual As) the contents of As increased after 40 days ($P < 0.05$), yet there was no significant difference between control and treatment groups ($P > 0.05$).

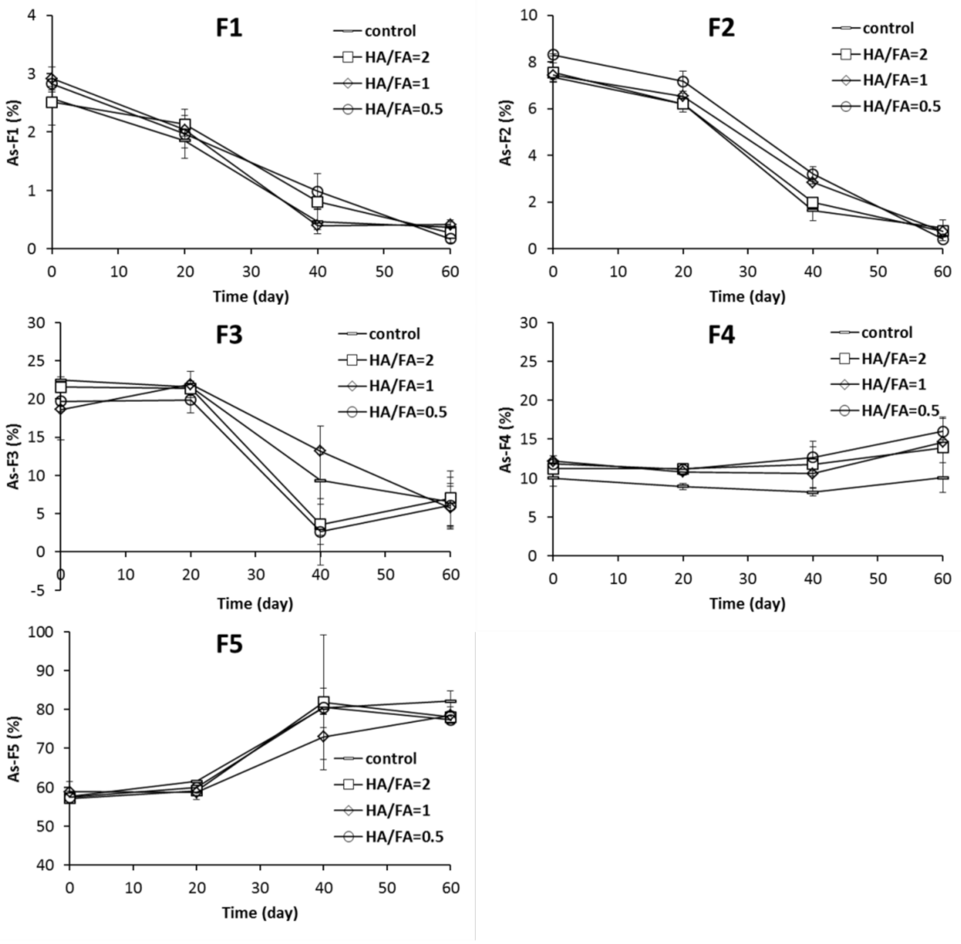

Figure 8. Temporal variations in As contents (in % of total content) in solution extracted by sequential extraction after treating with different quality of humic substances: control, HA/FA=2, HA/FA=1, and HA/FA=0.5. Error bars indicate standard errors of triplicates.

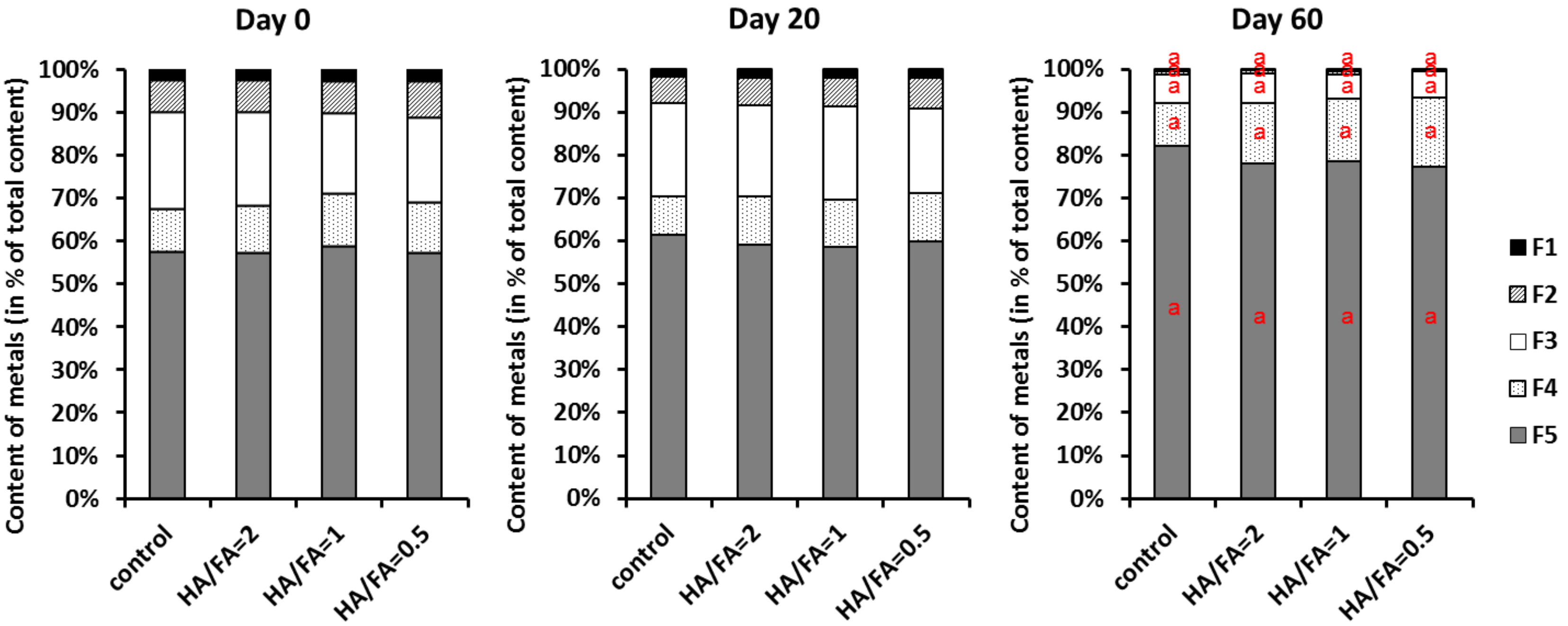

Figure 9. Temporal variations in As contents distribution (in % of total content). Bars with different letters are significantly different ($P < 0.05$) for each metal fraction across different quality of humic substances.

3-3. Chemical speciation of copper (Cu)

Compared to As, the distribution of Cu fraction in smelter area soils was significantly affected by humic substances (Fig. 10, 11). The contents of water soluble, exchangeable, and carbonate bound Cu (F1, F2) gradually increased during the incubation ($P < 0.05$) and they were lower in the humic substances-treated soils than in the control soil. There was significant difference in F1 between control and treatment groups, and between treatment groups ($P < 0.05$). Among treatment groups, the higher contents of FA, the higher contents of Cu in F1. However, F2 showed a significant difference only between control and treatment groups ($P < 0.05$). The contents of Fe-Mn oxide bound Cu (F3) decreased until 40 days and slowly increased after that. The treatment of humic substances increased the contents of insoluble Cu (F4, F5) compared to those in the control. The Cu content of F4 in control soil diminished dramatically while those in humic substances-treated soils decreased slightly. The significant difference between control and treatment groups was found in F3 and F4 ($P < 0.05$), but there was no difference between treatment groups ($P > 0.05$). Residual Cu (F5) gradually increased but slightly decreased after 60 days. In addition, F5 did not show any significant difference between control and treatment groups ($P > 0.05$).

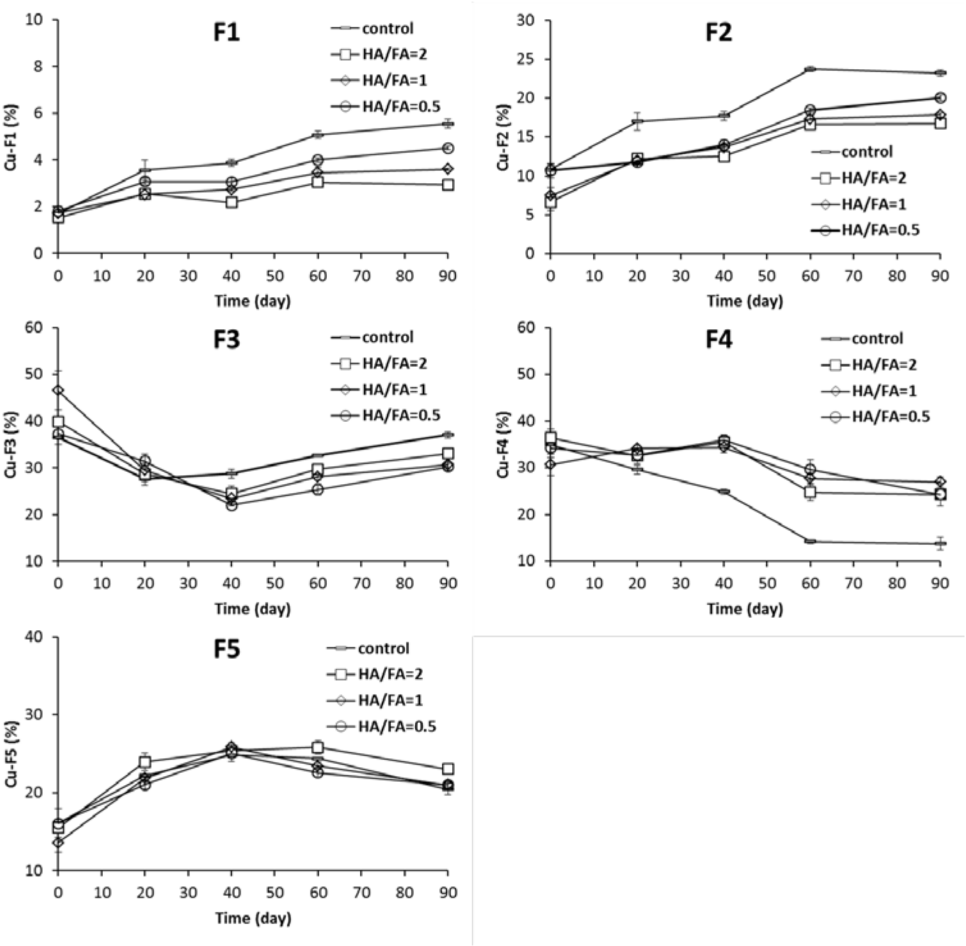

Figure 10. Temporal variations in Cu contents (in % of total content) in solution extracted by sequential extraction after treating with different quality of humic substances: control, HA/FA=2, HA/FA=1, and HA/FA=0.5. Error bars indicate standard errors of triplicates.

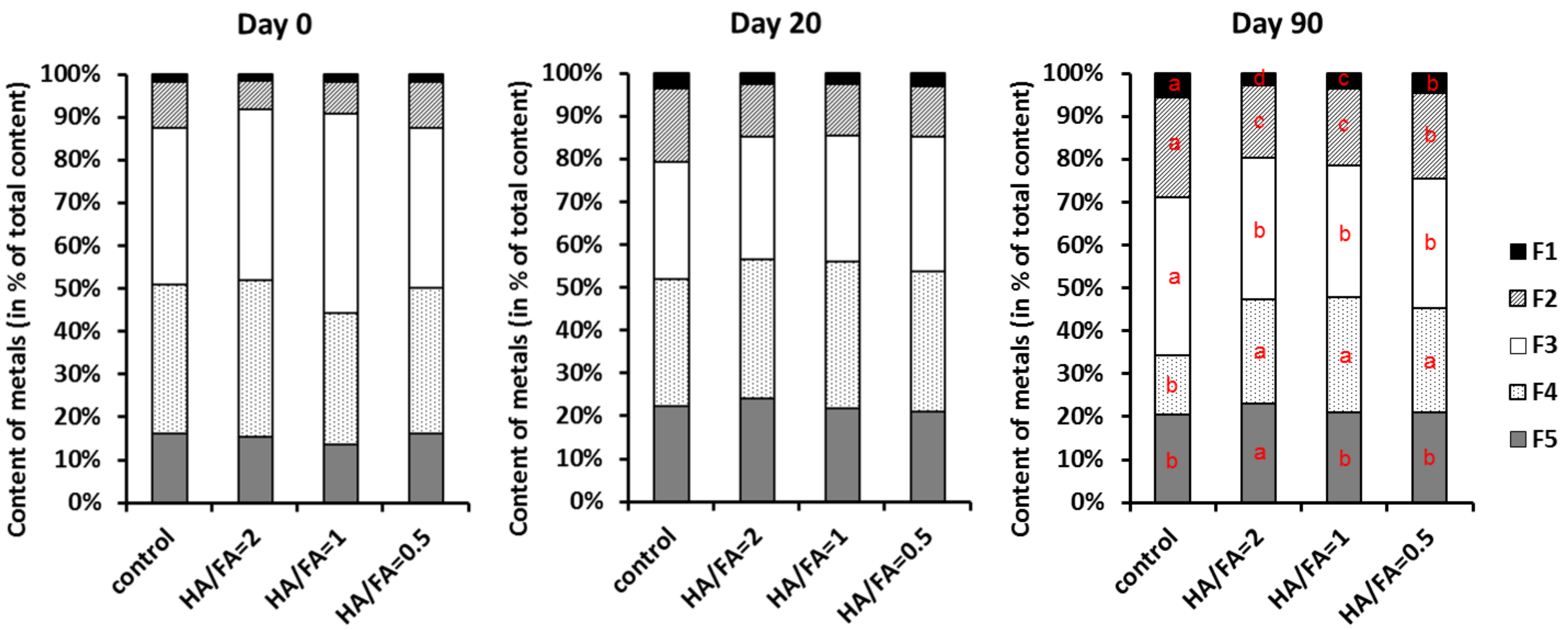

Figure 11. Temporal variations in Cu contents distribution (in % of total content) in solution extracted by sequential extraction after treating with different quality of humic substances: control, HA/FA=2, HA/FA=1, and HA/FA=0.5. Bars with different letters are significantly different ($P < 0.05$) for each metal fraction across different quality of humic substances.

3-4. Chemical speciation of lead (Pb)

The distribution of Pb fraction was also influenced by humic substances treatment along with that of Cu (Fig. 12, 13). The content of water soluble and exchangeable Pb (F1) in the control soil was steady until 40 days but gradually decreased after that, however, the Pb contents in the humic substances-treated soils increased in 40 days and slightly declined during the rest of incubation. The contents of carbonate bound Pb (F2) showed the similar pattern with that of F1, but there were no significant differences between control and treatment groups in both fractions ($P > 0.05$). In F3 (Fe-Mn oxide bound Pb), the temporal patterns of both control and treatment groups were similar, but the Pb contents were lower in the humic substances-treated soils than in the control soil throughout the incubation. In contrast, the contents of organic bound Pb (F4) in the humic substances-treated soils were significantly higher than that in the control soil. There were significant differences between control and treatment groups in F3 and F4 ($P < 0.05$). The more FA exists in the soil, the more similar the distribution pattern is in F3, but the opposite phenomenon was found in F4. The residual Pb (F5) contents had a similar pattern as those of F1 and F2, and it was hard to find any significant difference between control and treatment groups ($P > 0.05$).

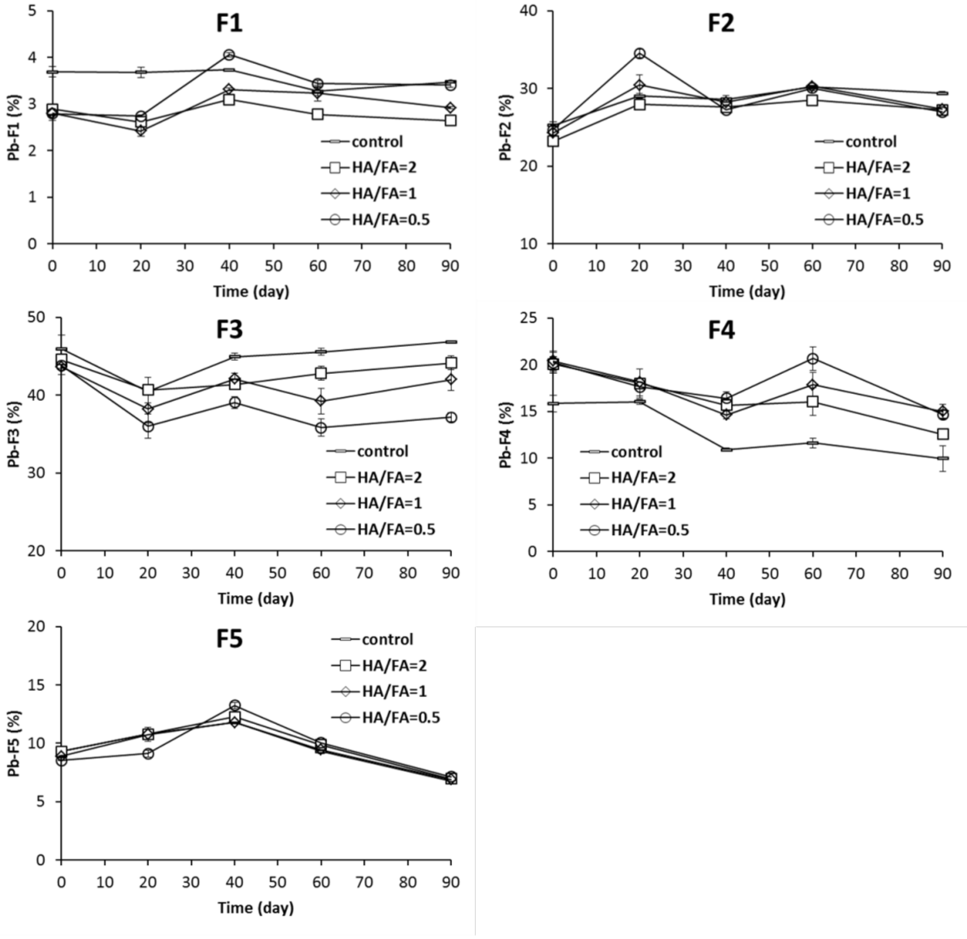

Figure 12. Temporal variations in Pb contents (in % of total content) in solution extracted by sequential extraction after treating with different quality of humic substances: control, HA/FA=2, HA/FA=1, and HA/FA=0.5. Error bars indicate standard errors of triplicates.

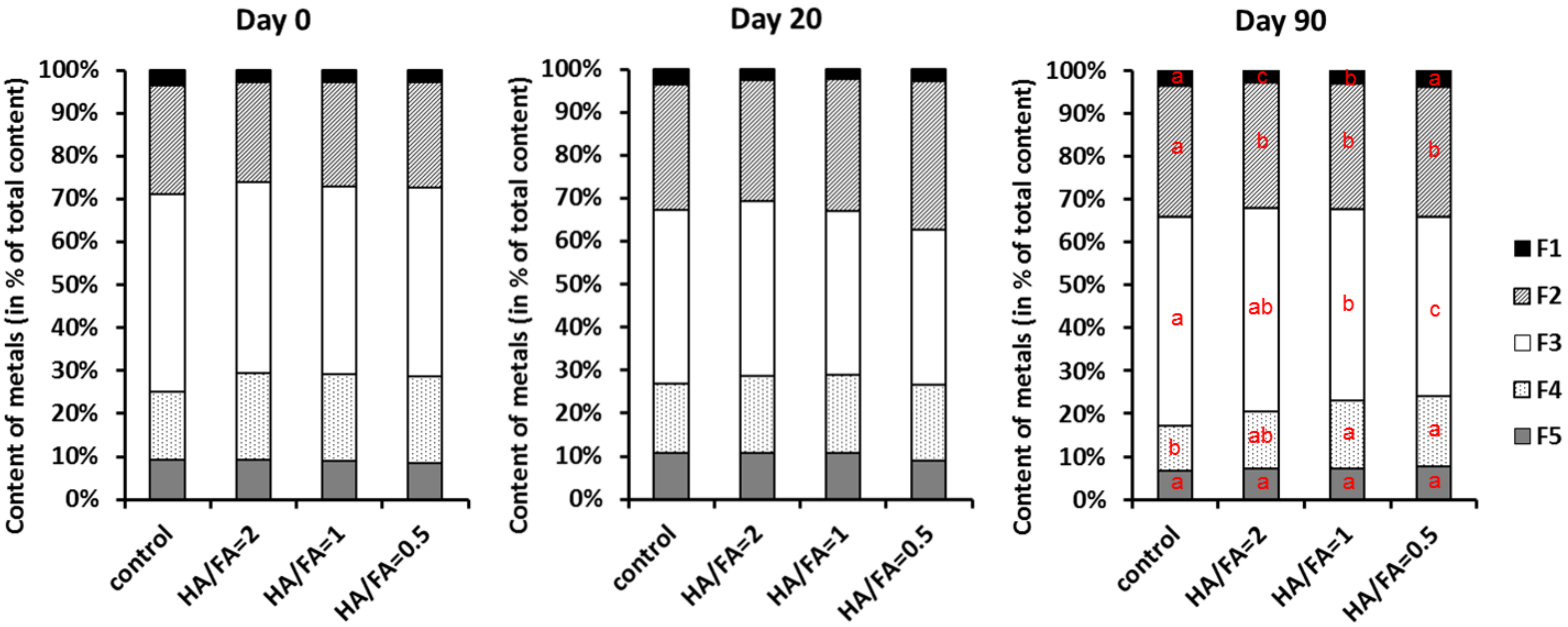

Figure 13. Temporal variations in Pb contents distribution (in % of total content) in solution extracted by sequential extraction after treating with different quality of humic substances: control, HA/FA=2, HA/FA=1, and HA/FA=0.5. Bars with different letters are significantly different ($P < 0.05$) for each metal fraction across different quality of humic substances.

3-5. Changes in concentrations of metals bound to humic substances

Figure 14 shows the concentration changes of metals bound on humic substances during the incubation. The concentrations of As-FA complexes slightly increased by time ($P < 0.05$), but there was no significant difference between control and treatment groups ($P > 0.05$). The As contents in As-HA complexes showed different patterns depending on the samples; one was steady and another decreased in 60 days and soared again after that. The humic substances-treated soil with the ratio of HA/FA=2 was similar to the control soil rather than to the other treatment group soils (HA/FA=1 and HA/FA=0.5). Interestingly, the As contents in As-HA complexes were higher in the control soil even though humic substances were treated to the soils. The concentrations of Cu in both Cu-FA complexes and Cu-HA complexes increased during the incubation, but there could not be found any significant differences between control and treatment groups ($P > 0.05$). The concentrations of Pb-FA complexes slightly decreased during the incubation and the Pb concentrations were higher in the humic substances-treated soils than that in the control soil ($P < 0.05$). The concentrations of Pb-HA complexes showed similar patterns to those of As-HA complexes.

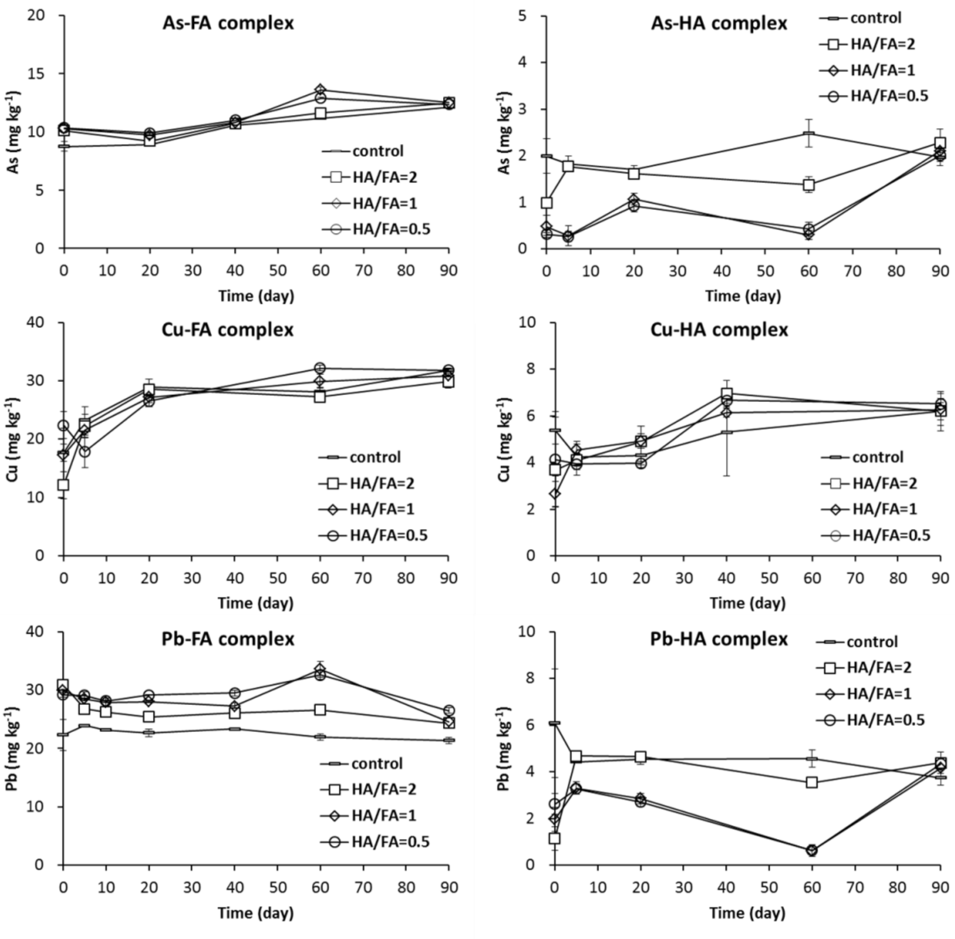

Figure 14. Temporal variations in concentrations of metals bound to humic substances in solution extracted by organo-metallic complexes extraction after treating with different quality of humic substances: control, HA/FA=2, HA/FA=1, and HA/FA=0.5. Error bars indicate standard errors of triplicates.

3-6. Changes in stability constants

The stability constant (log K) values for metal-humic substance complexes are listed in Table 11. The log K values for the As-FA complexes decreased during the incubation, but there was no significant difference ($P > 0.05$). As-FA complexes were more stable in the control soil than in the treatment group soils. The log K values for the As-HA complexes also decreased by time except for the HA/FA=2 treatment group. Both log K values for the Cu-FA complexes and Cu-HA complexes decreased significantly ($P < 0.05$). At the first time of incubation Cu-FA complexes were more stable in the control soil than in the treatment group soils except for the case of HA/FA=2 treated soil; however all three humic substances-treated soils had higher log K values than that of control soil after 90 days. In contrast, the log K values for the Cu-HA complexes were lower in the treatment group soils than in the control soil during the incubation. In case of Pb-humic substance complexes, the log K values had a tendency to decrease except for HA/FA=2 treatment group in Pb-FA complex and HA/FA=0.5 treatment group in Pb-HA complex. Pb-HA complexes showed higher stabilities in the control soil than in the humic substances-treated soils except for the HA/FA=0.5 treatment group. Most metal-humic substance complexes had higher stabilities when the ratio of humic acids was higher. On the contrary to this, Pb-HA complexes were getting stable with a higher fulvic acid ratio.

Table 11. Temporal variations in stability constants for metal-humic substance complexes.

	As						Cu						Pb					
	log K																	
	FA			HA			FA			HA			FA			HA		
	0	90	△▽	0	90	△▽	0	90	△▽	0	90	△▽	0	90	△▽	0	90	△▽
control	16.2	15.4	▽	2.5	1.9	▽	0.9	-0.7	▽	8.4	5.8	▽	3.1	3.0	▽	8.1	7.4	▽
HA/FA=2	14.3	13.8	▽	1.8	2.5	△	1.3	0.5	▽	6.9	5.5	▽	3.2	3.6	△	7.3	6.4	▽
HA/FA=1	14.2	12.5	▽	2.2	1.3	▽	0.8	0.7	▽	7.2	5.0	▽	-	-	-	7.3	6.8	▽
HA/FA=0.5	13.3	12.4	▽	2.6	1.6	▽	0.7	-0.5	▽	7.8	5.1	▽	-	2.4	-	6.8	7.6	△

Ⅳ. DISCUSSION

1. Stability constants experiment

The greater the association is, the greater the stability of the compound, qualitatively. The log stability constants for the Pb-HA complexes and Pb-FA complexes were considerably higher than those of the other metal complexes. This might be attributed to the high reactivity of humic substances towards Pb than As or Cu. While log K values for As-FA complexes were greater than those for As-HA complexes, log K values for Cu-HA complexes were almost twice greater than those for Cu-FA complexes. From these results, it could be possibly considered that As is more reactive with FA but Cu is with HA in contrast. Because stability constants cannot provide enough information on the mechanisms of metal and humic substance interactions and on the chemical structures of the complexes formed, it was suggested that the reactions with metal ions proceeded via functional groups (Schnitzer and Skinner, 1966).

Schnitzer and Skinner found that two types of reactions occurred: a major one, in which both acidic carboxyls and phenolic hydroxyls participated simultaneously, and a minor one, which involved acidic carboxyls only (Buffle, 1984). There was found a notable slow migration of metal ions within the humic structures by Hering and Morel (1989). According to their research, the coulombic interactions with negatively charged moieties on the humic acid and fulvic acid chains (especially -COO^-) were followed by the addition of any metal ions initially. After that, the configuration of humic and fulvic acid gradually changed as metal ions found their thermodynamically favorable positions within the humic substance structure, probably entailing the formation of relatively stable inner sphere complexes (Pandey et al., 2000).

The complexes formed between one mole of humic substance and one mole of metals are more stable compared to the complexes formed between one mole of humic substance and two moles of metals. Moreover, it was found that the number of moles of humic substances that reacted with the number of moles of metals depends on the characteristics of metal ions. Especially, the molar FA/Cu ratios were relatively lower than the other ratios. If metal-FA reactions are visualized to occur via functional groups, numerically more functional groups in each FA molecule reacted with one mole of Cu than with one mole of any of the other metal ions (Schnitzer and Skinner, 1966). However, the stabilities of complexes formed between fewer functional groups (As-FA, Cu-HA, Pb-HA, and Pb-FA complexes) were considerably greater than those of complexes formed between more numerous functional groups (Cu-FA complexes). Therefore, it could be concluded that it is not the number of bonds but the strength of bonds that determines the magnitude of the stability constants. This investigation indicated that the order of stabilities of complexes formed between humic substances and metal ions was not consistent with the Irving-Williams series, but that they were Pb > Cu > As with HA and Pb > As > Cu with FA, respectively.

However, the dependence of stability constants on many different factors supports the fact that these are conditional stability constants and are reasonable only when the environmental conditions under which they were characterized are same (Pandey et al., 2000). For instance, the stability constants were slightly higher at pH 5.0 than at pH 3.5 in the study of Schnitzer and Hansen (1970), which is due to the higher dissociation of functional groups, particularly carboxyl groups, at higher pH. According to their research, H^+ and the metal ion are likely to compete for binding sites on the ligand and less metal is bound at lower pH. In addition, Zn^{2+} and Cu^{2+} formed 1:1 molar complexes with what so called as the "natural ligand" of the soil solutions (Geering and Hodgson, 1969). However, because the pH in this study was around 6.6, it could be thought that the stability constants were higher than those from the result of Schnitzer and Hansen, and the competition between H^+ and the metal ion for the binding sites were less than their results.

2. Batch incubation experiment

It is well known that toxic metals in different fractions of soil have different remobilization behaviors under changing environmental conditions (Leschber et al., 1985) and geochemical forms of these metals are relevant to their solubility, which directly influence their bioavailability (Xian, 1987). Therefore, determining total content of toxic metals is not enough to assess the environmental impact of contaminated soils on ecosystems (Salomons and Förstner, 1980), so it is important to figure out the chemical forms of metals when determining metal behavior in the environment and its remobilization ability (Tessier and Campbell, 1988; Tessier et al., 1979). It is assumed that metal bioavailability decreases with each successive extraction step in the procedure. Xian (1989) reported that metals in water soluble and exchangeable fractions would be readily bioavailable to the environment, while the metals in the residual fraction are tightly bound and would not be released easily under natural conditions (Ma and Rao, 1997). Barančíková and Makovníková (2003) found that the main factors to affect the mobility of toxic metals in soil could be pH, content and quality of soil organic matter, content and quality of clay fraction, and Fe-Mn oxides. Especially, as one of the significant proportion of total organic matter in the global carbon pool, it is well known that the predominant fractions of humic substances are humic acids (HA) and fulvic acids (FA), which are very active in chelating with inorganic pollutants. The major ligand sites for metal ions in HA and FA are oxygen-containing functional groups such as carboxyl groups, which are thought to play a conspicuous role in the interactions with metal ions (Barančíková et al., 1997).

As the incubation was progressed, the pH of the soils gradually decreased (Fig. 7). This happened not only in the humic substances-treated soils but also in the control soil. The reason for this might be due to the effect of emissions from copper smelting. The air emissions level is presented in Table S2 in the Supplemental Data (WBG et al., 1999). Furthermore, according to the research by Aryes et al. (2002) the copper smelter has used a variety of copper ore minerals (see Table S3 in the Supplemental Data) and the minerals including other elements can be washed off an exposed surface by wind or flowing water over time. There are some sulfide minerals among these and they could cause acid rock drainage by the oxidation. This is a natural chemical reaction in case that the minerals are exposed to air or water. Jennings et al. (2008) suggested that the reaction of sulfide containing minerals with oxygen and water produces a solution of ferrous sulfate and sulfuric acid. Ferrous iron, after that, can be oxidized producing additional acidity. When this happens, the minerals with sulfide are oxidized, resulting in hydrogen ions, sulfate ions, and soluble metal ions release as shown in equation (3). Further oxidation of Fe^{2+} (ferrous iron) to Fe^{3+} (ferric iron) is processed when sufficient oxygen is dissolved in the water or when the water is exposed to sufficient atmospheric oxygen (equation 4). Ferric iron can either precipitate as $Fe(OH)_3$ or it can react directly with pyrite to produce more ferrous iron and acidity as shown in equations (5) and (6). The acidification of the soils can be explained from the reactions below.

$$2FeS_2\,(s) + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H^+ \tag{3}$$

$$2Fe^{2+} + \tfrac{1}{2}\,O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \tag{4}$$

$$2Fe^{3+} + 6H_2O \leftrightarrow 2Fe(OH)_3\,(s) + 6H^+ \tag{5}$$

$$14Fe^{3+} + FeS_2\,(s) + 8H_2O \rightarrow 2SO_4^{2-} + 15Fe^{2+} + 16H^+ \tag{6}$$

The variation in concentration of five fractions of metals, which were extracted by sequential extraction method was different among As, Cu, and Pb. As was mostly associated with residual and Fe-Mn oxide bound fractions at first but residual and organic bound fractions later (Fig. 8, 9). In case of Cu, the Fe-Mn oxide bound and organic bound fractions were dominant throughout the incubation (Fig. 10, 11), and Pb showed prevalent associations with Fe-Mn oxide bound and carbonate bound fractions (Fig. 12, 13). Generally, metal cations such as Cu, Pb become mobile under acidic conditions and the pH increase results in reducing their availability, on the contrary some metal cations such as arsenite (As^{3+}) and chromium (Cr^{6+}) become mobile under alkali conditions (Alloway, 1995). Because of the soil acidification as explained above the proportion of mobile phase (F1, F2) of Cu and Pb should be increased but decreased in As. The acidification of soil might be the reason for the chemical distribution shift to stable form of As. Also Lindsay (1979) reported that the H^+ dissociation from the exchange sites of mineral surfaces results in an increase of the metal bonding to soil particles followed by a solubility decrease of the metals.

From this research, it is possibly expected that the decrease in soil pH could increase the soluble form of metals and decrease other forms (F3, F4). However, the humic substances application led to a dramatic decrease in soluble metals in soils as Gardea-Torresdey et al. (1996) reported. The effect of soil acidification was lessened by the treatment of humic substances even though every soil pH decreased by time both control and treatment groups. In case of Cu and Pb, the mobile fractions increased due to the soil acidification but the contents of available forms were lower in the humic substances-treated soils than in the control soil. This reflected the decrease in Fe-Mn oxide bound fraction, organic bound fraction of Cu and Pb and the increase in organic bound fraction and residual fraction of As.

However, the residual fraction of Cu increased whereas that of Pb decreased and this might be explained by differences in chemical characteristics between Cu and Pb. Compared to Pb, Cu is co-precipitated with Fe-Mn oxide, which is consistent with Alloway (1995) and Cu was combined with organic matter preferentially as Luo and Christie (1998) reported. Zalidis et al. (1999) described that humic substances can play an important role in retaining toxic metals in an exchangeable form and in forming complexes as well. Therefore, even though the concentration of water soluble, exchangeable and carbonate bound Cu increased, Cu was mainly associated with the Fe-Mn oxide bound and organic bound fraction and the decrease in these two fractions (F3, F4) was reduced by the humic substances treatment.

Humic substances also adsorb metals by forming chelate complexes (Alloway, 1995). The chelate complexes with metals can be formed by organic ligands and prevent metals from being adsorbed on soil particles. Arias et al. (2002) also reported that binding on humic substances may enhance the metal sorption on mineral particle. This explanation suits well with the phenomena that the organic bound fraction of three metals increased than control and that the mobile Cu fractions decreased than control after the treatment of humic substances. According to Borûvka and Drábek (2004), complexes of Cu and Pb with humic substances were not only immobile but also more stable than other metal complexes such as Cd and Zn. This effect was attributed to the forming of coordination complexes in the case of Cu and Pb, whereas the reaction of Cd and Zn could be characterized by the adsorption type through ion-exchang (Ladonin and Margolina, 1997). It is believed that metals associated in residual fraction do not change readily during the short term experiment under natural conditions (Su and Wong, 2003). The result of this study was also consistent with the discovery of Su and Wong for short time, but the variation in content of residual fraction was observed after long term period.

The distribution of toxic metals between humic and fulvic fraction is presented in Table 11. All studied metals were more abundant in the fulvic than in the humic fraction. Here are some points should be dealt with as mentioned by Donisa et al. (2003):

(a) The sodium pyrophosphate extraction is used to extract the humic and fulvic acids from the soil selectively. However, other water soluble material can be present in the soils. In that reason, the total contents of pyrophosphate extractable metals are probably overestimated.

(b) The HA fraction was separated from the solution at pH 1.5. Stevenson (1976, 1977) suggested that the metal–HA complexes could be instable at such low pH, so the stability constants for them might be invalid. By considering thermodynamically, therefore, most metals bound to HA in the original pyrophosphate-extracted solution might remain in solution after precipitation of HA.

However, if the organo-metallic complexes are inert enough, the metal may still be retained in the HA precipitate. Österberg et al. (1999) reported that Cu-HA complexes were characterized by a very slow rate of dissolution and it would seem because Cu ions diffuse into humic acid particles, where bind to sites from which they may not be readily released. This intraparticle binding can possibly give humic acids the capacity to store metal ions at relatively high acidity. The distribution of the metals between HA and FA fractions was in accord with the results of Donisa et al. (2003). This might be explained from the fact that a large number of reactive groups on FA took effect. Borůvka and Drabek (2004) found that the FA fractions of metals are controlled by the total metal content in soil that is the level of pollution. The higher number of reactive groups on FA could also explain unlimited capacity of FA for metal binding. Furthermore, it also means that metal sorption on FA is not restricted to specific sorption sites. On the other hand, the HA fractions of metals were irrelevant to total metal content. This indicates that HA has a limited number of specific sorption sites for metals. Moreover, based on the study of Stevenson (1994) because FA has smaller molecular weight and larger contents of acidic functional groups, they can form more soluble, bioavailable, and mobile metal complexes than those formed with HA. Plaza et al. (2005) concluded that FA can form metal complexes that are more soluble, bioavailable and mobile than those formed by HA. Thus, FA can act as carriers of Cu and Pb in soil solution, whereas HA tends to immobilize and accumulate the metals in soil solid phases (Stevenson, 1994).

The log stability constants gradually decreased after the incubation (Table 11). According to Schnitzer and Hansen (1970), the stability constants were slightly higher at higher pH which is related to the higher dissociation of functional groups, particularly carboxyl groups. Likewise, this could be attributed to the soil acidification. The tendency of magnitude of stability constants from batch incubation was similar to that of stability constants experiment. The results showed that As became more reactive with FA, but Cu and Pb were more reactive with HA. Interestingly, the stability constants with higher portion of HA were greater than those with more FA. This could be explained by the characteristics of HA that is more stable in terms of organic matter decomposition. The number of bonds between humic substance and metal is also considered to act as an important role in stability. It could be thought that As-FA complexes had great stability constants because their FA/As mole ratio is around 5.

Furthermore, Ram and Raman (1984) tried to explain the higher stability of

Cu complexes with humic substances than other metals. They believed that could be attributed to the existence of coordinate covalent bond between the complexing agents (humic substances) and the Cu^{2+}. Because Cu^{2+} is a metal of the transitional series with $3d^9$ electronic configuration, it can accept the electrons from the complexing agents. However, because Zn^{2+} has $3d^{10}$ electronic configuration which is a completely filled d orbital, it has the lower stability. Therefore, there can’t be attraction of the complexing ligand either through octahedral or square planar hybridization (Mellor and Maley, 1948). However, it should be considered more to explain with this theory how other metals such as As and Pb have stability constants differently.

Ⅴ. CONCLUSIONS

The results of this study give novel information in the influence of humic substances quality on chemical speciation and stability of metals. The differences in the concentrations and distributions of five fractions of metals by the sequential extraction method could be explained by the effect of humic substances on soil pH, formation of chelating complexes with metals, and differences in metal properties. The application of humic substances was effective to lessen the acidification of soils compared to the control soil, so reduce the mobility of metals, resulting in the increase in relatively stable fractions of metals. Especially the humic substances treatment with higher portion of humic acid showed a greater ability to reduce the contents of mobile phases and enhance those of stable forms in Cu and Pb. However, even though the chemical distribution of As turned to stabilized direction, it did not appear any significant differences between control and treatments, and this might have been attributed to the effect of soil acidification itself. In terms of stability of metal-humic substance complexes, As was more stable when complexing with FA, whereas Cu and Pb became stabilized with HA on the other hand. Moreover, the incubation results for 90 days provided that the log K values for metal-HA complexes increased while those for metal-FA complexes decreased. The results of this study suggested that the addition of humic substances with higher proportion of humic acid could contribute to the immobilization and stabilization of metals in heavily polluted soils.

Ⅵ. REFERENCES

Alloway, B.J., 1995. Heavy Metals in Soils. Blackie Academic & Professional.

Arias, M., Barral, M., Mejuto, J., 2002. Enhancement of copper and cadmium adsorption on kaolin by the presence of humic acids. Chemosphere 48(10), 1081-1088.

Ayres, R.U., Ayres, L.W., Råde, I., 2002. The life cycle of copper, its co-products and by-products. International Institute for Environment and Development (IIED) and World Business Council for Sustainable Development (WBCSD).

Barančíková, G., Makovníková, J., 2003. The influence of humic acid quality on the sorption and mobility of heavy metals. Plant Soil Environ 49, 565-571.

Bjerrum, J., Lamm, C.G., 1972. Metal Ammine Formation in Solution. Acta Chem. Scand 26(7), 11.

Borůvka, L., Drabek, O., 2004. Heavy metal distribution between fractions of humic substances in heavily polluted soils. Plant, Soil and Environment 50(8).

Buffle, J., 1984. Natural organic matter and metal-organic interactions in aquatic systems.

Carrizales, L., Razo, I., Téllez-Hernández, J.I., Torres-Nerio, R., Torres, A., Batres, L.E., Cubillas, A.-C., Díaz-Barriga, F., 2006. Exposure to arsenic and lead of children living near a copper-smelter in San Luis Potosi, Mexico: Importance of soil contamination for exposure of children. Environmental Research 101(1), 1-10.

Chapman, H.D., 1965. Cation-exchange capacity. Methods of soil analysis. Part 2. Chemical and microbiological properties (methodsofsoilanb), 891-901.

Chen, Y., 1996. Chapter 13 - Organic Matter Reactions Involving Micronutrients in Soils and Their Effect on Plants. In: P. Alessandro (Ed.), Humic Substances in Terrestrial Ecosystems. Elsevier Science B.V., Amsterdam, pp. 507-529.

Choudhry, G.G., 1984. Humic substances. Structural, photophysical, photochemical and free radical aspects and interactions with environmental chemicals. Gordon & Breach Science Publishers, Inc.

CL:AIRE, 2007. Understanding Soil Washing. CL:AIRE (Contaminated Land: Applications in Real Envionments) technical bulletin TB13.

Day, P.R., 1965. Particle Fractionation and Particle-Size Analysis. Methods of Soil Analysis. Part 1. Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling agronomymonogra(methodsofsoilana), 545-567.

Donisa, C., Mocanu, R., Steinnes, E., 2003. Distribution of some major and minor elements between fulvic and humic acid fractions in natural soils. Geoderma 111(1), 75-84.

Elliott, H., Dempsey, B., Maille, P., 1990. Content and fractionation of heavy metals in water treatment sludges. Journal of environmental quality 19(2), 330-334.

Fan, T.W.-M., Higashi, R.M., 2001. Mechanisms of Heavy Metal Sequestration in Soils: Plant-Microbe Interactions and Organic Matter Aging, University of California, Davis, CA; University of California, Riverside, CA (US).

Gardea-Torresdey, J., Polette, L., Arteaga, S., Tiemann, K., Bibb, J., Gonzalez, J., 1996. Determination of the content of hazardous heavy metals on Larrea tridentata grown around a contaminated area, Proceedings of the Eleventh Conf. on Hazardous Waste Research,(HSRC/WERC Joint Conference on the Environment), Edited by LR Erickson, DL Tillison, SC Grant, and JP McDonald, Albuquerque, NM, pp. 660-669.

Geering, H., Hodgson, J., 1969. Micronutrient cation complexes in soil solution: III. Characterization of soil solution ligands and their complexes with Zn2+ and Cu2+. Soil Science Society of America Journal 33(1), 54-59.

Gupta, S.K., Vollmer, M.K., Krebs, R., 1996. The importance of mobile, mobilisable and pseudo total heavy metal fractions in soil for three-level risk assessment and risk management. Science of The Total Environment 178(1–3), 11-20.

Hering, J.G., Morel, F.M., 1989. Slow coordination reactions in seawater. Geochimica et Cosmochimica Acta 53(3), 611-618.

Huang, S.S., Liao, Q.L., Hua, M., Wu, X.M., Bi, K.S., Yan, C.Y., Chen, B., Zhang, X.Y., 2007. Survey of heavy metal pollution and assessment of agricultural soil in Yangzhong district, Jiangsu Province, China. Chemosphere 67(11), 2148-2155.

Irving, H., Williams, R.J.P., 1953. 637. The stability of transition-metal complexes. Journal of the Chemical Society (Resumed) 0(0), 3192-3210.

ISO, 1995. Soil Quality-Extraction of Trace Elements Soluble in Aqua Regia. ISO.

Jennings, S.R., Blicker, P.S., Neuman, D.R., Group, R.R., Fish, U.S., 7, W.S.R., 2008. Acid Mine Drainage and Effects on Fish Health and Ecology: A Review. Reclamation Research Group.

Ladonin, D., Margolina, S., 1997. Interaction between humic acids and heavy metals. Eurasian soil science 30(7), 710-715.

Leschber, R., Davis, R., L'Hermite, P., 1985. Chemical methods for assessing bio-available metals in sludges and soils.

Li, X., Poon, C.-s., Liu, P.S., 2001. Heavy metal contamination of urban soils and street dusts in Hong Kong. Applied Geochemistry 16(11–12), 1361-1368.

Li, Y., Gou, X., Wang, G., Zhang, Q., Su, Q., Xiao, G., 2008. Heavy metal contamination and source in arid agricultural soil in central Gansu Province, China. Journal of Environmental Sciences 20(5), 607-612.

Lindsay, W.L., 1979. Chemical equilibria in soils. Wiley.

Luo, Y., Christie, P., 1998. Bioavailability of copper and zinc in soils treated with alkaline stabilized sewage sludges. Journal of Environmental Quality 27(2), 335-342.

Ma, L.Q., Rao, G.N., 1997. Chemical fractionation of cadmium, copper, nickel, and zinc in contaminated soils. Journal of Environmental Quality 26(1), 259-264.

Manz, M., Weissflog, L., Kühne, R., Schüürmann, G., 1999. Ecotoxicological Hazard and Risk Assessment of Heavy Metal Contents in Agricultural Soils of Central Germany. Ecotoxicology and Environmental Safety 42(2), 191-201.

Martell, A.E., Calvin, M., 1952. Chemistry of the metal chelate compounds. Soil

Science 74(5), 403.

Mellor, D., Maley, L., 1948. Order of stability of metal complexes. Nature 161, 436-437.

Miller, M., Ohlrogge, A., 1958. Water-soluble chelating agents in organic materials: I. Characterization of chelating agents and their reactions with trace metals in soils. Soil Science Society of America Journal 22(3), 225-228.

Mládková, L., Rohosková, M., Borûvka, L., 2006. Methods for the assessment of humic substances quality in forest soils. Soil & Water Res. 1(1), 3-9.

Moon, S.Y., Oh, M., Jung, J., Choi, S.I., Lee, J.Y., 2011. Assessment of soil washing efficiency for arsenic contaminated site adjacent to Jang Hang Refinery. Journal of Soil and Groundwater Environment 16(1), 71-81.

Österberg, R., Wei, S., Shirshova, L., 1999. Inert copper ion complexes formed by humic acids. Acta Chemica Scandinavica 53, 72-80.

Pandey, A.K., Pandey, S.D., Misra, V., 2000. Stability constants of metal–humic acid complexes and its role in environmental detoxification. Ecotoxicology and Environmental Safety 47(2), 195-200.

Peña-Méndez, E.M., Havel, J., Patočka, J., 2005. Humic substances-compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. J. Appl. Biomed 3(1), 13-24.

Peters, R.W., 1999. Chelant extraction of heavy metals from contaminated soils. Journal of hazardous materials 66(1-2), 151-210.

Plaza, C., Senesi, N., García-Gil, J., Polo, A., 2005. Copper (II) complexation by humic and fulvic acids from pig slurry and amended and non-amended soils. Chemosphere 61(5), 711-716.

Podlešáková, E., Němeček, J., Sirový, V., Lhotský, J., Macurová, H., Ivanek, O., Bumerl, M., Hudcová, O., Voplakal, K., Hálová, G., Blahovec, F., 1992. Analysis of soils, waters and plants. Institute for Soil and Water Conservation, Prague.

Ram, N., Raman, K., 1984. Stability constants of complexes of metals with humic and fulvic acids under non-acid-conditions. Zeitschrift für Pflanzenernährung und Bodenkunde 147(2), 171-176.

Randhawa, N.S., Broadbent, F., 1965. Soil organic matter-metal complexes: 6 stability constants of zinc-humic acid complexes at different pH values. Soil Science 99(6), 362-366.

Rashid, M.A., 1985. Geochemistry of marine humic compounds. Springer-Verlag.

Salbu, B., Krekling, T., 1998. Characterisation of radioactive particles in the environment[dagger]. Analyst 123(5), 843-850.

Salomons, W., Förstner, U., 1980. Trace metal analysis on polluted sediments: part II: evaluation of environmental impact. Environmental Technology 1(11), 506-517.

Schnitzer, M., Hansen, E., 1970. Organo-metallic interactions in soils: 8. An evaluation of methods for the determination of stability constants of metal-fulvic acid complexes. Soil Science 109(6), 333-340.

Schnitzer, M., Skinner, S., 1965. Organo-metallic interactions in soils: 4. Carboxyl and hydroxyl groups in organic matter and metal retention. Soil Science 99(4), 278-284.

Schnitzer, M., Skinner, S., 1966. Organo-metallic interactions in soils: 5. Stability constants of Cu++-, Fe++-, and Zn++-fulvic acid complexes. Soil Science 102(6), 361-365.

Schnitzer, M., Skinner, S., 1967. Organo-metallic interactions in soils: 7. Stability constants of Pb++-, Ni++-, Mn++-, Co++-, Ca++-, and Mg++-fulvic acid complexes. Soil Science 103(4), 247.

Schubert, J., 1948. The Use of Ion Exchangers of the Determination of Physical-Chemical Properties of Substances, Particularly Radiotracers, in Solution. I. Theoretical. The Journal of Physical Chemistry 52(2), 340-350.

Shuman, L.M., 1999. Organic Waste Amendments Effect on Zinc Fractions of Two Soils. J. Environ. Qual. 28(5), 1442-1447.

Sidle, R.C., Chambers, J.C., Amacher, M.C., 1991. Fate of heavy metals in an abandoned lead-zinc tailings pond: II. Sediment. Journal of environmental quality 20(4), 752-758.

Stevenson, F., 1976. Stability constants of Cu2+, Pb2+, and Cd2+ complexes with humic acids. Soil Science Society of America Journal 40(5), 665-672.

Stevenson, F., 1977. Nature of divalent transition metal complexes of humic acids as revealed by a modified potentiometric titration method. Soil Science 123(1), 10-17.

Stevenson, F.J., 1994. Humus Chemistry: Genesis, Composition, Reactions. Wiley.

Su, D., Wong, J., 2004. Chemical speciation and phytoavailability of Zn, Cu, Ni and Cd in soil amended with fly ash-stabilized sewage sludge. Environment International 29(7), 895-900.

Sud, D., Mahajan, G., Kaur, M., 2008. Agricultural waste material as potential adsorbent for sequestering heavy metal ions from aqueous solutions–a review. Bioresource Technology 99(14), 6017-6027.

Sumner, M., Miller, W., Sparks, D., Page, A., Helmke, P., Loeppert, R., Soltanpour, P., Tabatabai, M., Johnston, C., 1996. Cation exchange capacity and exchange coefficients. Methods of soil analysis. Part 3-chemical methods., 1201-1229.

Tessier, A., Campbell, P., 1987. Partitioning of trace metals in sediments: relationships with bioavailability. Hydrobiologia 149(1), 43-52.

Tessier, A., Campbell, P.G., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Analytical chemistry 51(7), 844-851.

USEPA, 1991. Guide for conducting treatability studies under CERCLA: Soil washing interim guidance.

Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil science 37(1), 29-38.

WBG, UNEP, UNIDO, 1999. Pollution Prevention and Abatement Handbook, 1998: Toward Cleaner Production. World Bank Group.

Wei, B., Yang, L., 2010. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchemical Journal 94(2), 99-107.

Wuana, R., Okieimen, F., Imborvungu, J., 2010. Removal of heavy metals from a contaminated soil using organic chelating acids. International Journal of Environmental Science and Technology 7(3), 485-496.

Xian, X., 1987. Chemical partitioning of cadmium, zinc, lead, and copper in soils near smelter. Journal of Environmental Science & Health Part A 22(6), 527-541.

Xian, X., 1989. Effect of chemical forms of cadmium, zinc, and lead in polluted soils on their uptake by cabbage plants. Plant and soil 113(2), 257-264.

Yusuf, K., 2007. Sequential extraction of lead, copper, cadmium and zinc in soils near Ojota waste site. Journal of Agronomy 6(2), 331.

Zalidis, G., Barbayiarinis, N., Matsi, T., 1999. Forms and distribution of heavy metals in soils of the Axios Delta of Northern Greece. Communications in Soil Science & Plant Analysis 30(5-6), 817-827.

Zende, N., Raman, K., 1978. Organic Matter-Metal Ion Complexes in Forested and Cultivated Soils. Journal of the Indian Society of Soil Science 26(3), 278-282.

APPENDIX. Supplementary data

Table S1. Ranges in concentration and summary statistics of elements in forest soils (Total, Aqua regia) (Yoon et al., 2009)

Parameter	Cd (n=13)	Cu (n=88)	Pb (n=88)	Cr (n=88)	Zn (n=88)	Ni (n=88)	As (n=88)
Mean	0.287	15.26	18.43	25.37	54.27	17.68	6.83
Standard Deivation	0.234	9.02	11.90	17.92	18.87	14.30	4.27
Standard Error	0.065	0.96	1.27	1.91	2.01	1.52	0.46
Coefficient of Variation (CV) (%)	81.600	59.10	64.60	70.60	34.80	80.90	62.50
Geometric Mean	0.190	12.98	16.04	21.29	51.04	14.31	5.72
Minimum	0.033	2.80	4.07	4.87	21.34	1.11	0.56
Median	0.233	13.00	15.73	20.48	52.00	14.44	5.99
Maximum	0.700	50.00	78.33	106.00	103.40	114.43	20.58

Table S2. Emissions from copper smelting (milligrams per normal cubic meter)

(World Bank Group et al., 1999)

Parameter	Maximum value
Sulfur dioxide	1,000
Arsenic	0.5
Cadmium	0.05
Copper	1
Lead	0.2
Mercury	0.05
Particulates, smelter	20
Particulates, other sources	50

Table S3. Copper Ore minerals and materials (Ayres et al., 2002)

Copper ore mineral	Composition
Native copper	Cu
Azurite	$2CuCO_3 \cdot Cu(OH)_2$
Bornite	Cu_5FeS_4
Chalcopyrite	$CuFeS_2$
Chalcolite	Cu_2S
Chrysacolla	$CuSiO_3 \cdot 2H_2O$
Cuprite	Cu_2O
Enargite	Cu_3AsS_4
Malachite	$CuCO_3 \cdot Cu(OH)_2$

Table S4. Mass balance of metal contents during 90 days of the batch incubation.

Metal	Time (day)				
	0	20	40	60	90
	mg kg-1 (% to total content in Day 0)				
As	70.77 (100)	67.34 (95)	66.38 (94)	52.23 (74)	-
Cu	163.01 (100)	130.07 (80)	163.34 (100)	119.31 (73)	116.87 (72)
Pb	235.24 (100)	275.85 (117)	236.18 (100)	300.26 (128)	279.68 (119)

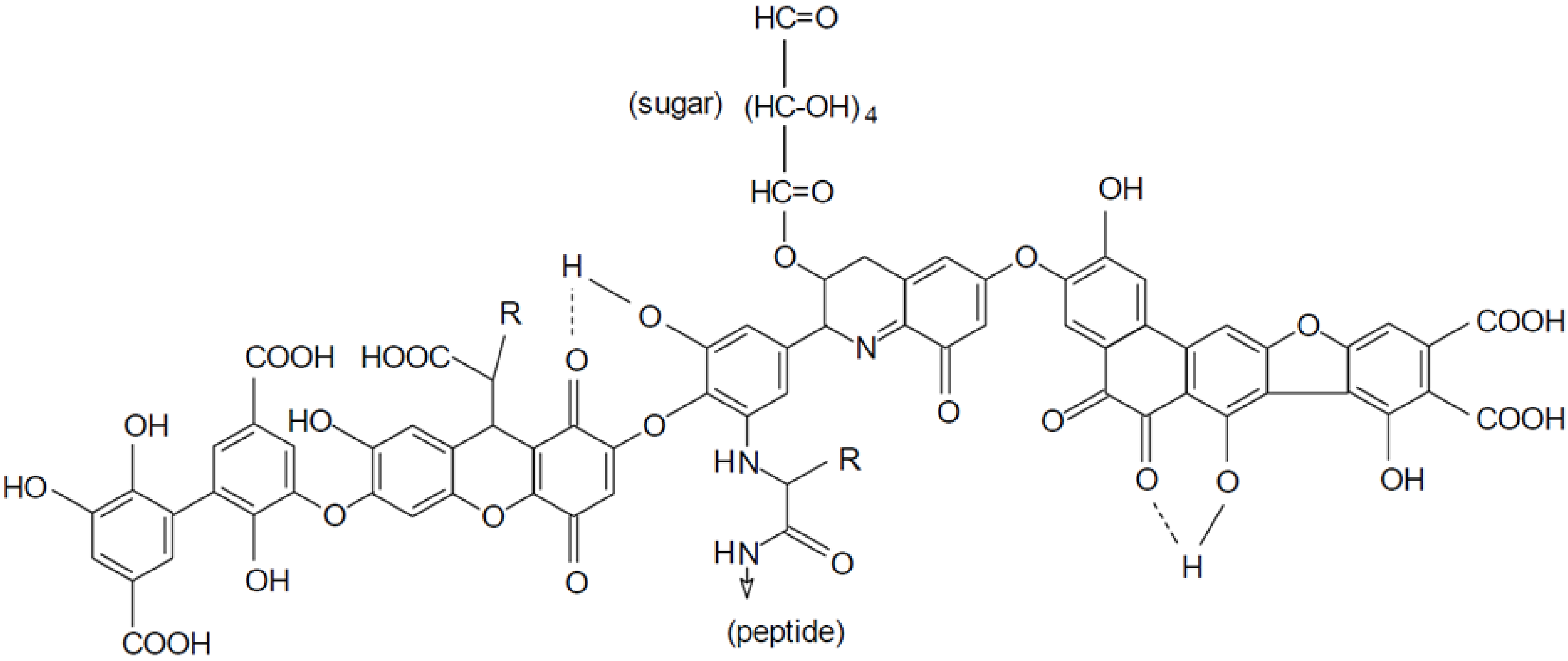

Figure S1. Model structure of humic acid according to Stevenson (1982).

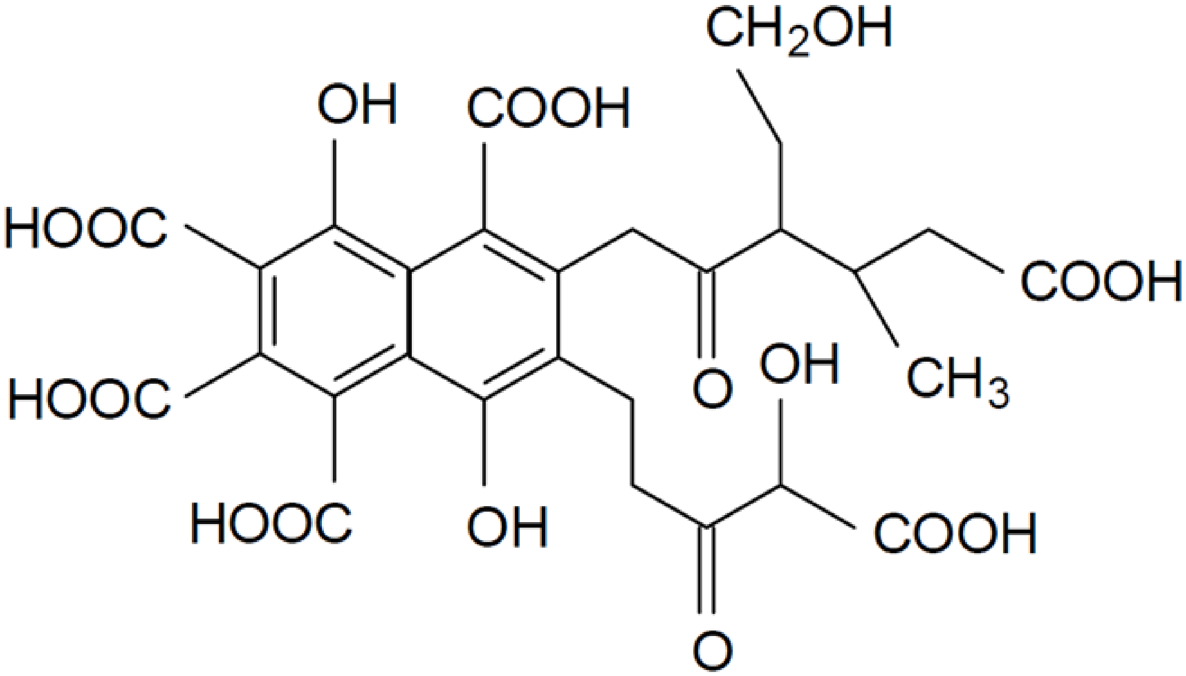

Figure S2. Model structure of fulvic acid according to Buffle et al. (1977).

SUMMARY (In Korean)

(구)장항제련소 지역은 오랜 제련활동으로 인한 비소, 구리, 납 등의 중금속 오염이 문제시 되어 왔다. 이에 본 연구에서는 토양 내 중금속의 이동성을 감소시키기 위하여 중금속 킬레이팅 능력이 뛰어난 펄빅산 (fulvic acid; FA)과 복합체의 안정도가 우수한 휴믹산 (humic acid; HA)의 비율을 달리하여 (HA/FA=2, HA/FA=1, HA/FA=0.5, 대조군) 토양에 처리한 후 90일 간 항온배양실험을 수행하여 화학종 변화와 안정도상수를 확인하였다. 실험 결과, 비소는 펄빅산과 복합체를 형성할 때 안정한 반면, 구리와 납은 휴믹산과 복합체를 형성할 때 더 안정했다. 대조군을 포함한 모든 토양이 산성화되었지만, 휴믹물질의 첨가로 인하여 토양의 산성화가 감소되었다. 이로 인하여 구리와 납은 대조군에 비하여 이동성이 적은 형태(철-망간 산화물 및 유기물 결합 분획)가 증가하였으며, 가용성 형태(수용성 및 교환성, 탄산염 결합 분획)가 감소하였다. 또한 휴믹산의 비율이 높을수록 이동성 분획의 감소와 안정한 형태의 증가가 가장 효과적임을 확인하였다. 하지만 비소는 대조군과 처리군 사이에서 유의적인 차이를 보이지 않았으며, 비소가 배양이 진행됨에 따라 안정화된 것은 토양 산성화의 영향으로 보인다. 중금속은 휴믹산보다 펄빅산과 더 많이 결합하였으며, 비소와 구리의 안정도상수는 휴믹산의 비율이 높을수록, 납의 경우 펄빅산의 비율이 높을수록 안정도상수가 커졌다. 본 연구 결과를 통하여 중금속으로 오염된 토양에서 휴믹물질이 중금속을 부동화하고 안정화하는데 기여할 수 있으며, 이들을 활용할 때 중금속과 휴믹물질의 특성이 고려되어야 함을 확인하였다.

Keywords : **중금속, 휴믹물질, 화학종 변화, 안정도상수, 제련소**

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