ISSN 1001—0742
Journal of Environmental Sciences Vol. 18, No. 6, pp. 1119—1123, 2006 CN 11—2629/X

Article ID: 1001-0742(2006)06-1119-05 **CLC number**: X131.3 **Document code**: A

Effect of incubation temperature and wet-dry cycle on the availabilities of Cd, Pb and Zn in soil

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Abstract: The effect of incubation temperature and wet-dry cycle on the availabilities of Cd, Pb and Zn was studied. Three soils with pH ranging from 3.8 to 7.3, organic carbon (OC) from 0.7% to 2.4%, and clay from 12.3% to 35.6% were selected. Soils were spiked with reagent grade $Cd(NO_3)_2$, $Pb(NO_3)_2$, and $Zn(NO_3)_2$ at concentrations of 30 mg Cd/kg soil, 300 mg Zn/kg soil and 2000 mg Pb/kg soil. The soils were incubated at 35, 60, 105℃, respectively and went through four wet-dry cycles. Metal availability in soils was estimated by soil extraction with 0.1 mol/L $Ca(NO_3)_2$. According to this study, the effect of the spiking temperature on the metal availabilities was different among the metals, soils and wet-dry cycles. Mostly, 35℃ was the first recommended spiking temperature for Cd and Pb while no spiking temperature was obviously better than others for Zn. Three wet-dry cycles was recommended regardless of the type of metals and incubation temperature.

Keywords: availability; wet-dry cycle; cadmium (Cd); zinc (Zn); lead (Pb)

Introduction

Spiked soils are commonly used in availability, toxicity and bioassay studies to calculate soil ecotoxicological parameters (Almas and Singh, 2001; Conder and Lanno, 2000; Hogg *et al.*, 1993; Holm *et al.*, 1996; Sheppard and Thibault, 1992). One advantage of using spiked soils is that any required concentrations can be obtained with this method. Another advantage lies in that research can be conducted on the ecotoxicity of almost any metals using the spiked soils. Finally, the spiking procedure is simple and easy to be carried out, and is very convenient for the lab research. The availability of metals freshly added as soluble metal salts exceed that of metals added with a complexing matrix (Logan and Chaney, 1983). Li *et al.* (2001) reported that the metals added to soil as the constituents of biosolids were less phytoavailable than metal salts added to soils. Lock and Janssen (2003) concluded that the use of spiked soils in toxicity assays could result in an over-estimation of the effects of Zn, especially at a high pH. Korcak and Fanning (1985) found the corn was grown better in biosolid-Cd soil than salt-Cd soil and the Cd bioaccumulation in corn was higher in salt-Cd soil, which meant that the availability of metal was higher in metal-spiked soil than in the soil mixed with biosolids.

Two methods are commonly used to minimize the salt effect when metals are added to soil: one is to incubate the spiked soil at elevated temperature. The other is to wet-dry spiked soil to force reactions with matrix precipitation. Almas *et al.* (2000) reported that the metal adsorption rate increased with increasing temperature. However, there has not been a well-accepted procedure so far. Jiang *et al.* (2003) used a paddy soil spiked with Zn at 28℃. Koster *et al.* (2005) stored the spiked soil for a minimum of four weeks at room temperature before using it in the experiments. In the study of Ivask *et al.* (2002), the spiked soil samples were homogenized, air-dried for 5 d and stored at 4℃. Vijver *et al.* (2003) stored the spiked soil in a sealed container for one month at 4℃ prior to bioassays. Schroder (2003) dried and rewetted the spiked soil three times at 70℃. In a word, the spiking temperature and the number of wet-dry cycles are different among different research groups, which could be a big problem about studies using spiked soils, for the availability and exposure to plants and vertebrate during the research periods may be greatly different. The same spiked metal concentration could have greatly different experimental results. Therefore, it is necessary to unify the procedure of spiked soil for the bioassay researches.

In this study, spiked soils were incubated at three different elevated temperatures and went through four wet-dry cycles. The effect of the wet-dry cycles on the availability of metals (Cd, Pb and Zn in this study) was evaluated, and so was the effect of spiking temperature on the availability of metals (Cd, Pb and Zn). Previous researches have shown that extractions using weak (<1 mol/L) $CaCl_2$ or $Ca(NO_3)_2$ solutions were as successful as toxicity-related measures of metal bioavailability to earthworms and lettuce in soils (Basta and Gradwohl, 2000; Conder and Lanno, 2000; Gradwohl, 1998; Peijnenburg *et al.*, 1997, 1999; Posthuma *et al.*, 1997; Weljte, 1998). These solutions are hypothesized to extract exchangeable or weakly

Foundation item: The Strategic Environment Research and Development Program, USA and Alcoa Foundation "Sustainable Development of Rural Area in China"; *Corresponding author

bound available metals in soil (Sloan *et al.*, 1997), which are believed to be available for uptake by soil organisms (Peijnenburg *et al.*, 1999; Posthuma *et al.*, 1997). In this study, neutral salt extraction 0.1 mol/L $Ca(NO_3)_2$ was used to extract the spiked soil sample to determine the metal availability.

1 Materials and methods

1.1 Selection of soils

Three soils (Richfield, Teller and Webster) with different physical/chemical properties including soil pH, organic carbon (OC) content and clay content were collected. The soil physical/chemical properties showed a wide range including soil pH (3.8, 5.5 and 7.3, respectively), organic carbon (0.7%, 1.4%, and 2.4%, respectively) and clay content (12.3%, 30.9%, and 35.6%, respectively). The Teller soil is acid and sandy with low absorption capacity. The Webster soil is a neutral soil with high organic carbon and clay content. The Richfield soil has a similar clay content, higher pH and lower organic carbon content compared to the Webster soil. All soils were air-dried and sieved to pass a 2-mm screen prior to analysis.

1.2 Soil physical and chemical properties

Soil pH was measured with 1:1 (soil: 0.01 mol/L $CaCl_2$; Sparks *et al.*, 1996). Acid dichromate digestion was used to determine soil organic carbon content (Heanes, 1984). The hydrometer method was used to determine soil texture (Gee and Bauder, 1986). Duplicate soil pH and soil organic carbon content were analyzed and triplicate analyses were conducted in the determination of soil texture on each soil.

1.3 Cd, Pb, and Zn spiking and incubation

Soils were spiked with reagent grade $Cd(NO_3)_2$, $Pb(NO_3)_2$ and $Zn(NO_3)_2$ at concentrations of 30 mg Cd/kg soil, 2000 mg Pb/kg soil and 300 mg Zn/kg soil. All spikes were calculated on a metal basis and 0.5 L of spiking solution was prepared using the metal salt and deionized distilled water. The interaction of heavy metals in soil could be antagonistic, synergistic or multiplicative (Peralta-Videa *et al.*, 2003; Moraghan, 1993). Therefore, soils were spiked with only one metal to avoid competitive adsorption effects (Basta and Tabatabai, 1992). 100 ml spiking solution was added and mixed with 300 g of soil in a big aluminum pan. Additional deionized distilled water was added and thoroughly mixed with the soil to make a saturated paste. Each spiked soil in a big aluminum pan was split into three 100 g subsamples and put into three different small aluminum pans. The soils in the small aluminum pans were oven-dried at 35℃ for 68 h, 60℃ for 20 h and 105℃ for 16 h, respectively. Then the dried soils were removed from the oven and crashed. Soil sample was randomly taken from each pan. Duplicate soil samples of 5 g were collected from all the pans. The soil samples were placed in 20 mg glass scintillation vials for the later extraction and analysis. Deionized water was added to each pan to make a saturated paste followed by drying under the same condition. All soils underwent four wet-dry cycles to achieve adequate reaction with the soil matrix and soil samples were taken after each wet-dry cycle.

1.4 Measurement of available metal using 0.1 mol/L $Ca(NO_3)_2$ extraction

All the soil samples were extracted with neutral salt extraction (0.1 mol/L $Ca(NO_3)_2$) to determine the availability. Soil (1.0 g) was weighed out in a 50-ml centrifuge tube and 20.0 ml of 0.1 mol/L $Ca(NO_3)_2$ was added. The samples were shaken on a reciprocal shaker for 16 h. The solution was then centrifuged at 10000 r/min for 15 min. The centrifuged samples were filtered with 0.45 mm syringe filters into 20 ml glass scintillation vials. 1.0 ml of trace metal concentrated hydrochloric acid (HCl) was added to each sample. All the samples were then stored at 4℃ until the analysis of metal was conducted by ICP-AES. Duplicate analyses were conducted for all the soils in this study.

1.5 Modeling and statistical analysis

Soil availability data were analyzed as a $3\times3\times4$ factorial design. The types of soils, metals and wet-dry cycles were used as the three factors. PROC MIXED was performed to evaluate the interaction effects of the three factors. As the interactions effects of the three factors were significant across all the four metals, PROC MIXED was performed to evaluate the interaction effects of inoculation temperatures and wet-dry cycles when the soil type was given. Simple effects of each individual factor were analyzed with a SLICE option in the LSMEANS statement given other two factors.

2 Results and discussion

2.1 Metal availability

The availabilities varied among the three metals. The overall mean values of all the three temperatures and three soils indicated that 35% (10.6 mg/L) of Cd, 13% (252 mg/L) of Pb and 30% (91.3 mg/L) of Zn were extracted by the 0.1 mol/L $Ca(NO_3)_2$. The results showed that the availabilities of spiked Cd were close to those of Zn, while the availabilities of Pb were much less. All the three metals demonstrated wide ranges of availabilities over different soils and wet-dry cycles. Extracted Cd, Pb and Zn varied from 9.4% to 76%, 0.19% to 49% and 0.27% to 74%, respectively.

2.2 Interaction effect of soil types, incubation temperatures and wet-dry cycles

The interaction effects of types of soils, incubation temperatures and wet-dry cycles were very significant across all the three metals ($p<0.01$ for all the three metals) (Table 1). In different soils, the interaction effects of incubation temperatures and wet-dry cycles depended on the type of metals and soils. In Teller soil, the interaction effects of incubation temperatures and wet-dry cycles were significant for Pb and Zn (p<0.01 across all the three metals) but were not for Cd (p=0.19). In Webster soil, the interaction effects of incubation temperatures and wet-dry cycles were significant for Pb and Cd (p<0.01 across all the three metals) but were not for Zn (p= 0.09). In Richfield soil, the interaction effects of incubation temperatures and wet-dry cycles were not significant for Cd, Pb and Zn (p=0.11, 0.05, 0.29, respectively) (Table 2). In sum, the effects of incubation temperature and wet-dry cycle on Cd, Pb and Zn were not the same and interacted in different soil, which might be resulted in by three reasons. Firstly, the effect of incubation temperature and wet-dry cycle on different reactions between soil component and metal is different. Incubation at elevated temperature could increase the speed of soil-metal reactions and wet-dry cycle could force reactions occur. The main reactions between soil components and metal include precipitation, specific adsorption, non-specific adsorption, complex, chelation and so on. Secondly, soil properties greatly influence the type and speed of different soil-metal reactions. For example, metal precipitation is controlled by soil pH and adsorption, complex and chelation are greatly affected by SOC and clay content. Thirdly, Cd, Pb and Zn are different metals and have different environmental activities in soil. For example, Pb can be specifically adsorbed by hydrous oxide but not for Cd and Zn. The effects of an individual factor were compared when the other two factors were given.

2.3 Effect of incubation temperatures on the availabilities of Cd, Pb and Zn

Incubation temperatures affected the availabilities of Cd, Pb and Zn. Generally, 35℃ resulted in lower Cd, Pb and Zn availabilities compared to other temperatures. The Cd availabilities at 35℃ were either lower or not significantly different compared to those at other temperatures. In Richfield and Webster soils, the Cd availabilities at 35℃ were generally significantly lower than those at other temperatures. In Teller soil, the Cd availabilities at 35℃ were generally not significantly different from those at other temperatures (Table 3). The effects of incubation temperatures on the availabilities of Pb were similar to those of Cd (Table 3). The Pb availabilities at 35℃ were either lower or not significantly different compared to those at other temperatures. After the 1st and 2nd wet-dry cycles, the Pb availabilities at 35℃ were not generally different from those at other temperatures. However, after 3rd and 4th wet-dry cycles, the Pb availabilities at 35℃ were generally significantly lower than those at other temperatures. The effects of incubation temperatures on the availabilities of Zn depended on the types of soil (Table 3). The Zn availabilities at 35℃ were generally not significantly different from those at other temperatures in Richfield and Webster soils. In Teller soil, the Pb availabilities at 35℃ were generally not much different from those at other temperatures after the 1st and 2nd wet-dry cycles. However, after 3rd and 4th wet-dry cycles, the Pb availabilities at 35℃ were generally significantly lower than those at other temperatures. In sum, the availabilities of Cd, Pb and Zn incubated at 35℃ were generally lower than those at other temperatures especially after three wet-dry cycles. The main reason might be that the wetted soils took longer time to be dried at 35℃ (68 h) than those at 60℃ (20 h), and 105℃ (16 h). Time is another important issue that affects the soil-metal reactions. Metal precipitations take seconds to days to occur. Adsorption, complex and chelation take hours to weeks to occur. The effect of time on soil-metal reactions might not be very obvious between 68 h to 20 h/16 h and 136 h to 40 h/32 h with the interaction influences of incubation temperatures. However, the results revealed that the availabilities of Cd, Pb and Zn were greatly affected by incubation time between 204 h to 60 h/48 h and above with the interaction influences of incubation temperatures.

Table 1 ANOVA results for the availabilities of Cd, Pb and Zn

Source of variation	df	Significance (p)		
		Cd	Pb	Zn
Cycle	3	<0.01	<0.01	<0.01
Temperature	2	0.01	<0.01	0.67
Cycle vs. temperature	6	0.02	0.02	<0.01
Soil	2	<0.01	<0.01	<0.01
Cycle vs. soil	6	<0.01	0.03	0.09
Temperature vs. soil	4	<0.01	<0.01	0.62
Cycle vs. temperature vs. soil	12	<0.01	<0.01	<0.01

Table 2 ANOVA results of the three metals′ availabilities in all soils

Metal	Source of variation	df	Significance (p)		
			Teller	Webster	Richfield
Cd	Cycle	3	0.02	<0.01	0.09
	Temperature	2	0.36	<0.01	<0.01
	Cycle vs. temperature	6	0.19	<0.01	0.11
Pb	Cycle	3	<0.01	<0.01	0.88
	Temperature	2	<0.01	<0.01	0.17
	Cycle vs. temperature	6	<0.01	<0.01	0.05
Zn	Cycle	3	0.59	<0.01	0.02
	Temperature	2	0.75	0.07	0.07
	Cycle vs. temperature	6	<0.01	0.09	0.29

Table 3 Available Cd, Pb and Zn comparison at three different incubation temperatures (mg/kg)

Soil	Cycles	35℃	60℃	105℃	Significant level (p)
		Available Cd			
Teller	1	228^{a}	176^{ab}	158^{b}	0.04
	2	181^{a}	156^{a}	165^{a}	0.61
	3	152^{a}	162^{a}	183^{a}	0.45
	4	174^{a}	113^{a}	149^{a}	0.45
Richfield	1	33.4^{a}	34.6^{a}	35.6^{a}	0.42
	2	27.6^{a}	34.1^{b}	36.4^{b}	<0.01
	3	28.2^{a}	31.5^{a}	38.9^{b}	<0.01
	4	28.1^{a}	30.8^{b}	40.3^{c}	<0.01
Webster	1	106^{a}	131^{b}	128^{b}	<0.01
	2	109^{a}	125^{b}	130^{b}	<0.01
	3	115^{a}	121^{a}	129^{b}	<0.01
	4	115^{a}	112^{a}	118^{a}	0.19
		Available Pb			
Teller	1	983^{a}	971^{a}	851^{b}	0.04
	2	715^{a}	681^{a}	792^{a}	0.12
	3	515^{a}	630^{b}	759^{c}	<0.01
	4	602^{a}	570^{a}	763^{b}	0.01
Richfield	1	7.65^{a}	6.75^{a}	7.83^{a}	0.30
	2	5.27^{a}	5.69^{a}	6.93^{a}	0.65
	3	7.05^{a}	4.10^{a}	7.27^{a}	0.20
	4	3.78^{a}	5.38^{a}	10.8^{b}	0.01
Webster	1	55.4^{a}	54.5^{a}	48.3^{a}	0.06
	2	41.2^{a}	53.1^{b}	48.8^{b}	0.01
	3	39.3^{a}	49.1^{b}	49.1^{b}	0.01
	4	37.0^{a}	43.9^{b}	49.0^{b}	0.01
		Available Zn			
Teller	1	181^{a}	169^{a}	193^{a}	0.71
	2	174^{a}	158^{a}	161^{a}	0.76
	3	188^{a}	185^{a}	116^{b}	0.01
	4	136^{a}	143^{a}	222^{b}	<0.01
Richfield	1	2.59^{a}	2.72^{a}	2.81^{a}	0.94
	2	1.53^{a}	0.85^{a}	2.05^{a}	0.22
	3	2.81^{a}	0.80^{b}	1.37^{b}	0.03
	4	1.75^{a}	0.95^{a}	1.85^{a}	0.51
Webster	1	141^{a}	150^{ab}	135^{ac}	0.03
	2	127^{a}	137^{a}	138^{a}	0.08
	3	119^{a}	127^{a}	128^{a}	0.18
	4	114^{a}	111^{a}	117^{a}	0.44

2.4 Effects of wet-dry cycles on the availabilities of Cd, Pb and Zn

The effects of the wet-dry cycles on the Cd, Pb and Zn availabilities were different according to the soil types and spiking temperatures. In Teller and Richfield soils, the Cd availabilities were generally not significantly different in different wet-dry cycles at the same inoculation temperature. In Webster soil, the Cd availabilities that went through 1 to 3 wet-dry cycles at the same incubation temperature were generally not significantly different from each other while those that went through 4 cycles showed significant decrease (Table 4). In the case of Pb availabilities, 4 wet-dry cycles did not result in significantly different Pb availabilities compared to 3 cycles under all the incubation temperatures and across all the soil types (Table 4). In Teller and Webster soils, the Pb availabilities that went through one wet-dry cycle were generally higher than those that went through more cycles. In Richfield soil, the wet-dry cycles did not result in significantly different Pb availabilities. For the availabilities of Zn, there were generally no significant differences after different wet-dry cycles in Teller and Richfield soils (Table 4). In Webster soil, the Zn availabilities after one wet-dry cycle were generally higher than those after more cycles. Across all the soil types and under all the incubation temperatures, the Zn availabilities that went through 3 and 4 wet-dry cycles were not significantly different.

2.5 Effect of soil types on the availabilities of Cd, Pb and Zn

The soil properties greatly affected the metal availabilities. However, the soil type itself cannot determine the appropriate incubation temperature and wet-dry cycles. Which temperature was the best really depended on the metal type and wet-dry cycle. Similarly, the best number of wet-dry cycles also depended on the metal type and the incubation temperature. During the bioassays, the soil types had great effects on the variances of Cd availabilities while they had few effects on those of Pb and Zn availabilities.

3 Conclusions

35℃ was the first recommended spiking temperature for Cd in clayey soils and 105℃ was the last recommended spiking temperature for Cd in clayey soils. In sandy soil, no significant differences were found among the spiking temperatures. 35℃ was the first recommended spiking temperature and there was no adequate support on which temperature was better regarding 60℃ and 105℃ for Pb. Since most of the Zn availabilities were not significantly different under all the temperatures and across all the wet-dry cycles, no spiking temperature was obviously better than others. In order to unify the spiking temperature, 35℃ was the first recommended temperature for all the three tested metals across all the soils.

Three wet-dry cycles were recommended regardless of the types of metals and spiking temperatures. In a few cases, the metal availabilities did not significantly decrease after one wet-dry cycle

Table 4 Available Cd comparison after one to four wet-dry cycles (mg/kg)

Temp.	Available Cd				Significant level (p)	Available Pb				Significant level (p)	Available Zn				Significant level (p)
	Cycle 1	Cycle 2	Cycle 3	Cycle 4		Cycle 1	Cycle 2	Cycle 3	Cycle 4		Cycle 1	Cycle 2	Cycle 3	Cycle 4	
	Teller soil					Teller soil					Teller soil				
35℃	228^{a}	181^{b}	152^{b}	174^{ab}	0.04	983^{a}	715^{b}	515^{c}	602^{c}	<0.01	181^{a}	174^{a}	188^{a}	136^{a}	0.12
60℃	176^{a}	156^{a}	162^{a}	113^{a}	0.27	971^{a}	681^{b}	630^{b}	570^{b}	<0.01	169^{a}	158^{a}	185^{a}	143^{a}	0.28
105℃	158^{a}	165^{a}	183^{a}	149^{a}	0.58	851^{a}	792^{a}	759^{a}	763^{a}	0.29	193^{a}	161^{ab}	116^{b}	222^{a}	<0.01
	Richfield soil					Richfield soil					Richfield soil				
35℃	33.4^{a}	27.6^{b}	28.2^{b}	28.1^{b}	<0.01	7.65^{a}	5.27^{a}	7.05^{a}	3.78^{a}	0.19	2.59^{a}	1.53^{a}	2.81^{a}	1.75^{a}	0.19
60℃	34.6^{a}	34.1^{a}	31.5^{a}	30.8^{a}	0.09	6.75^{a}	5.69^{a}	4.10^{a}	5.38^{a}	0.54	2.72^{a}	0.85^{b}	0.80^{b}	0.95^{b}	0.04
105℃	35.6^{a}	36.4^{a}	38.9^{a}	40.3^{a}	0.05	7.83^{a}	6.93^{a}	7.27^{a}	10.76^{a}	0.05	2.81^{a}	2.05^{a}	1.37^{a}	1.85^{a}	0.22
	Webster soil					Webster soil					Webster soil				
35℃	106^{a}	109^{ab}	115^{b}	114.5^{b}	0.04	55.4^{a}	41.2^{b}	39.3^{b}	37.0^{b}	<0.01	141^{a}	127^{b}	119^{b}	114^{b}	<0.01
60℃	131^{a}	125^{a}	121^{a}	112^{b}	<0.01	54.5^{a}	53.1^{a}	49.1^{ab}	43.9^{b}	0.01	150^{a}	137^{b}	127^{b}	111^{c}	<0.01
105℃	128^{a}	130^{a}	129^{a}	118^{b}	0.01	48.3^{a}	48.8^{a}	49.1^{a}	49.0^{a}	0.99	135^{a}	138^{a}	128^{ab}	117^{b}	0.01

compared to those after more wet-dry cycles. However, more cycles generally resulted in lower metal availability especially in the first three cycles. Most of the time, four wet-dry cycles were not better than three because the metal availabilities were not significantly different between those after the 3rd and 4th wet-dry cycles.

References:

Almas A R, Salbu B, Singh B R, 2000. Changes in partitioning of cadmium-109 and zinc-65 in soil as affected by organic matter addition and temperature[J]. Soil Sci Soc Am J, 64: 1951—1958.

Almas A R, Singh B R, 2001. Heavy metals in the environment: Plant uptake of cadmium-109 and zinc-65 at different temperature and organic matter levels[J]. J Environ Qual, 30: 869—877.

Basta N T, Tabatabai M A, 1992. Effect of cropping systems on adsorption of metals by soils. III. Competitive adsorption[J]. Soil Sci, 153: 331—337.

Basta N T, Gradwohl R, 2000. Estimation of Cd, Pb, and Zn bioavailability in smelter-contaminated soils by a sequential extraction procedure[J]. J Soil Contam, 9(2): 149—164.

Conder J M, Lanno R P, 2000. Evaluation of surrogate measures of cadmium, lead and zinc to *Eisenia Fetidia*[J]. Chemosphere, 41: 1659—1668.

Corder J M, Lanno R P, Basta N T, 2001. Assessment of metal availability in smelter soil using earthworms and chemical extraction[J]. J Environ Qual, 30: 1231—1337.

Gee G W, Bauder J W, 1986. Paricle-size analysis[M]. In: Methods of soil analysis. Part 1. Physical and mineralogical methods (A. Klute ed.) . 2nd ed. Agronomy Monograph 9, Soil Science Society of American, Madison, WI. 383—411.

Gradwohl R, 1998. Heavy metal bioavailability of contaminated soils, remediation methods and long-term stability [D]. M.S. thesis. Oklahoma State Univ, Stillwater.

Hogg D S, McLaren R G, Swift R S, 1993. Desorption of copper from some New Zealand soils[J]. Soil Sci Soc Am J, 57: 361—366.

Holm P E, Andersen B B H, Christensen T H, 1996. Cadmium solubility in aerobic soils[J]. Soil Sci Soc Am J, 60: 775—780.

Ivask A, Virta M, Kahru A, 2002. Construction and use of specific luminescent recombinant bacterial sensors for the assessment of bioavailable fraction of cadmium, zinc, mercury and chromium in the soil[J]. Soil Biol & Biochem, 34: 1439—1447.

Jiang X J, Luo Y M, Liu S L *et al.*, 2003. Changes in soil microbial biomass and Zn extractability over time following Zn addition to a paddy soil[J]. Chemosphere, 50: 855—861.

Korcak R F, Fanning D S, 1985. Metal salt vs. biosolids metal on Corn (*Zea mays* L.)[J]. Soil Sci, 140: 23—34.

Koster M, Reijnders L, van Oost N R *et al.*, 2005. Comparison of the method of diffusive gels in thin films with conventional extraction techniques for evaluating zinc accumulation in plants and isopods[J]. Environ Pollut, 133: 103—116.

Li Z B, Ryan J A, Chen J L *et al.*, 2001. Adsorption of cadmium on biosolids-amended soils[J]. J Environ Qual, 30: 903—911.

Lock K, Janssen C R, 2003. Influence of ageing on zinc bioavailability in soils[J]. Environ Pollut, 126: 371—374.

Logan T J, Chaney R L, 1983. Utilization of municipal wastewater and sludge on land-metals[C]. In: Proceedings of the 1983 Workshop on utilization of municipal wastewater and sludge on land (L. Page, T.L. Gleason, J.E. Smith *et al.*, ed.). Riverside, CA: University of California. 235—323.

Moraghan J T, 1993. Accumulation of cadmium and selected elements in flax seed grown on a calcareous soil [J]. Plant Soil, 150: 61—68.

Peijnenburg W J G M, Posthuma L, Eijsackers H J P *et al.*, 1997. A conceptual framework for implementation of bioavailability of metals for environmental management purposes [J]. Ecotoxicol Environ Saf, 37: 163—172.

Peijnenburg W J G M, Posthuma L, Zweers P G P C *et al.*, 1999. Prediction of metal bioavailability in Dutch field soils for the Oligochaete *Enchytraeus crypticus*[J]. Ecotoxicol Environ Saf, 43B: 170—186.

Peralta-Videa J R, Gardea-Torresdey J L, Walton J *et al.*, 2003. Effects of zinc upon tolerance and heavy metal uptake in alfalfa plants (*Medicago sativa*)[J]. Bull Environ Contam Toxicol, 70: 1036—1044.

Posthuma L, Baerselman R, van Veen R P M *et al.*, 1997. Single and joint toxic effects of copper and zinc on reproduction of *Enchytraeus crypticus* in relation to sorption of metals in soils[J]. Ecotoxicol Environ Saf, 38: 108—121.

Schroder J, 2003. Bioavailability and toxicity of heavy metals in contaminated soils to human and ecological receptors [D]. Ph.D dissertation. Oklahoma State University, Stillwater.

Sheppard M I, Thibault D H, 1992. Desorption and extraction of selected heavy metals from soils[J]. Soil Sci Soc Am J, 56: 415—423.

Sloan J J, Dowdy R H, Dolan M S *et al.*, 1997. Long-term effects of biosolids applications on heavy metal bioavailability in agricultural soils[J]. J Environ Qual, 26: 966—974.

Sparks D, Page A L, Helmke P A *et al.*, 1996. Methods of soil analysis [M]. Part 3. SSSA Book Soil Science Society of American. Madson, WI.

Vijver M, Jager T, Posthuma L *et al.*, 2003. Metal uptake from soils and soil-sediment mixtures by larvae of *Tenebrio molitor* L. (Coleoptera)[J]. Ecotoxicol Environ Saf, 54: 277—289.

Weljte L, 1998. Mixture toxicity and tissue interactions of Cd, Cu, Pb and Zn in earthworms (Oligochaete) in laboratory and field soils: A critical evaluation of data[J]. Chemosphere, 36: 2643—2660.

(Received for review November 21, 2005. Accepted January 23, 2006)