Effect of Drying on Heavy Metal Fraction Distribution in Rice Paddy Soil

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Abstract
An understanding of how redox conditions affect soil heavy metal fractions in rice paddies is important due to its implications for heavy metal mobility and plant uptake. Rice paddy soil samples routinely undergo oxidation prior to heavy metal analysis. Fraction distribution of Cu, Pb, Ni, and Cd from paddy soil with a wide pH range was investigated. Samples were both dried according to standard protocols and also preserved under anaerobic conditions through the sampling and analysis process and heavy metals were then sequentially extracted for the exchangeable and carbonate bound fraction (acid soluble fraction), iron and manganese oxide bound fraction (reducible fraction), organic bound fraction (oxidizable fraction), and residual fraction. Fractions were affected by redox conditions across all pH ranges. Drying decreased reducible fraction of all heavy metals. Curesidual fraction, Pboxidizable fraction, Cdresidual fraction, and Niacid soluble fraction increased by 25%, 33%, 35%, and >60%, respectively. Pbresidual fraction, Niacid soluble fraction, and Cdoxidizable fraction decreased 33%, 25%, and 15%, respectively. Drying paddy soil prior to heavy metal analysis overestimated Pb and underestimated Cu, Ni, and Cd. In future studies, samples should be stored after injecting N2 gas to maintain the redox potential of soil prior to heavy metal analysis, and investigate the correlation between heavy metal fraction distribution under field conditions and air-dried samples.

Introduction
Soil heavy metals Cu, Pb, Ni, and Cd, are regarded as “chemical time bombs” because of their propensity for accumulation in the soil and uptake by crops. This ultimately causes human toxicity in both the short- and long-term [1–2], making farmland ecosystems dangerous to health [3].

Morphological characteristics and processes of heavy metals have been studied to better understand heavy metal occurrence in various environments, transport pathways, and crop uptake. There are a wide range of soil inorganic and organic substances affected by redox conditions. Redox changes the valence of ions, subsequently affecting the forms of various elements and compounds and their transport processes. Furthermore, heavy metal behavior is strongly correlated with redox potential (Eh) [4].

Many studies of river and lake sediments have reported the effect of redox conditions on the distribution of heavy metals. For example, such changes in redox conditions affect heavy metal association with organic matter (OM) [5], and iron (Fe) and manganese (Mn) oxidized fractions are unstable in reduced environments [3,6]. Kelderman and Osman [2] reported that in river sediments, exchangeable and carbonate bound fractions of Cu, Zn, and Pb increased as Eh increased, and organic fractions, oxidizable Cu, and oxidizable Pb decreased. Lu et al. [7] found that in reduced Iraqi River sediment in Changchun City, Mn oxide fractions increased significantly, organic bound Cu decreased by 40%, but decrease in organic bound Pb, Zn, and Ni was less than Cu.

Soil heavy metal studies which include plant available indices, total concentrations, and fraction distributions have also been conducted on many agricultural systems, including rice (Oryza sativa) production. Rice has a high water requirement compared to other crops and during the growing season, Eh can reach roughly 300–200 mV [4]. Soil samples, including from rice paddies under submerged conditions, are routinely air-dried prior to heavy metal analysis [8–12]. Zheng and Zhang [13] studied the effect of moisture regimes on paddy soil heavy metals and found that soil moisture did not affect the direction or pathways of fractionation distribution (from active to stable fractions), but did affect the transformation rate. Zheng and Zhang [13] dried rice paddy soil samples after collection and then reconstituted three moisture regimes under controlled conditions in the lab. It does not appear that sample preparation was conducted in an anaerobic environment, therefore this result does not represent in situ soil moisture regime that controls the distribution of heavy metals.

Redox conditions are known to have a significant effect on heavy metal speciation in sediments [14]. As indicated by Calmano et al. [5], if anoxic sediments are exposed to atmosphere, redox condition change and redistribution and transformation of heavy metal fractions in the sediments takes place. A few studies show the effect of redox conditions on heavy metal availability [15–17]. Paddy soil has the anoxic condition during rice growing.
season similar to the river sediment. Thus, we hypothesize that drying prior to analysis for paddy soil changes the anoxic condition to redox condition as a consequence mobility and uptake of heavy metal by crop are changes. To date, the effect of drying on soil heavy metal fraction distribution in rice paddies is largely unknown.

In order to fill this knowledge gap and work towards a better methodology for sampling and handling paddy soil prior to heavy metal analysis, we conducted a study in Zhangjiagang County, a typical rice production region of China, to 1) understand heavy metal distribution in paddy soil under various redox and pH conditions, and 2) determine the effect of soil air-drying on heavy metal fractions. This study intends to provide useful information for heavy metal assessment in situ. It is hoped that through a better understanding of heavy metal fractions in situ, a better understanding of correlation of heavy metal between soils and crops can be drawn, and a suitable set of corrective measures to prevent heavy metal pollution can be put in place.

Materials and Methods

Ethics Statement

All the sample sites were distributed in the private land and permission were approved by the land owner in each site (Zhigang Wang can be contacted for the future permissions). The field studies did not involve endangered or protected species because all the sample sites were in the farmland with rice planted. The coordinates of sample sites ranged from 120°35’ to 120°42’E and from 31°44’ to 31°51’N.

Sample Collection

Zhangjiagang County was selected as the research area because of two reasons, firstly, the soil is typical paddy soil with rice planting history of more than 100 years, secondly, heavy metals have been accumulated slightly in this area because of sparkly distributed chemical factories. Zhangjiagang County has a humid monsoon climate in the north subtropical zone, with four distinct seasons, and average annual temperature and precipitation is 15.0°C and 1045.9 mm, respectively.

An investigation of soil physical and chemical characteristics of Zhangjiagang County, Jiangsu, was conducted in 2004 [18–19]. In the current study, ten locations from the 2004 investigation, each with a long history of rice production, were selected to represent a wide pH range (4.31–8.16).

Topsoil (0–20 cm) was collected in October, 2007 from rice paddies before the harvest and after a full season of submersion. At the time of collection, there was still a 1–2 cm water layer covering the soil. Each soil sample was a composite of sub-samples taken from 6–8 points within 50 m² with a stainless steel soil probe. Half of the sample was quickly placed in polyethylene plastic tubes and stored at −4°C until analysis. For the Fe and Mn oxide bound fraction (reducible fraction), the residue from the step above was shaken with hydrogen peroxide (20 ml, acidified to pH 2 with nitric acid). This was also centrifuged, decanted, and stored as described above. For the organic bound fraction (oxidizable fraction), the residue from reducible fraction was digested using the method described above for the total digestion of heavy metals.

The entire extraction procedure for soil samples in polyethylene tubes was the same as described for the air-dried samples except that samples were handled in an anaerobic chamber, using N as the purge gas. All analysis of heavy metal concentrations was determined using inductively coupled plasma-atomic emission spectrometry (ICP-AES).

Statistical Analysis

Analysis of variance was performed using the GLM procedure. Mean separations were performed using Duncan’s Multiple Range test soil pH and redox on soil heavy metal fractions. Linear relationship (correlation) within fractions and regression within the percentage distribution of each metal fraction in oxidized and reduced condition were conducted. All statistical analysis was conducted using SPSS (version 13.0).

Results

Soil Physicochemical Properties

Soil physicochemical properties of rice paddies in Zhangjiagang are shown in Table 1. Average soil moisture of submerged soil was 30.2% and ranged from 24.6% to 33.9%. Soil OM ranged from 19.40 g kg⁻¹ to 31.30 g kg⁻¹ and had a mean of 25.47 g kg⁻¹. The most abundant metal was Pb, which had a mean concentration of 43.27 mg kg⁻¹. The least abundant metal was Cd, which had a mean concentration of 0.20 mg kg⁻¹. The coefficient of variation (CV) for Cd (41.3%) was higher than Cu, Pb, and Ni (25.6%, 8.8% and 9.3%, respectively).

Soil Heavy Metal Fraction Distribution

Heavy metal fraction distribution varied among elements (Fig. 1 and 2). Redox conditions had a significant effect on all Cu fractions (Fig. 1, Table 2), soil pH did not affect Cu fractions, and there was only a significant interaction effect of redox conditions and pH with reducible fraction. The predominant fractions were reducible fraction and oxidizable fraction (35–40%), followed by residual fraction (15–18%) and acid soluble fraction (<1%). Air-dried samples had significantly different Cu fraction distribution. Compared with reduced soil, acid soluble fraction was still the smallest fraction accounting for 5% of the total Cu. Reducible fraction and oxidizable fraction had significantly lower proportions.
while residual fraction had significantly higher proportion and was the predominant fraction.

Redox conditions affected all Pb fraction distributions except reducible fraction, and pH did not affect fraction distribution (p>0.05) (Table 2). There was also no interaction effect of redox conditions and pH with Pb fractions. In reduced soil, the predominant fraction was residual fraction (60%), followed by reducible fraction (20%), oxidizable fraction and acid soluble fraction. In oxidized soil, acid soluble fraction was 3% higher than in the reduced soil, oxidizable fraction was 20% higher and residual fraction was 33% lower, respectively, than in reduced soil, and reducible fraction was not significantly different.

Redox conditions affected all Ni fraction distributions and pH affected all fraction distributions except oxidizable fraction (Table 2). In reduced soil with pH 6 to 7, Ni oxidizable fraction had the lowest proportion, and Ni residual fraction had the highest (Fig. 1). There was significant interaction of redox conditions and pH with acid soluble fraction. In oxidized soil, acid soluble fraction decreased from 30% to 5% and reducible fraction decreased from 35% to 13%. The residual fraction increased by more than 60% and oxidizable fraction increased less than 5%.

Redox conditions and pH affected Cd oxidizable fraction and Cd residual fraction (Table 2). In reduced soil the predominant fraction was reducible fraction (55%), followed by oxidizable fraction (25%), acid soluble fraction and residual fraction. In oxidized soil, residual fraction increased by 15% and oxidizable fraction decreased by 15%. Treatments had no significant effect on acid soluble fraction or reducible fraction.

Regression of Reduced and Oxidized Samples
Regression analysis did not reveal significant relationships between soil heavy metal distribution of oxidized and reduced soil. None of the Cu fractions had significant regression results. For Pb, only residual fraction was significant, and for Ni, only reducible fraction was significant (Fig. 3). For Cd fractions, regression of oxidized and reduced samples had $R^2$ values of 0.26, 0.69, 0.48, and 0.66 for acid soluble fraction, reducible fraction, oxidizable fraction, and residual fraction, respectively (Fig. 4). Regression analysis between relative concentration in oxidized soil and relative concentration in reduced heavy soil showed significant correlation for reducible fraction, oxidizable fraction and residual fraction (Fig. 4). Regression analysis separately by pH and OM did not improve correlation between the dependent and independent variable in Fig. 4.

Discussion

Total Heavy Metal Concentrations in Rice Paddies
Soil heavy metals originate from both the environment and anthropogenic sources. Although all heavy metal concentrations in this study, except three Cd samples, were higher than typical background concentrations [24], they are lower than the critical limits established by the Soil Environmental Quality Standard of China (GB 15618–1995) [25]. The results of our study are consistent with previous research in the area [25]. In rice paddies of China, there is some evidence that this accumulation is a result of heavy metal rich irrigation water [9]. However, low concentrations of heavy metals suggest that although heavy metal concentrations in Zhangjiagang have experienced accumulation from anthropogenic sources, it is not yet a pressing environmental concern.
Heavy Metal Fractionation in Reduced Rice Paddies

Generally, rice paddies are submerged during rice cultivation and have lower Eh values during this part of the year. Reduced soil had lower Cu acid soluble fraction and Pb acid soluble fraction and higher Ni acid soluble fraction, Ni reducible fraction, and Cd reducible fraction than air-dried soil. This is similar to heavy metal fractionation changes that occur in various redox states of sewage sludge [26–28]. Förster and Wittmann [3] explain that for Cu and Pb the most unstable fraction, acid soluble fraction, is replaced by reducible fraction and oxidizable fraction under reduced conditions.

For Ni and Cd, acid soluble fraction accounted for a relatively high proportion (15%–50%), which is not consistent with heavy metal fractionation ranges in sewage sludge. Several studies address changes in soil heavy metal fraction distribution in the rhizosphere of rice paddies, or both. Generally, heavy metal acid soluble fraction increases as Eh increases, however, in our study, Ni and Cd did not respond accordingly. The reason for this is not clear and further study is needed. As Eh increases, reducible fraction generally decreases, most likely because of heavy metal bonding with Fe and Mn oxides [2, 4–5, 39]. Our study bears this out as well.

Effect of Drying on Heavy Metal Fractionation

When soils are routinely dried in the lab prior to chemical analysis, Eh rapidly increases and metals are oxidized. While this may not have a significant effect on heavy metal fractions in agriculture soil which is not submerged, this procedure can drastically change the heavy metal chemistry of reduced soils.

Previous research showed that the relationship of Eh with heavy metal oxidizable fraction and residual fraction is not clear [2–5,7,39]. In our study, increasing Eh led to increased Pb oxidizable fraction and decreased Cu oxidizable fraction. Collavini et al. [40] suggested that decreased Cu oxidizable fraction as soil oxidizes occurs due to the interaction of copper sulfide oxidation and Fe and Mn oxides. Released Cu$^{2+}$ is then redistributed to other fractions such as acid soluble fraction and residual fraction. Part of the obscurity may be because of the complex nature of bonding in copper sulfides, which

![Figure 1. The percentage distribution of each metal fraction in reduced condition in paddy soil samples in Zhangjiagang County.](http://example.com/figure1.png)

L means lower pH (<6), M means moderate pH (6–7), H means higher pH (>7). Note: significant interactions within and between factors are presented in Table 2. doi:10.1371/journal.pone.0097327.g001
have both covalent and ionic bonding characteristics and a high occurrence of delocalized electrons [41–42]. In this study, increasing Eh led to increased Cu reducible fraction, which is in agreement with Kelderman and Osman [2], who reported a decrease in reducible fraction for river sediments under submerged conditions, and with Saeki et al. [14] who reported an increase in Cu reducible fraction after drying of lake sediments. Drying caused an increase in Pb oxidizable fraction which contradicts previous literature [2,7]. As Eh increases, Pb reducible fraction slightly decreases, mainly due to bonding of Pb with Fe and Mn oxides [7]. Dried soil had lower Ni reducible fraction and Cd oxidizable fraction and higher Ni residual fraction and Cd residual fraction than reduced soil. This was consistent with the results of other studies [2,7,40].

Figure 2. The percentage distribution of each metal fraction in oxidized condition in paddy soil samples in Zhangjiagang County. L means lower pH (<6), M means moderate pH (6–7), H means higher pH (>7). Note: significant interactions within and between factors are presented in Table 2.

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Table 2. P-values from the analysis of variance of redox conditions and pH for heavy metal fraction distributions.

| Heavy Metal | Factors  | P value     | Acid soluble fraction | Reducible fraction | Oxidizable fraction | Residual fraction |
|-------------|----------|-------------|-----------------------|--------------------|---------------------|-------------------|
| Cu          | Redox    | <0.001      | 0.050                 | <0.001             | <0.001              |
|             | pH       | 0.685       | 0.109                 | 0.325              | 0.555               |
|             | Interaction | 0.308     | 0.034                 | 0.873              | 0.111               |
| Pb          | Redox    | 0.001       | 0.901                 | <0.001             | 0.000               |
|             | pH       | 0.230       | 0.544                 | 0.124              | 0.068               |
|             | Interaction | 0.123     | 0.092                 | 0.345              | 0.970               |
| Ni          | Redox    | 0.000       | 0.000                 | 0.000              | 0.000               |
|             | pH       | 0.023       | 0.009                 | 0.314              | 0.009               |
|             | Interaction | 0.040     | 0.304                 | 0.356              | 0.164               |
| Cd          | Redox    | 0.08        | 0.316                 | 0.000              | 0.024               |
|             | pH       | 0.810       | 0.261                 | 0.015              | 0.142               |
|             | Interaction | 0.089     | 0.359                 | 0.277              | 0.413               |

P-values <0.05 show significant difference within factors and significant interaction between factors for each heavy metal fraction, separately.

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There is strong evidence that drying significantly affects soil heavy metal fractionation of submerged rice paddy soil samples but effects are different than lake sediment, river sediment, and sewage sludge. One of the main reasons most likely is the differences in Eh of the sample sources. Generally, sediment Eh is $<-100\text{mv}$, while rice paddy soil Eh ranged from 200–300 mv [4]. Abundant roots in rice paddies may be another important factor. Root exudates can affect many soil properties such as pH and microbial activity. Changes in these properties can then indirectly affect soil heavy metal fraction distribution. Chen et al. [43] reported that abundant rhizosphere can significantly increase Cu_{acid soluble fraction} and significantly decrease Cd_{acid soluble fraction}. Shuman and Wang [44] reported that rice rhizosphere significantly increased Zn_{reducible fraction} and Cu_{oxidizable fraction}. Similarly, Lin et al. [24] reported that rice rhizosphere significantly increased Cd_{reducible fraction} and Cd_{oxidizable fraction}.

**Effects of Drying on Heavy Metal Availability**

The distribution, migration, and plant availability of soil heavy metals are reflected not in the total concentrations, but in specific
fractures. With the BCR sequential extraction method, heavy metal bioavailability decreases with each progressively stronger extraction [45]. Acid soluble fraction has the highest bioavailability and residual fraction has the lowest. Acid soluble fraction, reducible fraction, and oxidizable fraction are considered active fractions and each contain some bioavailable heavy metals [46]. In this study, Ni and Cd have large active fractions (80%), followed by Cu (>60%), and Pb (<40%). Drying submerged paddy soil samples caused decreases in the active fractions of Cu, Ni, and Cd and increased active fractions of Pb. Zheng and Zhang [13] found an increased bioavailability with increased Eh across all heavy metals measured (Cu, Pb, Cd, and Hg). Our results suggest that when samples are dried prior to heavy metal bioavailability analysis, Pb is overestimated and Cu, Ni, and Cd are underestimated.

Regression analysis results showed that samples which have been dried prior to analysis are not suitable indicators of reduced soil conditions and cannot be used to predict field conditions. However, adding pH and OM did not improve the predictive power, particularly for Cd (data not shown).

Conclusions
Almost all heavy metal fractions were significantly affected by redox conditions, and few heavy metal fractions were affected by pH. Drying the soil prior to analysis caused metal ions to change valence and a redistribution of heavy metal fractions. Some redistribution occurred as metals moving from active fractions to stable fractions. Therefore, drying decreases the representativeness of in situ conditions and the availability of some heavy metals are misestimated. We suggest that soil heavy metal fractionation procedures for rice paddies ensure anaerobic conditions from the time of sampling until analysis. Methodology studies to improve sampling and lab handling techniques would be beneficial, as would further investigation of possible correlation of non-dried sampling and analysis using bioavailable extractions with air-dried samples. For some heavy metals, the processes driving redistribution trends are still unclear. Because the relationship between soil Eh and heavy metal fractionation distribution directly affects heavy metal mobility and bioavailability, a better understanding of the soil heavy metal chemistry under various redox conditions is imperative.

Author Contributions
Conceived and designed the experiments: YQ. Performed the experiments: YQ, JLD. Analyzed the data: YQ, BH. Contributed reagents/materials/analysis tools: BH. Wrote the paper: YQ.

References
1. Gong ZT, Huang B (1998) Studies on potential “chemical inner bombs” and their igniting factors in soils. Adv. Earth Sci. 23: 184–191.
2. Kelderman P, Osman AA (2007) Effect of redox potential on heavy metal binding forms in polluted canal sediments in Delhi (the Netherlands). Water Res. 41: 4251–4261.
3. Förstner U, Wittmann G (1983) Metal pollution in the aquatic environment. Second ed. Springer, Berlin.
4. Li YG, Xue SG, Wu XY (2004) Transport and transformation of heavy metals in the soil–paddy system. Prog. Geogr. China, 31(supp);: 87–92.
5. Calmano W, Hjort N, Förstner U (1993) Binding and mobilization of heavy metals in contaminated sediments affected by pH and redox potential. Water Res. 26: 225–235.
6. Stumm W, Morgan J (1996) Aquatic chemistry: an introduction emphasizing chemical equilibria in natural water, third ed. Wiley, New York.
7. Lu YZ, Dong DM, Fu Y (2006) Comparison of speciation patterns of heavy metals in Yitong River sediment under reduced and oxidized conditions. Chem. J. Chinese University, 27: 449–453.
8. Andreu V, Gimeno-Garcia E (1999) Evolution of heavy metals in marsh areas under rice farming. Environ. Pollution 104: 271–282.
9. Luo YM, Jiang XJ, Wu LH, Song J, Wu SC, Lu RH, Christie P (2003) Accumulation and chemical fractionation of Cu in a paddy soil irrigated with Cu-rich wastewater. Geoderma, 115: 113–120. 
10. Li JX, Yang XE, He ZL, Jilani G, Sun CY, Chen SM (2007) Fractionation of lead in paddy soils and its bioavailability to rice plants. Geoderma, 141: 174–180.
11. Zhao KL, Liu XM, Xu JM, Selim HM (2010) Heavy metal contaminations in a soil–rice system: Identification of spatial dependence in relation to soil properties of paddy fields. J. Hazard. Mater. 181: 78–78.
12. Ngoc MN, Dulz S, Khasholm J (2009) Simulation of retention and transport of copper, lead and zinc in a paddy soil of the Red River Delta, Vietnam, Agr. Ecosyst. Environ. 129: 8–16.
13. Zheng SA, Zhang MK (2011) Effect of moisture regime on the redistribution of heavy metals in paddy soil. J. Environ. Sci. 23: 434–443.
14. Seuki K, Okazaki M, Matsuzo S (1999) The chemical phase changes in heavy metals with drying and oxidation of the lake sediments. Water Res. 27: 1243–1251.
15. Reddy CN, Patrick WHJ (1977) Effect of redox potential and pH on the uptake of cadmium and lead by rice plants. J. Environ. Qual. 6: 259–262.
16. Sajwan KS, Lindsay WL (1986) Effects of redox on zinc deficiency in paddy rice. Soil Sci. Soc. Am. J. 50: 1264–1269.
17. Kashem MA, Singh BR (2001) Metal availability in contaminated soils: I. Effects of flooding and organic matter on changes in Eh, pH and solubility of Cd, Mn and Zn. Nutr. Cycl. Agroecosys. 61: 247–255.
18. Qj YB, Huang B, Gu QZ, Sun WX, Zhao YC (2008) Spatial and temporal variation of C/N ratio of agricultural soils in typical area of Yangtze Delta region and its environmental significance. B. Miner. Petro. Geochem. 25: 53–30.
19. Moore P, Coale F (2000) Phosphorus fractionation in flooded soils and sediments. In: G. M. Pervezski, Ed.), Methods of phosphorus analysis for soils, sediments, residual, and waters. Available online at http://www.soil.nrcsu.edu/sera17/publications/sera17-2/ppm_cover.htm.
20. Lu RK (2004) Soil and agro-chemical analytical methods, China Agricultural Science and Technology Press, Beijing.
21. Nelson DW, Sommers LE (1996) Total carbon, organic carbon, and organic matter, In: D. L. Sparks (Eds.), Methods of soil analysis, part 3: Chemical methods, Madison, Wisc.: SSA and ASA, 961–1010.
22. Ure AM, Quevauviller PH, Frateur H (1993) Speciation of heavy metals in soils and sediments: an account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of European Communities. Int. J. Environ. Anal. Chem. 51: 135–151.
23. Lin Q, Zeng CR, Chen HM (1998) Transformation of cadmium species in rhizosphere. Acta Pedologica Sinica 35: 461–467.
24. Shao XX, Huang B, Zhao YC, Sun WX (2008) Pollution assessment of soil heavy metals in a representative area of the Yangtze River Delta region. Environ. Chem. 27: 218–226.
25. Tsai LJ, Yu KC, Chang JS (1998) Fractionation of heavy metals in sediment cores from the Ell-En River, Taiwan. Water Sci. Tech. 37: 217–224.
26. Tessier A, Campbell PG, Bisson M (1979) Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51: 844–851.
27. Angese E, Bettiol C (2003) Heavy metal partitioning in sediments from the lagoon of Venice (Italy). Toxicol. Environ. Chem. 79: 157–170.
28. Hinsinger P, Passard C, Tang C, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 240: 41–59.
29. Tao S, Chen YJ, Xu FL, Cao J, Li BG (2003) Changes in copper speciation in maize rhizosphere soil. Environ. Pollut. 122: 447–454.
30. Martinez-Alcala I, Walker DJ, Bernal MP (2010) Chemical and biological properties in the rhizosphere of Lupinus alba under soil heavy metal fractionation. Environ. Toxicol. Saf. 73: 595–602.
31. Singh SP, Ma LQ, Taek FMG (2000) Trace metal leach ability of land-disposed dredged sediments. J. Environ. Qual. 29: 1124–1132.
32. Wu HS, Zhang AQ, Wang LS (2004) Immobilization study of biosorption of heavy metal ions onto activated sludge. J. Environ. Sci. 16: 640–645.
33. Wang G, Li XC, Ma HT, Qian J, Zhai JB (2006) Distribution of extractable fractions of heavy metals in sludge during the wastewater treatment process. J. Hazard. Mater. 137: 1277–1283.
34. Surjia B, Branica M (1995) Distribution of Cd, Pb, Cu and Zn in carbonate sediments from the Krka River estuary obtained by sequential extraction. Sci. Total Environ. 170: 101–118.
35. Li RY, Yang H, Zhou ZG, Lu J, Jia JS, Zhao YC, Jin F (2007) Fractionation of heavy metals in sediments from Dianchi lake, China. Pedosphere, 17: 265–272.
36. Shirvastaia SK, Banerjee DK (2004) Speciation of metals in sewage sludge and sludge-amended soils. Water, Air, Soil Pollut. 152: 219–232.
37. Telcira E, Ortiz L, Alves M, Sanchez J (2001) Distribution of selected heavy metals in fluvial sediments of the coal mining region of BiaxJacui, RS, Brazil. Environmental Geol. 41: 145–154.
39. Kazi TG, Jamali MK, Kazi GH, Arain MB, Afridi HI, et al. (2005) Evaluating the mobility of toxic metals in untreated industrial wastewater sludge using a BCR sequential extraction procedure and a leaching test. Anal. Bioanal. Chem. 383: 297–304.
40. Collavini F, Zonta R, Novelli AA, Zaggua L (2000) Heavy metals behavior during resuspension of the contaminated reduced sludge of the Venice canals. Toxicol. Environ. Chem. 77: 171–187.
41. Zheng SA, Zheng XQ, Chen C (2012) Leaching Behavior of Heavy Metals and Transformation of Their Speciation in Polluted Soil Receiving Simulated Acid Rain. PLoS ONE 7(11): e49664. doi:10.1371/journal.pone.0049664.
42. Goh SW, Buckley AN, Lamb RN, Rosenberg RA, Moran D (2006) The oxidation states of copper and iron in mineral sulfides, and the oxides formed on initial exposure of chalcopyrite and bornite to air. Geochim. Cosmochim. Ac. 70: 2210–2228.
43. Chen YJ, Tao S, Deng BS, Zhang XQ, Huang Y (2001) Effect of root system on metal fractionation in rhizosphere of contaminated soil. Acta Pedologica Sinica 38: 54–59.
44. Shuman LW, Wang J (1997) Effect of rice variety on zinc, cadmium, iron and manganese contents in rhizosphere and non-rhizosphere soil fractions. Commun. Soil Sci. Plant 28: 23–36.
45. Kashem MA, Singh BK, Kowai S (2007) Mobility and distribution of Cadmium, Nickel, and Zinc in contaminated soil profiles. Earth Environ. Sci. 77: 187–198.
46. Wang CQ, Dai TF, Li B (2007) The speciation and bioavailability of heavy metals in paddy soils under the rice-wheat cultivation rotation. Acta Ecologica Sinica 27: 889–897.