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RESEARCH ARTICLE

The respective effects of soil heavy metal fractions by sequential extraction procedure and soil properties on the accumulation of heavy metals in rice grains and brassicas

Ling Xiao[1,2] · **Dongsheng Guan**[1,2] · **M. R. Peart**[3] · **Yujuan Chen**[1,2] · **Qiqi Li**[1,2]

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Abstract This study was carried out to examine heavy metal accumulation in rice grains and brassicas and to identify the different controls, such as soil properties and soil heavy metal fractions obtained by the Community Bureau of Reference (BCR) sequential extraction, in their accumulation. In Guangdong Province, South China, rice grain and brassica samples, along with their rhizospheric soil, were collected from fields on the basis of distance downstream from electroplating factories, whose wastewater was used for irrigation. The results showed that long-term irrigation using the electroplating effluent has not only enriched the rhizospheric soil with Cd, Cr, Cu, and Zn but has also increased their mobility and bioavailability. The average concentrations of Cd and Cr in rice grains and brassicas from closest to the electroplating factories were significantly higher than those from the control areas. Results from hybrid redundancy analysis (hRDA) and redundancy analysis (RDA) showed that the BCR fractions of soil heavy metals could explain 29.0 and 46.5 % of total eigenvalue for heavy metal concentrations in rice grains and brassicas, respectively, while soil properties could only explain 11.1 and 33.4 %, respectively. This indicated that heavy metal fractions exerted more control upon their concentrations in rice grains and brassicas than soil properties. In terms of metal interaction, an increase of residual Zn in paddy soil or a decrease of acid soluble Cd in the brassica soil could enhance the accumulation of Cd, Cu, Cr, and Pb in both rice grains and brassicas, respectively, while the reducible or oxidizable Cd in soil could enhance the plants' accumulation of Cr and Pb. The RDA showed an inhibition effect of sand content and CFO on the accumulation of heavy metals in rice grains and brassicas. Moreover, multiple stepwise linear regression could offer prediction for Cd, Cu, Cr, and Zn concentrations in the two crops by soil heavy metal fractions and soil properties.

Keywords Heavy metal accumulation · Heavy metal fractions · Soil properties · Wastewater irrigation · Rice · Brassica

Abbreviations

SOM	Soil organic matter
CEC	Cation exchange capability
AFO	Amorphous Fe oxides
CFO	Crystalline Fe oxides
ASF	Acid soluble fraction
RDF	Reducible fraction
OXF	Oxidizable fraction
RSF	Residual fraction

Introduction

Rice is the dominant agricultural crop in China and it accounts for more than 42 % of crop yield (Yu et al. 2012), while at the global scale, it is the second most important crop by quantity of production and a staple food for more than half the world's

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✉ Dongsheng Guan
eesgds@mail.sysu.edu.cn

[1] School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China

[2] Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology (Sun Yat-sen University), Guangzhou 510275, China

[3] Department of Geography, The University of Hong Kong, Hong Kong, China

population (Lam et al. 2013). Brassicas are important leafy vegetable crops that are grown all over the world with a variety of species being utilized (Kapusta-Duch et al. 2016). In China, brassicas are widely grown especially in home gardens and in market gardens near urban areas. It is clear that the quality of the two crops is of great concern to society. However, some environmental stresses, soil heavy metal contamination for example, may pose a threat to their production and human health. With respect to China, Lu et al. (2015a) report that Cd and Pb contamination in rice is the major concern, while Niu et al. (2013) indicate that brassicas are contaminated with Cd, Cr, and Pb, most of which result from the contamination of agricultural soil. There are a number of anthropogenic sources of heavy metals in soils used for agriculture. They include metal mining and smelting, industrial activities, irrigation, fertilizer, and herbicide/pesticide use in agriculture and urban development (Liu et al. 2011; Teng et al. 2014; Chen et al. 2015; Lu et al. 2015a). In particular, long-term irrigation with wastewater is a significant source of soil heavy metal pollution (Gupta et al. 2012; Lu et al. 2015b). For example, Liu et al. (2016) indicated that irrigation with industrial effluent caused an elevation of Cd, Cu, Pb, and Zn in soils, while Meng et al. (2016) reported that long-term irrigation with sewage resulted in significant Cd, Zn, and Hg pollution in soils and the accumulation of Cd and Pb in vegetables. The bioavailability of heavy metals and soil properties have been considered as the two most important environmental factors influencing heavy metal accumulation in plants (Kidd et al. 2007; Zeng et al. 2011). The bioavailability and mobility of heavy metals largely depend on the form of the heavy metals (Zhong et al. 2011), which can be measured by sequential extraction. Kubová et al. (2008) observed that the metal fractions that are separated in early stages of a sequential extraction are those metals that retained on the soil surface by relatively weak electrostatic interactions and which can be released by an ion exchange process. The heavy metal fractions that are extracted in later stages can be bioavailable, and uptaken by plants, when Eh or pH changes. Soil properties, such as pH, organic matter, particle size, cation exchange capacity (CEC), and Fe oxides, have all been shown to have a noticeable effect on the bioavailability of heavy metals in the soil and also to influence plant growth (Acosta et al. 2009; Dong et al. 2015; Sungur et al. 2015). Consequently, soil properties might have a great impact on metal accumulation in plants. Understanding the respective effects of heavy metal fractions and soil properties on metal accumulation in rice grains and brassicas should facilitate agricultural soil management and help with food safety. Therefore, there is a need to figure out the respective effects and contributions of metal fractions and soil properties on heavy metal accumulation in rice grains and brassicas.

The interaction of heavy metals in their accumulation by plants has been documented by a number of studies (Wu et al. 2005; Qiu et al. 2011; Mendoza et al. 2015). However, most of the studies focused on the interaction between two elements, namely, Cd and Zn. In contrast, the interaction among other heavy metals such as Cu, Pb, and Cr was rarely reported (Qiu et al. 2011; Wu et al. 2005). Moreover, in a study in the Baiyin region of China, Nan et al. (2002) observed that “interactive effects of multiple metal pollution on plant metal accumulation are common but not consistent”. There is a need, therefore, for a multi-element approach to investigate heavy metal interaction and its role in heavy metal uptake and accumulation by plants. Many of the studies on the interactions of different elements and plant uptake of heavy metals were carried out either by hydroponic experiment or using pot culture (Nan et al. 2002). Moreover, with respect to the latter, the metal concentrations of soil gradient employed may be quite different from those in the real-world, larger scale field situation. Thus, regarding the uptake of heavy metals by plants, there is a need to analyze the interaction of multiple elements, especially the interaction of the metal fractions in soil and the metal concentrations in plants, under real-world field conditions.

This study was carried out in Guangdong Province, South China, through field investigation of the heavy metal in soil, rice grains, and brassicas. The sampling was based upon the distance downstream from the electroplating factory outlets, whose wastewater was used for irrigation, so that a range of soil and plant heavy metal concentrations could be obtained. The objectives of this paper were (1) to identify the respective effects of heavy metal fractions and soil properties on metal concentrations in both rice grains and brassicas by means of large scale field investigation and multivariate statistical analysis and (2) to determine the interaction of soil heavy metal fractions on their accumulation in rice grains and brassicas.

Materials and methods

Study area

The study area is located in Huizhou, Jiangmen, and Zhaoqing of Guangdong Province in South China (Fig. 1). The region experiences a subtropical monsoon climate with the average annual precipitation ranging from 1650 to 2146 mm and a mean annual temperature range of 19~22 °C. The soils in the study areas are mainly latosolic red soils, and they are developed from Quaternary red clay and shale (Shi et al. 2010). The electroplating industry in the region has emerged since the 1990s and many, often small scale workshops, have operated for a long time. The effluent from the electroplating factories and workshops, which is often used to irrigate farmland in the surrounding areas, contains 3.38–7.35, 0.02–0.03, 0.03–0.05, 3.24–5.35, and 3.85–8.35 mg L^{-1}, for Cu, Cd, Pb, Zn, and Cr, respectively.

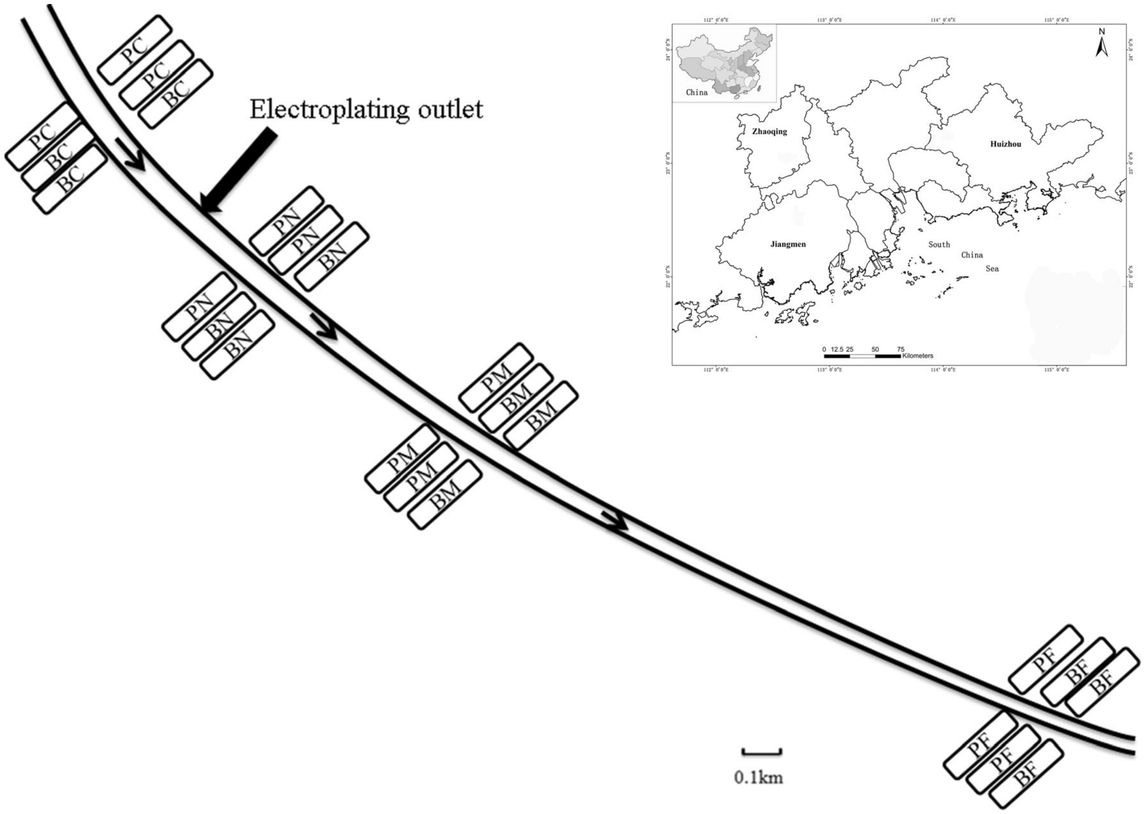

Fig. 1 Map of sampling sites in Pearl River Delta

Around 15 % of the land is under cultivation in Guangdong Province and the agriculture tends to be intensive and irrigation is important, along with the use of chemical fertilizers. Rice is the most important crop, accounting for over 70 % of the cultivated area with two crops per year often being grown. Brassicas are one of the most important vegetable crops in China (Rackow 2004) and Guangdong, being commonly grown on farms and market gardens near urban areas and also in kitchen gardens (Rackow 2004).

Sampling and preparation

The sampling was carried out during the maturity of the rice and brassica plants in November. Based upon the work of Liu et al. (2011) and in order to obtain a range of soil heavy metal concentrations, three sampling areas were identified and three sampling sites in each area were selected on the basis of their distance downstream from each electroplating factory effluent outlet (0.1, 0.5–1, >3 km). These electroplating factories provided a source of wastewater containing heavy metals, for the irrigation of the fields used to grow rice and brassicas. At least five subsamples of rice plants (*Oryza sativa* L. subsp. *indica* Kato) and brassica plants (*Brassica campestris* L. ssp. *chinensis* var. *utilis* Tsen et Lee) were obtained at each sampling site. In total, 44 rice plants and 36 brassica plants were obtained from the farmland downstream of the electroplating effluent outlets. In addition, three samples of soil, rice plants, and brassica plants were collected from three control sites, giving a total of nine samples, on which no wastewater had been used for the irrigation of crops. These control site samples served to (a) increase the range of metal concentrations to be utilized in the statistical analysis of this study, (b) provide a comparison against the irrigated fields, and (c) provide background data on the heavy metal content of the soil in the study area.

Both rice and brassica plants were removed from the ground with the soil intact in their root systems. The rhizospheric soil was separated from the plant root systems by shaking gently by hand. Each sample (soil, rice, and brassica plants) was placed in a polythene ziplock bag and transported to the laboratory on the day of sampling. In the laboratory, the rice grains were separated from the ears and hulled, and the roots of the brassica plants removed. The fresh leaves of the brassica plant samples were washed with tap water, rinsed with deionized water, and then dried to a constant weight in a drying oven. Lastly, both the rice grains and brassica leaves were milled into a powder for the determination of heavy metal concentrations. After being dried in an air-circulating room, the sampled soils were treated to remove stones and plant residues, ground with a wooden grinder, and passed through a 2-mm nylon sieve. The sieved soil samples were collected and split into two subsamples, one of which was used to measure soil properties, such as pH and particle size distribution, and the other was ground to pass through a plastic 0.15-mm sieve for the analysis of heavy metals and soil organic matter (SOM).

Chemical analysis

Soil pH was determined using 1:2.5 soil-to-water ratio, while SOM was determined by the dichromate digestion method (Walkley and Black, 1934). CEC was measured by the ammonium acetate saturation method (Kitsopoulos 1999). Particle size distributions were measured by the pipette method (Konert and Vandenberghe 1997) and classified according to the USDA soil texture classification system. Amorphous Fe oxides (AFO) were quantified by extracting Fe with 0.2 M Tamm solution at pH 3 in dark conditions, and crystalline Fe oxides (CFO) were measured by extraction with a sodium dithionite–citrate–bicarbonate (DCB) solution (Silva et al. 2010).

The total concentrations of Cu, Pb, Zn, and Cr were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, PerkinElmer Optima 5300DV) after aqua regia digestion of about 0.5 g of ground rice grains, brassica leaves, or soil sample in a microwave digestion instrument. Cadmium was analyzed by graphite furnace atomic absorption spectrophotometer (GFAAS, HITACHI Z5000).

Some previous studies have used a soil heavy metal fraction that was extracted by means of single-step protocols such as DPTA, $CaCl_2$, EDTA, and NH_4NH_3 extraction to predict the bioavailability of heavy metals (Bose and Bhattacharyya, 2008; Jamali et al. 2009; McGrath et al. 1997). However, Kubová et al. (2008) and Mendoza et al. (2006) demonstrated that the bioavailability of heavy metals analyzed by a single-step extraction was underestimated through comparing the effects of both single and sequential extractions on metal accumulation in plants. Therefore, this study utilized soil heavy metal fractions obtained by Community Bureau of Reference (BCR) sequential extraction protocol to examine the effects of soil heavy metals upon plant uptake and which has been used by other studies (Li et al. 2014; Monterroso et al. 2014).

The metal fractionation was carried out by a BCR sequential extraction procedure, which was modified from Nemati et al. (2011), and performed on 1 g of soil sample in a 100-ml polypropylene centrifuge tube. The metal fractions comprise of the acid soluble, reducible, and oxidizable fractions and residues. After each extraction procedure, the tubes were centrifuged at 4000×*g* for 20 min. After filtering, or acidifying with super pure HNO_3, Cr, Cu, Pb, and Zn in the various extracts were measured by FAAS, and the extracts of Cd were determined by GFAAS.

Quality control of the analysis was undertaken by the use of blank samples and replicates and the standard reference material (GSS-16 for soils) of the CRM/RM Information Center of China. The recovery ((species sum / total metal) × 100 %) was 83–117 %, showing a satisfactory extraction of metals from the soil with the aqua regia digestion and BCR sequential extraction.

Statistical analysis

Descriptive statistics were calculated for the data sets of the soils, rice grains, and brassicas. SPSS Software (20.0) was employed to calculate the correlation between total heavy metals and the various metal fractions as determined by BCR fractionation and also a range of soil properties such as SOM. It was also used to statistically analyze the difference of the data sets by ANOVA test and to estimate the concentration of metals in rice grains and brassicas through multiple stepwise linear regressions. CANOCO 4.5 was used for detrended correspondence analysis (DCA), hybrid redundancy analysis (hRDA), and redundancy analysis (RDA), to analyze the distribution of the different heavy metals in the rice grains and brassicas and also to identify the different controls, such as soil properties and heavy metal fractions on their accumulation.

The DCA was used to measure the gradient length of the variables because if the gradient length of the variables is lower than 4, then linear methods such as RDA and hRDA are appropriate for further analysis of the data. RDA is a constrained multivariate analysis method, which allows analyzing the simultaneous responses of many dependent variables to several explanatory factors (Sadyś et al. 2015). hRDA illustrates the influence of the environmental variables on response variables with the first axis, as opposed to variation due to other unmeasured factors on the second axis. Through RDA and hRDA, the significance of metal fractions and soil properties, along with the interaction of different elements in the soil–plant system, upon metal uptake by rice grains and brassicas can be identified. For the hRDA, heavy metal concentrations in rice grains and brassica leaves were selected as the response variables, hereafter named species, while the heavy metal fractions were treated as explanatory variables and labeled as environmental factors. Soil properties, including pH, SOM, soil particle size distribution, AFO, and CFO, were set as co-variables in order to eliminate their influence, thereby permitting only the influence of the metal fractions in the soil upon the metal concentrations in the rice grains and brassicas to be identified. Those explanatory variables whose inflate factor was higher than 20 were removed from further hRDA in order to eliminate the problem of multicollinearity of the variables. In addition, RDA was carried out in order to determine the relationship between soil properties and metal concentrations in rice grains and brassicas. In the RDA, the metal concentrations in the rice grains and brassica leaves were set as species, while soil properties were the explanatory environmental factors. In summary, the hRDA permits evaluation of the influence of heavy metal fractions, while the RDA permits investigation of the control exerted by soil properties, upon heavy metal uptake by rice grains and brassicas. The original data was transformed to Lg(x + 1) and was standardized to avoid the influence of different data dimensions.

Results

Soil physicochemical properties and heavy metal concentrations at the sampling locations

The values of soil physicochemical properties of the samples are shown in Table 1. About 48 % of the rhizospheric soil samples were classified as silt loam and 23 % of those were loam, according to the USDA classification. The values of soil CEC were all less than 9 cmol kg^{-1}, with an average value of 5.13 cmol kg^{-1}. The SOM of paddy soil ranged from 21.71 to 57.09 g kg^{-1} and that of brassica vegetable soil ranged from 11.01 to 82.57 g kg^{-1} in the sampled locations. These results indicated that the soil samples had a wide range of physicochemical properties, thereby facilitating investigation of the influence of soil properties on metal fractions in the soil and also plant accumulation of metals.

The data of total heavy metal concentrations for the control sites provides background data for agricultural soils of the study area and it also affords a baseline against which to examine the influence of irrigation with wastewater from electroplating factories. The concentrations of Cd in the rhizospheric soil samples from the fields irrigated with wastewater were all higher than the Chinese national standard (GB 15618-1995), which is 0.3 mg kg^{-1}. In particular, the Cu, Zn, and Cr concentrations of the rhizospheric soil samples in some of the sample sites were much higher than the Chinese national standard (GB 15618-1995), which are 50, 250, and 250 mg kg^{-1} for Cu, Zn, and Cr, respectively. According to the study of Wong (2003), the heavy metal contamination from atmospheric deposition was relatively low in the study area which may indicate that electroplating wastewater is the major resource of soil heavy metal contamination. However, fertilizers along with herbicides and pesticides are also a potential source of heavy metals in soils used for agriculture (Liu et al. 2011; Teng et al. 2014; Chen et al. 2015; Lu et al. 2015a). In this study, both the control sites and those sites irrigated with electroplating wastewater have been exposed to their application, and consequently the differences in heavy metal concentration between the control and other sites are most likely to reflect the quality of water used for irrigation given that the parent materials are predominantly Quaternary red clays and shales. Table 2 shows that the concentrations of Cd, Cr, Cu, and Zn are significantly higher in the rhizospheric soil that was sampled from closest to the electroplating factories than those from the control areas ($P < 0.05$), indicating that the long-term irrigation by electroplating effluent has enriched these heavy metals in the soils at these sites. In addition, Table 2 shows that the concentrations of Cd, Cr, Cu, and Zn in the irrigated fields showed a declining trend with increasing distance downstream from the electroplating factory outlets. Regarding Pb, Table 2 reveals that it mostly has higher mean concentrations at the control sites in comparison to the others. This is in contrast to the other heavy metals of this study. Compared to previous studies of soil heavy metal pollution in the Pearl River Delta by Wong et al. (2002) and Chen et al. (2005), the Cu, Cd, Pb, and Zn concentrations in this study are higher.

Fractioning of soil heavy metal by BCR

The chemical fractionations of Cu, Cd, Pb, Zn, and Cr in the soil samples are shown in Fig. 2. Generally, the sum of the acid soluble, reducible, and oxidizable fractions, which are considered bioavailable (Zeng et al. 2011), contributed 72.32 % (Cu), 59.80 % (Pb), 53.13 % (Zn), 29.88 % (Cr), and 55.68 % (Cd) of the total metals. There is a contrast between Cr, for which the bioavailable fraction accounts, on average, for a comparatively small percentage (30 %) of the total metal concentration and in which the residual fraction is dominant, and the other four metals where the bioavailable fraction always exceeds 50 % and in the case of Cu is greater than 70 % of the total metals. The sum of the acid soluble, reducible, and oxidizable fractions for most of the studied metals decreased with the increase of sampling distance away from the electroplating factories. Figure 2 shows that the residual fraction of the studied metals in the soil that was sampled from closest to the electroplating outlets was much lower than that in the control sites, suggesting that irrigation with electroplating effluent could enhance the bioavailability and mobility of heavy metals. With regards to the acid soluble fraction of Zn, Fig. 2 shows a declining trend in paddy soils but a contrasting increasing trend in the brassica rhizospheric soil, with the increase of sampling distance downstream from the electroplating factory wastewater outlets.

Heavy metal concentrations and their distribution in rice grains and brassica vegetables

Regarding rice, 64.9 and 45.9 % for Cd and Cr, respectively, of the samples were higher than the national food safety standards (GB 2762-2005, GB 2762-2012), which are 0.2 and 1.0 mg kg^{-1}, respectively. The brassica had 64.2 and 35.7 % of the samples for Cd and Cr, respectively, with concentrations that were much higher than the national food safety standards (GB 2762-2005, GB 2762-2012), which are, for Cd, 0.2 mg kg^{-1} and, for Cr, 0.5 mg kg^{-1}. Generally, the

Table 1 Physicochemical properties of soils from different sampling locations (range and mean)

Location	N	pH	SOM (g· kg^{-1})	CEC (cmol· kg^{-1})	Clay^a (%)	Silt^a (%)	Sand^a (%)	AFO (g· kg^{-1})	CFO(g· kg^{-1})
Paddy rhizospheric soil									
Close	16	4.97–6.62 (5.49)	24.08–57.09 (36.45)	3.87–7.93 (5.34)	12.83–67.66 (29.75)	12.31–69.33 (40.23)	5.82–56.69 (30.02)	0.28–1.53 (0.68)	16.07–61.60 (39.36)
Mid-distance	15	4.96–6.28 (5.50)	25.60–52.67 (38.34)	3.45–7.79 (5.43)	12.30–40.31 (24.33)	23.20–68.24 (48.06)	13.52–47.52 (27.61)	0.23–1.49 (0.68)	24.02–81.77 (47.42)
Far away	13	5.34–5.90 (5.60)	21.71–47.45 (29.04)	3.84–5.63 (4.53)	7.62–23.24 (16.41)	21.28–63.64 (37.92)	16.75–64.24 (45.67)	0.16–1.18 (0.60)	16.97–56.13 (34.21)
Control sites	9	4.94–6.51 (5.57)	23.11–41.23 (29.18)	2.61–8.40 (4.81)	8.63–24.23 (16.76)	17.05–57.35 (36.65)	27.63–51.34 (46.59)	0.22–1.03 (0.54)	18.18–57.13 (35.73)
Brassica rhizospheric soil									
Close	13	4.74–6.85 (5.34)	21.71–82.57 (47.21)	3.58–8.47 (6.01)	7.62–57.86 (30.25)	20.95–58.22 (38.26)	5.15–49.12 (31.49)	0.53–1.28 (0.78)	29.68–96.15 (53.75)
Mid-distance	11	4.82–6.43 (5.64)	25.75–39.32 (29.21)	3.89–7.39 (5.29)	8.11–32.29 (16.39)	19.03–57.39 (38.71)	26.10–65.86 (44.89)	0.45–1.19 (0.74)	33.87–73.59 (48.25)
Far away	12	5.02–6.48 (5.33)	14.14–31.94 (24.47)	3.82–5.39 (4.67)	11.25–25.76 (19.62)	41.69–53.53 (48.79)	21.85–42.07 (31.59)	0.32–1.01 (0.67)	23.58–75.87 (43.84)
Control sites	9	7.13–7.86 (7.54)	11.01–18.30 (12.92)	3.03–6.47 (3.91)	13.04–21.36 (17.21)	24.09–42.25 (34.04)	44.70–57.06 (48.75)	0.11–0.49 (0.20)	16.96–50.76 (37.75)

^{a} Clay < 2 μm, silt = 2–50 μm, and sand >50 μm

concentrations of Cu in the rice grains were higher than that of the brassicas, while the Cr concentrations in the rice grains were lower than that of the brassicas, indicating the different physiology of rice grains and brassicas. Table 3 shows that the average concentrations of heavy metals in rice grains and brassicas from the sampling sites closest to the electroplating factories were higher than those observed from the control areas, although they may not be significantly different at the $P < 0.05$ level. This is further evidence of the role of wastewater from the electroplating factories being the source of Cd, Cr, Cu, and Zn enrichment in soil. Statistically, Cd, Cr, and Pb concentrations in the rice grains, along with Cd, Cr, and Cu concentrations in the brassicas that were sampled from closest to the electroplating factories were significantly higher than those from the control sites ($P < 0.05$), illustrating that irrigation with electroplating effluent would increase the accumulation of these metals in both rice grains and brassicas. In addition, the concentration of Cd, Cu, Cr, and Pb in rice grains and brassicas presented a declining trend with distance away from the electroplating factories, suggesting the influence of soil environmental factors, such as soil heavy metal concentrations and soil properties, on the metal accumulation in rice grains and brassicas.

The influence of soil properties and the BCR fractions of heavy metals on heavy metal concentrations in rice grains and brassica leaves

Table 4 presents the results of correlation analysis between heavy metal concentrations in rice grains and brassicas and various soil properties: only significant correlations are presented. It can be seen that soil pH, AFO, CFO, and sand content are associated with the concentrations of Cu, Cd, Cr, Pb, and Zn in the rice grains. In general for rice grains, higher correlations are observed for Cu and Zn in comparison the other three metals while Table 4 reveals that the influence of AFO, CFO, and sand content upon metal concentrations in rice grains is generally negative. For the brassicas, correlation analysis reveals that AFO, CFO, and SOM, along with clay, silt, and sand content are associated with metal concentrations in the plants. In general, Table 4 suggests that the influence of SOM on metal accumulation in brassicas is positive while that of sand content is predominantly negative. Table 5 presented significant correlation coefficients between the various heavy metal fractions in the soil and heavy metal concentrations in the rice grains and brassicas. For the rice grains, only three fractions of heavy metals correlate with heavy metal concentrations of the rice grains and brassicas, namely, reducible fraction (RDF)-Cd, RDF-Pb, and residual fraction (RSF)-Zn. In terms of the brassicas, Table 5 reveals that acid soluble fraction (ASF)-Cd, ASF-Cr, and ASF-Zn along with oxidizable fraction (OXF)-Cd and RDF-Pb all significantly correlate with metal concentrations in the brassicas. Some contrasts are

Table 2 Total concentrations of heavy metals in the soils of the sampling locations (mg kg^{-1})

Sampling location	Cu	Cd	Pb	Zn	Cr
Paddy rhizospheric soil					
Close (n = 16)	122.07 ± 72.72*	0.64 ± 0.20 *	46.91 ± 16.78	117.50 ± 27.61*	172.21 ± 84.98*
Mid-distance (n = 15)	42.29 ± 9.19	0.51 ± 0.17 *	36.58 ± 19.29	74.54 ± 17.43	65.26 ± 13.48*
Far away (n = 13)	19.56 ± 1.82	0.24 ± 0.09	25.43 ± 11.54*	61.35 ± 21.91	48.01 ± 6.67
Control sites (n = 9)	15.54 ± 3.90	0.15 ± 0.07	31.65 ± 19.96	58.06 ± 25.56	25.87 ± 8.79
Brassica rhizospheric soil					
Close (n = 13)	40.08 ± 21.89*	0.84 ± 0.28 *	41.53 ± 19.83	103.41 ± 64.46*	72.84 ± 34.02*
Mid-distance (n = 11)	26.01 ± 4.97*	0.38 ± 0.11 *	28.80 ± 14.67 *	74.87 ± 19.28	39.42 ± 7.94
Far away (n = 12)	15.58 ± 3.06	0.18 ± 0.07	19.09 ± 3.43 *	42.14 ± 20.43	39.31 ± 8.38
Control sites (n = 9)	10.92 ± 1.52	0.17 ± 0.07	19.98 ± 11.76	39.45 ± 17.77	25.82 ± 7.73

*$P < 0.05$, significant difference compared with unpolluted sampling area, through multiple comparisons of LSD and ANOVA. For control area, $n > 3$, while for polluted area $n > 10$

evident with more metal fractions correlating with metal concentrations in the brassicas than for the rice grains and also the fact that the ASF fraction dominates in the brassicas but the RDF fraction is predominant for the rice.

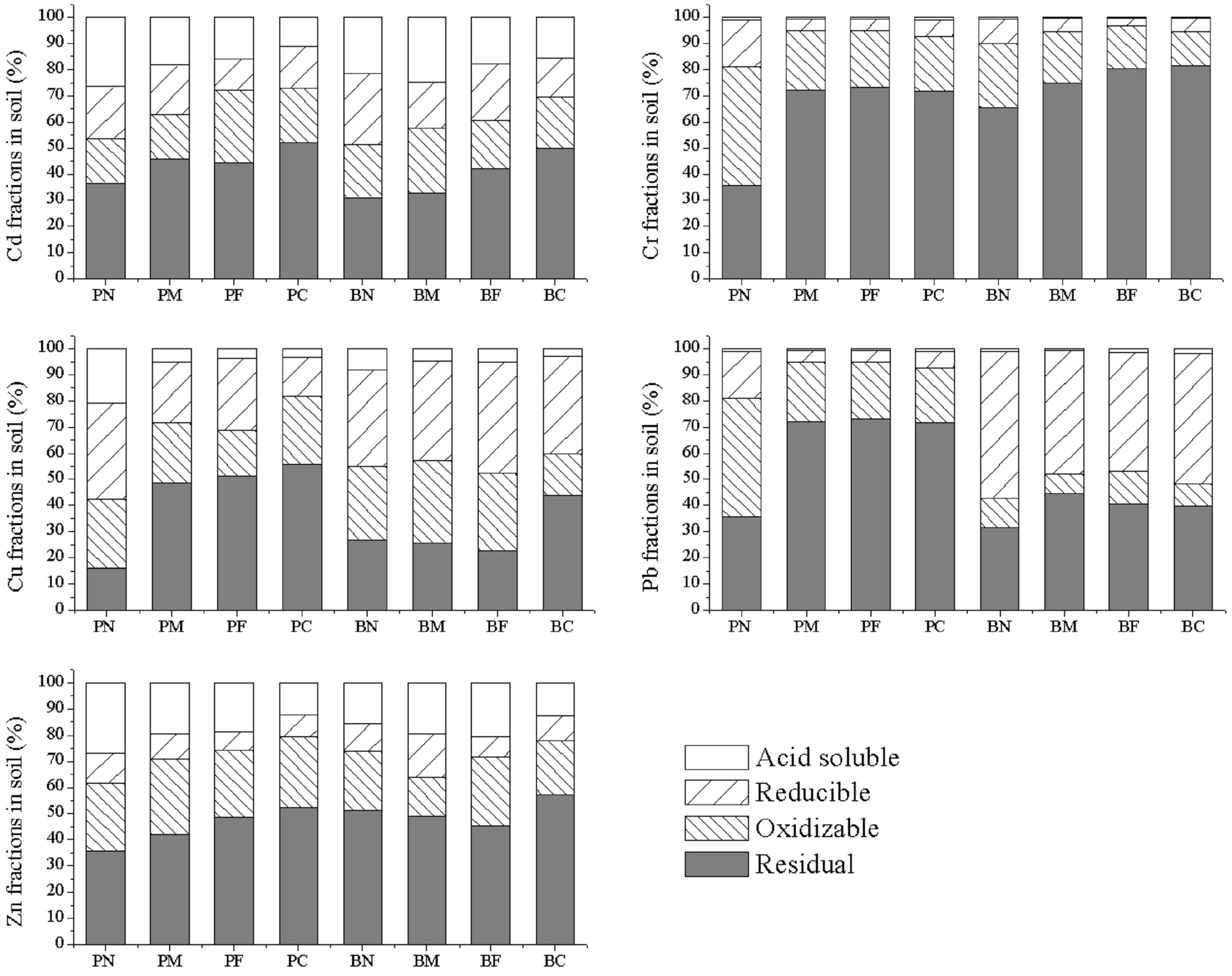

Fig. 2 Average proportions of the four fractions in the total concentrations of five metals from the three locations

Table 3 Heavy metal concentrations in rice and brassicas at the different sampling locations (mg kg^{-1} dry weight)

Sampling location	Cu	Cd	Pb	Zn	Cr
Rice grain					
Close (*n* = 16)	4.48 ± 0.83	0.53 ± 0.31*	0.48 ± 0.30*	40.99 ± 5.70	2.32 ± 1.99*
Mid-distance (*n* = 15)	4.19 ± 0.96	0.31 ± 0.06	0.34 ± 0.14	39.23 ± 3.79	1.13 ± 0.72
Far away (*n* = 13)	4.08 ± 1.35	0.27 ± 0.09	0.29 ± 0.12	37.00 ± 6.53	1.13. ± 0.63
Control sites (*n* = 9)	3.68 ± 0.45	0.18 ± 0.09	0.18 ± 0.07	39.15 ± 5.49	0.64 ± 0.21
Brassica					
Close (*n* = 13)	6.94 ± 3.24*	0.55 ± 0.40*	0.89 ± 0.59	47.94 ± 21.72	1.33 ± 0.75*
Mid-distance (*n* = 11)	7.23 ± 2.50*	0.34 ± 0.25	0.48 ± 0.37	32.53 ± 28.78	0.63 ± 0.41
Far away (*n* = 12)	5.87 ± 2.66	0.24 ± 0.17	0.40 ± 0.19	37.80 ± 15.29	0.71 ± 0.35
Control sites (*n* = 9)	4.23 ± 0.29	0.12 ± 0.07	0.12 ± 0.20	41.09 ± 21.58	0.43 ± 0.14

*$P < 0.05$, significant difference compared with unpolluted sampling area, through multiple comparisons of LSD and ANOVA. For control area, $n > 3$, while for polluted area $n > 10$

The result of DCA showed that the first axis's length of gradient for the metal concentrations in rice grains and brassicas was 0.504 and 1.144, respectively, indicating that hRDA and RDA were appropriate for further analysis of the data. Accordingly, hRDA and RDA were employed to analyze the data to gain further insight into the influence of soil properties and soil heavy metal fractions on the metal content of rice grains and brassicas. The results of the hRDA and RDA are shown in Figs. 3a, b and 4a, b, respectively. According to results from the hRDA, after running a Monte Carlo test 999 times ($P < 0.05$), only RDF-Cd, RSF-Cu, and RDF-Pb remained in explaining the concentration of the study metals in the rice grains, and they could explain 29.0 % of the total eigenvalue, and these metal fractions were also identified by the correlation analysis. The first axis of the hRDA could explain 27.3 % of response variable change and 94.3 % of the relationship between response variables and explanatory factors in hRDA$_{\text{-rice}}$. In Fig. 3a, it can be observed that soil RSF-Zn was positively associated with Cd, Cu, Cr, and Pb in rice grains, indicating that the increase of the stability of Zn, as indicated by the residual fraction, is beneficial for the accumulation of these elements in rice grains. In hRDA$_{\text{-brassica}}$, the selected variables, after running a Monte Carlo test 999 times ($P < 0.05$), were ASF-Cd, OXF-Cd, ASF-Cr, RDF-Pb as well as ASF-Zn, which could explain 46.5 % of the total eigenvalue. These fractions were also identified by correlation analysis. The first axis of hRDA-$_{\text{brassica}}$ could account for 44.4 % of response variables' change and 95.3 % of the relationship between mental concentration in brassica leaves and environment. Figure 3b shows that soil ASF-Zn was negatively correlated with Cd, Cu, Cr, and Pb in brassicas, suggesting that the active fraction of Zn inhibits the accumulation of the other studied metals in brassicas.

Table 4 Correlation coefficients of heavy metal concentration in rice grains and brassica soil with various soil physicochemical properties

	Cu	Cd	Cr	Pb	Zn
Rice grains					
pH	0.92**	−0.33*	0.37*	0.58**	−0.91**
AFO	−0.98**	−0.30*	−0.33*	−0.42**	0.72**
CFO	−0.88**	−0.35*	−0.44**	−0.65**	0.88**
Sand	0.34*	−0.98**	−0.90**	−0.74**	−0.31*
Brassicas					
AFO	0.51**	0.94**	0.78**	0.39*	−0.46**
CFO	−0.86**	−0.53**	−0.47**	0.32*	0.86**
SOM	−0.30*	0.68**	0.44**	0.98**	−0.31*
Clay	−0.32*	0.46**	0.37*	0.92**	−0.32*
Silt	0.95**	0.66**	0.77**	−0.32*	−0.95**
Sand	−0.53**	−0.70**	−0.86**	−0.78**	0.42**

SOM soil organic matter, *CFO* crystalline Fe oxides, *AFO* amorphous Fe oxides

*$P = 0.05$, **$P = 0.01$ probability levels; correlation is significant

Table 5 Correlation coefficients of heavy metal concentration in rice grains and brassica soil with soil heavy metal fractions

	Cu	Cd	Cr	Pb	Zn
Rice grains					
RDF-Cd	−0.48*	−0.41*	0.82**	0.61**	−0.38*
RDF-Pb	−0.55**	−0.72**	−0.59**	−0.40*	0.83**
RSF-Zn	0.81**	0.93**	0.40**	0.57**	−0.92**
Brassicas					
ASF-Cd	−0.82**	−0.32*	−0.32*	−0.45*	0.71**
OXF-Cd	0.43**	0.90**	0.89**	0.69**	−0.46*
ASF-Cr	−0.48**	−0.88**	−0.89**	−0.96**	0.42**
RDF-Pb	−0.87**	−0.34*	−0.35*	−0.37*	0.81**
ASF-Zn	−0.40*	−0.94**	−0.94**	−0.68**	0.37*

ASF-metal ASF-metal acid soluble fraction, *RDF-metal* reducible fraction, *OXF-Metal* oxidizable fraction and residual fraction

*$P = 0.05$, **$P = 0.01$, probability levels; correlation is significant

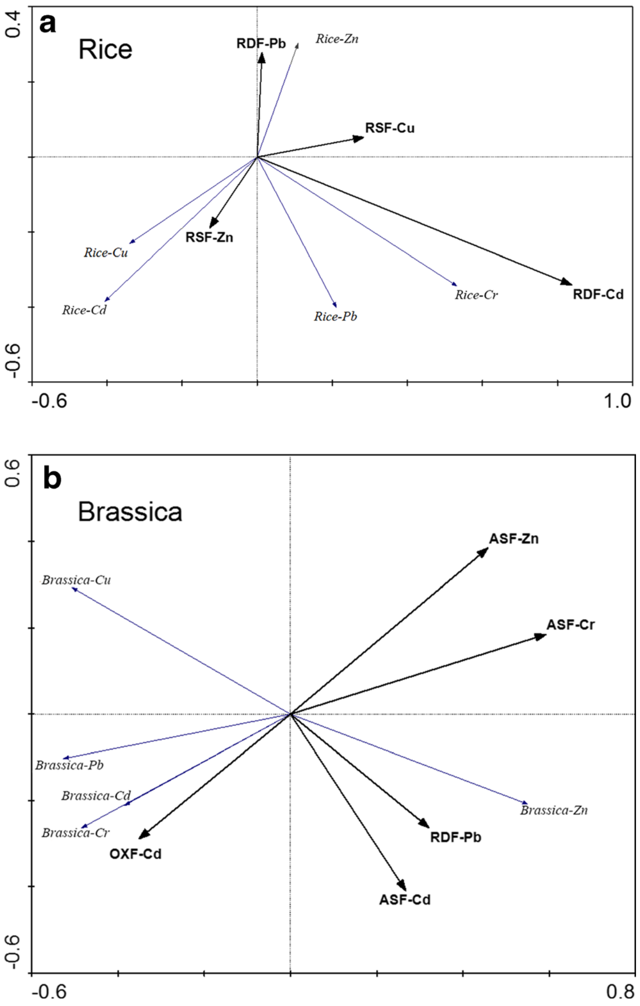

Fig. 3 hRDA biplot of heavy metal fractions in rhizospheric soil and heavy metal concentrations in rice grain (**a**) or in brassicas (**b**)

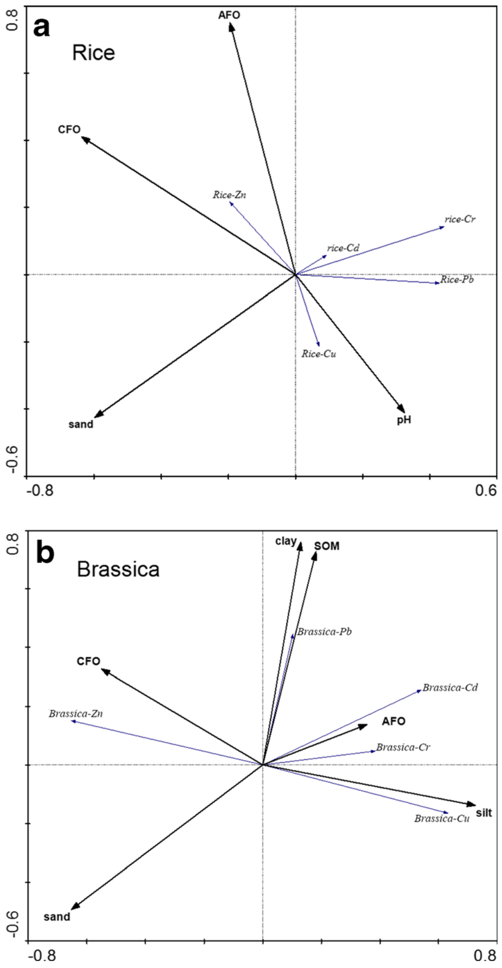

Fig. 4 RDA biplot of soil properties and heavy metal concentrations in rice grain (**a**) or in brassicas (**b**)

RDA was applied to examine the relationship between soil properties and metal concentrations in rice grains and brassica plants (Fig. 4a, b). After running a Monte Carlo test 999 times ($P < 0.05$), the selected variables of RDA$_{\text{-rice}}$ were AFO, CFO, sand, and pH, while those of RDA$_{\text{-brassica}}$ were CFO, SOM, AFO, and soil particle distribution as represented by the clay, silt, and sand content. The selected variables could explain 11.1 % of the total eigenvalue for RDA$_{\text{-rice}}$ and 33.4 % of the total eigenvalue for RDA$_{\text{-brassica}}$, which were much less than those of hRDA$_{\text{-rice}}$ and hRDA$_{\text{-brassica}}$, respectively. This indicated that the soil heavy metal fraction had more effect on metal accumulation in the studied plants compared to soil properties. In RDA$_{\text{-rice}}$, although the first two axes could only explain 10.9 % of the variation of metal concentration in rice grains, it could offer 98.6 % explanation of the relationship between metal concentration in rice grains and soil properties. In RDA-brassica, the first two axes could account for 31.8 % of change of metal concentration in brassicas and 95.3 % of the relationship between it and soil properties. It is worth noting, based on the results of both the hRDA and RDA, that the soil heavy metal fraction was more significant for estimation of the heavy metal concentrations in rice grains and brassicas than were soil properties.

To further predict the metal concentration in rice grains and brassicas using the metal fractions in the soil and soil properties as independent variables, multiple stepwise linear regression has been employed. The results of the analysis are as shown in Table 6. With the exception of Pb, all the heavy metal concentrations in rice grains and brassica plants could be fitted with equations by stepwise multiple regression. The multiple regressions showed that the reducible fraction of both Cd and Cr was retained as a variable for explaining their concentrations in rice grains, demonstrating that the reducible fractions of Cd and Cr have a more fundamental effect on the accumulation of these two metals in rice grains than the other factors. Although the variables are distinct for different metals, it is interesting to note that the uptake of Cd, Cr, and Cu by brassicas was related only to soil properties based upon the regression analysis. This indicates that improving soil properties is important in controlling heavy metal uptake in brassicas, which has important implications for food safety.

Table 6 Summary of multiple regressions between heavy metal concentration in rice grain or brassicas and soil heavy metal fractions and physicochemical properties

Element	Equation	R^2	$R^2_{\text{adj.}}$	*P* value
Rice				
Cd	$\mathrm{Log}_{10}(\mathrm{CdR}) = 0.174 + 0.550\mathrm{Log}_{10}(\mathrm{Cd}_{\text{-RDF}})$	0.544	0.498	0.006
Cr	$\mathrm{Log}_{10}(\mathrm{CrR}) = -0.128 + 0.308\mathrm{Log}_{10}(\mathrm{Cr}_{\text{-RDF}})$	0.441	0.385	0.019
Cu	$\mathrm{Log}_{10}(\mathrm{CuR}) = -0.646 + 0.653\mathrm{Log}_{10}(\mathrm{SOM}) - 0.215\mathrm{Log}_{10}(\mathrm{AFO}) + 0.141\mathrm{Log}_{10}(\mathrm{CFO})$	0.936	0.912	<0.001
Pb	No stepwise regression equation	–	–	–
Zn	$\mathrm{Log}_{10}(\mathrm{ZnR}) = 1.143 – 0.273\mathrm{Log}_{10}(\mathrm{AFO}) + 0.103\mathrm{Log}_{10}(\mathrm{Zn}_{\text{-RSF}}) - 0.140\mathrm{Log}_{10}(\mathrm{clay}) + 0.171\mathrm{Log}_{10}(\mathrm{CEC})$	0.963	0.942	<0.001
Vegetable				
Cd	$\mathrm{Log}_{10}(\mathrm{CdV}) = 2.479\mathrm{Log}_{10}(\mathrm{CEC}) - 2.090$	0.478	0.42	0.018
Cr	$\mathrm{Log}_{10}(\mathrm{CrV}) = 0.814\mathrm{Log}_{10}(\mathrm{SOM}) - 1.208$	0.431	0.368	0.028
Cu	$\mathrm{Log}_{10}(\mathrm{CuV}) = -0.086\mathrm{pH} + 1.315$	0.489	0.432	0.017
Pb	No stepwise regression equation	–	–	–
Zn	$\mathrm{Log}_{10}(\mathrm{ZnV}) = 1.986\mathrm{Log}_{10}(\mathrm{Zn}_{\text{-OXF}}) - 0.559$	0.377	0.307	0.045

$Metal_{-ASF}$ acid soluble fraction, $Metal_{-RDF}$ reducible fraction, $Metal_{-OXF}$ oxidizable fraction, $Metal_{-RSF}$ residual fraction, *SOM* soil organic matter, *CFO* crystalline Fe oxides, *AFO* amorphous Fe oxides

Discussion

The effects of heavy metal fraction on metal accumulation in rice grains and brassica plants

Based upon the results presented in Fig. 2 and Table 3, which, respectively, show that the active metals, as represented by the acid soluble, reducible, and oxidizable fractions, in the soil decrease with distance away from the electroplating factories and that the metal content of rice grains and brassicas decreases with distance from the factories, it can be inferred that the increase of heavy metal bioavailability in soil enhances the accumulation of heavy metal accumulation in rice grains and brassicas. Table 6 presents the results of the multiple regression analysis on controls upon metal accumulation in rice grains and brassica leaves. The results show that for rice grains the reducible fractions of Cd and Cr in soil were important and dominant as a control upon them as they were the only variables included in the predictive equation. Regarding the metal content in brassicas, Table 6 reveals that the soil oxidizable fraction of Zn is the major factor that affects Zn accumulation in brassicas. In addition, the bioavailable fractions of heavy metals in Table 6 were all positively correlated with the concentration of metals in plants, which is similar to the results of Fig. 2 and Table 3, indicating that soil heavy metal fractions have a direct and important effect on metal accumulation in rice grains and brassicas.

By means of hRDA and RDA, this study has identified that the soil heavy metal fractions have higher eigenvalues compared to soil properties in the estimation of metal accumulation in both rice grains and brassica plants, confirming the major controlling effects of soil heavy metal fractions on heavy metal accumulation in rice grains and brassicas. This reflects the fact that, for most plants, the uptake of heavy metals is mainly through the root system (Clemens and Ma, 2016) and it is the soil heavy metal fractions which determine the behavior and fate of metals and hence dictate their availability and mobility (Roussiez et al. 2011). Consequently, the accumulation of heavy metals in plants depends on soil heavy metal bioavailability.

In more detail, both the multiple regression and hRDA for the rice grains identify that the reducible fraction is important for Cd accumulation, and the former also identifies it as being controlling for Cr while the latter (hRDA) identifies reducible Pb and Zn in the soil as influencing their content in rice grains. In contrast, for brassica leaves, the reducible fraction was not identified as controlling for any of the five studied metals. In the literature, Agrawal et al. (2016) found that the reducible fraction of heavy metals could release from Fe/Mn oxyhydroxides and become bioavailable under certain circumstance. Khaokaew et al. (2012) demonstrated that paddy soil, which is anaerobic due to flooding and reducing tillage, is beneficial to the releasing of heavy metals from Fe/Mn oxyhydroxides. As a result, reducible fractions of Pb and Zn in the paddy soil could become bioavailable and play an important role in their accumulation in rice grains.

As regards to the brassicas, multiple regression analysis only identified the oxidizable fraction for Zn as being controlling, while the hRDA identified oxidizable Cd as important. This oxidizable fraction of metals is regarded as bioavailable since it can be bound to organic matter which has a high affinity to plants (Ondrasek and Rengel 2012; Anjum et al. 2015), while Liu et al. (2008a) and Shahid et al. (2012) have reported that such metals combine with low molecular weight organic acids which is beneficial for their transport in plants. In addition, the redox condition of brassica soil is aerobic due to the frequent turning over of the soil during planting, which favors the oxidation of organic matter and hence enhances metal release from organic matter (Kumar et al. 2013). Thus, any metal combined with relatively high molecular weight organic matter in brassica soil could also be bioavailable which is favorable to its accumulation by plants. The hRDA results of the brassica leaves reveal that the acid soluble metal fraction in the soil was also very important for Cd, Cr, and Zn accumulation, demonstrating the significant effect of soil heavy metal fractions.

The effects of soil properties on metal accumulation in rice grains and brassica plants

Soil properties may affect metal concentration in rice grains and brassicas and, as noted previously, the multiple regression analysis (Table 4) identified their influence. The relative importance of metal fractions and soil properties can be evaluated using the results of the hRDA and RDA, with percentage explanations for metal content in rice and brassicas of 29 and 46.5 %, respectively, from available metals and 11.1 and 33.4 %, respectively, for rice grains and brassicas from soil properties, showing that the importance of soil properties is lower compared to soil heavy metal fractions, in explaining the metal accumulation.

Accepting the lesser importance of soil properties as compared to soil heavy metal fractions, it is still of interest to see what soil properties are identified in the RDA as being important controls upon metal content of rice and brassicas. In the present study, pH and soil organic matter had minor effect on metal accumulation in brassicas and rice grains, respectively, which is different from some previous studies (Clemente et al. 2003; Steiner et al. 2007; Zhang et al. 2010), which recognized soil pH and organic matter as vital factors that influence the accumulation of heavy metal in plants. In this study, sand content and CFO could inhibit the accumulation of heavy metals in rice grains and brassicas, while AFO inhibited their accumulation in rice grains but enhanced their accumulation in brassicas. The study of Acosta et al. (2009) and Ljung et al. (2006) showed that increased sand content in soil indicates the decrease of surface area, and less negative charges and surface energy, which is unfavorable for the retention of metals in soil. Cheng et al. (2014) found that AFO and CFO in the forms of ferric hydroxides, geothites, and lepidocrocites could form an iron plaque, by the oxidation of ferrous iron to ferric iron, leading to the precipitation of iron oxide on the plant root surface. Such plaque on the root surface of aquatic plants (e.g., rice) can inhibit the migration of Cd and Cu and partly inhibit the migration of Pb into both plant roots and shoot tissues (Chang et al. 2014b; Zhang et al. 2013). On one hand, AFO and CFO can bind with toxic heavy metals and result in co-precipitation outside of the plant tissues (Liu et al. 2008b; Zhou et al. 2015), while on the other hand, the plaques can supply a large amount of Fe^{2+} to compete with heavy metals for metabolic sensitive sites (Cheng et al. 2014). In addition, AFO and CFO, as inorganic colloids, can reduce the bioavailability of soil heavy metals (Komárek et al. 2013). It was noted in the introduction that Chang et al. (2014a) state that the role of soil pH upon metal availability in the soil and plant uptake is conflicting and controversial. In this study, the RDA picked out pH as influencing metal content in rice grains, but it was not important in brassicas. However, the multiple regression analysis for Cu in brassicas identified it as being influential in metal concentration, but it was not included for the other four metals. This is somewhat supportive of the observation of Chang et al. (2014a) that the effect of soil pH on metal accumulation in leaf vegetable is not clear, in a study also undertaken in South China. Despite the influence of soil properties upon metal accumulation in plants identified in this study, it should be noted that soil properties are indirect factors that affect the bioavailability of soil heavy metals or the physiology of the plants (Clemente et al. 2003; Zhang et al. 2010). The comparison of the eigenvalues associated with the hRDA and RDA for metals and soil properties, respectively, in this study, revealed the effect of the soil heavy metal fractions upon metal accumulation in rice grains and brassicas to be most important.

The interaction between soil heavy metal fractions and metal concentrations in rice grains and brassica

In Fig. 3a, b, which presents the results of the hRDA, it was concluded that the increase of Zn bioavailability reduced the accumulation of Cd, Cu, Cr, and Pb in both rice grains and brassica. The interaction between soil Zn and other heavy metals in rice and brassica observed in this study has been well documented by previous studies. Yoneyama et al. (2015) indicated that Zn and Fe concentrations in rice grains under different levels of soil/hydroponic metals are known to change only within a small range while the concentrations of Cd show greater change. Balen et al. (2011), however, found that with a supplement of Zn, the accumulation of Cd in plants reduced, which is in agreement with the present study. The mechanism that causes the antagonistic effect of Zn and Cd is because on one hand, as a non-essential element, the uptake of Cd by plants is non-specific; instead, they enter plant cells via uptake systems for essential cations (Clemens 2006). Zn and Cd are transported by a common carrier at the root plasma membrane, which has a higher affinity for Cd than for Zn, indicating that Cd and Zn should experience competitive inhibition (Hart et al. 2002). On the other hand, the number of intercellular metal-binding sites and their affinity results in different distributions for Cd and Zn in plant tissues (Clemens et al. 2002). The residual Zn in paddy soil was positively related to the Cd concentration of rice grains in this study, and the acid soluble Zn was negatively associated with the Cd concentration in brassicas, confirming that the bioavailability of Zn in soil is a key factor influencing Cd accumulation in the rice grains and brassicas. Hafeez et al. (2013) noted that Zn could strongly depress Cu absorption within plants in a pot experiment, due to a competitive inhibition of Zn on Cu absorption in plants. In Fig. 3a, b, it was observed that under field conditions, the accumulation of Cu in rice grains and brassicas was negatively correlated with the bioavailability of Zn, indicating that the available Zn in soil inhibits the accumulation of Cu in both rice grains and brassicas.

This study also found that the concentration of Pb and Cr in rice grains and brassicas was positively associated with reducible Cd and oxidizable Cd, respectively. Zeng et al. (2008) found that the concentration of Pb in rice grains was significantly and positively correlated with soil Cd concentrations; however, Cr in rice grains was not correlated with Cd in soil. Xu et al. (2014) reported that Cd enhances the activity of phytochelatin synthase which would further increase the tolerance and the accumulation of Pb by plants. Thus, a synergistic effect should exist between soil Cd concentrations and the Cr and Pb concentrations in rice grains and brassicas. Furthermore, our observations suggest that it was the reducible fraction of Cd in paddy soil and oxidizable Cd in brassica rhizospheric soil that tended to be released and become a major factor that influenced the metal concentrations in plants. This is supported by the study of Fulda et al. (2013) who observed that Cd may be mobilized by reductive dissolution of Mn(III/IV)- and Fe(III)-(oxyhydroxide) sorbent phases and concomitant increases in dissolved Mn^{2+} and Fe^{2+} that compete with Cd for sorption sites. In addition, Gerringa (1990) indicated that soluble Cd could be released by the degradation of particulate organic carbon in an aerobic environment such as is found in soils used to grow vegetables. Kumar (2013) also demonstrated that brassica soil is aerobic due to frequent turning over of the soil during the planting, which benefits the oxidation of organic matter and hence enhances metals that release from organic matter.

From Figure 2 and Table 3 which, respectively, present the results of the BCR metal fractionation for the soil and the metal concentration of rice grains and brassicas with distance downstream from the electroplating factory outlets, it can be seen that similar trends exist for the residual Zn in paddy soil and the concentrations of Cd, Cu, Cr, and Pb in rice grains. For the brassicas, soil acid soluble Zn decreases with the increase of distance downstream from the factory outlets but the concentrations of Cd, Cu, Cr, and Pb in brassicas increase. Meanwhile, the trends of soil Cd fractions and the concentrations of Pb and Cr in rice grains and brassicas are very similar. These results demonstrate the interaction of soil heavy metal fractions and heavy metal concentrations in rice grains and brassicas and also indicate soil heavy metal fractions should have a significant impact on metal accumulation in the crops.

Conclusions

Long-term irrigation with electroplating effluent enriched soils in the study area with Cd, Cd, Cr, and Zn and also increased the mobility and bioavailability of these metals. In addition, the concentration and the bioavailability of these metals in both paddy and brassica rhizospheric soil showed a declining trend with the increase of sampling distance away from the electroplating factories. With respect to Cd, some 65 and 64 %, respectively, of the rice grain and brassica plant samples exceeded national food safety standards, while for Cr, the equivalent values are 46 and 36 %, respectively, for rice and brassicas. Concentrations of heavy metals in rice grains and brassicas from the closest areas (0–0.1 km) to the electroplating factories were significantly higher than those from the control areas, similar to the result of total heavy metal concentrations in soils of the study area ($P < 0.05$). Similar trends of metal concentration with distance downstream from the electroplating outlets could be observed in both rice grains and brassicas, indicating the potential influence of soil factors.

Correlation analysis identified some controlling heavy metal fractions, derived from a BCR fractionation, and soil properties upon the heavy metal concentrations of rice grains and brassicas. Their influence and relative importance were clarified using hRDA, RDA, and stepwise multiple regression analysis. The hRDA and RDA results showed that 29.0 and 46.5 % of the metal concentration variation in the rice grains and brassicas, respectively, were determined by the BCR fractions of soil heavy metals and that 11.1 and 33.4 %, respectively, of metal concentration variation were controlled by soil properties, suggesting that metal fractionation was much more significant for the estimation of metal concentrations than were soil properties. Sand content and CFO were the major factors that reduced the accumulation of heavy metal in rice grains and brassicas in this study. In terms of metal interactions, an increase of residual Zn in soil could enhance the accumulation of Cd, Cu, Cr, and Pb accumulation in rice grains while an increase of acid soluble Zn could reduce the accumulation of these metals in brassicas. Cd could be released from reducible Cd in paddy soil and oxidizable Cd in vegetable soil, and this enhanced the accumulation of Cr and Pb in both rice grains and brassicas. Multiple stepwise linear regression could offer prediction for Cd, Cu, Cr, and Zn concentrations in rice grains and brassicas by soil environmental factors. The reducible fraction of Cd and Cr was retained as a variable for explaining the concentrations of Cd and Cr in rice grains, demonstrating that the reducible fractions of Cd and Cr have a more fundamental effect on the accumulation of these two metals in rice grains than the other factors do. Although the variables are distinct for the different metals, it is interesting to note that the uptake of Cd, Cr, and Cu by brassicas was related only to soil properties based upon the multiple regression analysis. Considering the significance of metal fractions and their interaction demonstrated in this study, further research should be conducted to improve estimation of the metal concentrations in plants based on the differential significance of the various metals in their uptake by plants.

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