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Effect of cadmium, lead, and arsenic from mining contamination on human HEPG2 and keratinocyte cell-lines

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Abstract

A mining district in south China shows significant metal(loid) contamination in paddy fields. In the soil, Pb, Cd and As were 516.7, 11.7 and 35.1 mg·kg^{-1} respectively. The content of Cd exceeded the environmental quality standard for agricultural soils in China by approximately 82%. The contents of Pb, Cd and As in rice were 22.7, 1.1 and 0.7 mg·kg^{-1} respectively. These concentrations are also higher than the agricultural industry standard of China for rice. The elevated contents of Pb, Cd and As detected in soils around the factories, indicated that their spatial distribution was influenced by anthropogenic activity. Greater concentrations of Cd in rice appeared in the northwest region of the factories, indicating that the spatial distribution of heavy metals was affected by natural factors. The metals affected the viability of HepG2 and KERTr cells, which decreased with increasing metal concentration. Co-exposure to heavy metals (Pb+Cd) increased the metals (Pb or Cd)-mediated MT protein induction in both human HepG2 and KERTr cells. Increased levels of MT protein will lead to greater risk of carcinogenic manifestations, and it is likely that chronic exposure to metals may increase the risk to human health. Nevertheless, when co-exposure to two or more metals occur (such as As+Pb), they may have an antagonistic effect thus reducing the toxic effects of each other.

Keywords: arsenic; cadmium; cell exposure effects; lead; soil contamination

1. Introduction

Soil pollution, especially in paddy fields, is an increasing concern for China. Pollution is predominantly from industrial and agricultural activities, whilst the soils in the south are more polluted than north China (Zhao 2015). Mining activities usually result in large volumes of waste materials, tailings, and acid mine drainage, which often contain high concentrations of potentially toxic elements (As, Cu, Zn, Cd, Pb etc). High concentrations of heavy metals can be found in and around abandoned and active mines due to the discharge and dispersion of mine waste into nearby air, water and soils (Witte et al. 2004; Liao et al. 2005). Metals accumulated in crops growing in the polluted soils potentially pose a health risk to residents in these areas (Wong et al. 2002; Galán et al. 2003; Zheng et al. 2007). Exposure routes differ, and may be through ingestion of vegetables grown on contaminated soils or through dust inhalation and dust adhering to plants (Seyfferth et al. 2014; Li et al. 2015).

Soil and crop pollution as a result of mining activities is now an important issue around the world (Candeias et al. 2014). Copper mining activities in the Vigonzano district of northern Italy has created multi-contamination of Cr, Ni and Cu (Dinelli and Tateo 2001). Karim et al. (2015) showed that soils in Karachi, Pakistan, which are influenced by intensive anthropogenic activities have exceptionally high concentrations of Pb. Furthermore, sediments in the Baixo Jacuí region, southern Brazil, are polluted with Cu, Fe, Ni, Pb, and Zn contamination from coal-related activities (Teixeira et al. 2001). Espinosareyes et al. (2014) showed mining activities caused severe pollution in the district of Villa de la Paz, Mexico, which showed that the concentrations of As and Pb in soils were higher than the national regulations for urban or agricultural areas. Candeias et al. (2014) investigated the environmental contamination impact on agricultural and residential soils in S. Francisco de Assis village due to mining and found that As in vegetable rhizosphere soils exceed 20 times the reference value for agricultural soils and some edible plants frequently used in the region could be enriched in metals/metalloids and may represent a serious hazard if consumed. Cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) in Australian grown rice and vegetables were investigated, and showed that brown rice was a potential source of dietary heavy metals (Rahman et al. 2014). The mean values for Pb and Cd in domestic cultivated and imported rice were considerably higher than limits set by FAO/WHO(Naseri et al. 2015). Mining activities around rice cultivation has increased the heavy metal contamination of paddy soils (Zhu et al. 2008; Williams et al. 2009), for example in Hunan province, where Liao et al. (2005) found that As in rice in Chengzhou, Huan contained concentrations of 7.5 mg/kg，significantly higher than China food standard maximum limits for rice（0.2 mg/kg. Zhou et al. (2004) investigated total contents and chemical species of heavy metals in tailings and soils in a vicinity of Dabaoshan mine, Guangdong province, and found that the paddy fields irrigated from the Hengshi River had been contaminated by heavy metals.

Average concentrations of Cu, Zn, As and Cd were 560.91, 1135.08, 218.07 and 2.453 mg/kg, greatly exceeding the recommended Environmental Quality Standard for soils.

Geostatistical techniques with variography and kriging have been commonly used to model the spatial structure and delineate the spatial variability of soil properties as well as physicochemical properties in soil environment (Lv et al. 2013). Geostatistical techniques were used to quantify the spatial heterogeneity of organic carbon and total nitrogen of a monsoon evergreen broadleaf forest soil in Dinghushan，Guangdong, China (Zhang and Ou et al., 2014). Zhang and Nie et al., (2014) used geostatistical techniques with kriging to investigate the spatial distribution, fractionation and contamination degree of heavy metals in soils of gold mine and tailings of Pinggu in Beijing, and found that As, Cd, Cu, Pb and Zn pollution was more serious in the gold mine and surrounding area, which was obviously affected by human activities. Furthermore, heavy metal spatial variations in agricultural soils of China obtained through Kriging showed that heavy metals have clear regional characteristics and the southwest of China has relatively high heavy metal concentrations in soils. The ordinary point kriging estimates of Pb concentration were mapped. White et al. (1997) created soil Zinc Maps of the USA using Geostatistics and Geographic Information Systems. They showed spatial correlation at distances up to 470 km. Rodríguez et al. (2008) characterized and mapped the spatial variability patterns of seven topsoil heavy metals (Cr, Ni, Pb, Cu, Zn, Hg and Cd) within the Ebro river basin in Spain by Multivariate Factorial Kriging. Ersoy et al.

(2004) determined the extent and severity of the pollution levels on land contaminated by past mining activity through using geostatistical techniques.

Based on the criteria of frequency of occurrence in the environment, toxicity, and potential exposure to humans, heavy metals (such as arsenic[As], cadmium [Cd] and lead [Pb]) are ranked highly as the most hazardous substances in the environment (ATSDR, 2007). Other studies also revealed that Cd (Achanzar et al. 2001), Pb (Martin et al. 2003) and As (Singh et al., 2015) were harmful to human liver, and endocrine and reproduction systems. The contaminant levels contained in adipose tissue of uterine fibroid disease patients revealed that, As (0.59 μg/kg fat; 0.32), Cd (0.38 μg/kg fat; 0.27) and Pb (5.24 μg/kg fat; 3.36) were significantly higher ($p<0.01$) in patients with uterine fibroid disease than their normal counterparts (Yan et al. 2010). It has also been revealed that elevated levels of Pb and Cd were detected in smokers' blood, milk and hair (Mortada 2004; Godschalk et al. 2005). However, the majority of published data concerning toxicity of Cd, Pb and As has been conducted separately (Martin et al. 2003; Yoshida et al. 2004; Singh et al., 2015). Only a few studies concerned the co-exposure effects of Cd, Pb and As in the environment (Vakharia et al. 2001a, b).

Metallothioneins (MTs) are ubiquitous low molecular weight proteins which contain 20 cysteine residues in mammalian MTs at invariant positions, and bind heavy metals such as Zn, Cu, Cd and Hg (Klaassen, Liu et al. 1999; Tapiero and Tew 2003; Chasapis, Loutsidou et al. 2012). Thus, the protein has been considered a suitable biochemical marker for metal exposure. Previous studies employed the MT proteins to monitor the coastal sediments which are contaminated by heavy metals (Wong et al. 2000; Kwok et al. 2010). In the present study, MT protein is used to assess the changes of co-exposure by heavy metals, and to investigate the influence of heavy metals on the induction of MT protein on human cell lines.

Human exposure around mining districts is mainly through oral intake of food and dermal contact. The HepG2 cell line has been used to evaluate the potential adverse effects of metals on human health via food ingestion (Huang et al., 2015; Darwish et al., 2016) whilst KERTr cell model has been used to evaluate house dust via dermal contact (Arlian et al. 2008; Kang et al., 2014). The objectives of the present investigation were firstly to investigate paddy soil and rice contamination spatial variability around a lead mining area of south China and secondly explore the co-exposure effects of heavy metals (Cd, Pb, As) on human HepG2 cells and human keratinocyte cells.

2. Materials and Methods

2.1. Sampling area

The sampling area is near a mining district of south China. The climate is subtropical and average rainfall is about 1300 mm. The average temperature is 18℃, with lowest temperature 9-3 ℃ in January and highest temperature 26-35 ℃ in August. The mine is one of the largest Pb in South China. The main metal products are as follows, Pb, Zn, Ag, Au, Cu, Mn and have caused significant pollution to paddy fields through discharge of waste gas, water and residues from the mining activities.

2.2 Sample collection and treatment

Soil samples were collected in the paddy fields around the mining area, on which samples of rice were also correspondingly collected. Soil samples were collected to a depth of 20 cm, from the paddy fields. In total, 147 soil samples and 129 rice samples were collected around the area of the mine. Ninety-five percent of the agricultural production in this areas investigated is for self-consumption. A mesh method （500×500m）was used for sampling, whilst sampling points were increased in the dense paddy field areas. The soils were air-dried and ground to pass through a 100 mesh screen. The plants were washed with tap water to remove adhering soil, rinsed with deionized water, oven dried at 60 ℃ for 48h and then ground to a fine powder using an agate mortar and pestle.

2.2. *Heavy metal determination in soils and rice*

Soil pH values were measured in a 1:2.5 soil-water suspension. The soils were digested by using HNO_3-HF-$HClO_4$ (Lu 2000; Lv et al. 2013), while Pb concentrations were determined by ICP-AES（Optima 5300DV, Perkin Elmer, USA）, and Cd contents were determined by graphite furnace atomic absorption spectrophotometer (Lu 2000).

For total As determination, rice samples were digested using HNO_3 (Wu et al., 2016), and determined using HG-AFS (AFS-8230, Beijing Jitian Instruments Co., China) (Shi et al., 2013; Wu et al., 2016). A certified reference material (bush branches and leaves, GBW07603) was used and As recovery ranged from 85.5% to 93.5% (n = 3).

2.3. *HepG2 and Keratinocyte Cell Cultures*

Sodium arsenite, lead nitrate, and cadmium chloride (all 99 to 100% pure) were obtained from Sigma-Aldrich, USA. A 10 to 20 mM stock solution of each salt was prepared using deionized water. Stock solutions of metals were stored at room temperature (25℃) and fresh dilutions were made prior to each experiment.

The HepG2 cell line (human hepatocellular liver carcinoma cell line) and CCD 1106 KERTr cell line (human skin derived keratinocyte) were obtained from the American Type Culture Collection (ATCC, Rockville, USA). The HepG2 cells were grown in Eagle's minimal essential medium, supplemented by 10% fetal bovine serum, which the KERTr cells were grown in Keratinocyte-serum free medium (Gibco, USA), supplemented with 0.05 mg/ml bovine pituitary extract (BPE) and 35 ng/ml epidermal growth factor (EGF). The HepG2 cell cultures were maintained in 25-cm^2 (Keratinocyte cell in 75-cm^2) surface area tissue culture flasks from Nunc (Denmark), in a 5% CO_2 incubator (2406-2, Shellab, USA) at 37 ℃.

During culture growth at around 80-100% confluence, the HepG2 cells were trypsinized, counted, and seeded onto 96-well tissue culture microtiter plates at a density of 2×10^4 cells/100µl/well. The KERTr cells were seeded onto 96-well tissue culture microtiter plates at a density of 1×10^4 cells/100 µl/well. After 24 h, the cell culture was removed and replaced by 100 µl culture media containing single metals or their mixtures. In each well, the final concentration of DMSO was limited to 0.5%.

2.4. Measurement of Cell Viability

HepG2 and KERTr cell viability after treatment with metals was determined by testing the capability of reducing enzymes present in viable cells to convert 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) to formazan crystals (Vakharia et al. 2001a; Vakharia et al. 2001b). After 24 h incubation, the culture media were removed and the cells washed twice with warm PBS. The cells were then incubated with serum free medium containing 0.5 mg MTT/ml at 37℃ for 4 h. The media were removed and replaced with 100 µl DMSO to dissolve the violet crystals. The plate was covered and shaken for 15 min. Then, the colored solution was detected at 540 nm and at 690 nm as a reference wavelength, using a spectrophotometer (Elx 800, BioTek, USA). 0.5% DMSO treated cells were used as the 100% viable control.

Plasma membrane integrity was assessed by measuring LDH leakage into the culture medium (Bergmeyer and Bernt 1974; Peters et al. 2004). The reduction of NADH in the presence of pyruvate was measured in the culture medium of cells that had been exposed to the metals for 72 h. In one cuvette 100 µl medium, 1 ml phosphate buffer containing 66 mg/l pyruvate and 20 µl NADH were added and measured spectrophotometrically (UV-1601, Shimadzu, Japan) at 340 nm (every 0.4s during 20s at room temperature). The control was performed with 0.1% (w/v) Triton X-100 and set as 100% LDH release.

2.5. Metallothionein Bioassay

The bioassay was based on the method described by Yang et al. (1993)(Yang et al. 1993). The cells were washed twice with 2 ml of the provided 'Wash Buffer' and then homogenized with 1 ml of 0.2M Na-phosphate buffer and 5 µl of protease inhibitor. After cooling on ice for 10 min, the homogenates were centrifuged at 1000 g at 4℃ for 10 min. The supernatant was then used for MT quantifications. All procedures were performed on ice to prevent denaturation of proteins.

For metallothionein determination, about 500 µl of supernatant was first transferred into a 1.5 ml micro-centrifuge tube and heated in a water bath at 80℃ for 5 min to destroy unwanted proteins. The tubes were then cooled in an ice bath and centrifuged at 9000 g at 4℃ for 5 min. Supernatant (230 µl) was added with 120µl of 4 mM EDTA (Sigma-aldrich) (added with 1N HCl (Riedel-de Haën) in a 1.5ml plastic cuvette). 150 µl of freshly prepared 2M NaCl + 0.43 mM DTNB (5,5′-Dithiobis(2-nitrobenzoic acid), Sigma-aldrich) and 1000 µl of phosphate buffer (Na_2HPO_4 + NaH_2PO_4(Riedel-de Haën), pH 8.0) were added into the cuvettes, mixed and incubated for 15 min. Absorbance of the mixtures were then measured at 412 nm using a spectrophotometer (Tecan Infinit F200, Tecan, Switzerland) (Ellman 1958).

2.6. Data Analysis

All data were represented as mean ±SD and analyzed using SPSS 23.0. Maps were generated using the ArcGIS V10.2 (ESRI Corporation). Figures were created using Origin 8.0. Statistical significance was tested by Student’s t-test or one-way analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test.

3. Results and Discussions

3.1 Contamination in paddy soils

The heavy metal concentrations and pH of the paddy soils are shown in Table 1. The average concentration of Pb, Cd and As in paddy soils were 516.7 mg·kg^{-1}, 11.7 mg·kg^{-1} and 35.1 mg·kg^{-1} respectively.

The coefficient of variation of Pb, Cd and As concentrations in the mine district were 130%, 253% and 121% respectively. The order for the metals were: As < Pb < Cd. The coefficient of variation (CV), also known as relative standard deviation (RSD), is a standardized measure of dispersion of a probability distribution, it reflects the average variance for the sampling points. The greater the coefficient of variance, the larger the variance between metal concentrations of the sampling points, thereby showing more influence from human activities. The lower the coefficient of variance, the smaller the variance between the metal concentrations of the sampling points, which shows less influence by human activities (Atalay et al. 2007). The coefficient of variance of Pb, Cd and As were larger than 100%, showing a high degree of variance (Zhang and Lei 2013). This demonstrated that the metal concentrations of surface paddy soils were uneven between sampling points, and mainly affected by human activities, not soil properties.

The skewness of metal concentrations was greater than 0, revealing that surface soil metal concentrations were affected by exterior factors. Sample Kurtosis reflects the variance of metal concentrations, showing Cd concentration variance was much more significant than Pb and As (Sun et al. 2014). According to the environmental quality standard for agricultural soils in China (GB15618-1995), average concentrations of Pb, Cd and As all exceeded the second level criterion for agricultural soils, especially for Cd contamination. Average concentrations of Pb, Cd and As were approximately 17, 92 and 2 times greater than background values, indicating that exterior input of these elements had important effects on the accumulation in soils, especially being significant for Cd.

3.2 Contamination in rice

Heavy metal concentrations in rice are shown in Table 2. The average concentrations of Pb, Cd and As were 22.7 $mg\cdot kg^{-1}$, 1.1 $mg\cdot kg^{-1}$ and 0.7 $mg\cdot kg^{-1}$ respectively. According to the China food safety quality standard for maximum levels of contaminants in foods (GB 2762-2012), average concentrations of Pb exceeded the criterion being approximately 114 times greater than the limit value. According to the China agricultural industry standard (NY 861-2004), Pb concentrations in rice were about 57 times greater than the limit value; Cd also exceeded the limit value, whilst As did not exceed the limit value (Table 2).

The coefficient of variation for Pb, Cd and As concentrations in rice were all greater than 100%, showing a high variance, and being significantly influenced by external inputs from human activities.

The bioconcentration factor (BF) of rice for the three metals were calculated according to the following equations (Mcgrath and Zhao 2003): $BF = [Metal]_{shoot} /[Metal]_{soil}$, which reflected the uptake and accumulation of metals in rice grains. The BF order was: As＞Pb＞Cd, with BF 1.06, 0.90 and 0.72 respectively (Table 2), revealing high risks for crops and humans. The variance of different metals may be due to metal speciation and the interaction between different metals (Moreno-Jiménez et al. 2006).

3.3 Spatial distribution of contaminants in paddy soils

Taking surface soil of the mine district, 147 surface soil samples were analyzed, and the statistical index, semi-variogram function model and kriging interpolation of ArcGIS software were used for the analysis. The spatial distribution of heavy metal concentrations in soils is shown in Fig. 1. In the district, the distribution of Pb, Cd and As was similar, with the concentration greater in the central area and lower in the surrounding areas; for Pb and As, the concentrations were higher in the north areas than the south areas. Lead concentrations in the central area were over 1000 mg·kg^{-1}, showing high contamination. Most areas were highly contaminated with Cd, especially in the north. Around the gold mine there is a deep red area showing As concentrations of 117 mg·kg^{-1} indicating a high risk. The higher contents of Pb, Cd and As detected in soils around the factories, indicates that their spatial distribution has been influenced by human activities.

3.4 Spatial distribution of contaminants in rice

According to their spatial distribution in rice, the concentrations of Pb, Cd and As were higher in the central area but much lower in surrounding areas (Fig. 2). The mining industry in this location is highly developed and as a consequence the wastes generated have caused serious contamination and metal accumulation in the surrounding soils (Zheng et al. 2007; Peng et al. 2014). The contents of Pb, Cd and As in soils near the industrial park are higher, and appear to form a center island spatial distribution tending to diffuse into the surrounding areas. Furthermore, the prevailing winds have re-entrained metals to accumulate in the south corner of the research district, whilst the use of pesticides and fertilizers in paddy fields has led to metal accumulation in soils (Chen et al. 2008; Aydin et al. 2010).

3.5 Effect of Metals on Human HepG2 and KERTr Cell Viabilities

The effects of the metals on HepG2 and KERTr cell viabilities were tested at 1, 5, 10, and 25 μM after a 24 h incubation period using the MTT assay. The metals differentially affected viabilities (Fig. 3), none of the metals affected HepG2 and KERTr cell viability at 1 μM. However, at 5 μM, cell viabilities as a result of the different contaminants were, As: (HepG2 cell 85.5%; KERTr cell 90.8%), Cd: (75.8%; 86.4%) and Pb: (96.2%; 94.0%) respectively. This indicated that 5 μM is a critical concentration, which can influence cell viability, even though the toxicity was not high. Cell viability studies with LDH (Fig. 4) yielded similar results as with MTT.

3.6 MT Protein Induction on Human HepG2 and KERTr Cells

Figure 5 presents the MT protein induction between mixed metal groups and single metals on human HepG2 cell-lines, with Pb (0.008 mg/ml), Cd (0.013), As (0.0155), Pb+Cd (0.015), As+Pb (0.012), As+Cd (0.016) and As+Pb+Cd (0.014) respectively.

Figure 6 compares the MT protein induction between metal groups and single metals on human KERTr cell-lines, with Pb (0.0075 mg/ml), Cd (0.005), As (0.0055), Pb+Cd (0.009), As+Pb (0.0085), As+Cd (0.005) and Cd+Hg+As (0.005) respectively. The experiment on MT protein induction on human HepG2 cells was conducted to investigate the effects of As, Pb and Cd induction of MT protein on human HepG2 cells. MT proteins are small, cysteine-rich heavy metal-binding proteins which participate in an array of protective stress responses (Andrews 2000; Chasapis, Loutsidou et al. 2012). Since the discovery of the metal-binding protein metallothionein (MT) in horse kidneys in the 1950s (Margoshes and Vallee 1957), there have been a vast number of studies focusing on this group of metal-chelating proteins in various organisms and cells, including human breast cancer cells (Jin et al. 2002), human ovarian cancer cells (Schilder et al. 2006), and aquatic invertebrates (Amiard et al. 2006).

It has been confirmed in mice that As can potentiate Cd nephrotoxicity during long-term, combined exposure (Liu et al. 2000). In another study it has been demonstrated that exposure to two or more heavy metals can considerably increase the mortality rate of nematode species, at low metal concentrations, than single metals (Wah and Chow 2002). However, according to our results, some heavy metal co-exposure induced less MT protein expression in both human HepG2 and KERTr cells than with single metals, which may be due to the antagonistic effects between these metals (Bellés et al. 2002). Garcia and Corredor (2004) proved that exposure to both Pb and Cd appears to protect against the toxicity produced by Pb or Cd separately in pregnant rats. Bellés et al. (2002) showed that exposure of Pb and As to pregnant mice were practically nontoxic, but concurrently with Hg caused supra-additive interactions.

4. Conclusion

Significant heavy metal contamination was observed in the mine fields with different degrees of heavy metal pollution. In the soil, the contents of Pb, Cd and As were 516.7, 11.7 and 35.1 mg·kg^{-1} respectively. The content of Cd exceeded 82% of the environmental quality standard for agricultural soils in China. The contents of Pb, Cd and As in rice were 22.7, 1.1 and 0.7 mg·kg^{-1} respectively. These concentrations are higher than the agricultural industry standard of China for rice. The higher contents of Pb, Cd and As may be detected in soils around the factories, indicating that the spatial distribution of heavy metals can be influenced by human activities.

The metals differentially affected cell viability, with viability decreasing with increasing metal concentrations. At 5 μM, the cell viabilities were As: (HepG2 cell 85.5%; KERTr cell 90.8%), Cd: (75.8%; 86.4%) and Pb: (96.2%; 94.0%) respectively. The MT protein induction between metal groups and single metals on human HepG2 cell-lines were Pb (0.008 mg/ml), Cd (0.013), As (0.0155), Pb+Cd (0.015), As+Pb (0.012), As+Cd (0.016) and As+Pb+Cd (0.014) respectively. The MT protein induction between metal groups and single metals on human KERTr cell-lines were Pb (0.0075 mg/ml), Cd (0.005), As (0.0055), Pb+Cd (0.009), As+Pb (0.0085), As+Cd

(0.005) and Cd+Hg+As (0.005) respectively.

Conversely, co-exposure to heavy metals (Pb+Cd) may increase the metal (Pb or Cd)-mediated MT protein induction in both human HepG2 and KERTr cells. Since the increased levels of MT protein will lead to increased carcinogenic incidence, it is likely that chronic exposure to metal mixtures may increase the risk of single metals on human health. However when co-exposure to two or more metals (such as As+Pb) occurs, they may have antagonistic effects reducing the toxic effects of these pollutants.

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Table 1 Soil metal(loid) contents

Element	Minimum mg·kg^{-1}	Maximum mg·kg^{-1}	Mean mg·kg^{-1}	Standard Deviation	Coefficient of variation %	Skewness	Kurtosis	GB 15618 -1995[1]	Background Value[2]
Pb	43.16	4961	516.7	674.2	130	4.2	23.9	250(pH < 6.5) 300 (pH 6.5-7.5) 350(pH>7.5)	29.7(pH 5.6)
Cd	< 0.001	248.5	11.7	29.5	253	6.4	46.3	0.30(pH < 6.5) 0.60(pH 6.5-7.5) 1.0(pH>7.5)	0.126(pH 5.6)
As	0.02	300.8	35.1	42.4	121	3.7	17.7	30(pH < 6.5) 25(pH 6.5-7.5) 20(pH>7.5)	15.7(pH 5.6)

[1] Environmental quality standard for agricultural soils in China，[2] [122]。

Table 2 Rice metal(loid) contents

Element	Minimum $mg \cdot kg^{-1}$	Maximum $mg \cdot kg^{-1}$	Mean $mg \cdot kg^{-1}$	Standard Deviation	Variation Coefficient %	GB 2762-2012[1]	NY 861-2004[2]	AF	Mean AF
Pb	< .001	1172	22.7	108.6	477	0.2	0.4	0.03-0.05	0.90
Cd	< .001	8.9	1.1	2.1	194	0.2	0.2	0.05-7.09	0.72
As	< .001	5.4	0.7	1.3	191	0.2	0.7	0.03-0.17	1.06

[1]the maximum safe contaminant concentration standard in food of China，[2] the agricultural industry standard of China，[3] Accumulation factor of heavy metals in rice

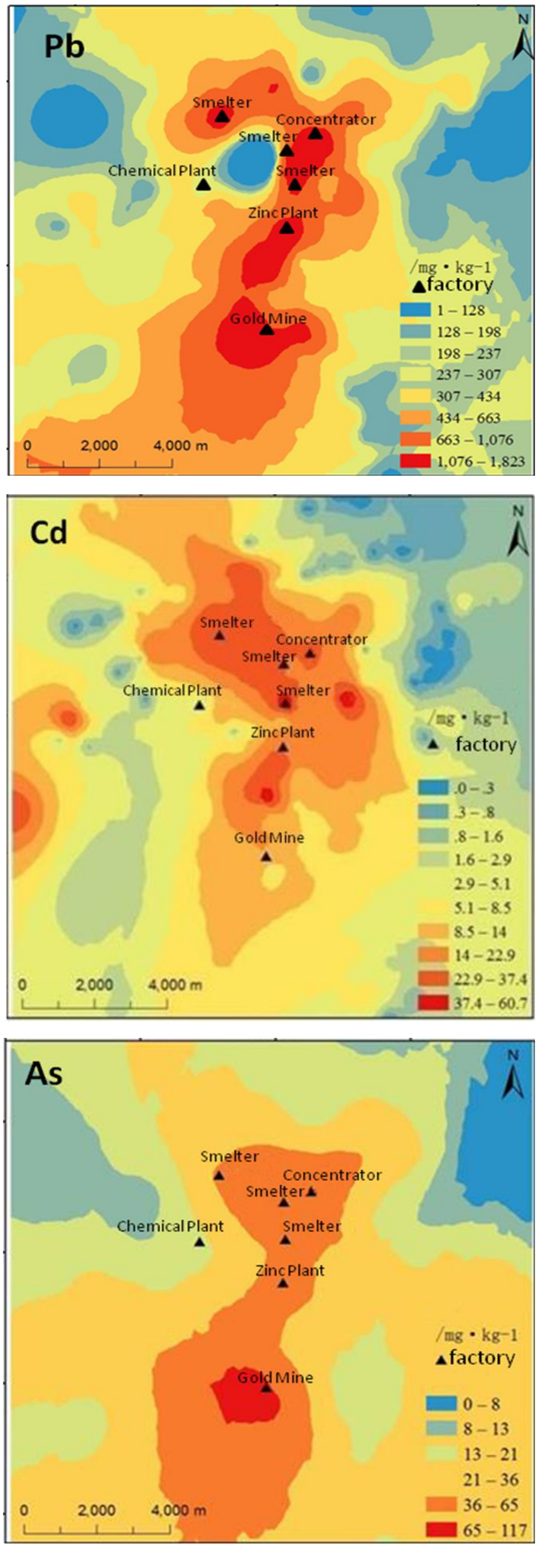
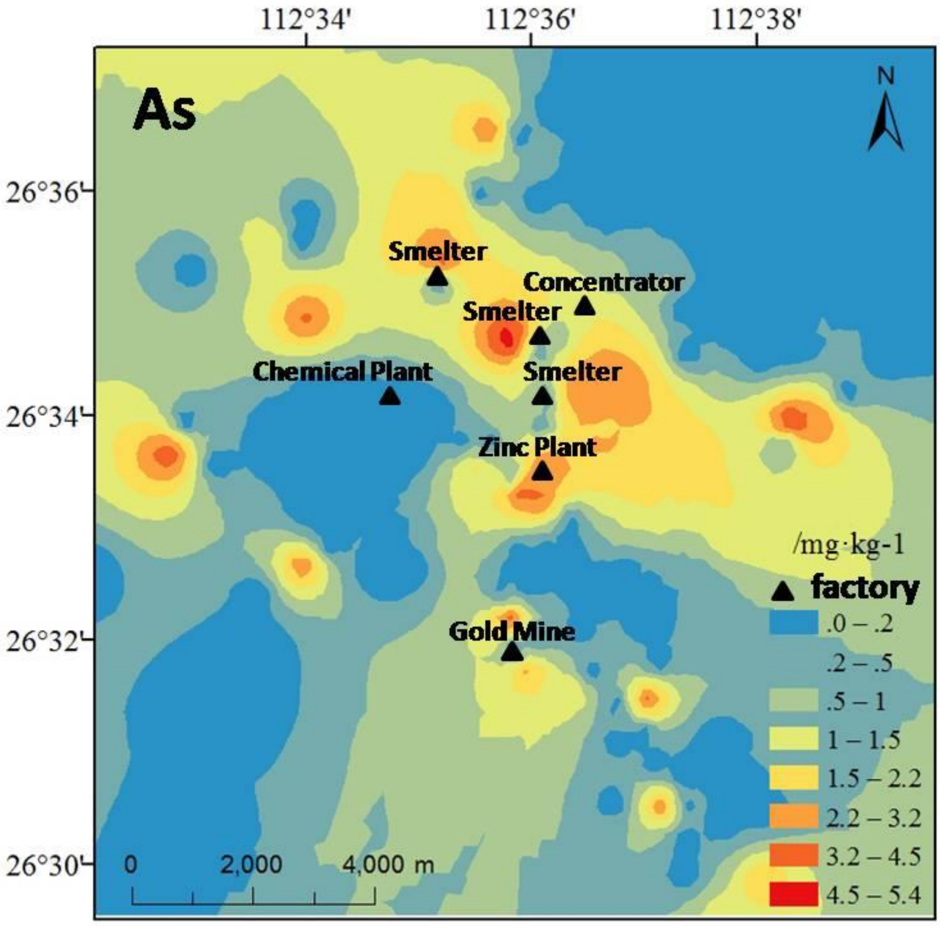


Fig. 1. Spatial distribution of contaminants in soils around the region surrounding the gold mine



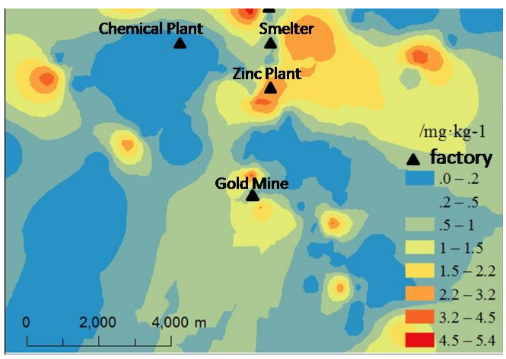


Fig. 2. Spatial distribution of contaminants in rice growing around the gold mine region

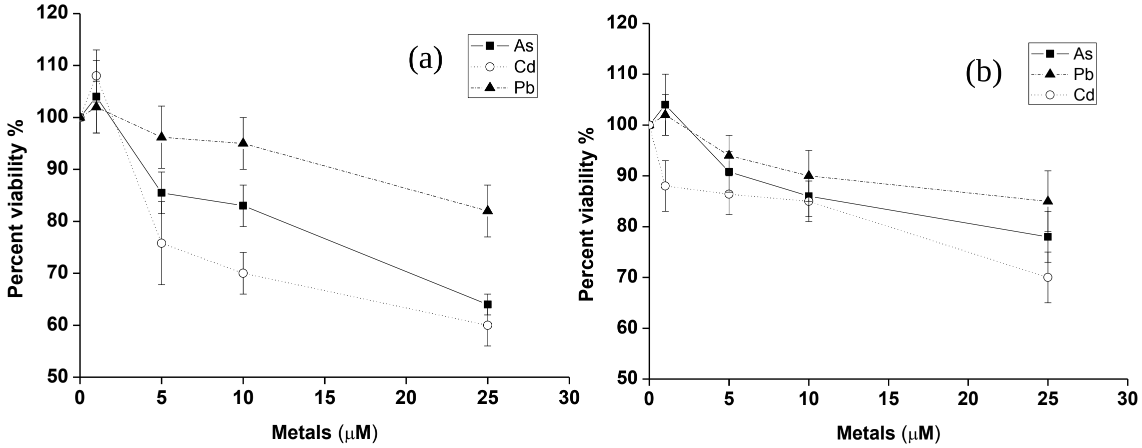


Fig. 3. Effects of lead nitrate (Pb), cadmium chloride (Cd) and sodium arsenite (As), on (a) human HepG2 cells and (b) KERTr cell viabilities. Viability was tested 24 h after treatment with a range (1-25 μM) of metals using the MTT assay. Values represent the mean ± SD of triplicate determinations on cells. At 5 μM, Pb, Cd, or As cell viability was 96.2%, 75.8%, 85.5%, respectively for HepG2 cells and 94.0%, 86.4%, 90.8% respectively for KERTr cells.

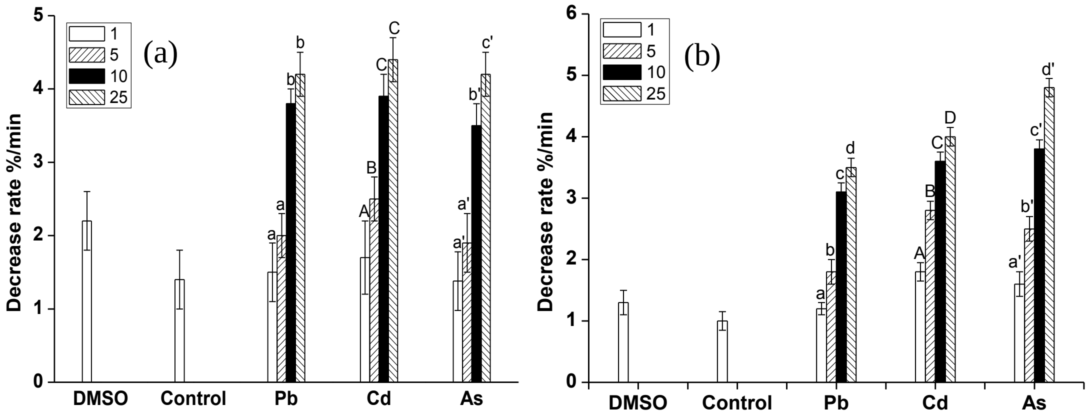


Fig. 4. Effects of lead nitrate (Pb), cadmium chloride (Cd), and sodium arsenite (As) on (a) human HepG2 cells and (b)KERTr cell viabilities. Viability was tested 24 h after treatment with a range (1-25 μM) of metal concentrations using the LDH assay. Values represent the mean ± SD of triplicate determinations on cells. 1 represent DMSO group, 2 represent control group.

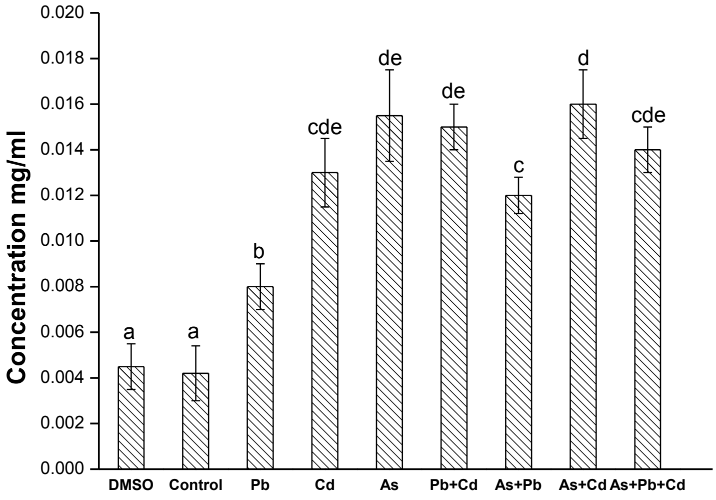


Fig. 5. Effects of Pb, Cd, and As induced metallothionein on human HepG2 cells over 24 h. *p<0.05, **p<0.01 represent the comparison of metal groups according to Student t-test.

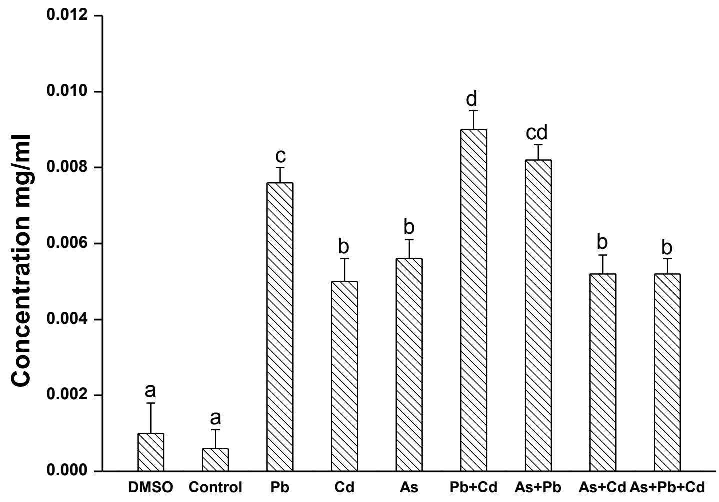


Fig. 6. Effects of Pb, Cd, and As induced metallothionein on human KERTr cells over 24 h. *$p<0.05$, **$p<0.01$ represent the comparison of metal groups according to Student t-test.