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ORIGINAL RESEARCH ARTICLE

Quantitative evaluation of ecological toxicity effect of real heavy metal combined pollution in site soil

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ABSTRACT

Quantitative evaluation of ecological effect of combined pollution of heavy metals in real site soil is considered as a great issue in ecological risk assessment of contaminated sites. In this work, a quantitative ecological assessment approach for combined contaminated soil in field by heavy metals was developed based on “top-down” and “bottom-up” knowledge, which was made up of three steps, namely, “screening of effective biomarkers-identification of dominant pollutants-evaluation of joint effect of different exposure types/contaminants”. Finally, taking an abandoned electronic planting site in Jiangsu Province as a case, the developed approach was verified using soil microcosm of earthworm. Results of the experiment by taking the biomarkers including malondialdehyde (MDA), metallothionein (MT), catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH)as effect endpoints, suggested that the bioaccumulation of main heavy metal contaminants including Cd, Cu, Zn, Ni, Pb and Cr by earthworms ranged in an order: Cd>Cu>Zn>Ni>Pb>Cr. Principal component analysis (PCA)revealed that GSH, CAT and MDA were screened as effective biomarkers, and heavy metals Cd and Zn were dominant contaminants. It was found that there was a significant multivariate linear relationship between the change of GSH and concentrations of total Cd and DTPA-Zn in soil. And the change of MDA could be predicted by DTPA-Cd in soil. The change of CAT activity was predictive by the total Zn in soil and the bioaccumulated Zn in earthworm. Evaluation of half effect dose (EC50) based on the site-specific soil properties and heavy metal contamination characteristics revealed that the sensitivity of the 3 screened effective biomarkers ranged in an order: GSH>CAT>MDA. Interactions will occur in between different heavy metals and exposure types (e.g., between soil total Cd and DTPA-Zn corresponding to GSH change), and (or) in between different exposure types of the same heavy metal (e.g., between soil total Zn and bioaccumulated Zn corresponding to the change of CAT activity).

Keywords: heavy metals; soil combined contamination; biomarkers; joint toxicity effect evaluation

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1. Introduction

In recent years, with the adjustment of China's economic structure and the transformation of the mode of economic growth, the problem of soil pollution in the sites left by the relocation of urban industrial enterprises has become increasingly prominent[1]. According to the 2014 survey report of the former Ministry of Environmental Protection, the rate of over standard industrial waste sites in China is 34.9%, mainly involving mining, non-ferrous metal smelting, electroplating, tanning, chemical production and processing industries[2]. The investigation found that the complex pollution of cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn) and nickel (Ni)in the electroplating contaminated site was serious[3,4]. The quantitative assessment of joint toxic effects of combined pollution is the key content of ecological risk assessment of contaminated soil, especially in site risk assessment, site specific is an important factor that must be considered.

At present, the "bottom-up evaluation" method is usually used to evaluate the ecological effects of compound pollution, that is, according to the mode of action of pollutants and based on the toxicity data of single pollutant, concentration addition (CA), independent action (IA)or composite index (CI)models are used to quantitatively estimate the toxic effects. However, this method requires a large amount of toxicity data, and since most of the data in the toxicity database are from laboratory simulation tests, there are often errors of order of magnitude in extrapolation to the actual contaminated soil in the field[5]. In contrast, the "top-down" effect evaluation method carries out the overall toxicity effect evaluation of the matrix based on the identification results of the dominant pollutants through the in-situ biological test of contaminated soil, combined with the analysis of the biological toxicity mechanism, and has a strong correlation with the actual soil pollution characteristics, which can meet the requirements for site specificity of the site risk[6,7]. However, the "top-down" effect evaluation method is rarely used in practice because it is difficult to quantitatively estimate the combined ecological effects of combined pollution and lacks a unified analysis program.

Earthworms are an important part of the soil ecosystem. They are widely distributed, large in number and sensitive to pollutants. They are often used as model organisms for toxicity assessment of soil pollutants[8]. The pollutants of earthworms are usually exposed through the skin or intestinal tract similar to the skin. The exposure route is simple, and the toxic effect is related to the internal exposure of earthworms and the soil environment exposure[9]. At the same time, a variety of biomarkers in earthworms, such as malondialdehyde (MDA), metallothionein (MT), catalase (CAT), superoxide dismutase (SOD)and reduced glutathione (GSH), The stress response relationship with pollutant exposure is obvious[10,13]. At present, the toxicological test of earthworms has been widely used in the ecological risk assessment of site soil pollution, the formulation of pollutant soil environmental quality standards and benchmarks, and the assessment of remediation effect of contaminated sites[14].

This study coupled "bottom-up" and "top-down" methods, and used multivariate statistical analysis methods to build a quantitative evaluation method for ecological effects of field actual site contaminated soil that can be quantitatively estimated and has site specificity. Taking an abandoned electroplating site in Jiangsu Province as the research object, the toxicity effect of the field actual heavy metal compound contaminated soil was quantitatively evaluated by earthworm soil microcosmic culture experiment. The purpose of this study is to establish a unified assessment procedure for ecological effects of actual contaminated soil in the field, and provide technical support for ecological risk assessment of soil pollution.

2. Materials and methods

2.1. Study site description and soil sample collection

The soil in this study was taken from an abandoned electroplating site in Jiangsu Province. This electroplating plant is a typical electroplating processing, metal and non-metallic surface treatment enterprise in the Yangtze River Delta region. It was built in 1985, including the electroplating workshop, zinc nitrate production workshop and wastewater treatment pool. It was closed in 2014. The plant has been abandoned until now. The site environment is poor. The plant is square, with a side length of 50 m, and an area of 2500 m^2. The land use type belongs to construction land. In accordance with the Technical Guidelines for Investigation of Soil Pollution on Construction Land (HJ 25.1-2019)[15], the method of point distribution in different areas was adopted. Considering the functional distribution of the site, sample points were arranged in electroplating workshops, wastewater treatment pools and silver nitrate production workshops, and 13 sample points (S1~S13) with different heavy metal pollution levels were selected for research.

The topsoil (0-20 cm) of each soil sample shall be collected by using the five points mixed sampling method, and the soil sample shall be put into a self-sealing bag, marked and taken back to the laboratory. The soil samples taken back to the laboratory shall be placed in a dry and ventilated place to dry naturally. The soil samples shall be milled after removing stones, plant roots and other impurities, and shall pass through 10 mesh and 100 mesh nylon sieves for standby.

2.2. Toxic effect test of earthworm

The earthworm tested in this experiment is Eisenia foetida, purchased from Tianjin Huiyude Biotechnology Co., Ltd. Before the experiment, earthworms were pre cultured in an artificial incubator for 14 days at (20 ± 1)℃, 75% humidity and 20% light. After pre culture, adult earthworms with obvious banding and body mass of 200~300 mg were selected for microcosmic experiment.

In this study, indoor soil microcosmic experiment was used. First, take 500g of dry soil, add a certain amount of deionized water to make its water content 35%, mix well and put it into a plastic beaker. Then place 15 earthworms in each beaker, seal them with plastic wrap, and pierce several holes in the plastic wrap to ensure that the earthworms can breathe normally and reduce soil moisture evaporation. Number each beaker and place it in an artificial climate box (the temperature of the incubator is set at (20 ± 1)℃, the humidity is 75%, the light is 20%, and day: night=12h: 12h. According to the amount of soil collected, 13 treatments (S1~S13) were set in the experiment, and 4 replicates were set for each treatment. After 14 days of culture, 8-10 earthworms were randomly taken out, washed with filter paper for 24 hours, and then the heavy metal content (dry weight) and enzyme activity in earthworms were determined. In this experiment, the survival rate of earthworms in each soil sample was more than 80%.

2.3. Analysis of soil physical and chemical properties and heavy metal content

Determination of soil physical and chemical properties

The soil pH was determined with a pH meter (Shanghai Yidian, China) of pHS-3C type after mixing the soil water ratio of 1: 2.5[16]. The total organic carbon in soil was pretreated with hydrochloric acid and directly determined with the element analyzer ElementarVario EL Ⅲ (Hanau, Germany)[17]. The soil cation exchange capacity is determined according to the Determination of Soil Cation Exchange Capacity Cobalt Hexamine Trichloride Extraction Spectrophotometry (HJ 889-2017)[18]. The available state of heavy metals in soil is determined by diethylenetriamine pentaacetic acid (DTPA)extraction method for 2mm air dried soil according to the Determination of Eight Available Elements in Soil - Inductively Coupled Plasma Atomic Emission Spectrometry (HJ 804-2016)[19].

Determination of heavy metals

Determination of heavy metals in soil: The content of heavy metals in soil was determined by HNO_3-HF-$HClO_4$-HCl tetraacid digestion method[20], the content of Cd, Ni, Cr and Pb in the sample was determined by ICPMS (7500A, Agilent, USA), and the content of Zn in the sample was determined by ICP-OES (Optima 8300, Perkin Elmer, USA). Each batch of samples is provided with 3 blank control groups, 10% sample repetitions and 3 reference materials. The reference materials are used for quality control with the national standard soil material GSS-27, and the recovery rate is between 83.0% and 119%.

Determination of earthworm heavy metal accumulation content: Put the earthworm to be tested into a petri dish with wet filter paper, spit mud for 24 hours, wash it with deionized water, freeze dry it for 48 hours, grind it with a mortar, and then use microwave digestion method (GB 5009.268-2016)[21] to digest it in a microwave digestion instrument (Multiwave PRO, Anton Paar, Austria), and use ICP-MS (7500A, Agilent, USA)to determine the content of Cd, Ni, Cr, Pb and Zn in the sample. Each batch of samples is provided with 3 blank control groups, 10% sample repeats and 3 reference materials, of which the reference materials are subject to quality control using the national reference material GBW10051 (GSB-29), and the recovery rate is between 85.1% and 123%.

2.4. Response determination of earthworm biomarkers

Take 3-4 earthworms after clearing intestines, weigh the mass, and freeze them quickly with liquid nitrogen. Add PBS buffer (pH=7.4) in the proportion of sample mass (g): buffer volume (mL)=1:9. Grind them fully with a tissue grinder under a 4℃ ice bath. Centrifuge 10% of the ground tissue homogenate for 10-15 minutes with a centrifuge 2000 r ·min-1. After centrifugation, take the supernatant and store it in a-80℃ refrigerator.

The earthworm biomarkers were determined with the kit, and the instructions in the kit were strictly followed. BCA method was used for total protein, WST-1 method for SOD, visible light method for CAT, microplate method for GSH, and enzyme-linked immunosorbent assay (ELISA) for MDA and MT. The total protein quantitative test kit (A045-3-2), SOD test kit (A001-3-2), CAT test kit (A007-1-1) and GSH test kit (A006-2-1)were purchased from Nanjing Jiancheng Biological Engineering Research Institute, and the insect MDA enzyme-linked immunosorbent assay (ELISA)kit (CD92025)and insect MT enzyme-linked immunosorbent assay (ELISA)kit (CD92144)were purchased from Wuhan Purity Biology Co., Ltd.

2.5. Data analysis

Screening of effective biomarkers

Firstly, KMO (Kaiser Meyer Olkin) test and Bartlett test were conducted for biomarkers to judge the feasibility of principal component analysis (PCA). Then, the biomarkers were analyzed by multivariate analysis. The different points were divided into multiple groups by PCA analysis and cluster analysis, and the grouping was tested by analysis of similarities (ANOSIM) to see whether it was statistically significant. Finally, BVSTEP method was used to screen effective biomarkers, and regression analysis was conducted between the selected effective biomarkers and the first principal component to obtain the relationship between effective biomarkers and earthworm health.

Identification of leading pollutants

Canonical correspondence analysis (CCA) is used to determine the correlation between biomarkers and pollutant exposure indicators. Variance inflation factor (VIF)is used to select redundant environmental factors in CCA modeling[22]. Finally, dominant pollutants are selected according to CCA results.

Data analysis

This study uses Microsoft Excel 2016 for raw data processing; SPSS 24.0 was used to statistically describe the physical and chemical properties of soil and the content of heavy metals in soil, and one-way analysis of variance (ANOVA)was conducted for earthworm biomarkers; Multivariate analysis was conducted on response of earthworm biomarkers with Origin 2018 software; ANOSIM analysis and BVSTEP analysis were conducted with R language;

Canoco5 software was used for VIF and CCA analysis; SPSS 24.0 was used for multiple regression analysis; Original 2018 software is used to complete the drawing.

3. Results and discussion

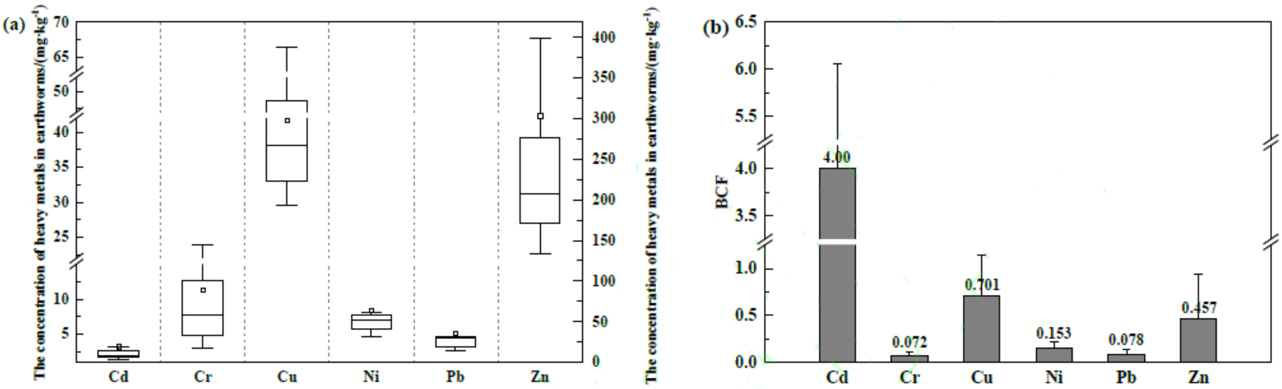
3.1. Physical and chemical properties of soil and content of heavy metals

As shown in **Table 1**, the total amount of Cd, Cr, Cu, Ni, Pb and Zn and the extracted content of DTPA in the soil samples of 13 sites vary greatly, and the coefficient of variation is 93.8%~309% and 114%~315% respectively, of which the total amount of Cd and Zn in the soil and the extracted content of DTPA are high. The variation of soil pH value and CEC is small, and the organic matter content is low as a whole, mostly below 1%.

Table 1. The total and DTPA extracted heavy metal concentration and the key physical-chemical properties of the tested soils

Parameters		Mean±SD	Coefficient of variation (CV)/%
Soil total heavy metal concentrations/(mg·kg-1)	Cd	4.74±14.7	309
	Cr	181±202	112
	Cu	96.9±98.9	102
	Ni	81.0±127	156
	Pb	100±94.2	93.8
	Zn	2 537±4 062	160
DTPA-extracted heavy metal concentrations/(mg·kg^{-1})	Cd	0.708±2.23	315
	Cr	0.0874±0.213	244
	Cu	8.80±15.1	172
	Ni	0.482±0.572	119
	Pb	9.06±14.6	162
	Zn	111±126	114
Physical-chemical properties	pH	7.34±0.492	6.71
	CEC/(cmol·kg^{-1})	8.32±2.45	29.5
	SOC/%	0.781±0.429	54.9

Note: CEC represents cation exchange capacity, and SOC represents soil organic carbon.



Note: In Fig.(a), Cd, Cr, Cu, Ni and Pb correspond to the ordinate on the left, and Zn corresponds to the ordinate on the right.

Figure 1. The concentrations (a) and the bioconcentration factors (BCF)(b)of heavy metals in earthworms

3.2. Bioaccumulation characteristics of heavy metals in earthworm tissues

The bioaccumulation characteristics of heavy metals in earthworm tissues are shown in **Figure 1**, which is consistent with previous research results[23,24]. Earthworms have different absorption and enrichment capacities for different heavy metals. The order of average enrichment coefficients for six heavy metals is Cd>Cu>Zn>Ni>Pb>Cr. Among them, the enrichment coefficients of Cu and Zn are close, which is consistent with most reported results[25,26], because both Cu and Zn are biologically necessary elements; The difference between the bioconcentration coefficients of Cr, Ni and Pb is also small, and these three elements are significantly lower than Cd, Cu and Zn, which is mainly due to the low bioavailability of these three elements in the soil. At the same time, the average BCF of earthworms to Cd is 4.00, while the average BCF of other elements is less than 1, which is due to the strong mobility of Cd in soil, which is easy to be absorbed and enriched by earthworms[27,28]. For example, the DTPA extraction rate of Cd in this study is 7.71%~22.0%, which is significantly higher than other five elements; In addition, earthworms can absorb and accumulate Cd in soil through feeding and direct skin absorption. For other elements, feeding and absorbing heavy metals combined with soil components is the main way for earthworms to enter earthworm tissue[29].

In addition, in this study, the actual contaminated soil in the field is used, and there is a phenomenon of polymetallic compound pollution. The interaction between the compound pollution elements can affect the absorption and accumulation of organisms. Traudt et al.[30] found in the plant experiment that under the combined pollution of heavy metals Ni, Cu and Cd, the plant Lemna minor has a competitive effect on the absorption of these three heavy metals, but after entering the plant tissue, only Cu and Cd have a competitive effect. Therefore, the bioaccumulation characteristics of pollutants must be considered in the assessment of ecological effects of combined pollution.

3.3. Screening of effective biomarkers

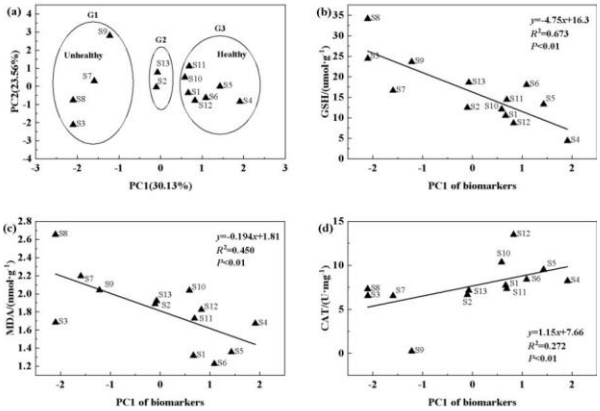
Single factor ANOVA was conducted on the toxicity response of earthworm biomarkers cultured in soil samples with different pollution levels. The results are shown in **Table 2**. The responses of six earthworm biomarkers were significantly different ($P<0.01$). KMO test and Bartlett test were used for toxicity response data of earthworm biomarkers. The results showed that KMO was 0.503 (>0.5) and Bartlett test was significant ($P<0.01$), indicating that the data could be analyzed by PCA. The results of PCA and cluster analysis (**Figure 2 (a)**)show that the variance contribution rates of PC1 and PC2 are 30.1% and 23.6% respectively; The response of biomarkers under different soil treatments can be divided into three groups, the first group (G1)includes S3, S7, S8 and S9, the second group (G2)includes S2 and S13, and the third group (G3)includes S1, S4, S5, S6, S10, S11 and S12. ANOSIM analysis of grouping results showed that there were significant differences among the sample points of the three groups ($P<0.01$). The comparative analysis of the total amount of heavy metals in the soil, the extracted content of DTPA and the bioaccumulative concentration of earthworms between the three groups showed that the three types of exposure indicators of heavy metals Cd and Zn in G1 were significantly higher than those in G3 ($P<0.05$), but not significantly higher than those in G2; Other heavy metals had no significant difference among the three groups (**Figure 3**). Therefore, it can be considered that the order of earthworm health status from good to bad under the soil culture of the three groups is as follows: G3>G2>G1; PC1 from left to right also reflects the trend of earthworm health from poor to good.

Effective biomarker screening was carried out by BVSTEP method. The results showed that the combination of GSH, MDA and CAT was significantly correlated with the combination of all other biomarkers. Therefore, their combination could be regarded as the minimum data set for response of all biomarkers. According to the regression analysis results of the response of three effective biomarkers and principal component 1 (PC1)(**Figure 2 (b)~(d)**), GSH and MDA content decreased significantly with the increase of earthworm health ($P<0.01$), while CAT activity increased significantly with the increase of earthworm health ($P<0.01$).

Table 2. ANOVA of biomarker responses in earthworms treated with different levels of heavy metal contaminated site soil

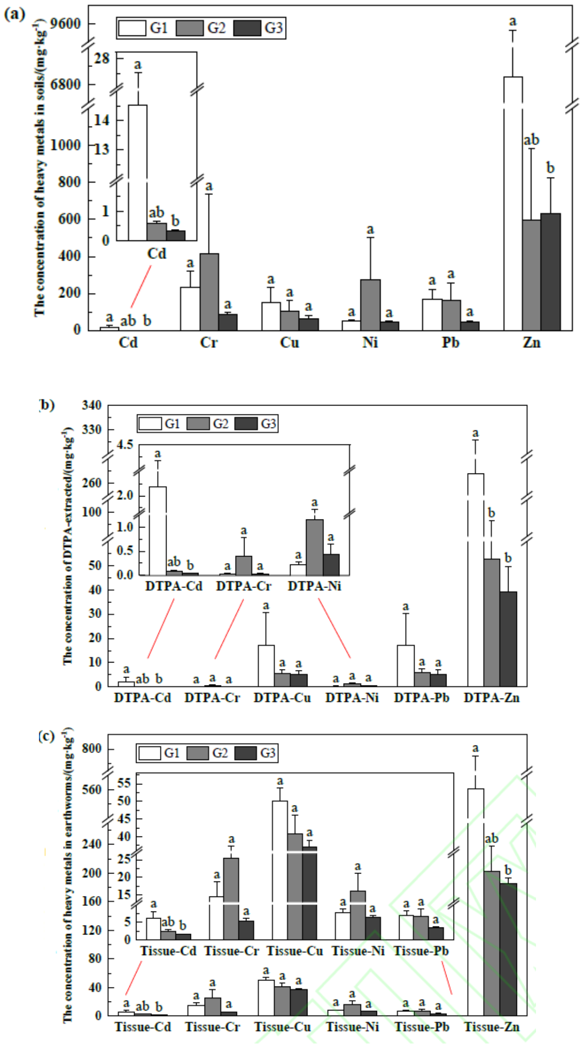
Biomarkers	Degree of freedom(df)	*F* value	Significance (P)
Total protein	twelve	5.18	<0.01
SOD	twelve	4.67	<0.01
GSH	twelve	5.05	<0.01
MDA	twelve	5.70	<0.01
MT	twelve	7.32	<0.01
CAT	twelve	9.81	<0.01

Note: SOD represents superoxide dismutase, GSH represents reduced glutathione, MDA represents malondialdehyde, MT represents metallothionein, and CAT represents catalase.



Note: (a)Principal component analysis (PCA)superimposed with cluster analysis; (b)～(d)Regression analysis between each effective biomarkers GSH, MDA and CAT and the first principle factor.

Figure 2. Multivariate analysis of biomarkers responses of earthworms



Note: (a)Soil total heavy metal concentrations; (b)DTPA-extracted heavy metal concentrations; (c)Earthworm bioaccumulation of heavy metal concentrations.

Figure 3. Comparison in heavy metal concentrations among the three divided groups (G1, G2, G3)

3.4. Identification of dominant pollutants in soil

CCA was further used to analyze the correlation between the response of biomarkers and the total amount of heavy metals in soil, the extracted content of DTPA and the bioaccumulation of heavy metals in earthworms. The VIF method was used to select the redundant environmental factors in CCA modeling. The results showed that only the VIF values of DTPA Cu and DTPA Pb were more than 10 (**Table 3**), indicating that there was a significant autocorrelation between them ($P<0.01$), while there was no multiple collinearity between the total amount of heavy metals in soil and the bioaccumulation of earthworms among the six heavy metals. Therefore, DTPA Cu was only excluded from CCA analysis of soil heavy metal DTPA extracted content and biomarker response. The CCA analysis results showed that, firstly, Cu, Cd, Zn and Pb were significantly correlated with the response of soil heavy metals to biomarkers, with the contribution rates of 3.90%, 54.0%, 17.3% and 11.8% respectively; Cr, Cd and Zn had significant correlation with DTPA extracted form of heavy metals in soil, and their contribution rates were 5.40%, 53.8% and 22.7% respectively; Cd, Cu and Zn are the elements with significant correlation with earthworm bioaccumulation of heavy metals, and their contribution rates are 41.1%, 14.6% and 28.2% respectively (**Figure 4**). It can be seen that the contribution rates of heavy metals Cd and Zn to the response changes of biomarkers are high under the three types of exposure modes. Based on the comparison results of heavy metal exposure in vivo and in vitro of the above three groups (G1, G2 and G3), it can be considered that heavy metals Cd and Zn are the leading pollutants causing the response changes of earthworm biomarkers.

Table 3. Variance inflation factor (VIF)analysis

Environmental factors	Cd	Cr	Cu	Ni	Pb	Zn
Total heavy metals in soil	3.24	7.26	4.15	3.97	1.50	3.53
DTPA-extracted heavy metals	4.03	1.77	508	1.27	497	5.23
Bioaccumulated heavy metals in earthworms	2.26	4.56	1.61	4.95	5.52	3.97

3.5. Toxicity effect evaluation of heavy metal combined pollution on earthworm biomarkers

In response to the three types of characteristic biomarkers, namely, GSH, MDA and CAT were stepwise regressed with the total amount of soil, DTPA extract content and earthworm bioaccumulation of the leading pollutants Cd and Zn respectively. The results are shown in **Table 4**. There was a multiple linear relationship between the change of earthworm GSH content and the total amount of soil Cd and the DTPA extract content of Zn; The change of earthworm MDA content can be predicted by the DTPA extracted content of soil Cd; The change of CAT activity can be predicted by the total amount of Zn in soil and the bioaccumulation content of earthworms.

Since the changes of GSH content and CAT activity of earthworms involve two types of pollutant exposure, based on the regression equation in Table 4, CA model is further used to comprehensively calculate the composite semi effect concentration of two types of exposure[31], and the results are shown in **Figure 5**. The compound semi effect concentration (EC50mix)of total Cd in soil and DTPA extract content of Zn with the change of GSH content in earthworm tissue as the end point of toxicity effect was lower than the measured concentration at each sample point (**Figure 5 (a)**); The EC50 value with the change of MDA content as the end point of toxic effect was lower than the measured value at S3, S7, S8 and S9 (**Figure 5 (b)**); The EC50mix values of total

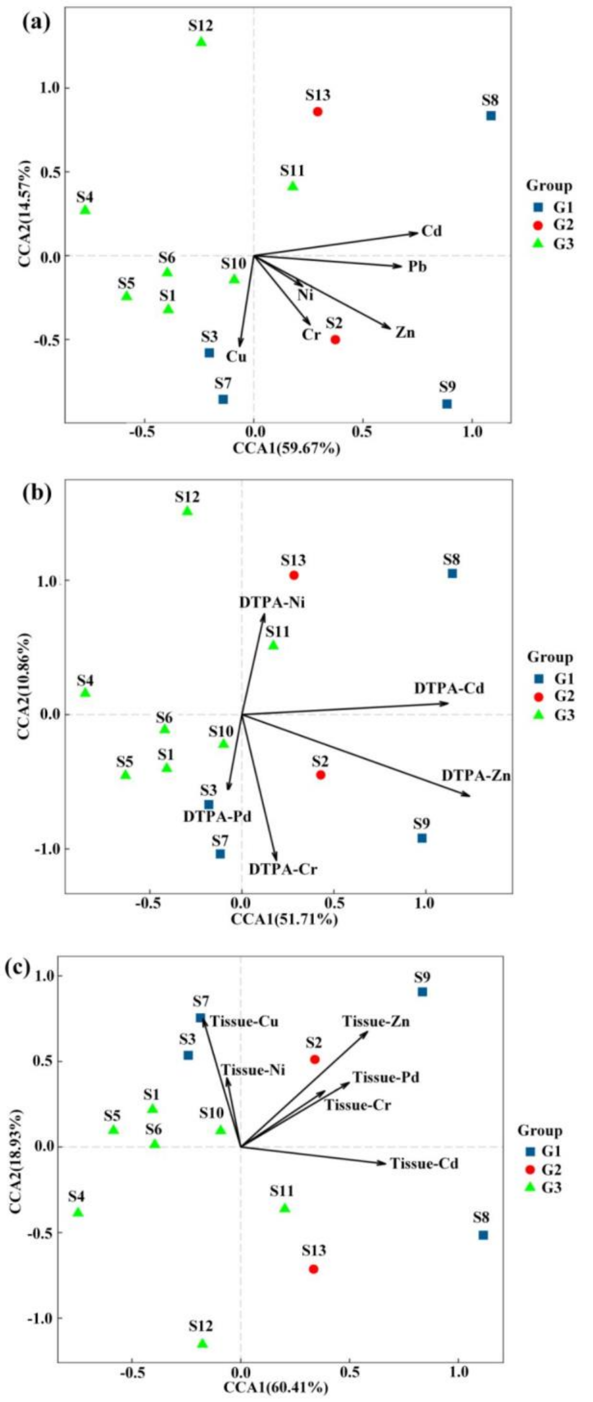


Figure 4. CCA analysis of biomarkers responses of earthworms and (a)the concentration of heavy metals, (b)the concentration of DTPA-extracted in soils, and (c)the concentration of heavy metals in earthworms

Zn in soil and earthworm bioaccumulative content with earthworm tissue CAT as the end point of toxicity effect

Table 4. Regression equations between the responses of effective biomarkers and heavy metal exposures

Biomarker	Regression equation	Coefficient of determination (R2)	P
GSH	GSH=7.58log(Cd)-2.89log(DTPA-Zn)+19.3	0.305	<0.01
MDA	MDA=0.245log(DTPA-Cd)+2.07	0.251	<0.01
CAT	CAT=-1.13log(Zn)-2.98log(Tissue-Zn)+17.2	0.359	<0.01

were lower than the measured values at 7 sampling points, including S2, S3, S4, S7, S8, S9 and S10. On the one hand, the above results indicate that the order of sensitivity of the three effective biomarkers from high to low is: GSH>CAT>MDA. These three kinds of biomarkers are all related to the biological antioxidant system. GSH, as a tripeptide containing sulfhydryl group, is easy to combine with heavy metal ions to reduce the damage of heavy metal pollutants to earthworm tissues; In addition, heavy metal pollutants will cause the rise of ROS in earthworms. As an important reductant substrate, GSH plays an important role in the biochemical reaction of removing ROS and is oxidized to oxidized glutathione (GSSG)[12, 32]. When GSH consumption suddenly increases, causing its content to decrease, as a stress mechanism for environmental pollution, organisms will induce synthesis of more GSH as feedback[33]. On the other hand, from the results of principal component analysis and multiple regression analysis, it can be found that there may be interaction between different elements and different types of exposure (such as total Cd and DTPA Zn corresponding to the change of GSH content), and between different exposures of the same element (such as total Zn in soil corresponding to the change of CAT activity and bioaccumulation Zn in earthworm tissues), However, there is no correlation between exposure of these pollutants. Especially between total Zn in soil and bioaccumulative Zn, due to the unique role of Zn element on organisms, the correlation between bioaccumulative Zn in earthworm and total Zn in soil is not significant, but both types of exposure are related to changes in CAT activity.

4. Conclusion

To sum up, the results of this study show that the quantitative evaluation of ecological effects can be achieved by means of multivariate statistical analysis, effective biomarker response, screening of dominant pollutants and joint effect estimation for the actual field soil heavy metal compound pollution.

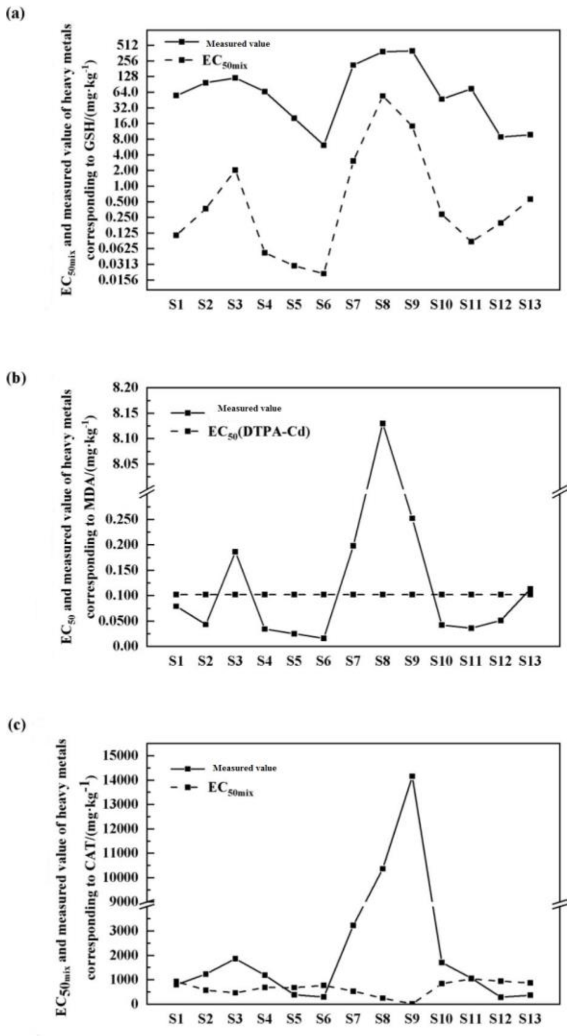


Figure 5. Comparison of calculated EC50 and measured concentration of dominant heavy metals

References

1. Luo Y M. Contaminated site remediation in China: Progresses, problems and prospects [J]. The Administration and Technique of Environmental Monitoring, 2011, 23(3): 1-6 (in Chinese)
2. The Ministry of Environmental Protection and the Ministry of Land and Resources of the People's Republic of China Bulletin on Investigation of Land and Soil Pollution [J] China's environmental protection industry, 2014 (5): 10-11
3. Chen Z L, Zhao S H, Zhou J M, et al. Pollution characteristics and ecological risk assessment of heavy metals in contaminated site of a typical electroplating factory [J]. Environmental Pollution ＆ Control, 2013, 35(10): 1-4, 11 (in Chinese)
4. Xue C J, Fang Z Q, Wang W. Review on remediation technology of composite polluted soil in electronic waste dismantling site [J]. Environmental Pollution ＆ Control, 2021, 43(1): 103-108 (in Chinese)
5. Wang M E, Ding S K, Guo G L, et al. Advances in ecological risk assessment of soil in contaminated sites [J]. Chinese Journal of Applied Ecology, 2020, 31(11): 3946-3958 (in Chinese)
6. Beyer J, Petersen K, Song Y, et al. Environmental risk assessment of combined effects in aquatic ecotoxicology: A discussion paper [J]. Marine Environmental Research, 2014, 96: 81-91
7. Li X Z, Wang M E, Jiang R, et al. Evaluation of joint toxicity of heavy metals and herbicide mixtures in soils to earthworms (Eisenia fetida)[J]. Journal of Environmental Sciences, 2020, 94: 137-146
8. Li Xuzhi Ecological effects of combined pollution of heavy metals and chlorsulfuron in urban soil on earthworms [D] Hefei: University of Science and Technology of China, 2018: 9-10
9. Davis B N K, French M C. The accumulation and loss of organochlorine insecticide residues by beetles, worms and slugs in sprayed fields [J]. Soil Biology and Biochemistry, 1969, 1(1): 45-55
10. Zheng L P, Wang G Q, Lin Y S, et al. Evaluation of toxicity effects of heavy metal contaminated soils on earthworm (Eisenia foetida)in a mining area of Guizhou Province [J]. Asian Journal of Ecotoxicology, 2015, 10(2): 258-265 (in Chinese)
11. Li X Z, Wang M E, Chen W P, et al. Ecological risk assessment of polymetallic sites using weight of evidence approach [J]. Ecotoxicology and Environmental Safety, 2018, 154: 255-262
12. Wang K. The ecological adaptability and detoxification mechanisms of earthworm in long-term heavy metal contaminated soil [D]. Beijing: China Agricultural University, 2018: 66-86 (in Chinese)
13. Beaumelle L, Hedde M, Vandenbulcke F, et al. Relationships between metal compartmentalization and biomarkers in earthworms exposed to field-contaminated soils [J]. Environmental Pollution, 2017, 224: 185-194
14. Yan Z G, He Q L, Li F S. The use of earthworm ecotoxicological test in risk assessment of soil contamination [J]. Research of Environmental Sciences, 2007, 20(1): 134-142 (in Chinese)
15. Ministry of Ecology and Environment of the People's Republic of China Technical Guidelines for Investigation of Soil Pollution on Construction Land: HJ 25.1—2019[S]. Beijing: Ministry of Ecology and Environment of the People's Republic of China, 2019
16. Ministry of Ecology and Environment of the People's Republic of China Determination of soil pH by potentiometric method: HJ 962—2018[S]. Beijing: China Environmental Science Press, 2018
17. International Organization for Standardization (ISO). Soil quality. Determination of organic and total carbon after dry combustion (elementary analysis): ISO 10694—1995 [S]. Geneva: ISO, 1995
18. Ministry of Environmental Protection of the People's Republic of China Determination of soil cation exchange capacity Cobalt hexachloride extraction spectrophotometry: HJ 889—2017[S]. Beijing: China Environmental Science Press, 2017
19. Ministry of Environmental Protection of the People's Republic of China Determination of 8 available elements in soil by diethylenetriamine pentaacetic acid extraction inductively coupled plasma atomic emission spectrometry: HJ 804—2016[S]. Beijing: China Environmental Science Press, 2016
20. Wang M E, Peng C, Chen W P. Impacts of industrial zone in arid area in Ningxia Province on the accumulation of heavy metals in agricultural soils [J]. Environmental Science, 2016, 37(9): 3532-3539 (in Chinese)
21. State Health and Family Planning Commission, State Food and Drug Administration Determination of multi elements in food of national food safety standard: GB 5009.268—2016[S]. Beijing: China Standards Press, 2017
22. Zuur A F, Ieno E N, Elphick C S. A protocol for data exploration to avoid common statistical problems [J]. Methods in Ecology and Evolution, 2010, 1(1): 3-14
23. Fu X Y, Qin S, Yang L, et al. Effects of earthworm accumulation of heavy metals in soil matrix [J]. Journal of Agro-Environment Science, 2009, 28(1): 78-83 (in Chinese)
24. Nannoni F, Rossi S, Protano G. Soil properties and metal accumulation by earthworms in the Siena urban area (Italy)[J]. Applied Soil Ecology, 2014, 77: 9-17
25. Wang K, Qiao Y H, Zhang H Q, et al. Bioaccumulation of heavy metals in earthworms from field contaminated soil in a subtropical area of China [J]. Ecotoxicology and Environmental Safety, 2018, 148: 876-883
26. Dai J, Becquer T, Henri Rouiller J, et al. Heavy metal accumulation by two earthworm species and its relationship to total and DTPA-extractable metals in soils [J]. Soil Biology and Biochemistry, 2004, 36(1):

91-98

27. Ernst G, Zimmermann S, Christie P, et al. Mercury, cadmium and lead concentrations in different ecophysiological groups of earthworms in forest soils [J]. Environmental Pollution, 2008, 156(3): 13041313
28. Chen X, Wang X R, Ji R. Bioaccumulation and oxidative stresses of cadmium in earthworm Eisenia foetida in a paddy soil [J]. Journal of Agro-Environment Science, 2015, 34(8): 1464-1469 (in Chinese)
29. Becquer T, Dai J, Quantin C, et al. Sources of bioavailable trace metals for earthworms from a Zn-, Pband Cd-contaminated soil [J]. Soil Biology and Biochemistry, 2005, 37(8): 1564-1568
30. Traudt E M, Ranville J F, Meyer J S. Acute toxicity of ternary Cd-Cu-Ni and Cd-Ni-Zn mixtures to Daphnia magna: Dominant metal pairs change along a concentration gradient [J]. Environmental Science & Technology, 2017, 51(8): 4471-4481
31. Xu X Q, Guo P, Wang X J, et al. Progress on CA and IA models in combined toxicity prediction of chemical mixtures [J]. Progress in Veterinary Medicine, 2020, 41(4): 91-94 (in Chinese)
32. Wang Q L, Wang H Y, Wang S R. Assessment of the state of oxidative stress [J]. Chinese Journal of Pathophysiology, 2005, 21(10): 2069-2074 (in Chinese)
33. van der Oost R, Beyer J, Vermeulen N P E. Fish bioaccumulation and biomarkers in environmental risk assessment: A review [J]. Environmental Toxicology and Pharmacology, 2003, 13(2): 57-14.