

Original Research

Interactive Effect of Radioactive and Heavy-Metal Contamination on Soil Enzyme Activity in a Former Uranium Mine

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Abstract

Radioactivity and heavy metal toxicity of multi-metal deposits coexisting with the element uranium (U) could have long-term adverse impacts on soil biological processes and the health of soil ecosystems. Soil enzyme activities are considered bioindicators for assessing soil health. An experiment was designed to investigate invertase and β -glucosidase activity in multi-metal deposits. Radioactivity and heavy metals were also investigated during this study. Our results showed that the invertase and β -glucosidase activities were significantly lower in the core mining area than the control area ($p < 0.05$). Activities of the two enzymes decreased with increasing metal concentrations and radioactivity. Cu and Zn showed significant negative effects on β -glucosidase and invertase activities in a multi-metal deposit at the study site. A significant nonlinear relationship was recorded between soil enzyme activities, radiation dose ($R^2 = 0.71, 0.63; p < 0.05$), Zn ($R^2 = 0.34, 0.41; p < 0.05$) and Cu concentrations ($R^2 = 0.46, 0.45; p < 0.05$). There were turning points at $1 \mu\text{Gy h}^{-1}$, $250 \mu\text{g g}^{-1}$, and $30 \mu\text{g g}^{-1}$ for radiation dose, Zn and Cu contents, respectively. The findings could provide more information regarding the toxic effects of radiation and heavy metals on the soil health of multi-metal deposits, which can more precisely guide environmental protection.

Keywords: multi-metal deposit, soil pollution, bioindicators, radiation

Introduction

Uranium (U) is widely distributed in rocks and soils. Because of their radioactivity and heavy metal toxicity, multi-metal deposits coexisting with the element U could have hazardous impacts on soil ecosystems and soil biological processes for a long time. U mining pollutes the environment through abandoned waste containing radioactive material [1]. It is considered the most widespread contaminant in the soils of mining areas [1]. In addition to the radionuclides from the U series, small quantities of non-radioactive heavy metals such as Mn, Cu, Mo, V, Ni, As, and Zn may also be present in ores [2]. These elements also should be considered when evaluating environmental quality [2]. These toxic heavy metals will be a threat for the soil environment, including adverse effects on soil structure and function as well as on microbial growth [3-4]. Thus, a relevant bioindicator of soil quality is needed to assess the environmental risk in multi-metal deposits.

As a bioindicator, soil enzymatic activities are widely used for monitoring the effect of soil management or contamination on soil health [5]. Soil enzyme activities are also highly sensitive to heavy metals. The methodology for enzyme determination is simple, rapid, and inexpensive [6]. Among the enzymes in soil, β -glucosidase and invertase are important for breaking down labile cellulose and carbohydrate polymers, releasing simpler sugars, and increasing soluble nutrients in soil. These enzymes produce the main energy sources for the growth of microorganisms in soil [7]. The inhibition of β -glucosidase and invertase activities by heavy-metal pollution has been reported by many scientists [8-10]. Most of them have suggested that heavy metals influenced the microbial community and soil respiration and have negative relationships with soil enzyme activities [11-12]. However, most of these studies have focused on the effect of individual heavy metals on soil enzyme activity or the

incubation of heavy metals in soils in a laboratory, but have neglected the radiation effect on the soil environment [12-13]. These results cannot determine or characterize the nature of combined pollution on the soil environment. Single-pollutant heavy metal is important for individual pollutants under certain conditions, but is not enough to reflect the real situation [14]. It is therefore necessary to study the natural change of soil enzyme activity under exposure to multi-metal deposits and radiation to make it a more accurate indicator for soil quality.

The present study aimed to: 1) examine the natural radiation and the content of heavy metals from former uranium mining areas, 2) investigate the effects of radiation and heavy metals on β -glucosidase and invertase activities, and 3) find the environmental threshold for radiation and heavy metals affecting β -glucosidase and invertase activities. This investigation could provide more information about radiation and heavy metals on the soil health of former U mining areas, and the results will help determine more precise environmental protection.

Materials and Methods

Study Site and Experimental Design

The study site is located at the western sector of the southern Qinling ore belt (Fig. 1). The ore deposit occurs in a set of epimetamorphic siliceous limestone, and the ore deposit is strictly controlled by its lithologic character, structure, deep hot water and geochemical environment [15]. The ore belt is developed from west to east and is accompanied by heavy metals, including Zn, V, Cu, Cr, Mn, and Ni. The total length of the ore belt is approximately 50 km, with a width of approximately 6 km, an area of 300 km², and an altitude in the range of 2,900-4,060 m [16]. There is a village downstream of the

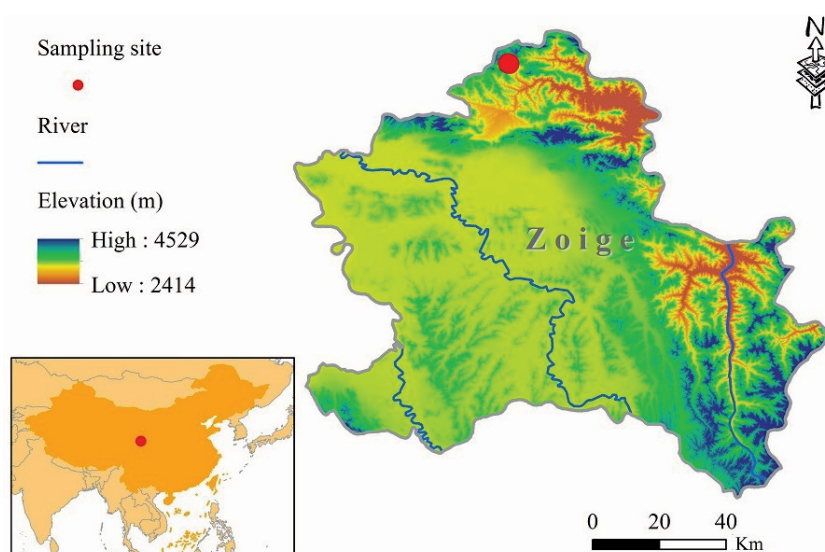


Fig. 1. Map of our study area.

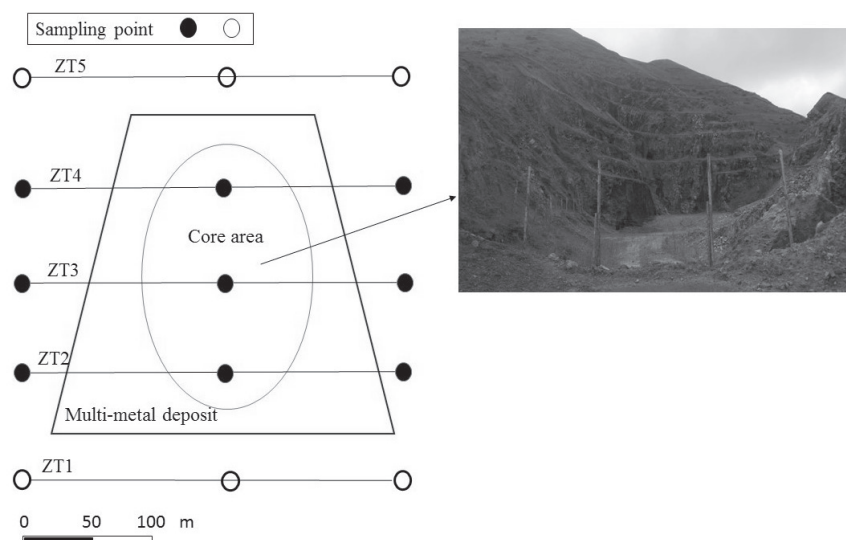


Fig. 2. Field experimental design. Five parallel transects ZT1 to ZT5 instead for our sampling line, each line with three sample points, were established across the multi-metal deposit. Solid circle instead of sample collected form core mine, hollow circle instead of sample collected from control area.

study site, and the distance to the village is approximately 2 km. This mining was explored during the 1970s-90s and was abandoned for approximately 20 years. This area is covered by a plateau meadow.

The fieldwork was undertaken during 13-20 August 2014 at the Zoige multi-metal deposit. The altitude of the sample region was between 3,282 m and 3,687 m. For the field sampling, a total of five transects, each with three sampling plots, were established crossing through the abandoned deposit (Fig. 2).

Among these five transects, ZT1 and ZT5 were considered control plots, and the other three were considered the core region of the deposit. The plots were located in the field using a handheld GPS receiver. In each plot, five individual soil cores were collected at a 0-10 cm depth and mixed as one sample. Soil samples were sealed in polyethylene Ziploc bags and delivered to the laboratory. All the soil samples were sieved through 2 mm and 0.25 mm sieves for later analyses. Samples sieved through the 2 mm sieve were stored at 4°C for analysis.

Soil Sample Measurement

The soil invertase and β -glucosidase activities were determined on the basis of the protocols described by Guan et al. [17-19]. Sucrose solution (8%) was used as a substrate to analyze invertase activity. Samples were incubated at 37°C for 24 h. A colorimeter reading at 508 nm was used for glucose production. A blank without a substrate was assessed for all experiments, and β -glucosidase activity was assessed using par-nitrophenyl- β -d-glucopyranoside (PNG) as a substrate. Soil sample (1 g fresh weight) in a 50 ml flask was treated with 0.25 ml toluene, 1 ml PNG, and 4 ml MUB (pH = 6), and then incubated at 37°C for 1 h. After incubation, CaCl_2 (0.5 M) and 4 ml 0.1 M Tris buffer was added to stop the reaction. The mixtures were

then filtered to determine β -glucosidase activity (expressed as $\mu\text{g PNG g}^{-1} \text{h}^{-1}$ dry weight) with colorimetry at 400 nm.

Soil samples were mixed with pure water at a ratio of 1:2.5 (g:ml) and stirred until the mixture was homogenized. After sitting for half an hour, the pH value was measured three times using a pH meter and reported as the mean value. Soil organic matter was determined using the wet oxidation of organic matter with a solution of potassium dichromate (KCr_2O_7) and H_2SO_4 , followed by back titration with ferrous sulfate [20]. The gamma dose was measured using a portable X- γ radiation dose rate meter (BH3103B). An inductively coupled plasma optical emission spectrometer (iCAP6500, ICP) was used to analyze trace elements (As, Au, Cd, Cr, Cu, Mo, Ni, Pb, Sr, U, V, and Zn). During analysis, samples were digested by acid ($\text{HCl} + \text{HNO}_3 + \text{HClO}_4 + \text{HF}$) using a constant temperature digestion instrument at 120-170°C. The detection limits of ICP device for Cr, Cu, Ni, V, and Zn are 0.05 mg/L, Mo, and Sr, and 0.01 mg/L U.

Data Analysis

Data for heavy metals and main elements are all presented as mean and standard deviation. Least significant difference (LSD) and one-way analysis of variance were used to assess the sample sites for soil enzyme activity. Regression analysis was used to examine the effects of heavy metals and radiation on soil enzyme activity. Pearson correlation method was used and the significance was defined at $p < 0.05$. Data for all variables were checked for normality using histograms. All data were found to satisfy the assumptions for ANOVA analyses. All analyses were performed using SPSS 18.0 (IBM, USA). Graphs were drawn using Sigmaplot 10.0.

Table 1. Characterization of the samples (the data shows mean ± sd).

	pH	Organic Matter %	As	Au	Cd	Cr	Cu	Mo	Ni	Pb	Sr	Zn	V	U
ZT1	7.7±0.3	9.7±0.2	903±713	8.6±2.7	11.8±7.3	62±14.2	17±3.5	99±19	19±1.1	55±38	117±18	101±15	72±22	92±128
ZT2	8.3±0.1	4.9±1.3	1500±400	6.2±1.5	18.5±3.2	48±19.5	28±17	70±22	23±4.0	71±47	125±24	190±69	108±48	96±68
ZT3	7.4±0.2	2.8±0.5	554±432	6.9±1.4	11.1±1.2	55±1.2	36±15	102±41	37±3.0	39±3	123±23	210±157	120±60	194±89
ZT4	6.9±0.1	4.5±1.2	353±294	6.9±0.5	5.3±2.4	44±0.2	22±7	69±5	18±0.5	40±15	85±18	97±10	28±33	121±143
ZT5	7.4±0.5	13.4±0.5	672±543	6.0±1.1	8.1±4.7	51±4.5	11±1	91±8	14±0.3	41±11	94±8	59±10	45±2	191±63

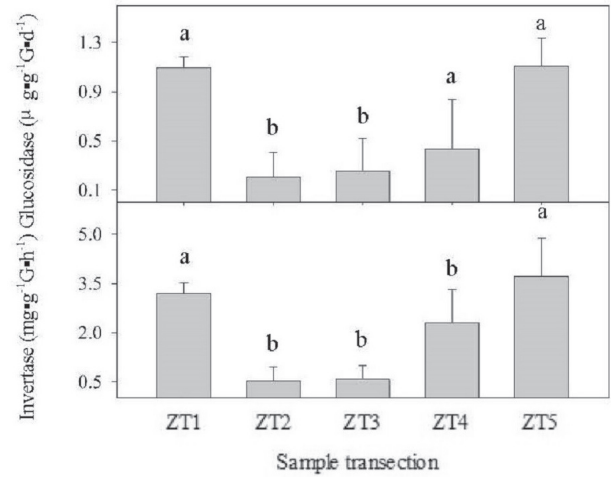


Fig. 3. β-glucosidase and invertase activities at different sample sites in uranium mining (different letters on the error bars indicate significant differences at p<0.05).

Results and Discussion

Soil pH, Radioactive, Heavy Metal, and Soil Enzyme Activities in the Multi-Metal Deposit Environment

Soil pH and heavy metals contents are presented in Table 1. The samples of tested soil were found to be slightly alkaline. The topsoil pH ranged from 6.9 to 8.3, with a mean value of 7.5. Organic matter in the core mining was lower in control plots.

Soil enzyme activities are used to evaluate the adverse effects of different pollutants on soil quality [11]. In the present study, β-glucosidase and invertase activities, which are related to soil carbon cycles, were significantly lower in the former core area (ZT2, ZT3, ZT4) of the multi-metal deposit than in the control

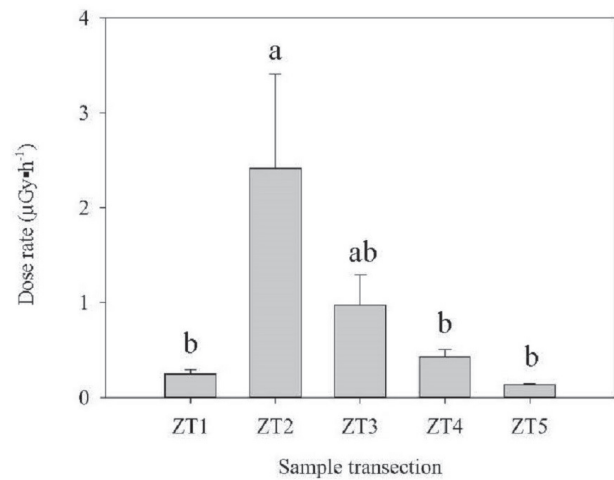


Fig. 4. Gamma radiation dose rates at different sample sites in the multi-metal deposits (different letters on error bars indicate significant differences at p<0.05).

Table 2. Correlation coefficients of soil enzyme activities (invertase and β -glucosidase) and environmental factors (**p<0.01, *p<0.05).

	Invertase	β g	Radioactivity	pH	As	Au	Cd	Cr	Cu	Mo	Ni	Pb	Sr	Zn	V	U
Invertase	1.00															
β g	0.80**	1.00														
Ra	-0.55*	-0.52*	1.00													
pH	-0.22	0.33	0.17	1.00												
As	0.06	-0.001	0.19	0.59*	1.00											
Au	0.16	0.157	-0.03	-0.02	-0.22	1.00										
Cd	-0.19	-0.25	0.32	0.65*	0.92**	-0.09	1.00									
Cr	-0.30	-0.42	0.08	-0.17	-0.43	0.20	-0.06	1.00								
Cu	-0.60*	-0.58*	0.49	-0.10	-0.28	0.24	-0.001	0.55*	1.00							
Mo	0.05	0.003	-0.02	-0.08	-0.51	0.24	-0.26	0.83**	0.33	1.00						
Ni	-0.45	-0.51	0.19	-0.06	-0.31	0.24	0.08	0.97**	0.66*	0.71**	1.00					
Pb	-0.11	0.09	-0.15	0.49	0.85**	-0.22	0.81**	-0.29	-0.37	-0.41	-0.16	1.00				
Sr	-0.51	-0.16	0.54*	0.58*	0.17	-0.10	0.40	0.24	0.23	0.30	0.29	0.25	1.00			
Zn	-0.63*	-0.56*	0.14	0.12	-0.06	0.09	0.29	0.76**	0.65*	0.44	0.87**	0.16	0.29	1.00		
V	-0.103	-0.56*	0.27	-0.69**	-0.05	0.20	0.27	0.54*	0.58**	0.41	0.52	-0.40	-0.16	0.43	1.00	
U	0.04	-0.13	-0.002	-0.10	-0.34	0.25	-0.22	0.31	0.38	0.16	0.29	-0.46	-0.21	0.12	0.15	1.00

plots (ZT1, ZT5; Fig. 3). The mean activity of glucosidase was $0.30 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in the core area and $1.10 \mu\text{g}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in the control plot. The mean activity of invertase was $1.14 \text{ mg}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in the core area and $3.45 \text{ mg}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$ in the control plot. In the mining area plots, β -glucosidase and invertase activities decreased by 73% and 67%, respectively, compared with the control plots. This result is in line with previous research that reported that mining always resulted in a series of eco-environment problems, leading to ecological degradation and environmental pollution in mining areas and decreased soil enzyme activities due to the leaching of heavy metals [7]. In the study site, most heavy metals (including Cd, Cr, Cu, As, Au, Mo, Ni, Pb, Sr, Zn, V, and U) were measured. The dominant heavy metals were As, Sr, Zn, and U, with concentrations of more than $100 \mu\text{g}\cdot\text{g}^{-1}$. The concentrations of Cu, Zn, and V were higher in the core region of the multi-metal deposit than in the control plots. The mean Cd and As concentrations in the sampled soil, 11 and $797 \mu\text{g}\cdot\text{g}^{-1}$, respectively, were 25-30 times higher than their maximum environmental quality evaluation standards in soil (Table 1). Although the As and Cd contents were much higher in the sample sites than the control site, they showed no significant effect on the measured soil enzyme activities. This was in agreement with the report that Cd-contaminated soils could exhibit reduced activities of soil enzymes such as alkaline phosphomonoesterase, arylsulfatase, and protease, although the β -glucosidase activity is not affected by Cd [21]. The lower soil enzyme activity in this study could be due to the combined impact of other heavy metals or radiation. Furthermore, the presence of U compound in the study site, with its radiation as an important factor in controlling the soil enzyme activity, could be a reason for lower soil enzyme activity in the mining area than in the control plots. The much higher radioactivity in the core region ($2.41 \mu\text{Gy}\cdot\text{h}^{-1}$, $0.97 \mu\text{Gy}\cdot\text{h}^{-1}$, and $0.43 \mu\text{Gy}\cdot\text{h}^{-1}$) of the multi-metal deposit than in the control plots ($0.25 \mu\text{Gy}\cdot\text{h}^{-1}$ and $0.13 \mu\text{Gy}\cdot\text{h}^{-1}$) can prove this point (Fig. 4).

Effects of Heavy Metals and Radioactivity on Soil Enzyme Activities

Soil pollution by heavy metals is an environmental problem, especially in mining areas, which impact soil quality and the regional environment [11]. Soil enzyme activities are indicators of the soil physico-chemical

Table 3. Stepwise linear regression analyses examining the relationships between measured soil enzyme activities (measured soil enzyme activities = dependent variables, heavy metals = independent variables, R^2 = coefficient of determination, P = probability). Bold numbers = $p < 0.05$.

Dependent variable	Independent variable	R^2	P
Invertase	= $3.85-0.08 \text{ Cu}$	0.43	<0.00
β -glucosidase	= $1.10-0.003 \text{ Zn}$	0.41	<0.00

conditions and microbial status, which are sensitive to soil pollution. Our results indicate that glucosidase activity was significantly negatively correlated with radiation, and with Cu and V contents ($p < 0.05$; Table 2). The invertase activity was negatively correlated only with radiation, Zn, and Cu ($p < 0.05$; Table 2). Stepwise linear regression analyses also showed that Cu and Zn are the main limitation factors affecting invertase activity and glucosidase activity in the mining area (Table 3). These results were consistent with the findings in previous studies, which reported that heavy metals inhibited the soil enzyme activity and metabolism of the microbial community [22-23]. The inhibition effects of Cu on hydrolytic activity have been reported by Guo [24]. However, no threshold of heavy metals was found in these studies. Our study measured a significant nonlinear relationship between Cu and Zn concentrations, glucosidase, and invertase activities. Interestingly, there was a threshold at $250 \mu\text{g}\cdot\text{g}^{-1}$ (C_{Cu}) and $30 \mu\text{g}\cdot\text{g}^{-1}$ (C_{Zn}). Glucosidase activity was also found to have a significantly negative relationship with the Cu content ($R^2 = 0.46$, $p = 0.03$). There are turning points at $1 \mu\text{Gy}\cdot\text{h}^{-1}$, $250 \mu\text{g}\cdot\text{g}^{-1}$ ($C_{\text{Cu}} = 250 \mu\text{g}\cdot\text{g}^{-1}$), and $30 \mu\text{g}\cdot\text{g}^{-1}$ ($C_{\text{Zn}} = 30 \mu\text{g}\cdot\text{g}^{-1}$) for radiation dose plus Zn and Cu contents, respectively (Figs 5-6). The turning point was determined when the curve smoothed by viewing the equation. When radiation dose and Zn and Cu contents exceed the thresholds, glucosidase and invertase activities will be inactivated. This might be useful for environmental models. Metal pollution has a negative impact on soil quality at a global scale on ecosystem services [25]. The toxicity mechanisms can influence enzyme activities in the following ways: 1) by reducing enzyme production through their toxic effects on soil microflora, 2) by forming a complex with the substrate, 3) by combining with the active enzyme protein groups, and 4) by reacting with the enzyme-substrate complex [26].

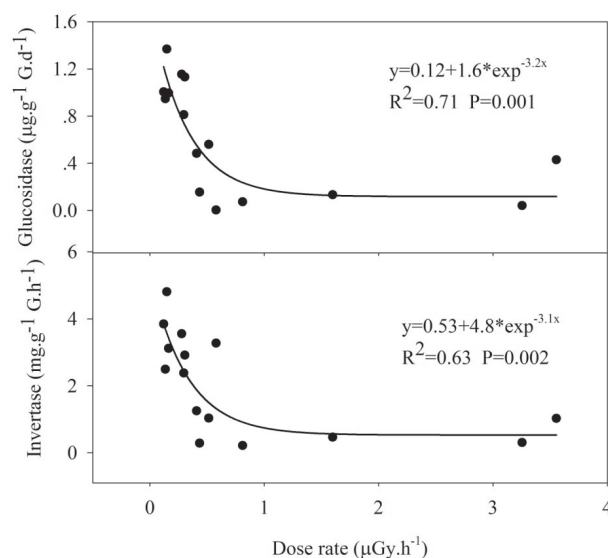


Fig. 5. Relationships between β -glucosidase, invertase activities, and gamma radiation dose rates.

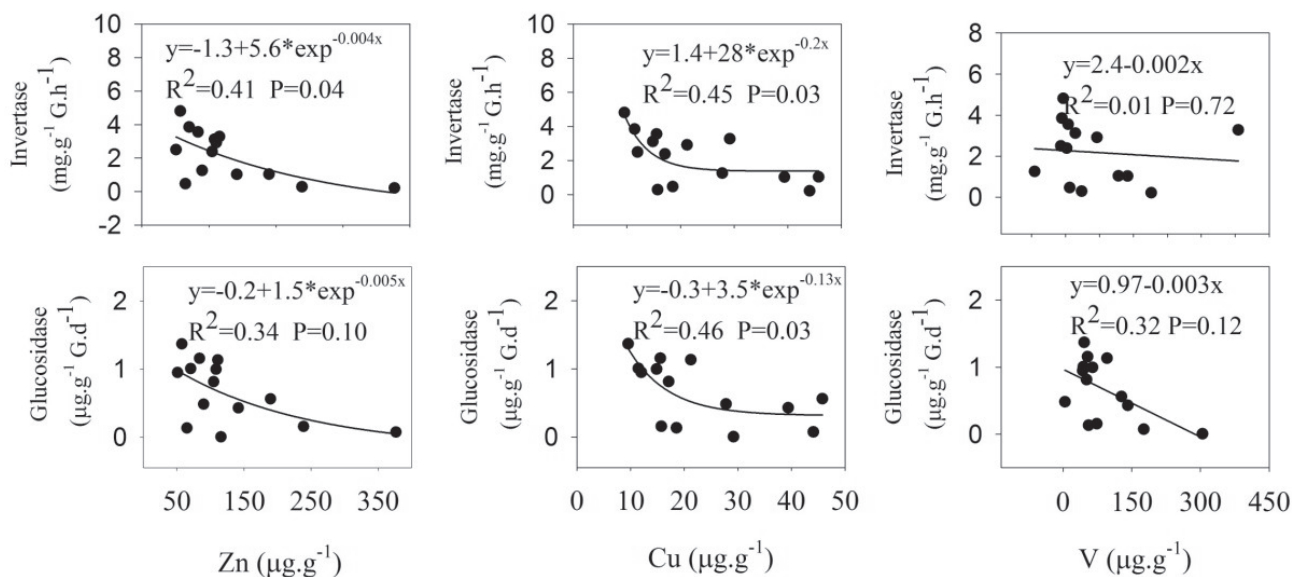


Fig. 6. Relationships between the β-glucosidase, invertase activities, and Zn, Cu, and V contents.

In this study, radiation exhibited a significant effect on soil enzyme activities. Generally, the radiation impact on soil enzyme activity is dependent on the radioactive material and the gamma radiation dose [27]. However, the natural radioactivity and terrestrial gamma dose essentially depend on geological conditions and the natural radioactivity in the soil [27]. In this study, since all the samples were taken from the same multi-metal deposit, they had similar radiation doses. But soil in the mining area had a higher radiation dose at more than 3 μGy h⁻¹. One of the reasons for the increase in radioactivity over the mining could be the dissolution of U ions in the area. With the increasing concentration of U and radiation, soil enzyme activity decreased, and the tipping point was found at 1 μGy h⁻¹ (Fig. 5). This indicates that if the radioactive dose exceeds 1 μGy h⁻¹, the enzyme protein could be deactivated. U is an adverse element due to its radioactivity and its toxicity as a heavy metal [28].

However, reports about the effects of U on soil enzyme activities are limited. This study measured a negative relationship between U and soil enzymes and provided the threshold radioactive dose.

Implications of Multi-Metal Deposits on Soil Environment

According to the national standard GB-15618-1995, which was issued in 1995, there are three grades for evaluating heavy metal pollution. Grade I aims to protect the natural ecosystem and with limiting values of soil environmental quality for natural background. Grade II aims to preserve human health by limiting values for agricultural production. Grade III aims to protect plant growth by limiting values for preserving forest and agricultural production (environmental quality standard for soil GB-15618-1995). As, Cd, and Zn are

the most abundant metals present in the environmental samples [29]. As and Cd were the highly measured metal pollutants in the mining area, and mean As concentration exceeded the grade III value (40 μg g⁻¹) by 10-37 times. Also, Cd content exceeded the grade III value (1.0 μg g⁻¹) by 5-19 times on the basis of the environmental quality standard (GB-15618-1995) and the environmental quality standards for farmland of edible agricultural products (Ministry of Environmental Protection of the PR China, 2007). In the control soil, the As and Cd concentrations were also higher than the environmental quality standard at grade III for soil. The Zn and Cu contents were below or close to the limits for the natural background.

Although As and Cd were significantly higher than the limit values, the effects of these two heavy metals on the soil enzymes were lower compared to those of the concentrations of Zn, Cu, and V. For example, the β-glucosidase and invertase activities negatively correlated with the concentrations of Cu, Zn, and V, with the exception of As and Cd. There are several possible reasons for this phenomenon. First, it may be due to the higher eco-toxicity of Cu, Zn, and V [30]. Second, it could be the modes of occurrence of heavy metals and minerals, not the concentration of the total metals controlling soil enzyme activities [8]. Because the total content of soil elements overestimate the toxicity encountered [6]. It is widely proved that the soil's biological and chemical properties are sensitive to ambient stresses [4, 11]. Soil enzyme activity plays an important role in soil and it has been proven that the soil enzyme has the potential ability to assess the effect of pollutants on the soil process. In this study, Cu and Zn had tipping points (30 μg g⁻¹ and 250 μg g⁻¹, respectively) for effects on the carbon cycle-related soil enzyme activities. This result was in line with a previous finding that soil enzyme activities are dramatically decreased when the concentration of Zn

and Cu in the soils reaches certain magnitudes [30]. Thus, Cu and Zn content should be controlled or reduced to below the threshold in order to maintain soil health.

Conclusions

The radioactivity and heavy metals toxicity of multi-metal deposits on soil enzyme activities were studied in a former U mining area. Cu and Zn were found to be the main compounds that have significant negative effects on β -glucosidase and invertase activities. The measured enzyme activities decreased with increasing concentrations of Cu, Zn, and radioactivity. The threshold for Cu, Zn, and radioactivity to cause enzyme inactivation was also found. The findings will be of great importance for researchers and policymakers.

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