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Original Research

Effects of Pb, Cd, Zn, and Cu on Soil Enzyme Activity and Soil Properties Related to Agricultural Land-Use Practices in Karst Area Contaminated by Pb-Zn Tailings

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Abstract

In order to study the impact of Pb, Cd, Zn, and Cu released by Pb-Zn tailings on soil enzymes and soil properties involving soil carbon and nitrogen cycle processes, 32 soil samples were collected from 2 different types of agricultural fields (one for growing corn and one for growing rice) contaminated by Pb-Zn tailings close to Sidi village in southwestern China. The results revealed that the paddy fields were seriously contaminated by Pb-Zn tailings compared with cornfields. Under the Pb-Zn tailings contamination, the population of fungi and actinomycetes as well as the activities of the soil enzymes (urease, invertase, and cellulase) in cornfields were significantly higher than those in the paddy fields. In addition, the results from path analysis showed that urease, invertase, and acid phosphatase were negatively correlated with DTPA-extractable Cd, Pb, and Zn (the direct path coefficients were -0.336, -0.314, and -0.591, respectively). Soil microorganisms and enzyme activities involving soil organic carbon and nitrogen decomposition and stabilization were decreased due to the toxic Pb-Zn tailings. Therefore, soil organic carbon and total nitrogen accumulate and an "elusive" carbon and nitrogen pool forms in the paddy fields compared with cornfields in the Pb-Zn tailings-contaminated karst area.

Keywords: Pb-Zn tailings, soil enzyme activity, path analysis, carbon and nitrogen cycle, "elusive" carbon and nitrogen pool

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Introduction

Pb-Zn tailings are considered the sustained-release source of Pb, Cd, Zn, and Cu in soil, which has serious influences on soil ecosystems and their biological processes [1]. Consequently, soil enzymes are usually used as an early indicator to monitor the effects of heavy metals on soil quality due to their sensitivity to environmental stress [2]. The main fraction of soil enzymes is contributed by soil microorganisms, which show high metabolic activity, large biomass, and a short lifetime [3].

To explore the toxic effects of heavy metals on soil, the relationships between heavy metals and soil enzymes have been extensively studied [4-8]. Some studies have demonstrated that heavy metals reduced enzyme activities [6-8]. In this respect, soil basal respiration and microbial biomass carbon can decrease 3-45% and 21-53%, respectively, due to heavy metal pollution [9]. In addition, based on data from 16S ribosomal RNA gene fragment sequencing, Zn and Pb pollution affected soil bacterial community [10]. However, the toxicity of several heavy metals (Pb, Cd, Zn, and Cu) originating from Pb-Zn tailings on soil enzyme activity and the soil carbon-nitrogen cycle is not well understood, especially in calcium-rich and alkaline karst soil [6, 11].

Based on our previous research [6-8, 12], we hypothesize that Pb-Zn tailings can affect the soil carbon/nitrogen cycle mediated by soil microbes and enzymes in karst areas due to different agricultural land-use practices. Therefore, Pb-Zn tailings-contaminated soil samples were collected from cornfields and paddy fields close to Sidi village in southwestern China, a region with karstic features. Consequently, we measured pH, soil organic carbon (SOC), cation exchange capacity (CEC), total nitrogen (TN), available nitrogen (AN), available phosphorus (AP), available potassium (AK), soil enzyme activities of invertase, cellulase, urease, protease, and acid phosphatase, as well as both total and diethylene triamine pentacetate acid (DTPA)-extractable Pb, Cd, Zn, and Cu. In addition, correlation analysis and path analysis were employed to evaluate their intrinsic relationships and the major factors controlling organic carbon/nitrogen decomposition and stabilization in Pb-Zn tailings-contaminated soil.

Materials and Methods

Study Region

Sidi village is on the peak forest karst system in southwestern China with the hydromorphic paddy soil [12]. As reported by us before, the underlying bedrock is composed of sandy shale and limestone, and an alpine valley river flows through the village [12-13]. In addition, a Pb-Zn mine located at the upper cliffs of Sidi village has been in use since the 1950s. In the 1970s the simple dam for collecting Pb-Zn tailings collapsed due to storm floods and Pb-Zn tailings contaminated the farmland of the village. Due to land shortage, the contaminated farmland was ploughed again for agricultural activities.

Sample Collection

Due to the non-homogeneous distribution of Pb-Zn tailings at this area [13], 32 soil samples from paddy fields (16 soil samples) and cornfields (16 samples) were collected in May and June 2013 (Fig. 1). Each land type contained 16 replicated samples and each soil sample was thoroughly mixed from 3 soil cores of 5 cm in diameter and 20 cm in depth (500 g). The soil samples were

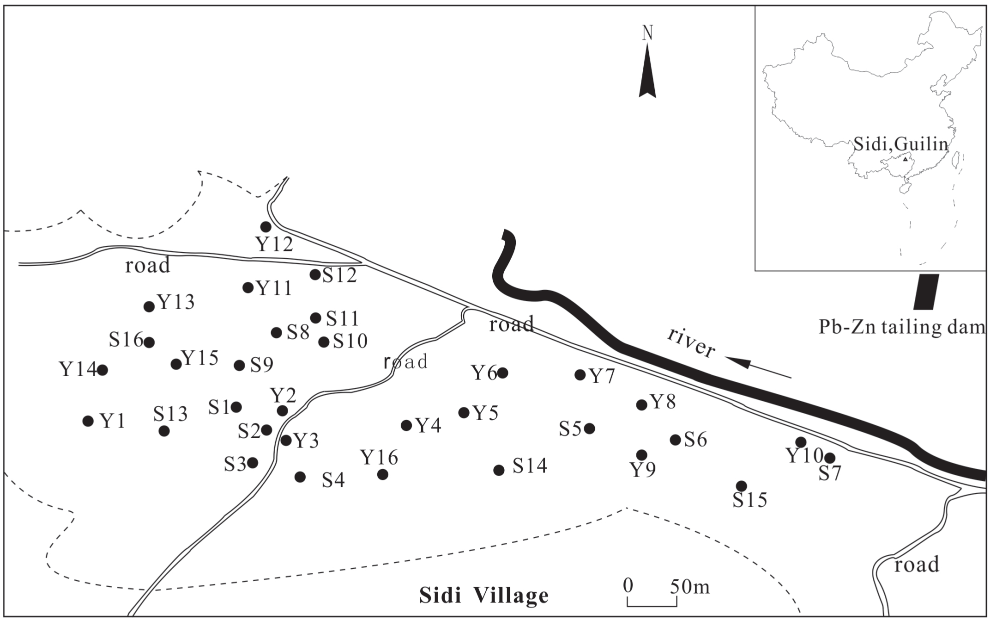

Fig. 1. Locations for collecting the soil samples (Y: corn field, S: rice paddy field).

homogenized and sieved (2 mm) to remove all rocks and roots. The homogenized fresh samples were stored at 4ºC for subsequent analysis [12-13].

Soil Physicochemical Properties and Enzyme Analysis

Air-dried and sieved soil samples were used for analysis of pH, SOC, CEC, TN, AN, AP, and AK as well as total and DTPA-extractable Pb, Cd, Zn, and Cu. SOC, pH, TN, and CEC were analyzed according to the method described by Jin et al. [13]. Total and DTPA-extractable Pb, Cd, Zn, and Cu were analyzed using flame atomic absorption spectrophotometry (AAS) [14]. AN, AP, and AK were analyzed according to Hu et al. [6].

Soil invertase, cellulase, urease, and acid phosphatase activities were measured by the methods described by Li et al. [15]. The enzyme activities of invertase and cellulase were expressed as mg glucose released 24 hours per gram of soil dry weight. The urease activity of soil was measured in mg (NH_4^+-N)/(24 h·g) of soil dry weight. The acid phosphatase activity of soil was given in mg *p*-nitrophenol-produced 2-hour per gram of soil dry weight. Soil protease activity was expressed in mg (NH_2-N)/(24h·g) of soil dry weight following a modified method [16].

Extraction and Enumeration of Cultured Microbes

The numbers of extracted and cultured bacteria and actinomycetes as well as fungi from the soil samples were enumerated by the modified soil-plate method [17]. The soil suspensions were serially diluted with sterile water and dispersed on solid culture media of nutrient agar, Gause'1 and Martin for culturing bacteria, actinomycetes, and fungi. They were incubated at 25ºC for 3 d. The mean values of 3 plates were obtained.

Data Analysis

To better explore the impact of Pb-Zn tailings on soil carbon/nitrogen cycle related to agricultural land-use practices, some data in May 2013 that have been reported were used for correlation analysis and path analysis [12]. SPSS 13.0 was used for correlation and path analysis. JMP version 5.0 was used to calculate the significant average differences ($P<0.05$). A path analysis was conducted according to Shipley [18].

Results and Discussion

Heavy Metals, Soil Enzyme Activities, and Soil Properties

Previously, we reported that total Pb, Zn, Cu, and Cd from cornfields and paddy fields had no statistical differences, although the distribution of Pb-Zn tailings in this area was non-homogeneous [13]. From Table 1, it can be seen that DTPA-extractable Zn, Pb, and Cd from cornfields are lower than those from paddy fields, though DTPA-extractable Cu is quite high in cornfields compared with paddy fields. Furthermore, DTPA-extractable Cd from cornfields and paddy fields show significant statistical differences, suggesting that DTPA-extractable Cd might be the dominant environmental factor controlling soil quality.

Based on the classification system of the coefficient of variation (CV) [13], the values except DTPA-extractable Zn from cornfields and paddy fields are considered to be moderately variable (Table 1). Moreover, the CV

Table 1. Descriptive statistical analysis of soil heavy metals in a Pb-Zn tailings-contaminated karst area (mg/kg).

Land use type	Analysis result	Total Pb	Total Zn	Total Cu	Total Cd	DTPA-Pb	DTPA-Zn	DTPA-Cu	DTPA-Cd
Cornfield (Sample numbers: 16)	Average	885.97a	1,211.79a	116.59a	3.67a	209.03a	141.20a	28.23a	2.37b
	Min.	330.20	621.29	55.13	1.69	112.90	44.61	10.80	0.36
	Max.	1,990.68	2,858.00	190.10	6.38	290.90	533.19	45.30	5.79
	SD	461.00	681.71	43.36	16.17	62.83	154.62	11.22	1.44
	CV	52.00	56.30	36.94	44.27	30.19	110.35	41.26	59.94
	Medians	748.63	1,009.88	111.94	3.46	195.32	79.85	24.45	2.13
Paddy field (Sample numbers: 16)	Average	1,133.34a	1,828.96a	131.07a	4.56a	223.37a	244.29a	25.37a	4.51a
	Min.	286.93	808.38	59.60	1.68	123.78	46.75	11.80	1.91
	Max.	1,977.19	3,210.00	169.30	9.91	337.30	597.27	48.37	7.47
	SD	469.34	845.46	39.34	29.94	72.19	218.61	9.16	1.80
	CV	41.45	46.22	30.22	65.85	32.57	89.56	36.52	40.64
	Medians	1,134.00	1,677.54	142.89	3.22	200.45	104.75	25.00	4.79

Min.: minimum (s); Max.: maximum (s); SD: standard deviation (s); CV: coefficient of variation (%)

Different letters (a, b) indicate a significant difference at the 0.05 probability level; same letters (a, a) indicate no significant difference at the 0.05 probability level of paddy fields is lower than that of cornfields (Table 1). The multiple influences on soil depend on topography, land use, human intervention, and vegetation type. The dominant influence is the seasonal irrigation and drainage in the paddy fields, which cause soil redox potential changes. The changing redox potential drives Pb-Zn tailings dissolution rate, which in turn controls the mobility, potential toxicity, and ultimate fate of heavy metals. Consequently, the concentrations of soluble Pb, Cd, Zn, and Cu from paddy fields with aerobic/anaerobic conditions are slightly higher than those aerated with oxygen [19]. In addition, the non-uniform distribution of Pb-Zn tailings related to land-use practices cause some anomalous soil properties.

The numbers of living bacteria, actinomycetes, and fungi from cornfields are higher than those from paddy fields (Table 2). The results further indicate that Pb, Cd, Zn, and Cu inhibit soil microorganisms relating to their germination and growth [12, 20]. Bacteria as the dominant population without significant differences from cornfields and paddy fields are related to highly significant differences of Pb, Cd, Zn, and Cu. Fungi and actinomycetes are more sensitive to Pb, Cd, Zn, and Cu than bacteria.

Knowing that soil enzymes, primarily produced by soil microorganisms, catalyze reactions in soil system relating to the decomposition of pollutants as well as wastes; consequently, they are used as potential and useful indicators for soil management and nutrient cycle processes due to their sensitivity [2]. Often, organic manure can increase the soil enzyme activities that usually show a positive and significant relationship with the C-N-P cycle [21]. Conversely, the C-N-P turnover and nutrient availability are reduced with the soil degradation processes [22]. In addition, the heavy metal ions inhibit these enzyme activities through binding to the substrate or combining with the protein-active groups [23]. So, the action mode depends on the substrate molecules used. Due to the inhibitory action of Pb-Zn tailings, soil enzymes (except protease) have low activities in the paddy fields compared with cornfields (Table 2). Therefore, protease, which can catalyze hydrolysis of proteins originating from microbes, plants, and animals, shows high activity in paddy fields compared with cornfields.

In addition, urease, secreted by microbes and plants, can catalyze the hydrolysis of urea to NH_3. Rodríguez-Caballero et al. demonstrated the importance of urease and proteases in the nitrogen cycle [24]. Cellulase catalyzes the endohydrolysis of 1, 4-ß-D glucosidic linkages in cellulose. The cellulase decomposition process is sensitive to heavy metals in soil and extremely slow in the polluted paddy soil [25]. Invertase catalyzes hydrolysis of sucrose into glucose and fructose. Invertase and cellulose, which are both involved in the carbon cycle, are quite important to carbon source supply [26]. The acid phosphatase involved in phosphorus nutrient cycling can catalyze the conversion of inorganic phosphorus from organic phosphorus compounds [26]. The activities of all 4 groups of enzymes, i.e., acid phosphatase, urease,

Table 2. Variation characteristics of soil enzymes and soil properties in a Pb-Zn tailings-contaminated karst area.

Land use type	SOC	TN	C/N	AN	AP	AK	pH	CEC	Urease	ACP	Invertase	Cellulase	Protease	Bacteria	Actinomycetes	Fungi
Corn field (Sample numbers:16)	13.68b	0.72b	19.21a	76.88a	59.55a	42.17a	5.44a	5.25a	0.26a	5.84a	8.37a	2.00a	153.69a	2.57a	6.71a	9.13a
Paddy field (Sample numbers:16)	18.23a	0.87a	22.23a	73.28a	45.40a	34.84a	5.81a	5.53a	0.19b	5.33a	3.82b	1.39b	205.43a	0.63a	3.31b	4.05b

g/kg is for SOC and TN; mg/kg is for AN, AP and AK; cmol/kg is for CEC; 10^5 cfu/kg is for bacteria; 10^3 cfu/kg is for actinomycetes; 10^4 cfu/kg is for fungi; mg(NH$_4$-N)/(24h·g) is for urease; mg (phenol)/(2h·g) is for acid phosphatase (ACP); mg (glucose)/(24h·g) is for invertase and cellulase; mg(NH$_2$-N)/(24h·g) is for protease. Different letters (a, b) indicate a significant difference at the 0.05 probability level; same letters (a, a) indicate no significant difference at the 0.05 probability level.

invertase, and cellulase, are lower from paddy fields than those from cornfields due to the inhibiting effect of Pb-Zn tailings related to land-use practices. When the differences in enzyme activities from the cornfields and paddy fields are evaluated, it is found that the urease, invertase and cellulase involved in the C-N cycle have significant differences. These enzyme activities (such as urease, invertase, and cellulase) could improve our understanding of the intimate relationship between soil microorganisms. However, acid phosphatase and protease have no significant difference in the two land-use practices (Table 2).

It should be noted that SOC and TN from cornfields are significantly lower than those from paddy fields, though no significant difference of C/N appears in cornfields and paddy fields (Table 2). The soil carbon/nitrogen balance can be assessed by the soil C/N ratio, which is important for the soil carbon/nitrogen cycle process [4]. The low C/N ratio can enhance microbial activity and accelerate the mineralization rate of SOC and TN. In view of the microbial response to Pb, Cd, Zn, and Cu inhibition, less inorganic nitrogen and more organic fertilizers will be input to maintain the balance of SOC and TN due to high C/N ratio in soils. The AN, AP, and AK in cornfields are higher than those from paddy fields, though no significant difference is found between cornfields and paddy fields. The results indicate that due to the low C/N ratio in cornfields, the local farmers will use more nitrogen, phosphorus, and potassium to increase crop yield, but neglect the input of organic fertilizer. From Table 2, it can be seen that the average soil pH from cornfields is 5.44 and the average soil pH from paddy fields is 5.81. The pH results from the complex interaction between the calcium-rich as well as alkaline karst soil and the Pb-Zn tailings [8]. However, no significant difference of pH appears in cornfields and paddy fields.

Often, heavy metal adsorption can be promoted by soil organic matter, which depends on soil properties or simulation experiment conditions, including pH and CEC [27]. The microorganism population can be reduced by Pb, Cd, Zn, and Cu, which in turn can decrease the activities of invertase, urease, cellulase, and acid phosphatase. Consequently, the decomposition rate of carbon, nitrogen, and phosphorus in soils would be blocked. The SOC and CEC from paddy fields are quite a bit higher than those from cornfields (Table 2), though no significant difference of CEC appears in cornfields and paddy fields.

Moreover, reduced microbial biomass as well as cellulase, invertase, urease, and acid phosphatase activities, which are related to C, N, and P transformations, most likely restrict nutrient uptake rate in paddy fields. Therefore, the accumulated SOC and TN in paddy fields, as previously reported [7, 12], is related to the decreased microbial biomass and reduced enzyme activities. Moreover, the relationship between enzyme activities associated with the C, N, and P cycles and long-term soil productivity in the Pb-Zn tailings-contaminated karst area need to be quantified in future research.

Correlation Analysis

The toxicity of heavy metals to soil enzymes and microbes and their relationships associated with soil physicochemical properties have been extensively studied [20]. For example, the effects of wastewater from mining operations, but not those of Pb-Zn tailings-contaminated water, in coarse-textured agriculture soils have been discussed [28]. However, a relationship between the magnitude of the adverse effects from tailings and soil properties such as pH or organic matter has not been established [4]. SOC showed a significant positive correlation with TN, AN, AP, CEC, total-Zn, total-Cu, DTPA-extractable Pb, and DTPA-extractable Cd at $P<0.05$ or $P<0.01$ (Table 3), which can be attributed to the restricted microbial communities and their activities. Similarly, other studies have shown that microbial biomass was significantly correlated with SOC content [17].

Soil pH can affect the adsorption/desorption of soil ions and soil nutrient transformation [8]. In our study we found a negative correlation between the microbiological characteristics (such as urease and protease activities, microbes) and soil pH. Similar results have been obtained by Taylor et al. [29] in studies on the relationship between soil pH and microbiological characteristics in Michigan (USA) sand soils with pH 5.5 and Iowa (USA) clay soils with pH 5.3.

CEC is related to heavy metal toxicity affecting microorganism activities in soils [30]. The CEC depends on the amount and type of hydrous metal oxides and organic matter. As a result of inhibition of sustained-release Pb, Cd, Zn, and Cu by Pb-Zn tailings, there is a significant positive correlation between CEC and SOC.

The negative correlations between some soil microbiological characteristics and total heavy metal content found in our research are shown in Table 3. Each toxic metal owns a different mechanism that inhibits enzymes, or reacts directly with DNA. Soil microbiological characteristics will show considerable differences relating to different heavy metals; the negative effects from heavy metal to soil microbiological characteristics have been reported recently, suggesting that soil microbiological characteristics are significantly inhibited by heavy metals [26, 31]. However, a significant increase in microbial biomass and activity was also found under the heavy metal stress because the soil enzyme activities in the acid environment can vary [2, 32]. This suggests that the microbial community structure as well as soil enzyme activities, depending on land-use practices and the type of heavy metals, can be an important factor explaining discrepancies among previous studies.

Path Analysis

Path analysis, a statistical technique differentiating between correlation and causation, has been increasingly used to define the best criteria for selection in biological and agronomical studies [18], which can partition the

Table 3. Correlation coefficients between soil enzyme activities and soil properties.

	TN	AN	AP	AK	CEC	pH	Ure	ACP	Inv	Cel	Pro	Bac	Fun	Act	TPb	TZn	TCu	TCd	APb	AZn	ACu	ACd
SOC	0.856**	0.357*	0.432*	-0.041	0.360*	-0.248	-0.016	-0.206	0.281	-0.330	0.264	-0.261	-0.234	-0.418*	0.301	0.361*	0.379*	0.267	0.416*	0.277	0.127	0.500**
TN		0.443**	0.256	0.178	0.335	-0.055	-0.017	-0.234	0.409*	-0.235	0.343	-0.388*	-0.358*	-0.474**	0.361*	0.463**	0.444**	0.175	0.358*	0.354*	0.163	0.569**
AN			0.238	0.202	0.084	-0.320	0.530**	-0.225	0.053	0.057	0.098	-0.177	0.230	-0.107	0.177	0.307	0.292	0.109	0.509**	0.208	0.155	0.368*
AP				-0.056	0.267	-0.310	0.108	-0.086	-0.434*	-0.242	-0.358*	0.263	-0.004	0.086	0.019	-0.230	0.027	0.335	0.471**	-0.319	0.175	-0.177
AK					0.344*	0.330	0.131	-0.180	0.066	0.024	0.286	-0.025	0.092	-0.148	0.007	0.283	0.087	-0.241	-0.281	0.239	-0.120	0.313
CEC						0.198	0.051	0.033	0.057	-0.157	0.179	0.090	-0.099	-0.112	-0.072	0.087	-0.003	0.123	-0.066	-0.010	-0.267	0.064
pH							-0.147	0.307	0.105	0.133	-0.026	-0.138	-0.253	-0.090	-0.277	-0.227	-0.430*	-0.033	-0.162	-0.204	-0.380*	-0.193
Urea								0.248	-0.301	0.415*	-0.161	0.166	0.620**	0.266	-0.276	-0.231	-0.266	-0.016	0.191	-0.182	-0.193	-0.199
ACP									-.187	0.105	-0.477**	0.203	0.103	0.095	-0.090	-0.637**	-0.486**	0.236	0.143	-0.577**	-0.125	-0.579**
Inv										0.005	0.697**	-0.476**	-0.460**	-0.445**	0.268	0.643**	0.339	-0.272	-0.133	0.738**	-0.134	0.658**
Cel											0.157	0.356*	0.146	0.404*	-0.258	-0.015	-0.059	-0.460**	-0.176	0.070	-0.107	-0.194
Pro												-0.313	-0.275	-0.287	0.153	0.794**	0.438*	-0.576**	-0.348*	0.901**	-0.148	0.668**
Bac													0.382*	0.534**	-0.258	-0.353*	-0.095	-0.178	0.029	-0.360*	0.155	-0.478**
Fun														0.308	-0.192	-0.298	-0.143	0.028	0.027	-0.248	0.079	-0.249
Act															-0.379*	-0.402*	-0.372*	-0.031	0.091	-0.309	-0.167	-0.465**
TPb																0.269	0.435*	0.230	0.255	0.280	0.366*	0.394*
TZn																	0.743**	-0.421*	-0.223	0.859**	0.162	0.890**
TCu																		-0.132	-0.016	0.522**	0.744**	0.634**
TCd																			0.562**	-0.500**	0.228	-0.240
APb																				-0.276	0.228	-0.109
AZn																					-0.047	0.786**
ACu																						0.136

*Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed) (Ure: urease, ACP: acid phosphatase, Inv: invertase, Cel: cellulose, Pro: protease, Bac: bacteria, Act: actinomycetes, Fun: fungi, T: total; A: DTPA)

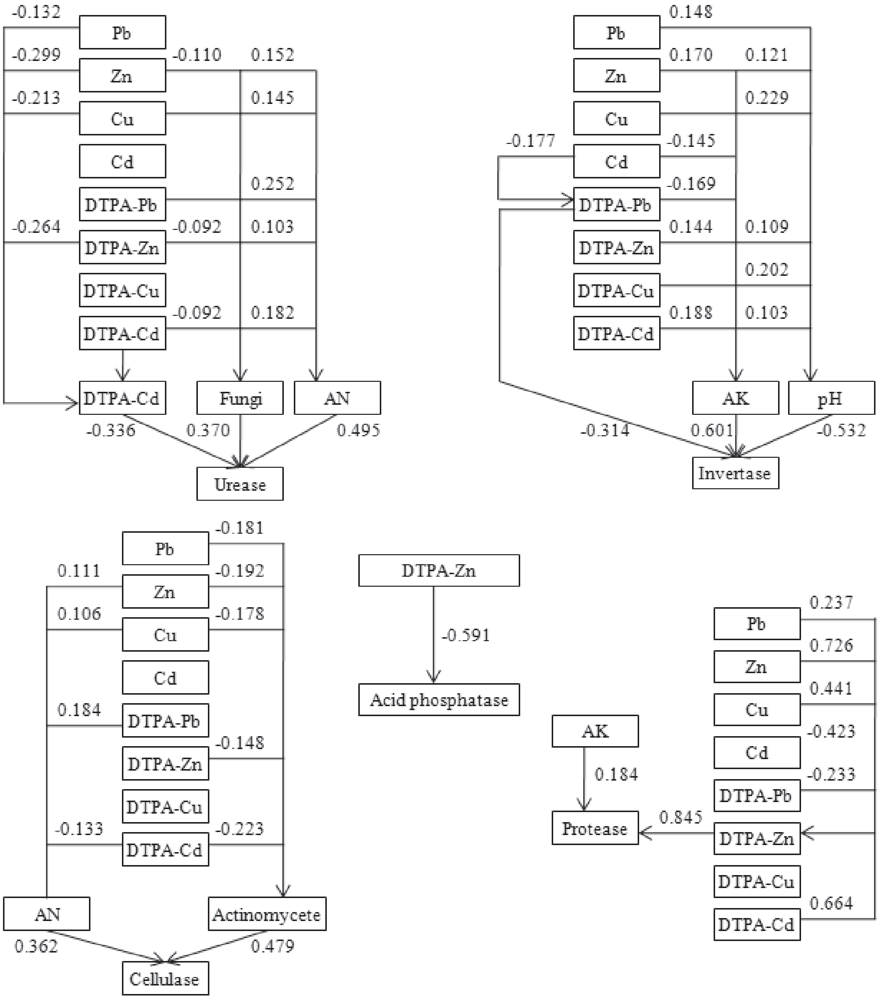

Fig. 2. Path coefficients of soil enzymes.

correlation coefficients into direct and indirect effects [33]. Moreover, path analysis allows for a direct comparison of causal relationships between soil enzymes and soil properties under interference of the Pb-Zn tailings. Therefore, in our study, path analysis can provide a somewhat different explanation than correlation analysis for soil enzyme activities and soil properties from the two different types of Pb-Zn tailings-contaminated agricultural fields. Moreover, path analysis reveals an additional relationship: namely, the relative importance of the concerted action of multiple heavy metals on soil enzyme activities. The results of path analysis on soil enzyme activities and soil properties from Sidi are given in Fig. 2.

Urease shows a direct positive correlation with fungi and AN, and a direct negative correlation with DTPA-extractable Cd (the corresponding direct path coefficients are 0.370, 0.495, and -0.336, respectively). This indicates that DTPA-extractable Cd inhibits urease activity secreted by fungi (the correlation coefficients are -0.199 and 0.620) and connecting with the N cycle (Table 3). Due to environmental stress, fungi in soil usually form a symbiotic association with plant roots, named mycorrhizal fungi, which colonize more than 80% of the terrestrial plant roots and provide a variety of benefits to their hosts, including increased nutrient uptake under low-input conditions, resistance to plant pests, improved water relations and drought resistance, and increased growth and yield of plants [34]. Though the mechanisms of translocation and transfer of some nutrients from fungi to the host are virtually unknown, fungi have the capacity to utilize and mineralize biodegradable organic compounds [35]. Hence, their role in soil carbon/nitrogen cycles can be explained due to the relatively low pollution of DTPA-extractable Cd in the cornfields.

The mycorrhizal fungi have been reported about nutrients and energy transfer between soil fungi and their host plant [36]. Their host plant can acquire mineral nutrients like nitrogen and carbon through symbiotic mycorrhizal fungi. Urease belongs to a group of enzymes acting on the carbon-nitrogen bond of urea. The mycorrhizal fungi gain nitrogen and carbohydrate from the degradable soil organic matter which, in turn, increases the supply of available nitrogen [37]. So, the TN and SOC are not easily accumulated in the cornfields and the “elusive” carbon and nitrogen pools appear in the paddy fields due to the different accumulation conditions under Pb, Cd, Zn, and Cu stress (Tables 1 and 2).

The fact that urease activity is greater in soil amended with DTPA-extractable Cd indicates that DTPA-extractable Cd has a negative effect on urease activity (Table 3). Similar results have been reported by He et al. [38], who discovered that soil enzyme activities could be stimulated by low Cd content, but inhibited by the addition of higher concentrations of Cd. Moreover, from our results, urease can be regarded as the dominant soil enzyme. However, more investigations are required to elucidate this. Moreover, cellulase shows a direct positive correlation with actinomycete and AN (the corresponding direct path coefficients are 0.479 and 0.362, respectively), indicating that cellulase is released by the actinomycetes and closely linked with soil N cycle. This result also proved that carbon and nitrogen cycles in terrestrial ecosystems had a complex relationship, and that nitrogen-carbon was released from the biodegradable soil organic matter by fungi and actinomycetes. Moreover, actinomycetes show an indirect negative correlation with total Pb, Zn, and Cu, as well as DTPA-extractable Zn and Cd (Fig. 2 and Table 3), as proven by Hu et al. [6].

Like other soil enzymes, acid phosphatase shows a direct negative correlation with DTPA-extractable Zn (the corresponding direct path coefficient is -0.591), indicating that acid phosphatase is inhibited by DTPA-extractable Zn. Invertase has a direct positive correlation with AK and a direct negative correlation with pH and DTPA-extractable Pb (the corresponding direct path coefficients are 0.601, -0.532, and -0.314, respectively) as well as protease showing a direct positive correlation with DTPA-extractable Zn and AK (the corresponding direct path coefficients are 0.845 and 0.184, respectively). The results indicate that invertase and protease jointly participate in the absorption of AK in the soil. Moreover, protease has a direct positive correlation and a significant positive correlation coefficient with DTPA-extractable Zn. This indicates that DTPA-extractable Zn can combine with the protein-active groups. As invertase, DTPA-extractable Pb may react with the invertase-substrate complex and inhibit its activity. Due to acidification from the tailings, soil pH is quite lower than that of karst soil [6]. So, invertase is inhibited by soil pH and DTPA-extractable Zn due to their complex relationship.

Conclusions

Due to the non-uniform distribution of Pb-Zn tailings and agricultural land-use practices in this area, the results indicate that Cd is the main potential ecological risk factor and appears to be significantly different in cornfields and paddy fields. Moreover, the morphology, metabolism, and growth of soil microorganisms related to protein denaturation, functional disturbance, and destruction of cell membrane integrity can be impaired by the sustained-released Pb, Cd, Zn, and Cu from Pb-Zn tailings. Therefore, sensitive fungi and actinomycetes as well as soil enzymes (urease, invertase, and cellulase) excreted by the restrained microorganisms from cornfields are significantly higher than those from paddy fields.

Path analysis provides a somewhat different explanation than correlation analysis for soil enzyme activities and soil properties. Protease has a direct positive correlation with DTPA-extractable Zn, although urease, invertase and acid phosphatase have a direct negative correlation with DTPA-extractable Cd, DTPA-extractable Pb, and DTPA-extractable Zn, respectively. Thus, from our study, the reduction of water-soluble Pb, Cd, Zn, and Cu contributes to the increase of enzyme activities and soil microorganisms. The positive correlations between soil enzyme activities and SOC as well as TN indicate that soil enzymes excreted by restrained microorganisms limit the soil C-N cycle process that affects SOC and TN concentration. Therefore, the "elusive" carbon and nitrogen pools appear in Pb-Zn tailing-contaminated karst paddy fields. To obtain a better understanding of the formatting process about "elusive" carbon and nitrogen pools [39], soil stable isotope labeling experiments should be applied to probe C-N cycle in the future.

Moreover, due to the alternating drought and flooding in paddy fields, soluble Pb, Cd, Zn, and Cu in paddy fields will increase and affect the quality of edible agricultural products. Therefore, a rice plantation is not recommended and adjusting cropping system is particularly acute in the still-used karst areas contaminated by Pb-Zn tailings.

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