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Rapid ecotoxicological assessment of heavy metal combined polluted soil using canonical analysis

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Abstract: Quick, simple to perform, and cheap biomarkers were combined in a rapid assessment approach to measure the effects of metal pollutants, Cu, Cd, Pb and Zn in meadow burozem on wheat. Analysis of orthogonal design showed that the significant zinc factor indicated both the inhibition rate of shoot mass and that of root elongation were affected by zinc($P < 0.05$ and $P < 0.01$, respectively). The first toxicity canonical variable (TOXI), formed from the toxicity data set, explained 49% of the total variance in the toxicity data set; the first biological canonical variable(BIOL) explained 42% of the total variation in the biological data set. The correlation between the first canonical variables TOXI and BIOL (canonical correlation) was 0.94 ($P < 0.0001$). Therefore, it is reliable and feasible to use the achievement to assess toxicity of heavy metal combined polluted soil using canonical analysis. Toxicity of soil combined polluted by heavy metals to plant community was estimated by comparing the IC_{50} values describing the concentration needed to cause 50% decrease with grow rate compared to no metal addition. Environmental quality standard for soils prescribe that all these tested concentration of heavy metals in soil should not cause hazard and pollution ultimately, whereas it indicated that the soils in second grade cause more or less than 50% inhibition rates of wheat growth. So environmental quality standard for soils can be modified to include other features.

Keywords: heavy metal; combined pollution; ecological toxicity; canonical analysis

Introduction

Elevated concentration of heavy metals such as copper and cadmium exist in many agricultural soils from mining activities (Chen, 2000; Dudka, 1997; Lambert, 1997) and from management practices including irrigation with sewage and utilization of sewage sludge(Clapp, 1994). Once heavy metals entered into soils, regardless the sources, they would not only be accumulated by organisms, but also circulate in the food chain (Uhlig, 2000; Barcan, 1998). Furthermore, their ramification would be retained in soil ecosystem with toxic concentration(Shaw, 1990), which often presents an unacceptable long-term risk to human and ecosystem health, especially to the sustainable food production(Adriano, 1997; Piexzynski, 1997). Thus it is important to fully assess the impact of metal releases into the soil.

In addition, it is easier for heavy metals to bring on concomitant harm to environment safety when they coexist at high doses in soils. As for combined pollution with heavy metals, the toxic effects of them on ecosystem are very different from those of single metal (Ross, 1996; Salt, 1998). In this field, no consistent results have been reported and even some researchers obtained opposite conclusions (Shehata, 1999; Yu, 1995). Owing to time, expertise, and cost constraints, it is very difficult to establish an assessment index of the combined pollution of heavy metals(Zheng, 1989). Toxicity testing using high plants, such as wheat(*triticum acstivnm*), is a rapid assessment approach to measure exposure and effects of metal pollutants in soils(Basta, 2001; Ulrich, 1999). By using some quick, simple methods to perform and inexpensive biomarkers, this study provided a useful means for rapid eco-toxicity diagnosis of heavy metal combined polluted soil. The joint influence of heavy metals on wheat growth in brown soil where combined pollution of exterior pollutant occurred was investigated. The work is probably useful to evaluate potential toxicity and provide information for editing some environmental criterion of heavy metals in soil.

1 Materials and methods

1.1 Test materials

Foundation item: The National Key Foundation Project of Research and Development Programme of China(No. G1999011808), the National Science Fundation for Distinguished Young Scholars(No. 20225722) by NSFC, and a special grant from Scientific and Social Practice for Graduates of Chinese Academy of Sciences

A 0—20 cm top layer soil was collected from main lead and zinc mining areas at Fengcheng City in Liaoning Province, China. The soil was typical brown soil, prevalent in northeast China. Its basic properties are as follows: pH 6.22; organic matters 1.65%; anion exchange capacity 12.26%; Kjedahl-nitrogen, total P and K 0.12%, 0.04% and 0.24% respectively. The background values of Cu, Zn, Pb and Cd were 32.9 mg/kg, 28.1 mg/kg, 11.1 mg/kg and 0.17 mg/kg, from which no pollution was discovered in this tested soil. All experiments employed air-dried soils, which were sieved through a 2 mm mesh.

Test crop was wheat (*triticum acstivnm*) whose variety is Liaoning Spring No.10, commonly planted in northern China.

Four kinds of heavy metals, Cu, Cd, Pb and Zn were selected as test pollutants because they are often presented in brown soil area as a result of active mining industry. Cu and Zn both as sulfate salts, Cd as chlorate salt and Pb as nitrate salt, were used in this study. All these metals were analytical reagents.

1.2 Experimental design

For the purpose of studying the relationship between various parameters affecting environment quality of soil and toxic effects they caused to wheat, a twenty five-run experiment using $L_{25}4^5$ Taguchi orthogonal design was employed. The tested concentration of Cu, Pb and Zn was respectively determined in accordance with Environmental Quality Standard for Soil about heavy metals in China (National Environmental Protection Bureau, 1995). They are listed in Table 1. All treatments were triplicated in order to decrease experiment errors.

Table 1 A $L_{25}4^5$ Taguchi orthogonal design of various parameters in toxicity test and measured results

No.	Experimental design Parameter, mg/kg dry soil				Measured results Inhibition rates, %	
	Copper	Cadmium	Lead	Zinc	Shoot mass	Root elongation
1	0	0	0	0	0	0
2	0	0.6	50	50	5.67	2.05
3	0	1.8	200	200	2.34	7.95
4	0	5.4	350	350	42.23	75.64
5	0	16.2	500	500	75.07	94.36
6	50	0	50	200	8.6	12.05
7	50	0.6	200	350	29.13	72.56
8	50	1.8	350	500	70.87	91.54
9	50	5.4	500	0	-0.68	11.79
10	50	16.2	0	50	-16.13	2.31
11	200	0	200	500	75.07	92.31
12	200	0.6	350	0	0.68	27.18
13	200	1.8	500	50	13.2	18.72
14	200	5.4	0	200	40.08	78.46
15	200	16.2	50	350	28.8	52.82
16	350	0	350	50	26.1	56.15
17	350	0.6	500	200	31.18	75.64
18	350	1.8	0	350	54.45	93.08
19	350	5.4	50	500	83.28	95.38
20	350	16.2	200	0	13.2	61.28
21	500	0	500	350	78.3	93.85
22	500	0.6	0	500	94.33	98.72
23	500	1.8	50	0	43.99	66.92
24	500	5.4	200	50	20.23	52.82
25	500	16.2	350	200	18.08	83.85

The significant factors affecting the inhibition rates of shoot mass and root elongation

Factor	The inhibition rates of shoot mass	The inhibition rates of shoot mass
Zn	$P < 0.05$	$P < 0.01$

According to the experimental design, different dosages of $CuSO_4$, $CdCl_2$, $Pb(NO_3)_2$ and $ZnSO_4$ were added to the tested soil after 50 mg of this soil was added to a plate with inner-diameter 90 mm. The water content of soil was adjusted to 60% of the water-holding capacity with 10 ml distilled water before twelve hours cultivation without disturbance. When the cultivation finished, fifteen seeds of wheat, disinfected with 7% formaldehyde solution, were uniformly exposed to the polluted soil. All treatments were incubated for 42 h without light at 25℃. Growth was stopped when the elongation of seminal roots and the rate of seed germination cultured in the soil without metal addition were up to 20 mm and 65% respectively.

Shoot mass and root elongation were measured simultaneously (Zhang, 1994). Means of triplicate were applied to calculate biological variables, including the inhabitation rates of shoot mass and root elongation. The inhabitation rates were used as an index of heavy metal tolerance of the wheat in the soil, and expressed as a percentage of the variable difference between samples with and without metal additions to the variables without metal addition $[(V_0 - V_t)/V_0] \times 100\%$ (Table 1).

1.3 Statistical analysis

To examine the statistical difference between the treatments, the results were subjected to the analysis of variance (ANOVA) as a 4^5-factorial experiment. The four main effects (Cu, Cd, Pb and Zn) and interaction were tested and their separate effects are shown in Table 2.

Canonical correlation analysis (CCA), performed for each treatment data separately, was used to investigate the relationships between soil toxicity and biological effects of plant variables. It generates pairs of linear combinations from two sets of original variables such that the correlation is maximal between the pairs of the new canonical variables (Gittins, 1985). A canonical variable is a linear summary of the set of input variables (Gittins, 1985). The data set of soil toxicity consisted of Cu, Cd, Pb, Zn, and the biological effects data set consisted of the inhabition rates of root elongation and shoot mass, depending on which variables were determined on the experiment in question. The new canonical variables are called TOXI and BIOL. Graphical presentations of CCA are scatter plot diagrams of each treatment on TOXI (x axis) and BIOL (y axis) (Fig. 1). Canonical structure (i.e., correlation between the original variables and canonical variables) was applied to the figure with the arrows of the original variables indicating the influence of the most important original variables on formation of the new canonical variable. Canonical correlation analyses were performed on SAS using the CANCORR procedure (SAS Institute, 1996).

Table 2 Summary of the canonical correlation analysis

Toxicity variable	TOXI	Biological variable	BIOL
Cu	0.46	Inhibition rate of shoot mass	0.65
Cd	-0.08	Inhibition rate of root elongation	0.38
Pb	0.08		
Zn	0.88		
Correlation *	0.94		
%T‡	49%	B%	42%
%B	46%		

Notes: * The correlations between the original and the first canonical variables TOXI (toxicity data set) and BIOL (biological data set). ‡%T, standardized variance of the toxicity variables explained by TOXI; %B, standardized variance of the biological variables explained by TOXI and BIOL

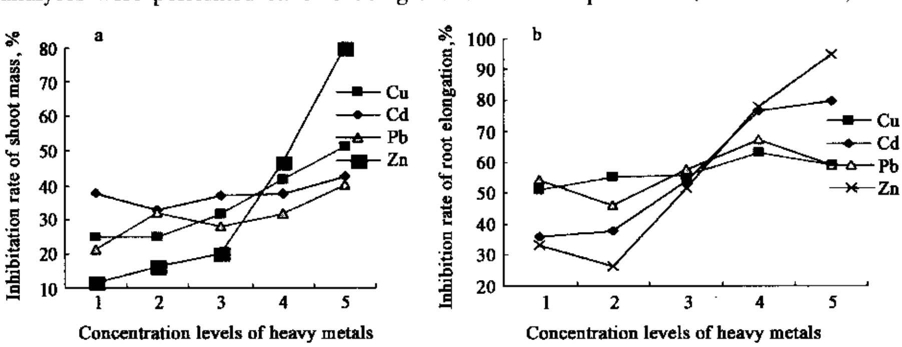

Fig. 1 Straightforward analysis of orthogonal design
a. the effects of heavy metals on the inhibition rate of shoot mass; b. the effects of heavy metals on the inhibition rate of root elongation mass

2 Results and discussion

2.1 Toxicity test

The results of the toxicity test are presented in Table 1. The significant zinc factor indicated both the inhibition rate of shoot mass and that of root elongation were affected by zinc($P < 0.05$ and $P < 0.01$ respectively). The other three metal factors have effects on them to some degree though they do not obtain significance($P > 0.05$). There is so little importance of their interaction that the variances of them were absorbed into the error.

As far as each metal, it has the same effects on the shoot and root. The inhibition rates of shoot mass and root elongation are low at low concentration while high at high concentration in general(Fig.1), which makes it possible for the biological variables to indicate the pollution situation of soil. However, because the added metal treatments had interaction effects on wheat tolerance even though the effects tended to have low values in this experiment, separate effects of these four metals are a bit different from those when they exist individually in soil(Song, 2000). The concentration of Cu, Cd, Pb and Zn should be about 500, 270, 1110 and 1200 mg/kg respectively if the inhibition rate of root elongation of wheat was up to 50% when there is no interaction(Song, 2000). While as for the combined pollution, the concentration of these metals was far lower compared to the precedent.

2.2 Canonical correlation analysis

The canonical correlation analysis(CCA) of the soil toxicity and biological effects of plant variables for these treatments is presented in Fig. 1. Cu, Cd, Pb and Zn were selected to represent the toxicity of polluted soil in CCA and the inhibition rates of shoot mass and root elongation represent biological effects caused by heavy metal pollution.

The canonical structure, providing the correlations of the original variables with their first canonical variables, and the proportion of explained variance, are presented in Table 2. The first canonical variable (TOXI), formed from the toxicity data set, explained 49% of the total variance in the toxicity data set, suggesting that the first canonical variable provided a fairly effective summary of the original toxicity variables. The first biological canonical variable (BIOL) explained 42% of the total variation in the biological data set. The correlation between the first canonical variables TOXI and BIOL (canonical correlation) was $0.94(P < 0.0001)$.

The interpretation of CCA with respect to the treatments is presented in Fig.1, where the sample plots are plotted along the first canonical axes TOXI and BIOL, the arrows describing the canonical structure (Table 2). Plots situated in the right upper corner, according to the canonical structure, are characterized by high toxicity and high biological effects on wheat. Low toxicity and low biological effects characterized the left lower plots.

Since both of the first canonical variables provide so much important information about the effects of the metal, and their interaction about the co-effects of these pollutants in soil, it is reliable and feasible to use the achievement to assess toxicity of heavy metal combined polluted soil using canonical analysis. Toxicity of soil combined polluted by heavy metals to plant community was estimated by comparing the IC_{50} values describing the concentration needed to cause 50% decrease with grow rate compared to no metal addition.

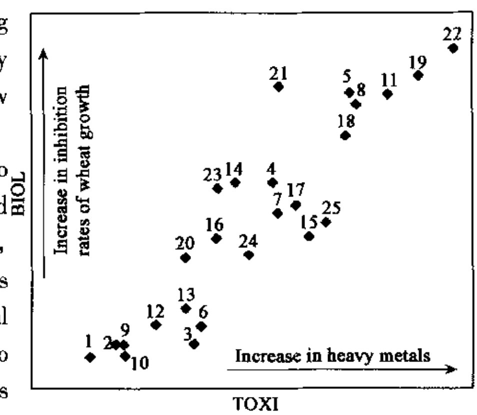

Fig. 2 Plot of treatments on the first canonical variables, TOXI(the toxicity data set) and BIOL(the biological data set) from canonical correlation analysis. The arrows indicate the effects of important variables on formation of the canonical variables

Depending on the applied function of soil and protection target, soils in China were roughly classified into three categories. Environmental quality standard for soils prescribe that all these three kinds of soils should not cause hazard and pollution ultimately, whereas it has been shown that the soils in second grade cause more or less than 50% inhibition rates of wheat growth, for example, the No. 4, 7, 14, 15 and 18 samples reached inhibition rates as 56.19%, 46.51%, 55.87%, 38.79% and 70.76% respectively.

3 Conclusions

Because the added metals had interaction effects on wheat tolerance even though the effects tended to have low values, separated effects of these four metals, Cu, Cd, Pb and Zn are a bit different from those when they exist individually in soil. The correlation between the first canonical variables TOXI and BIOL (canonical correlation) was 0.94 ($P < 0.0001$). Therefore, it is reliable and feasible to use the achievement to assess toxicity of heavy metal combined polluted soil using canonical analysis. Environmental Quality Standard for Soil can be modified to include other features(i.e., soil types, pH, concomitant objects, available content of pollutants, etc.).

It should be noted that the methodology could be implemented to any type of environmental assessment where multivariables play a great role in the environmental quality and give rise to biological effects on biota.

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