Joint Toxicity of Zinc, Cadmium and Plumbum on *S.obliquus*

Fan Zhang[1,a], Xin Zhang[1,a], Nan Xiao[1,a], Minyi Zheng[1,a] , Litang Qin[2,a], Lingyun Mo[2,a]*

[1]College of Environmental and Engineering, Guilin University of Technology, Guilin 541004, China

[2]Guangxi Key Laboratory of Environmental Pollution Control Theory and Technology, Guilin University of Technology, Guilin 541004, China

[a]Email: meizictgu@163.com

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Abstract. In this article, the study assessed $ZnSO_4 \cdot 7H_2O$ [Zn], $Pb(NO_3)_2$ [Pb], $CdCl_2 \cdot 2.5H_2O$ [Cd], and set the *S.obliquus* as biological indicators to study the toxicity of binary mixtures after 96h by trace analysis methods. The results showed that the relationship of toxicity among these compounds as follows: Cd> Zn> Pb. In addition, Zn, Pb and Cd experienced interactions with each other, which mainly showed additive effects and synergy effects. And with the changes of concentration, the types of the effect are also changed accordingly.

Introduction

The situation of soil heavy metal contamination in China is grim now, which is mainly caused by the pollution of Hg, Cd, Pb, As, Zn and Cu. Heavy metal pollutants can not be degraded by chemical or biological methods, and are easy to accumulate inside plants， animals and even human beings through the food chain cycle, resulting in the growth of toxicity, which threats the ecological environment, food safety and human health [1-3]. In recent years, heavy metal contamination of soil is serious, not only influenced the quality of agricultural products ， but also disrupted the arable land.

Alga is a kind of primary producer in aquatic ecosystems, and it can be an indicator water quality [4]. *S.obliquus* is a freshwater unicellular chlorella, widely distributed in the river area. It has strong vitality, and breed quickly. Also, it is sensitive to the changes of environmental conditions, when the impact on the water environment of the detection of pollutants, usually the impact of pollutants on *S.obliquus* as an important indicator test. In this study, *S.obliquus* as indicator organisms, Acute toxicity analysis of heavy metals Zn, Pb, Cd single and binary mixtures on *S.Obliquus*. In order to more accurately assess the ecological risk of contamination.

Material and methods

Experimental materials

The freshwater microalgae used was *S.obliquus* FACHB-12, which was supplied by Freshwater Algae Culture Collection at the Institute of Hydrobiology, FACHB, upon receipt of algae, in a clean workbench, algae will be transferred to the prepared culture media, *S.obliquus* culture medium using BG11. The Tecan M200 PRO purchased from TECAN Switzerland company, 96-well

flat-bottomed microplate purchased from greiner company. The properties of three heavy metals, $ZnSO_4 \cdot 7H_2O$ [Zn], AR, $CdCl_2 \cdot 2.5H_2O$ [Cd], RG and $Pb(NO_3)_2$ [Pb], RG, used in the test are listed in table 1. All of them were prepared in distilled water and stored at 4ºC.

Table 1. Chemical properties of three heavy metals

Compound	CAS	MW	Purity/ %
$ZnSO_4 \cdot 7H_2O$	7446-20-0	287.58	≥99.5
$Pb(NO_3)_2$	10099-74-8	331.21	99
$CdCl_2 \cdot 2.5H_2O$	7790-78-5	228.36	98

Experimental Methods

The cultivation of *S.obliquus*

In a clean bench sterile inoculation algae and then placed in artificial light incubator thermostat cultured, set the temperature 22 ° C, light intensity about 3000lux, light-dark cycle of 12h: 12h. Algae cell metabolism in the most productive period of inoculation, before the toxicity test, the adapter in the logarithmic phase of algae to fresh culture medium, culture is carried out after the experiment 2d.

Toxicity Test

Before the start of the experiment, when the test algae by microplate absorbance greater than 0.24, Referring to conduct experiments Chlorella microplate toxicity analysis (MTA)[5], the experiment was repeated 3 times per plate. Microplate after adding transparent cover at a temperature of 22℃, illumination 3000lux, Light - dark cycle than 12h:12h. measured exposure time t = 0, 24, 48, 72 and 96h microplate by Microplate reader, and the wavelength was 682 nm. The toxicity of a treatment was expressed as a percentage inhibition of the cell growth showed as Eq. 1.

$$I=1-(OD_{t,i}-OD_{t,0})/(OD_{0,i}-OD_{0,0}) \quad (1)$$

Where E was the inhibition at a time, i was expose time, $OD_{t,i}$ was measured optical density of treated cells at time i, $OD_{t,0}$ was measured optical density of treated cells at time 0, $OD_{0,i}$ was measured optical density of controlled cells at time i, $OD_{0,0}$was measured optical density of controlled cells at time 0.

The mixture experimental design

Binary mixtures used is Equivalent-Effect concentration Ratio (EECR)[6], to explore the mixed heavy metal toxicity. In this paper, we have a total of three sets of Zn-Pb, Zn-Cd, Pb-Cd binary mixtures, using the Mix-EC20, Mix-50, Mix-NOEC mixed system, for mixture toxicity testing and analysis.

Combination Index

Combination Index[7] calculated as follows Eq. 2:

$$CI_x=\sum_{i=1}^{n}\frac{c_i}{EC_{x,i}} \quad (2)$$

In the formula, CI_x is the Cl value when the effect is x%; Ci is the concentration of the i-th compound mixture which producted the effect of x%; ECx, i is the concentration of the i-th compound mixture which alone producted the effect of x%; n is the mixture fraction.

CI=1, >1 <1, said mixture interaction were additive, antagonistic and synergistic effect, this paper introduces observations based on the 95% confidence interval, if the confidence limit is

greater than 1, at the same time, the lower confidence limit of less than 1, the mixture interaction is added.

Results and disscussion

The toxicity of single heavy metal compounds on *S.obliquus*

Three kinds of heavy metals on *S.obliquus* 96h Toxic effects can be fitted with a Logit or Weibull function, showed in table 2. Three compounds CRC fitting parameters R> 0.95 and RMSE <0.08. The toxicity of a single compound is: Cd> Zn> Pb. The 96h concentration-response curves of three heavy metals on *S.obliquus* were showed in Fig.1.

Table 2. The 96h statistics of toxicity of single heavy metals on *S.obliquus*

Compound	model	NOEC	EC_{20}	EC_{50}	pEC_{50}
Zn	W	4.582E-07	1.468E-06	1.449E-05	4.839
Pb	W	7.046E-07	5.984E-06	4.455E-05	4.351
Cd	W	4.582E-07	2.196E-06	5.504E-06	5.259

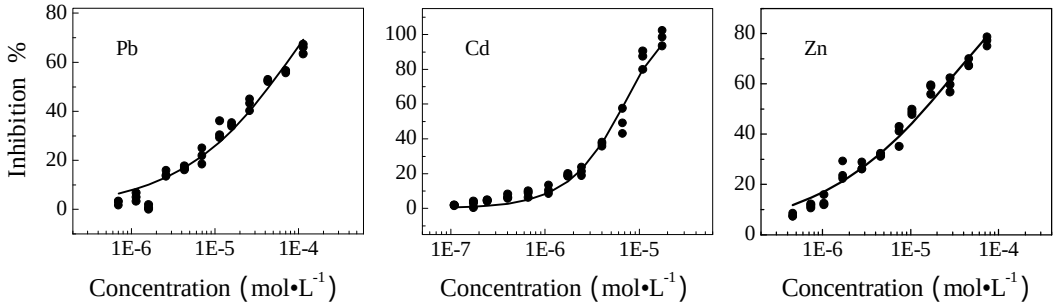

Fig.1 The 96h concentration-response curves of Zn, Cd and Pb on *S.obliquus*

The toxicity of binary mixture on *S.obliquus*

Zn, Pb, Cd three compounds build three binary mixed system, using Equivalent-Effect concentration Ratio design 3-ray binary mixtures for each system: Mix-EC20、Mix-50、Mix-NOEC. All data can be fitted with a Logit or Weibull function, each of rays obtained fitting parameters α, β, R, RMSE and pEC_{50} are in Table 3. The response-Index curves of Pb-Cd, Zn-Cd and Zn-Pb were showed in Fig.2, Fig.3 and Fig.4, respectively.

Table 3. The 96h statistics of toxicity of binary mixture heavy metals on *S.obliquus*

Binary system	Component	Pi		model	α	β	R^2	*RMSE*	pEC_{50}
		Pb	Cd						
Pb-Cd	EE-20	0.7315	0.2685	W	4.700	1.090	0.9619	0.0368	4.669
	EE-50	0.8900	0.1100	L	8.590	1.960	0.9481	0.0599	4.383
	EE-NOEC	0.6361	0.3639	L	12.790	2.500	0.9898	0.0188	5.116
		Zn	Cd						
Zn-Cd	EE-20	0.4007	0.5993	L	18.340	3.700	0.9620	0.0528	4.957
	EE-50	0.7247	0.2753	L	13.550	2.890	0.9750	0.04247	4.689
	EE-NOEC	0.5320	0.4680	L	14.310	2.740	0.9819	0.0233	5.223
		Zn	Pb						
Zn-Pb	EE-20	0.1970	0.8030	L	11.910	2.530	0.9828	0.0314	4.706
	EE-50	0.2454	0.7546	L	11.570	2.690	0.9852	0.0319	4.301
	EE-NOEC	0.3940	0.6060	L	13.800	2.550	0.9942	0.0182	5.412

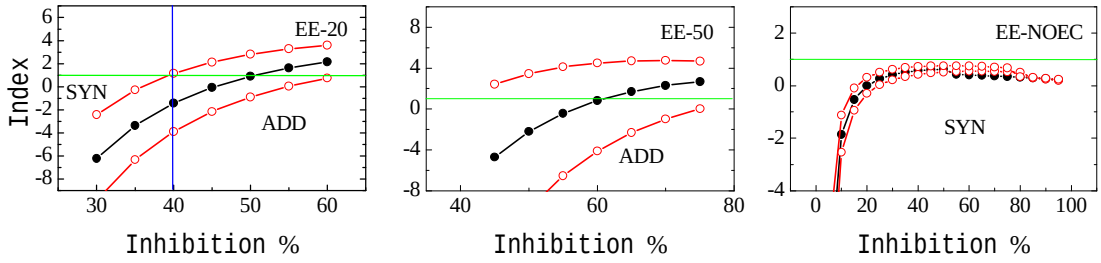

Fig.2 The 96h response-Index curves of Pb-Cd on *S.obliquus*.

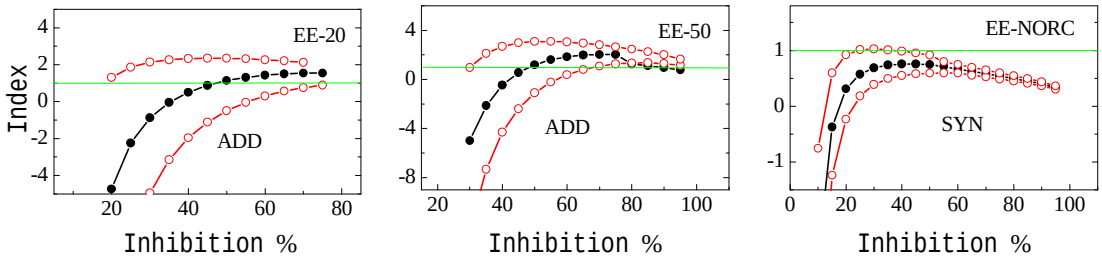

Fig.3 The 96h response-Index curves of Zn-Cd on *S.obliquus*.

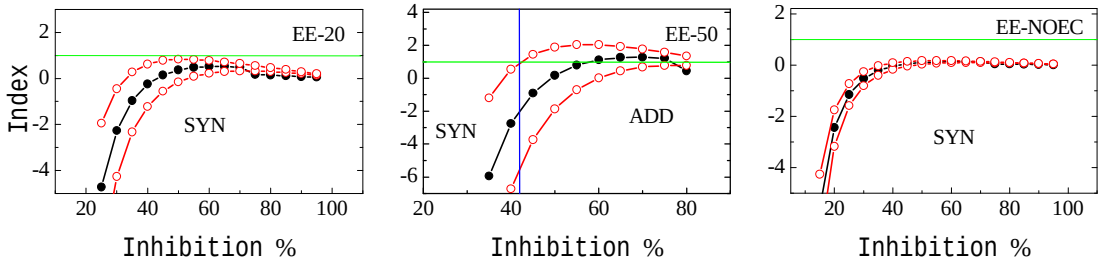

Fig.4 The 96h response-Index curves of Zn-Pb on *S.obliquus*.

From the figure shows, for Pb-Cd systems, EE-NOEC showed a synergistic effect, EE-20 showed synergistic effect in the range of 0-30 and the addition effect in the range of 30-100.EE-50 showed additive effect. For Zn-Cd system, EE-NOEC showed a synergistic effect, EE-20 and EE-50 showed additive effect. For Zn-Pb system, EE-NOEC and EE-20 showed a synergistic effect, EE-50 showed synergistic effect in the range of 0-40 and the addition in the range of 40-100.

Conclusion

The heavy metal for the study, the algae as biological experiments. The 96h toxicity on *S.obliquus* were studied, the results showed that the toxicity of a single compound is: Cd> Zn> Pb. Moreover, Zn-Pb, Zn-Cd, Pb-Cd have interaction, Three systems of binary mixtures at low concentrations of the mixed region have shown a synergistic effect and at high concentration of the mixed performance of regional additive effect.

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