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Original Research Paper

Modelling of the Joint Toxicity of Heavy Metals (Ni^{2+}, Co^{2+}, Cr^{3+} and Pb^{2+}) on Photobacteria Based on the Factorial Experiment (2^4)

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ABSTRACT

The joint toxicity of heavy metals (Ni^{2+}, Co^{2+}, Cr^{3+}, Pb^{2+}) and trends in toxicity were analysed by multiple linear regression and back propagation-artificial neural network models, using photobacteria as an indication organism in factorial experiments. The joint toxicity of Ni^{2+}, Co^{2+}, Cr^{3+} and Pb^{2+} mainly occurs through multiple interactions. Interactions between Ni^{2+}, Co^{2+} and Cr^{3+} weaken the single toxicity and binary interaction of Pb^{2+}. Binary or quaternary heavy metal mixtures exert mainly antagonistic effects, while ternary interactions are mainly synergistic. Increased concentrations of Pb^{2+}, Cr^{3+} and Ni^{2+} corresponded with increased toxicity of the mixed system, but Co^{2+} showed the opposite trend. The toxic effects of the mixed system were greatest with high Cr^{3+} concentration, while Pb^{2+} exerted the smallest effect.

INTRODUCTION

Heavy metals in the environment show high stability and slow degradation, and are cumulative and toxic. They can directly affect the human body through drinking water and domestic water, and can be absorbed by aquatic animals and plants, thereby entering the food chain and endangering humans and animals. Heavy metal pollution has become one of the major environmental problems throughout the world (Luo et al. 2011, Zhang et al. 2008). Pollutants in the environment do not appear singly and usually occur as mixtures, such that toxic effects in organisms can be complex. Köneman (1981) found that in some mixtures, even though the level of a single toxic pollutant is low, the joint toxicity may be significant. Some studies have examined the joint toxicity of heavy metals in China and throughout the world. Sun et al. (2009) found that the joint toxicity of copper and zinc in sea cucumber *Apostichopus japonicus* occurred by an antagonistic effect, whereas Yang et al. (2003) found that the joint toxicity of copper and cadmium against tadpole occurred through a synergistic effect. Other studies on joint toxicity investigated the effects of uranium and copper on *Lemna paucicostata* (Charles et al. 2006), and the effects of cadmium, lead, and zinc on fish (Wang et al. 2003). The results indicated that the joint toxicity of heavy metals is not simply the sum of single metal toxicities. However, most studies concentrated on the toxicity of binary, ternary, or quaternary mixtures, and little attention has been paid to the evaluation of joint toxicity.

Given that it is not feasible to assess pollutant mixtures with all possible compositions, we used a factorial design to fully explore the joint toxicities of pollutant mixtures with many concentrations of all components. A factorial experiment is a crossed experiment that consists of two or more factors. The experiment studies the effect of each factor on the response variable, as well as the effects of interactions between factors on the response variable (Montgomery 1998, Wei et al. 2007). In studying the joint toxicity of heavy metals, the factorial experiment can further analyse the degree of pollution and the toxicity mechanism of heavy metal pollutants.

This study used Ni^{2+}, Co^{2+}, Cr^{3+} and Pb^{2+} as the components of the mixed system, and used the factorial experiment to study the joint toxicity of the mixed system. The experimental results were used to establish a multiple linear regression model and a back propagation (BP)-artificial neural network model to analyse the joint toxicity effects.

MATERIALS AND METHODS

Reagents and instruments: The toxicity testing program used the following equipment: biological toxicity test instrument (DXY-2, Nanjing Soil Research Institute, Chinese Academy of Sciences), a vortex oscillator (5PCFT-IR, Ronghua), a cyclotron oscillator (HJ-6, Ronghua), a magnetic stirrer (HY-5, Ronghua), a vortex mixer (QL-901, Haimen Lindberg), an electronic balance (TP-220A, Xiangyi), and a digital analysis balance (TG328B, Shanghai Balance). Photobacterium phosphoreum lyophilized powder T3 (Nanjing Soil Research Institute, Chinese Academy of Sciences) was used as a biological indicator. The compounds selected as heavy metal pollutants were $Pb(NO_3)_2$, $Cr(NO_3)_3{\cdot}9H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$, and $Co(NO_3)_2$, and obtained as analytically pure reagents.

Experimental method: Toxicity testing of heavy metals on photobacteria was based on the Chinese Government Standard GB/T 15441-1995 (*Water quality-Determination of the acute toxicity-Luminescent bacteria test*). The toxicity of heavy metals was characterized by the rate of inhibition of photobacteria, and the relative deviation of the repeated detection was not higher than 15%.

Experimental design: The joint toxicity of Ni^{2+}, Co^{2+}, Cr^{3+} and Pb^{2+} was studied in two-level factorial experiments. In each experiment, each metal represented a "factor". Given that two-level factorial experiments were used in this study, each factor had a high level (EC30) and a low level (EC10). Another 16 sets of experiments were randomly mixed with the concentration of heavy metals outside the factorial experimental level. Design parameters are given in Table 1.

RESULTS AND DISCUSSION

Construction and analysis of multiple linear regression model: The experimental results are presented in Table 2. Datasets 1-16 were used for analysis of variance, and the results are given in Table 3. The main effect of the four heavy metals and the interaction between the heavy metals on the inhibition rate of the photobacteria were statistically significant. To further clarify the relationship between the dose concentration of heavy metals and the rate of inhibition of the photobacteria a multiple linear regression model was established with concentration as an independent variable and the inhibition rate as the dependent variable. A goodness-of-fit test is shown in Table 4, an *F*-test is shown in Table 5, and a *t*-test is shown in Table 6.

According to Tables 3-5, the correlation coefficient of the multiple linear regression model was 0.997, which suggests consistent agreement between the experimental and predicted results. All variables were evaluated via the *t*-test and *F*-test at a confidence level of 95%. The resulting relation is defined by Eq. 1:

$$T = 6.82 \times 10^2 \times Cr + 5.55 \times 10^3 \times Co + 244.992 \times Ni - 2 \times 10^6 \times Pb \times Co - 7.08 \times 10^4 \times Cr \times Co - 1.67 \times 10^3 \times Cr \times Ni - 1.38 \times 10^4 \times Co \times Ni + 3.019 \times 10^7 \times Pb \times Cr \times Co + 5.14 \times 10^6 \times Pb \times Co \times Ni + 1.44 \times 10^5 \times Cr \times Co \times Ni - 4.6 \times 10^7 \times Pb \times Cr \times Co \times Ni - 14.95 \quad ...(1)$$

where *T* represents inhibition rate of heavy metals on the photobacteria (%), and Pb, Cr, Co and Ni represent the concentrations of the respective metal ions (mmol/L).

According to Eq. 1, the joint toxicity of Ni^{2+}, Co^{2+}, Cr^{3+} and Pb^{2+} mainly occurs through multiple interactions. The independent effects of Ni^{2+}, Co^{2+} and Cr^{3+}, and the interactions of Pb^{2+}-Co^{2+}, Cr^{3+}-Co^{2+}, Cr^{3+}-Ni^{2+}, Co^{2+}-Ni^{2+}, and Pb^{2+}-

Table 1: Factorial design levels for joint toxicity of Pb^{2+}, Cr^{3+}, Co^{2+} and Ni^{2+} on photobacteria.

Level (mmol/L)	Pb^{2+}	Cr^{3+}	Co^{2+}	Ni^{2+}
Low(-)EC10	0.0001	0.0432	0.0001	0.0166
High(+)EC30	0.0012	0.1075	0.0095	0.3552

Table 2: Joint toxicity to photobacteria with different heavy metal contents.

Group	Pb^{2+} (mmol/L)	Cr^{3+} (mmol/L)	Co^{2+} (mmol/L)	Ni^{2+} (mmol/L)	Inhibition ratio (%)
1	0.0001	0.0432	0.001	0.0166	15.57
2	0.0012	0.0432	0.001	0.0166	19.52
3	0.0001	0.1075	0.001	0.0166	60.92
4	0.0012	0.1075	0.0095	0.0166	53.01
5	0.0001	0.0432	0.0095	0.0166	38.97
6	0.0012	0.0432	0.0095	0.0166	29.48
7	0.0001	0.1075	0.0095	0.0166	40.65
8	0.0012	0.1075	0.0001	0.0166	47.06
9	0.0001	0.0432	0.0001	0.3552	78.25
10	0.0012	0.0432	0.0001	0.3552	77.32
11	0.0001	0.1075	0.0001	0.3552	79.51
12	0.0012	0.1075	0.0001	0.3552	82.85
13	0.0001	0.0432	0.0095	0.3552	74.49
14	0.0012	0.0432	0.0095	0.3552	79.36
15	0.0001	0.1075	0.0095	0.3552	68.88
16	0.0012	0.1075	0.0095	0.3552	79.03
17	0.0001	0.0432	0.0095	0.3552	85.37
18	0.0001	0.0654	0.0017	0.3552	87.33
19a	0.0001	0.0833	0.0008	0.1265	73.16
20	0.0001	0.1075	0.0001	0.2373	58.08
21	0.0004	0.0833	0.0056	0.1265	55.60
22a	0.0004	0.0432	0.0008	0.2373	68.24
23	0.0004	0.0654	0.0017	0.0166	40.52
24	0.0004	0.1075	0.0001	0.3552	78.71
25a	0.0008	0.0432	0.0001	0.0166	22.94
26	0.0008	0.0654	0.0056	0.0833	54.79
27a	0.0008	0.0833	0.0008	0.1265	55.78
28	0.0008	0.1075	0.0095	0.2373	68.30
29a	0.0012	0.0432	0.0001	0.0833	32.05
30	0.0012	0.0654	0.0017	0.0166	40.20
31a	0.0012	0.1075	0.0095	0.3552	80.30
32a	0.0012	0.0833	0.0008	0.1265	71.33

Table 3: Variance analysis of joint toxicity of Pb^{2+}, Cr^{3+}, Co^{2+} and Ni^{2+} to photobacteria.

	Quadratic sum	df square	Mean	F	Sig.
Calibration model	16124.595a	15	1074.973	107.497	0.000
Intercept	106926.949	1	106926.95	314.56	0.000
Pb	13.509	1	13.509	7.948	0.012
Cr	1224.046	1	1224.046	720.180	0.000
Co	10.245	1	10.245	6.028	0.026
Ni	12364.167	1	12364.167	7274.588	0.000
Pb*Cr	23.089	1	23.089	13.585	0.002
Pb*Co	22.736	1	22.736	13.377	0.002
Pb*Ni	74.790	1	74.790	44.004	0.000
Cr*Co	653.361	1	653.361	384.412	0.000
Cr*Ni	1182.290	1	1182.290	695.613	0.000
Co*Ni	67.841	1	67.841	39.915	0.000
Pb*Cr*Co	103.545	1	103.545	60.922	0.000
Pb*Cr*Ni	3.787	1	3.787	2.228	0.155
Pb*Co*Ni	17.163	1	17.163	10.098	0.006
Cr*Co*Ni	274.512	1	274.512	161.512	0.000
Pb*Cr*Co*Ni	89.515	1	89.515	52.667	0.000
Error	27.194	16	1.700		
Sum	123078.738	32			
Correction sum	16151.789	31			
R^2=0.998 Adjust R^2=0.997					

Note: Significant effect for the research object in bold.

Table 4: Goodness-of-fit test for regression model.

Correlation coefficient R	R^2	Adjust R^2	Error
0.997	0.994	0.989	2.341

Table 5: F test for regression model.

Model	Quadratic sum	df	Mean square	F	P
Regression	16054.595	14	1146.739	200.050	0.000
Residuals	87.448	17	5.732		
Sum	16151.789	31			

Cr^{3+}-Co^{2+}, Pb^{2+}-Co^{2+}-Ni^{2+}, Cr^{3+}-Co^{2+}-Ni^{2+}, and Pb^{2+}-Cr^{3+}-Co^{2+}-Ni^{2+} were the main factors to affect the photobacteria activity. Ni^{2+}, Co^{2+}, Cr^{3+}-Co^{2+}, Cr^{3+}-Ni^{2+}, and Pb^{2+}-Co^{2+} produce antagonistic effects, Pb^{2+}-Co^{2+}-Ni^{2+}, Cr^{3+}-Co^{2+}-Ni^{2+}, and Pb^{2+}-Cr^{3+}-Co^{2+} produce synergistic effects, while Pb^{2+}-Cr^{3+}-Co^{2+}-Ni^{2+} also exert antagonistic effects. Among the four heavy metal pollutants, Co^{2+} was most likely to interact with other pollutants, followed by Cr^{3+} and Ni^{2+}. Although the analysis of variance showed some toxicity attributable to Pb^{2+}, Pb^{2+}-Cr^{3+}, and Pb^{2+}-Ni^{2+}, these contributions to the toxicity was very small.

Construction and analysis of BP-ANN model: Artificial neural networks (ANNs) were recently developed as a powerful modelling tool, and have been used for many engineering applications such as prediction, optimization, classification, and pattern recognition (Lou 2002). ANNs have a highly interconnected structure similar to biological neural networks and consist of a great number of processing elements called neurons that are arranged in different layers. Each network comprises an input layer, an output layer and one or more hidden layers (Morgan & Chow 2004). The BP-ANN is a typical ANN, and can implement any complex nonlinear mapping functions. They are increasingly used in applications that include environmental science, prediction of Pb^{2+} in aqueous solution and chlorophenol removal effects, algal content in lakes, groundwater chloride migration, and insecticide concentration studies (Zhang 1995, Qu et al. 2004). A BP-ANN model was established to study the effects of heavy metals on the joint toxicity against photobacteria in a factorial experiment.

The training and test datasets for the ANN model (Table 1) were randomly selected; 27 of 32 data points were used for training and the remaining 5 were used for testing. The model used four input variables (Ni^{2+}, Cr^{3+}, Co^{2+}, Pb^{2+} concentrations: X1, X2, X3, X4; mmol/L), inhibition rate (Y; %) as the single output variable, eight hidden layers, a structure of 4-8-1 for good performance, train epochs $E = 4300$, train goal $G = 0.004$, and learning efficiency $L = 0.01$.

The correlation coefficient (R^2), the nash-sutcliffe coefficient (NSC), and the mean square error (MSE) were used to assess the validity of the model. If the predictive value and the experimental value of $R^2 > 0.90$, the predictive ability is high, and if $R^2 < 0.90$, the predictive ability is limited. According to Fig. 1, $R^2 = 0.9923 > 0.90$, and the distribution of scattered points in the straight line indicates that the model shows good agreement between the predicted and experimental values.

Equation 2 gives the relation for the NSC:

$$NSC = 1 - \frac{\sum(Y_{cale} - Y_{test})^2}{\sum(Y_{test} - \overline{Y_{test}})^2} \quad ...(2)$$

Where Y_{test} is the experimental value, Y_{cale} is the predicted value, and $\overline{Y_{test}}$ is the mean experimental value. An NSC of 1 indicates that the predicted value is equal to the experimental value. When $0 \le NSC \le 1$, the predicted value is close to the experimental value, when the NSC is close to 1, and when NSC $\le$ 0, the credibility of the predicted value is lower than the experimental value (Zhang 2003). The calculated NSC value of 0.9437 shows good correlation between the predicted and experimental data, and that the model shows

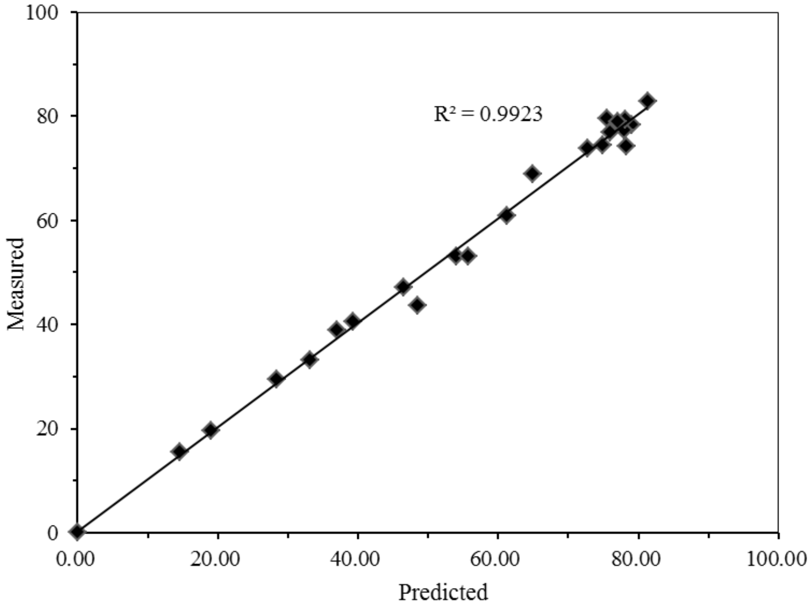

Fig. 1: Correlation curve for experimental and model-predicted values.

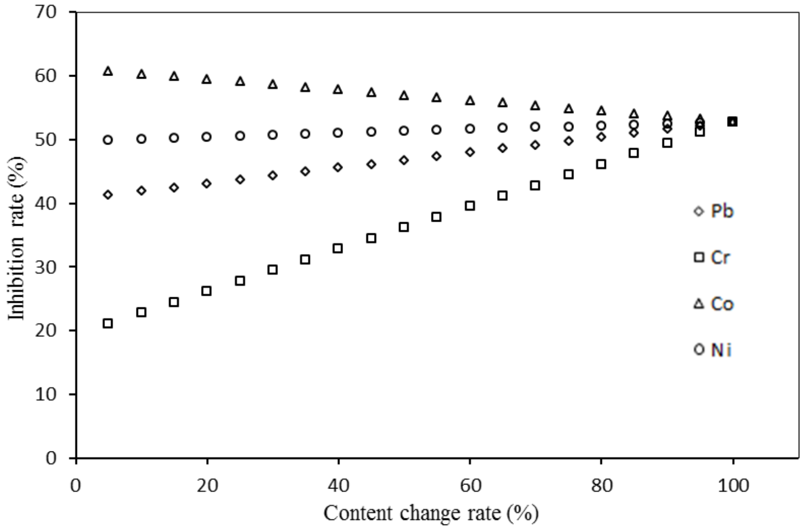

Fig. 2: Inhibition rate of the mixed system with different contents of Pb^{2+}, Cr^{3+}, Co^{2+} and Ni^{2+}.

good prediction performance.

Equation 3 shows the relation for MSE:

$$MSE = \frac{1}{N}\sum_{i=1}^{N}(Y_{cale,i} - Y_{test,i})^2 \quad ...(3)$$

Where N is the number of input data, and $Y_{cale,i}$ and $Y_{test,i}$ are the predicted and experimental values, respectively. The MSE values were 0.0052 for training and 0.0489 for testing, indicating that the model has good prediction ability and generalization ability.

To determine the toxic effects of different concentrations on the photobacteria and clarify the mechanism of joint toxicity, we used the BP-ANN model to simulate and predict the relationship between the four heavy metal compounds and their effects on the inhibition rate. This required changes to the mixture concentrations, with change ranges of 0-0.0012 mmol/L for Pb^{2+}, 0-0.1082 mmol/L for Cr^{3+}, 0-0.0100 mmol/L for Co^{2+}, and 0-0.0360 mmol/L for Ni^{2+}. The maximum content of each heavy metal element was used as the initial value. According to the same change rate, the inhibition rate of the photobacteria was simulated when

Table 6: *T* test for regression model.

Model	Unstandardized coefficients		Standardized		
	Γ_i	Standard deviation	coefficients	*t*	*P*
Constant	-14.958	2.962		-5.05	0.000
Pb	2265.991	2983.882	0.055	0.759	0.458
†Cr	681.979	34.918	0.976	19.531	0.000
†Co	5545.587	479.032	1.160	11.577	0.000
†Ni	244.992	9.813	1.846	24.966	0.000
Pb*Cr	-55855.960	33449.789	-0.123	-1.670	-0.113
†Pb*Co	-2259749.113	529490.735	-0.482	-4.268	0.001
Pb*Ni	8385.927	6352.101	0.066	1.320	0.204
†Cr*Co	-70810.170	5761.865	-1.307	-12.289	0.000
†Cr*Ni	-1666.287	108.667	-1.120	-15.334	0.000
†Co*Ni	-13795.551	1774.657	-0.881	-7.774	0.000
†Pb*Cr*Co	3.019E7	6278879.345	0.545	4.808	0.000
†Pb*Co*Ni	513669.822	1816011.930	0.301	2.829	0.012
†Cr*Co*Ni	144366.098	20989.008	0.777	6.878	0.000
†Pb*Cr*Co*Ni	4.596E7	2.058E7	-0.224	-2.234	0.039

Note: † indicates significant effect for the research object.

the heavy metal content was changed. The simulation results are shown in Fig. 2.

As shown in Fig. 2, as Pb^{2+}, Cr^{3+} and Ni^{2+} content increased, the inhibition rate of the mixed system gradually increased. The change of Cr^{3+} content in the mixed system was the most influential, while the change of Pb^{2+} content showed the smallest effect. In contrast, Co^{2+} showed the opposite trend where increased Co^{2+} content saw the inhibition rate of the mixed system decrease.

Comparison of multiple linear regression model and BP-ANN model: The study found that the multiple linear regression model and the BP-ANN model of the effects of four heavy metals on photobacteria had better characterization and prediction function. Both models were able to analyse the joint toxic effects of heavy metals. According to the BP-ANN model, in a certain range, the concentration of Co^{2+} increased and the toxicity of the mixed system decreased. In contrast, the other three heavy metals showed the opposite phenomenon with changes of Cr^{3+} content being the most influential, and Pb^{2+} the least influential. Promotion of inhibition was not an effect of a single metal, with the interaction of heavy metal components playing an important role. Changes in Pb^{2+} content had the smallest effect on the mixed system, because the interaction between Cr^{3+}, Co^{2+} and Ni^{2+} weaken the single toxicity of Pb^{2+}. In the multiple linear regression model, the regression coefficients for Pb^{2+}, Cr^{3+}, Co^{2+} and Ni^{2+} were all positive, and they were promoted in the system. This conclusion does not conform with the BP-ANN model, because the study of the interaction between the heavy metals hides the separate effects. Therefore, the BP-ANN model is advantageous in analysis of the effect of the single heavy metal concentration on the toxicity of the mixed system, while the multiple linear regression model is a more comprehensive analysis of the internal interactions of the mixed system.

CONCLUSIONS

Factorial experimental design was used to study the toxicities of binary, ternary, and quaternary metal mixtures. High-quality models were fitted to the experimental data, and interactions between the components in metal mixtures were indicated by the interaction terms in the models. The joint toxicity of Ni^{2+}, Co^{2+}, Cr^{3+}, and Pb^{2+} occurs through multiple interactions. Ni^{2+}, Co^{2+}, Cr^{3+}-Co^{2+}, Cr^{3+}-Ni^{2+}, Pb^{2+}-Co^{2+}, and Pb^{2+}-Cr^{3+}-Co^{2+}-Ni^{2+} exert antagonistic effects, while Pb^{2+}-Co^{2+}-Ni^{2+}, Cr^{3+}-Co^{2+}-Ni^{2+}, and Pb^{2+}-Cr^{3+}-Co^{2+} act synergistically. The separate coefficients of heavy metals in the model were small, so the single toxicities were less affected than the joint effects of the heavy metals. By the BP-ANN model, the increased concentration of Co^{2+} lowered the inhibition rate of the mixed system. For the three other heavy metals, the effect of Cr^{3+} on the mixed system was the largest, and increasing the concentration of Cr^{3+} gave the highest toxicity in the mixed system. These findings are expected to provide useful background in efforts to control of heavy metal pollution.

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