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Study on toxicity of wastewater from mining activities on some biota

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Abstract — Dawu River water was taken as the wastewater of copper mine and used to study its toxicity on algae and *Daphnia*. It was found that wastewater affected the growth and photosynthesis of algae, and also affected the growth and breeding of *Daphnia*. The results through SOS chromotest showed that there was no apparent genotoxins from the sediments.

Keywords: toxicity; copper mine; wastewater; algae.

INTRODUCTION

From the ecological survey of aquatic organisms in Le An River, it was found that the acidic wastewater from the mine polluted Le An River and obviously affected the species and quantity of the aquatic organisms. The toxicity of wastewater on some biota is presented.

As algae are the main aquatic organisms in Le An River, the effect of wastewater on inhibiting growth, photosynthesis and respiration of algae and the bioaccumulation of heavy metals were discribed. Zooplankton *Daphnia magna straus* was found to be more sensitive to the heavy metal than fish, LC_{50} and reproduction of *Daphnia* were studied as well.

SAMPLES AND MATERIALS

1. Wastewater

According to the data from Xiangtun Hydrological Station located downstream of Gukou, the annual average flow rate of Le An River is 122 m^3/ s and the flow rate of Dawu River is about 1/ 60 that of Le An River. Diluted Dawu River water was taken as the wastewater of copper mine and used to study its toxicity on the biosystem.

As there are suspended matters in Dawu River, river water was keep still for 24h and toxicities of its upper layer solution were used. Based on the ratio of the flow rates in Dawu River and Le An River, riverwater and its upper layer solution were diluted with test medium (Zhou, 1987) or Le An River water from upstream.

2. Water soluble fraction of the sediments

Metals in wastewater containing suspended matter were settled partly on the river bed and can release into river water under strong acidic condition, toxicity was studied by using water soluble fraction of sediments. Sediment samples were collected by Schmits W. and Mao Meizhou (in this issue) at Daicun (D.), Caijiawan (C.) and Shuangang (S.). Samples were dried, screened and extracted by pH 7 medium. Shaken 175 times/ min for 2h, then set still for 1h, decanted and centrifuged (3000 r/ min) for 10 minutes, the upper layer solutions were used for the algae inhibition growth test.

3. Biota

Chlorella pyrenoidosa (supplied by Institute of Hydrobiology, Chinese Academy of Sciences), *Ankistrodesmus bibraianus* Korshikov (supplied by Technical University of Hamburg-Harburg, Germany), *Escherichia coil* K12 PQ37 (supplied by Technical University of Hamburg-Harburg, Germany) and *Daphnia magna* were selected in the tests.

EXPERIMENTAL METHODS

1. Determination of EC_{50} (LC_{50}; Zhou, 1987)

Using wastewater (or upper layer solution) at different ratio, proper amount of algae suspension was added into bottles. Initial cell concentration was made into 5×10^5/ ml, growing at 25± 1℃. Using 1 cm colorimetric cell, the optical density determined at 660 μm, EC_{50} of waste water and upper layer solution on algae was calculated.

The effects of wastewater on photosynthesis and respiration of algae by the black and white bottle test were determined and EC_{50} was calculated.

For LC_{50} of wastewater (or upper layer solution) on *Daphnia*, each concentration was repeated in 3 bottles, setting 8 same aged young *Daphnia* into each bottle, culturing at 20 ± 1℃ for 24 and 48h, respectively. Recording the death number, calculated the LC_{50} of wastewater (or upper layer solution) on *Daphnia*.

2. Chronic toxicity of wastewater on *Daphnia magna* (Zhou, 1987)

On the basis of LC_{50} determination, the genital date, genital times and number of foetus of *Daphnia* within 2 weeks were observed.

3. Toxicity of water soluble fraction of the sediments

Ankistrodesmus bibraianus Korshikov was used for algae inhibition growth tests (Entwurf, 1987; OECD, 1984). The extracted liquid of sediment (water soluble fraction of sediments), test medium (Miller, 1987) and precultured algae suspension were added separately in proportion (Table 1).

There are about 10^6–10^7 cells/ ml algae in test tube, cultivated at 20℃, illuminated at 6 000 lx, and shaken 175 times/ min. After cultivation about 0, 24 and 48 hours, optical density

of solution was determined separately at 660 μm with 1 cm colorimetric cell. According to the growth calibration curve $N = 12 \times 10^6$ E (N-cells/ ml, E-optical density, taken by Michael Dahm in the laboratory), a number of algae cells was calculated.

Table 1 Mixing ratio of extracting liquid in each test

Test	Medium volume, ml	Extract volume, ml	Algae suspension volume, ml	Repeating times
Contrast	10	0	1	4
Test 1	9.5	0.5	1	2
Test 2	8.0	2.0	1	2
Test 3	6.0	4.0	1	2
Test 4	2.0	8.0	1	2

4. SOS chromotest

Quillardet P. (1982) suggested a new bacterial assay known as SOS chromotest after SOS responses in *Escherichia coli* was first reported by Radman M. (1975) and Witkin E. M. (1976). It was applied here to determine the genotoxins. Genotoxins of water soluble fraction of sediment were tested with *Escherichia coli* K12 PQ37 strain in SOS chromotest.

Procedure for the SOS chromotest: 200 μl freezed stored bacterium *E. coli* K12. PQ37 and 1 ml *Ampicillin* (1 mg/ ml) were added to 50 ml LB medium, cultured and shaken at 37℃ for 16 hours. Add 1 ml proliferous bacterial suspension to 50 ml fresh LB medium, and renewed culture for 2 hours, adjusted cell concentration with the medium. Adjusted pH of the Miller medium to 4, 5 or 6 with HCl or NaOH solution. Then extracted sediments by different pH medium, shaken 175 times/ min for 2 hours, set still 1 hour and the upper layer liquid was centrifuged at 3 000 r/ min for 10 min. These upper layer solutions were liquids extracted at different pH. Extracted liquid with different pH diluted with relevant pH medium was added to microcell plate A and plate B with 96 cells. Took different concentration of 4-NQO (4-nitroquinoline-1-oxide) for positive control at the same time. 100 μl *E. coli* suspension was added to each cell, at 37℃, 175 times/ min, shaken for 2 hours, then 100 μl Tris buffer (containing 4-nitrophenyl phosphat) was added to each cell of plate A, 100 μl phosphate buffer (containing o-nitrophenyl-β-D-galactopyranoside) to each cell of plate B, determined OD_{405} of plate A immediately and B was determined as T_0, shaken and cultivated at 37℃. After 20 min OD_{405} of plate A was determined as T_{20}, after 60 min OD_{405} of plate B was determined as T_{60}.

5. Bioaccumulation of heavy metal on algae

Different dilution of wastewater was added into algae suspension. The samples were nurtured for ten days, then algae and sediment were filtered and weighed. The concentrations of copper in filtrate and algae on dried filter were determined.

RESULTS AND DISCUSSION

1. Wastewater (Dawu River water) or its upper layer solution was diluted with clean Le An

River water or algae medium, made into 1/ 30, 1/ 45, 1/ 67.5. 1/ 101.25 and 1/ 151 five diluted grades. Diluent was taken for contrast. After *Chlorella pyrenoidosa* was cultivated for 96 hours, optical density was determined, and EC_{50} calculated was given in Table 2. Using least square method, it was found that data fit fairly in lincar relationship.

Table 2 Toxicity of wastewater and its upper layer solution on *Chlorella Pyrenoidosa* (EC_{50})

Diluent	Wastewater, EC_{50}	Upper layer solution, EC_{50}
Clean Le An River water	1/ 60.67	1/ 102.78
Algae medium	1/ 15.29	1/ 32.71

In case of diluting the wastewater (Dawu River water) with clean upstream river water, EC_{50} is equal to 1/ 60.67, nearly to be the ratio of annual average flow rate of Dawu River and Le An River, while in case of diluting wastewater with algae medium, the toxicity appeared to be lower. It might be due to the better nutrient conditions. As in the case of upper layer solution, higher toxicity may be due to the adsorption of toxic metal with suspended matter. Further observation is needed.

2. Effects of Copper Mine wastewater on algal photosynthesis and respiration by black and white bottle test were studied (Table 3).

In the case of ratio 1/ 60, algal photosynthesis and respiration were obviously inhibited. With such dilution ratios EC_{50} calculated was about 1/ 61.56, similar to the result by the previous method (Table 2).

Table 3 Effects of wastewater on algal respiration and photosynthesis (*Chlorella pyrenoidosa*)

Dilution ratio	Respiration, O_2mg/ L	Net productivity, O_2mg/ L
Contrast	1.080	13.962
1/ 120	0.929	13.292
1/ 90	0.408	12.488
1/ 60	0.518	6.329
1/ 45	0.842	2.506
1/ 30	0.303	−0.292
1/ 15	0.054	−0.054

3. Toxicity of wastewater (Dawu River water) on *Daphnia magna* was determined. Wastewater diluted with medium at 1/ 13.33, 1/ 20, 1/ 30, 1/ 45, 1/ 67.5, 1/ 101.5 and 1/ 151.9 seven levels, and the medium was taken as contrast at 24h and 48h. Death number were recorded and LC_{50} were calculated respectively. Results are shown in Table 4.

Similar results were observed as algae. In the tests of diluting wastewater with clean Le An

River water, LC_{50} was equal to 1/ 48.43. While ratio of diluting wastewater with algae medium decreased, toxicity of the upper layer solution of same wastewater increased.

Table 4 Acute toxicity of wastewater on *Daphnia magna*

Diluent	Wastewater		Upper layer solution, LC_{50}	
	24h	48h	24h	48h
Clean Le An River water	1/ 22.02	1/ 48.43	1/ 32.48	1/ 78.57
Algae medium	1/ 6.23	1/ 11.86	1/ 9.3	1/ 26.41

4. Chronic effects of wastewater on *Daphnia magna*

Diluting wastewater (Dawu River water) with clean Le An River water, samples with lower concentration 1/ 90, 1/ 450, 1/ 900, 1/ 4500 and 1/ 9000 (clean river water sample as contrast) were tested. The growing, breeding and death of *Daphnia* were observed at 12 days internal. Results showed that, as diluting ratio of wastewater was increased, the number of larvage was decreased and at certain duration the breeding time was decreased, and the first breeding date was delayed. The number of larvae in each foetus of the contrast was never greater than ten, but that of the test sample at same time was as high as 16~17. In the 1/ 90 test, there was no foetus. It showed that the Dawu River water affected the growing and breeding of *Daphnia*, even at 1/ 9000 dilution.

5. Toxicity of water soluble fraction of the sediment on some biota

(1) In the algal inhibition growth test, based on the growth calibration curve, the optical density was converted into algae per ml cell quantity as the biomass (as N_0 , N_1 , N_2 and N_3 at 0, 24, 48 and 72h). The increased accumulation of biomass B was calculated according to the following formula:

$$B = 0.5 \times [N_0 + (N_1 + N_2) + N_3] - 3 \times N_0.$$

Finally, the inhibition growth ratio, Hb was calculated:

$$Hb = \frac{Bk - Bt}{Bk} \times 100\%,$$

where Bk — accumulation biomass in control group; Bt — accumulation biomass in test group.

Results were listed in Table 5. If the inhibition growth ratio is positive, it is indicated that the samples have capability of inhibition on algal growth, that meas the sample has a poisoning effect on algal growth. If the inhibition growth ratio is zero or negative, it shows that the samples have no inhibition effect on algal growth. Data showed that all extracts of three sediment samples almost have no inhibition effect on the growth of algae.

Table 5 Inhibition growth ratio of water soluble fraction of sediments

Test		Accumulation of biomass (B), 1×10^6 cell/ ml	Inhibition growth ratio (Hb), %
Contrast		5.663	0
Daicun	1	6.363	−12.37
	2	6.084	−7.44
	3	5.700	−0.66
	4	6.360	−12.32
Caijiawan	1	8.469	−49.56
	2	7.119	−25.72
	3	7.515	−32.72
	4	6.261	−10.57
Shuangang	1	8.028	−41.77
	2	5.400	4.64
	3	7.119	−25.72
	4	7.512	−32.66

(2) Optical density of cell of plate A was determined at T_0 and T_{20} separately. $OD_{T20}-OD_{T0}=OD_{AN}$ calculated for each sample is given in Table 6. If the sample has cell toxicity, the alkaline phosphatase will be disrupted, a little of PNPP was converted, the color was darker, it is needed to calculate modulated factor $C.F.=OD_{AO}/OD_{AN}$, and to modify the data of plate B with $C.F.$ As OD_{AN} approximate to OD_{AO} in this test, it is not necessary to calculate the $C.F.$ The optical density of each cell of plate B at T_0 and T_{60} was recorded separately. $OD_{T60}-OD_{TO}=OD_{BN}$ calculated for each sample is given in Table 7.

Table 6 Optical density (OD) of cells before and after cultivation on plate A

Diluting	Daicun, OD				Caijiawan, OD	Shuangang, OD				4-NQO
ratio	pH4	pH5	pH6	pH7	pH7	pH4	pH5	pH6	pH7	10ppm
1/ 1	490	554	465	918	759	475	469	442	784	611
1/ 2	509	480	454	988	797	473	416	431	783	877
1/ 4	527	464	464	963	908	499	478	447	854	866
1/ 8	498	460	460	911	881	466	530	479	979	989
1/ 16	522	495	517	931	950	482	490	491	897	992
1/ 32	522	438	463	927	979	476	565	462	949	948
1/ 64	534	480	398	943	929	472	503	577	989	876
Contrast	575	579	516	972	950	579	538	561	1004	918

The curve obtained from the concentration of samples and corresponding OD_{BN}, SOS induction potency can be calculated according to the following formula:

$$SOSIP=\frac{10\times(OD_{B2}-OD_{B1})}{C_2-C_1},$$

where, C_1, C_2— concentration of sample on the linear fraction of the curve; OD_{B1}, OD_{B2}— corresponding to optical density. Except the positive 4-*NQO*, there is almost no difference between OD_{B2} and OD_{B1} of the samples, that means apparently there is no genotoxins from the three kinds of sediment.

Table 7 Optical density (*OD*) of cells before and after cultivation on plate B

Diluted	Daicun, *OD*				Caijiawan, *OD*	Shuangang, *OD*				4-*NQO*
ratio	pH4	pH5	pH6	pH7	pH7	pH4	pH5	pH6	pH7	10ppm
1/ 1	198	181	174	220	239	172	174	160	258	1322
1/ 2	193	171	167	228	225	158	157	150	249	1382
1/ 4	187	166	164	250	219	170	163	161	248	1070
1/ 8	182	165	159	245	217	152	153	154	237	665
1/ 16	182	162	162	250	219	131	148	163	229	494
1/ 32	189	167	165	248	218	142	159	161	245	362
1/ 64	198	179	170	270	239	159	158	164	254	323
Contrast	201	184	175	275	247	167	142	175	268	270

6. Bioaccumulation of copper on algae

The suspended matter and algae in the samples were weighed and the copper contents in suspended matter and algae were determined (Table 8). The results show that the copper was enriched by algae in the wastewater (i. e. Dawu River water). It showed that higher the ratio of wastewater in the medium, more copper was enriched in algae.

Table 8 Heavy metal enriched by *Chlorella pyrenoidosa* from wastewater medium

Diluted ratio	Algae suspension	Suspender, g	Algae+ suspender, g	Algae, g	Cu/ sample, μg/ ml	Cu/ algae, μg/ dry g
Contrast 0	−	0.0002			0.1186	
Contrast 0	+		0.0190	0.0188	0.2210	136.2
Test 1/ 200	−	0.0006			0.1814	
Test 1/ 200	+		0.0283	0.0277	0.3770	176.5
Test 1/ 25	−	0.0103			0.6876	
Test 1/ 25	+		0.0341	0.0238	2.423	1822.9

Toxicity test results (representing copper mine wastewater by Dawu River water) showed that, the EC_{50} , (96h) of wastewater on algae was 1/ 60.67, the LC_{50} (48h) of wastewater on *Daphnia* was 1/ 48.43 (nearly to the same ratio of annual flow rate of the rivers). Copper can be enriched by algae. Even at lower diluted ratio, wastewater may poison the genital function of *Daphnia*. Due to the instability of the discharged effluents of the copper mine wastewater and of the flow rate of Dawu River and Le An River, the ratio of wastewater in Le An River may be

increased in fatality, and further damages on aquatic biomass is possible. Wastewater from mining activity will be the vital factor effecting the biomass qualitatively and quantitatively from Gukou downstream in Le An River. Further study is needed.

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REFERENCES

Deutsche Industrie Norm 38412 Deutsche Einheitsverfahren zur Wasser-, Abwasser-, und Schlammuntersuchung, Bestimmung der Hemmwirkung von Wasserinhaltsstoffen auf Grunalgaen, Entwurf Teil 9: Testverfahren mit Wasserorganismen (Gruppe L), 1987

Miller, W. E., Greene, J. C. and Shiroyama, T., The *Selenastrum capricornutum* printz algal assay bottle test, EPA-600/ 9-78-018, Corvalis, Oregon, USA, 1987

OECD, Guideline for testing of chemicals, Paris: OECD Pub., 1984:201

Quillardet, Ph., O. Huisman, Ari, R. D'and Hotnung, M., SOS Chromotest, Proc. Natl. Acad. Sci. USA, 1982, 79:5971

Radman, M., SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis, In: Molecular mechanisms for repair of DNA (Eds. by Hanawalt, P. and Setlow, R. B.), New York: Plenum Press, 1975:355

Witkin, E. M., Bacteriol. Rev., 1976, 40:869

Zhou Yongxing and Zhang Zongshe, Method of toxicity test on hydrobios, Beijing: Agriculture Press, 1987

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