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Effects of lead, cadmium, mercury chlorides and aquatic environmental samples on the growth of *Escherichia coli*

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Abstract—In this paper it was reported that $PbCl_2$, $CdCl_2$, $HgCl_2$, and aquatic environmental samples containing Al, Cd, Cu, Pb, Sr, Zn and other heavy metal ions as well as organic chemicals suppressed the growth of *E. coli* only at higher exposed concentrations. The stimulative effects of $PbCl_2$ and $HgCl_2$ on the growth of *E. coli* were clearly showed at lower concentrations and longer time. It suggested that the toxic effects of heavy metal ions and other pollutants on the growth of *E. coli* are variable according to the different exposed levels.

Keywords: heavy metal ions; *Escherichia coli*; two - way growth effects.

1 Introduction

With the increased world - wide natural resources exploitation and industrialization for recent years, both the developed and developing countries face increasing ecological and toxicological problems from the release of pollutants. The presence of pollutants can interact with natural biological system and some direct or indirect consequence are possible on both human or animal health and the ecological field (Becking, 1979; Bitton, 1983; 1986). Microorganisms act as an important role in the biosphere and may be widely exposed to the pollutants in natural waters, soils, and wastewater treatment processes. The growth of microorganisms may be significantly suppressed or promoted by heavy metal ions in different conditions. Although effects on bacterial, particularly to *Escherichia coli*, has been observed as a consequence of salinity, heat treatment, freezing, irradiation, pH, and exposure to toxic compounds, and so on (Przybylski, 1979; Anderson, 1979; Roller, 1980; Wenlian, 1993), little attention have been given to the effects of heavy metal ions which, at long term and lower concentrations, could act as stressors of water - born indicator bacteria (Gadd, 1978; APHA, 1980; McFeters, 1982; Cenci, 1985; 1987).

In the present study, the effects of lead, cadmium, mercury, and aquatic environmental samples on the growth of *E. coli* were observed in wide ranges of concentrations and long exposure durations. *E. coli* was chosen as test organism base on account of its importance in public health (sanitary indicators) and for a possibility of using it to detect aquatic environmental toxicity.

2 Materials and methods

The test microorganism *Escherichia coli* is a common laboratory bacterial strain. The cultures were previously grown in 10 ml salt glucose medium (SGM), NaCl 5 ‰, $MgSO_4$0.2 ‰, $NH_4H_2PO_4$1 ‰, $K_2HPO_4$1 ‰, with glucose 5 ‰ as the carbon source (Cenci, 1987) in the deionized and organic chemical - free water, atmospheric CO_2, pH 7.0, for 12h at 37°C, 120 r/min (approximately 10^6 bacteria per ml). All of bacterial suspensions were freshly prepared prior to experiment and resuspended in SGM and diluted to $3\times10^2-4\times10^2$ alive bacteria per ml by reference to a calibration curve.

The metals were dissolved in the deionized and organic chemical - free water, and diluted with the water to suitable concentration. The solutions were filtered through 0.45 μm membrance and stored in sterile glass screw - capped tubes at 4°C.

The environmental samples were collected from city river, lake, and industry wastewater. The samples were filtered through 0.45 μm membrane within 1 hour after the sample collected, and then stored in sterile glass screw - capped tube at 4°C until used within 1 week. Aquatic environmental samples were collected and their pH value, COD and BOD_5 were determined according to the standard methods for the examination of water and wastewater, and the concentrations of metal ions were screened by inductively coupled plasma (ICP) quantometer method (APHA, 1989). In the toxicity test, 1 ml bacterial suspension ($3\times10^2-4\times10^2$ alive bacteria per ml) and 2 ml metal solution were mixed at the reported final concentrations in the sterile 90×18 mm Petri dish. After 20 min, 12 ml ready nutrient agar media was added into the dish to mixed and incubated at 37°C for different exposed durations. All cultures were grown in triplicate. Results were obtained comparing, at the different time interval, mean values of colony forming units (*CFU*) in nutrient agar media. The relative in inhibition percentage (*RIP*) were obtained respectively from the mean values of *CFU* in nutrient agar media by following equation:

$$RIP(\%) = \frac{CFU\text{ control} - CFU\text{ treatment}}{CFU\text{ control}} \times 100\%$$

3 Results and discussion

3.1 The toxic effects of heavy metal ions on the growth of *E. coli*

Growth activity, as a measure for cell division, of *E. coli* cells in the presence of different heavy metal concentrations specifies the range of dose - response relationship in the toxicity test system. The data in Table 1 show that $PbCl_2$ has a significant two - way effect on the growth of *E. coli*. Below and at the 312 ppm $PbCl_2$ concentration, no effects were observed before the incubation for 48 h. However, a stimulative effects were markedly shown at the same lead exposed concentrations after the incubation for 72h. At the 625 ppm group of $PbCl_2$, it showed the suppressive effect of $PbCl_2$, but it was gradually and hardly decline and it may be disappear at a later time, about half - month forecasted. The result suggested that the two - way effect of lead on the growth of *E. coli* was related to the exposed concentration and time; between 78 and 312 ppm, the higher of the dose, the lower of stimulation, and it was finally changed into thorough inhibitory state when lead - exposed concentration exceeded 1250 ppm.

Table 1 Relative inhibition percentage of $PbCl_2$ on the growth of *E. coli*

Unit: %

Concentration of $PbCl_2$, ppm	Exposure time, hours			
	24	48	72	96
78	3.5	4.1	-16.3^{ac}	-21.9^{ad}
156	0.4	2.9	-15.8^{ad}	-11.1^{ad}
312	5.3	3.2	-9.7^{ac}	-10.3^{ad}
625	43.0^{b}	38.7^{b}	21.7^{ac}	20.2^{ad}
1250	97.7^{b}	97.8^{b}	97.8^{b}	97.1^{b}
2500	98.5^{b}	98.6^{b}	98.6^{b}	98.4^{b}

a: $P<0.05$ vs o ppm group b: $P<0.01$ vs o ppm group

c: $P<0.01$ vs the group at the previous exposure

The effects of $CdCl_2$ were observed in a narrow ranges and short exposure time as it also show the basic pattern of the toxic effect. The results in Table 2 show a continually suppressive effects of $CdCl_2$ on the growth of *E. coli*. It suggested that *E. coli* cell was sensitive to $CdCl_2$ and it also had the ability to resist the toxic effect of $CdCl_2$ for its growth. It can not be ascertained whether the stimulative effect may be appeared at the lower concentration.

Table 2 Relative inhibition percentage[a] of $CdCl_2$ on the growth of *E. coli*

Unit: %

Concentration of $PbCl_2$, ppm	Exposure time, hours		
	12	24	36
0.8	48.9	33.4	51.6^{b}
1.6	42.9	47.1	63.0^{b}
3.2	58.9	57.1	69.7^{b}
6.4	57.1	65.0	75.1^{bc}
12.8	71.4	78.6	80.6

a: All of *RIP* values of each group in Table 2 is $P<0.01$ vs o ppm group

b: $P<0.05$ vs the group at previous exposure

c: $P<0.01$ vs the group at previous exposure

The stimulative effects of $HgCl_2$ on the growth of *E. coli* appeared at lower concentration and disappeared in shorter exposed time. However, the suppressive effects of $HgCl_2$, as well as $CdCl_2$, was kept up a longer time once the suppressive effects appeared. The result indicated that the basic pattern of the toxic effects on the growth of *E. coli* are significant difference among $HgCl_2$, $CdCl_2$ and $PbCl_2$(Table 3).

Table 3 Relative inhibition percentage of $HgCl_2$ on the growth of *E. coli*

Unit: %

Concentration of $HgCl_2$ ppm	Exposure time, hours		
	24	36	60
0.06	0.3[a]	−21.1[b]	−9.0[b]
0.27	91.9	93.9	93.5
1.34	91.5	89.2	90.5
6.67	99.8	100.0	99.8
33.34	100.0	100.0	99.9

a: Except the group, the *RIP* values of each group in Table 3 are $P<0.05$ or $P<0.01$ vs o ppm group

b: $P<0.01$ vs the group at previous exposure

From these data, the maximum no-observed suppressive effect levels (MNOSEL), the 50 percent inhibition concentrations (IC_{50}) of growth activity, and the regression equations were calculated (Table 4).

Table 4 The MNOSEL, IC_{50} values, and the regression equations of $PbCl_2$, $CdCl_2$ and $HgCl_2$ for the growth of *E. coli* at the incubation for 24h

Metals	MNOSEL, ppm[a]	IC_{50}, ppm[b]	Regresiion equations[c]
$PbCl_2$	357.00	569.00	$Y=-4.185+3.334X$
$CdCl_2$	0.440	2.280	$Y=4.663+0.940X$
$HgCl_2$	0.140	0.320	$Y=5.955+1.886X$

a: Maximum no-observed suppressive effect level: It was calculated by the regression equation to set $Y=4.3255$ for 25% *RIP* values

b: 50% inhibition concentration: It was calculated by the regression equation to set $Y=5$ for 50% *RIP* values

c: Regression equation: Y is the probit units of *RIP*, X is thelogarithm values of the metal ion concentrations

3.2 The toxic effects of aquatic environmental samples on the growth of *E. coli*

The data of physicochemical properties and toxic effects of aquatic environmental samples showed a critical pollution to the water. There are a general correspondence between the physicochemical levels and the toxic effects of the environmental samples.

The data in Table 5 and Table 6 indicate that the sample 3 and sample 6 have higher concentrations of metal ions (such as Cr, Pb, Cd, Cu) and organic chemicals, as response to it the toxic effects of the samples are also more serious than others. In the sample 1 and the sample 8, however, the metal ions and organic chemicals are lower than others, but its toxicity is also higher yet. It suggested that the survivor and growth of *E. coli* is significantly affected by the polluted aquatic environment even at the normal levels of the physicochemical index. The stimulation of aquatic environmental samples was not observed at the tested concentrations. Therefore, it needs to take the environmental samples to be tested further at lower concentrations.

Table 5 The physicochemical properties and the *RIP* of environmental samples

Unit: %

No. of Samples	pH	COD_{Cr}, ppm	DOD_5, ppm	Exposure time, hours 24	48	72
1	7.17	3.96	4.64	12.87	13.49	30.36[a]
2	6.81	13.80	33.60	27.90	28.40	32.40
3	8.50	1080.00	281.00	67.86	65.71	79.52[a]
4	7.50	150.00	18.00	35.72	22.30[a]	57.22[b]
5	7.20	13.50	21.00	32.71	34.00	38.10
6	7.80	27077.00	556.00	100.00	100.00	100.00
7	7.00	271.00	5.56	100.00	97.70	93.10
8	6.80	27.00	0.56	83.00	50.20[b]	51.40

a: $P<0.05$ vs the *RIP*(%) at the previous exposed level

b: $P<0.01$ vs the *RIP*(%) at the previous exposed level

Table 6 The concentrations of heavy metal ions in the environmental samples determined by inductively coupled plasma (ICP)

Unit: ppm

No. of samples	Al	Cd	Cr	Cu	Pb	Sr	Zn
1	2.540	<0.002	0.040	0.046	0.140	1.310	1.250
	(2)[a]	(4.5)	(4)	(3)	(3)	(2)	(4)
2	2.040	<0.002	0.037	0.008	0.097	0.669	1.780
	(3.5)	(4.5)	(5)	(6)	(4)	(3)	(2)
3	1.400	0.024	0.121	0.028	0.203	0.251	0.810
	(5)	(1)	(2)	(4)	(2)	(5)	(6)
4	1.370	<0.002	0.100	0.061	0.061	0.180	1.10
	(6)	(4.5)	(6)	(5)	(6)	(4)	(3)
5	2.040	<0.002	<0.005	0.021	<0.025	0.275	1.360
	(3.5)	(4.5)	(6)	(5)	(6)	(4)	(3)
6	8.610P	0.002	0.290	0.085	0.296	1.430	2.600
	(1)	(2)	(1)	(1)	(1)	(1)	(1)
7	0.090	0.000	0.003	0.001	0.003	0.001	0.026
	(7)	(7.5)	(7)	(7)	(7)	(7)	(7)
8	0.011	0.000	0.000	0.000	0.000	0.000	0.003
	(8)	(7.5)	(8)	(8)	(8)	(8)	(8)

a: The ordinal number of metal ions concentration in the samples

4 Conclusion

The results of study reported here demonstrated that: the survival and growth of *E. coli* can be significantly affected by environmental heavy metal ions and organic pollutants; the effects of heavy metal ions and other pollutants on the growth of *E. coli* are variable according to the different exposed levels (concentration and time), the stimulations of growth occurred at lower concentrations and longer time exposure to lead and mercury; there are a possibility of using the *E.*

coli test system to detect environmental toxicity.

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