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Effects of some heavy metals on cell suspension cultures of *Catharanthus roseus*

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Abstract—The effects of Cd(II), Cr(III), Cr(VI), Cu(II), Hg(II), Pb(II) and Zn(II) on cell suspension cultures of *Catharanthus roseus* (*C. roseus*) were investigated in detail. Biomass yields of the cells in the presence of these heavy metals relatively to that of control cultures were obtained. It was indicated that heavy metals were uptaken and bioconcentrated by *C. roseus*. The toxicities of heavy metals in the cell culture were interrelated to their oxidation states and pH of the media.

Keywords: *Catharanthus roseus*; cell culture; heavy metals; toxicity test.

1 Introduction

Heavy metals are common pollutants. The primary source of heavy metals in the environment is from naturally occurring geochemical materials. This occurrence may be enhanced by a human activity. It is difficult to eliminate a heavy metal pollution after being released in the environment. On the other hand, heavy metals may transport from water, air and soil to plants, engender impacts on the growth and development of plants. These elements may also have impacts on human via the food chain, drinking water and inhalation. So heavy metals have received considerable attention. Usually, the impacts of heavy metals on plant growth were studied by adding them to soil. It took a long time and the results reflected on the indirect impacts because of the interaction of these metals with the soil. We have chosen to investigate plant - tissue cultures, this offers a more convenient and direct system.

Catharanthus roseus, commonly the Madagascar Periwinkle, is a member of the alkaloid rich family *apocynaceae*. Many types of compounds, including abscisic acid (Smith, 1987) vanadyl sulphate (Smith, 1987) and various arsenicals (Cullen, 1989), have been investigated for activities in *catharanthus* cultures. In this paper, the effects of some heavy metals on cell suspension cultures of *C. roseus* were investigated from the point of view of environmental biology and toxicology.

2 Experimental

Reagents: Cd(II), Cr(III), Cr(VI), Cu(II), Hg(II), Pb(II) and Zn(II) solutions were

prepared by dissolving $CdCO_3$, $KCr(SO_4)_2 \cdot 12H_2O$, $K_2Cr_2O_7$, $CuSO_4 \cdot 5H_2O$, $HgCl_2$, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$ and $ZnSO_4$ respectively in distilled water.

Catharanthus cultures: To each 500 ml Erlenmeyer flask was added 100 ml of alkaloid production medium (Table 1), various amounts of heavy metals and distilled water to a total of 200 ml, adjusted the media to pH 5.5. The flasks were autoclaved at 121℃, 20 Psi for 15 min, cooled and inoculated with 15 ml of 10 day old *C. roseus* suspension culture in 1-B5 medium (Gamborg, 1982). The cultures were incubated at 25℃ in gyratory shakers at 135 r/min for 17 days. The growth was monitored by determining a refractive index of the residual media. Upon completion of the incubation, the suspension was filtered through a Miracloth filter under vacuum. The isolated cells were washed with distilled water. The wet weights and dry weights after freeze-drying were recorded. The percentage biomass yields of the dry cells in the presence of heavy metals were obtained relatively to that of the control cultures. The residual media were analyzed for obtaining percentage uptakes of heavy metals by *C. roseus*. The cultures of *C. roseus* were done twice.

Table 1 Medium for alkaloid production by periwinkle cell culture

Ingredient	mg/g	ml	Ingredient	mg/g	ml
KH_2PO_4	68		Micronutrients		1.0
KNO_3	950		Vitamins		5.0
NH_4NO_3	720		Sucrose	50g	
$MgSO_4 \cdot 7H_2O$	185		Folic acid		1.0
$CaCl_2 \cdot 2H_2O$	220		Biotin		1.0
Iron	55.9		Indole-3-acetic acid		1.0
KI		0.5	6-benzylaminopurine		1.0

Notes: Adjust pH to 5.5

Stock solutions: Micronutrients (mg/100ml): $MnSO_4 \cdot H_2O$, 700; $ZnSO_4 \cdot 7H_2O$, 405; H_3BO_3 240; glycine, 200; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 9.25; $CuSO_4 \cdot 5H_2O$, 1; KI: 75 mg/100ml; Vitamins (mg/ml): myo-inositol, 2000; nicotinic acid, 100; pyridoxine·HCl, 10; thiamine·HCl, 10; Folic acid: 25 mg/100ml; Biotin: 5 mg/100 ml; Indole-3-acetic acid: 17.52 mg/100ml; 6-Benzylaminopurine: 112.5 mg/100ml

When studying the effect of mercury on the cultures of *C. roseus*, the procedure was adjusted because of its volatility. After the flask were autoclaved and cooled, the mercury solution filter-sterilized using a 0.45 μm Milipore HA filter was added.

Digestion of cell sample: Approximately 0.2 g of the dried material was placed into a 100 ml beaker with 5.0 ml concentrated HNO_3 and 1 ml concentrated H_2SO_4. The beaker was laid up for 1 h at room temperature, then heated on a hot plate until the solution was clear. The sample was cooled and made up volumetrically to 50 ml with distilled water. The digested samples were then analyzed by atomic absorption spectrometry. The results were compared with that found by determining the filtrates.

Measurement method: A varian techtron model AA 1275 atomic absorption spectrometer

was used for analysis. Cd(II), Cr(III), Cr(VI), Hg(II), Pb(II) in the dried cells or the residual media were analyzed by graphite furnace atomic absorption spectrophotometry. Cu(II), Zn (II) in samples were determined by flame - atomic absorption spectrophotometry.

3 Results and discussion

3.1 Effects of heavy metals on the cultures of *C. roseus*

Heavy metals are found in all living organism, where they play a variety of roles. Some of heavy metals are essential in plant nutrition. However, some are not vital for plants. All elements are toxic if absorbed in excess. The effects of Cd(II), Cr(III), Cr(VI) on the growth of *C. roseus* culture are shown in Fig. 1. The biomass yields decreased with increasing Cd(II), Cr (III) or Cr(VI) concentrations in the culture media. Cadmium is more toxic than chromium, Cr (VI) is more toxic than Cr(III). When Cd(II), Cr(VI) are 10.0 and 20.0 μg/ml respectively, the cells do not grow.

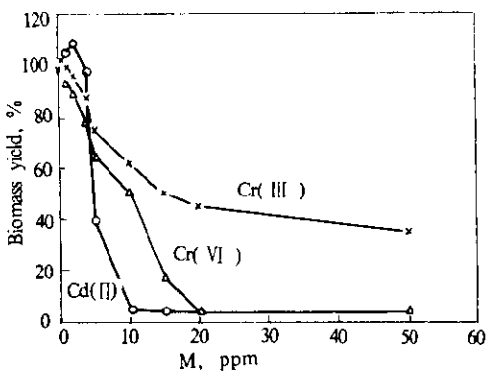

Fig. 1 Effects of Cd(II), Cr(III) and Cr(VI) on growth of *C. roseus*

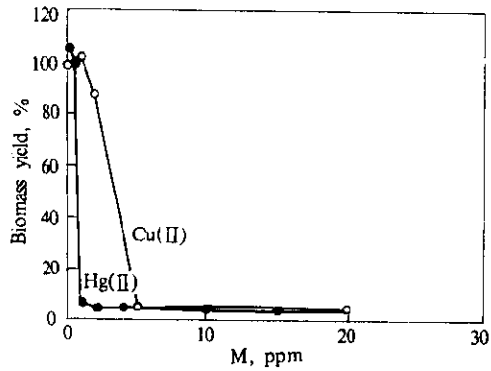

Fig. 2 Effects of Cu(II) and Hg(II) on growth of *C. roseus*

Fig. 2 is the effects of Cu (II), Hg(II) on the growth of *C. roseus*. Hg(II) is very toxic in the cultures of *C. roseus*. Biomass yield suddenly drops with increasing Hg(II) concentration in the media. When Hg(II) is 1.0 μg/ml, the cells hardly grow. Cu(II) is less toxic than Hg(II), when Cu(II) is 5.0 μg/ml, the cells stop growing. The effects of Pb(II) and Zn(II) on the growth of *C. roseus* are shown in Fig. 3. When Pb(II) initial concentration in the media at pH 5.5 is below 10.0 μg/ml, cultures of *C. roseus* healthy and the biomass yields are comparable with those of control cultures grown in the absence of Pb(II); however, when Pb(II) is over 10.0 μg/ml there is a gradual decrease in biomass yields with increasing Pb(II) concentration. Pb(II) has not a marked impact on the growth of *C. roseus* because at higher concentrations Pb (II) formed the precipitation in the culture media at pH 5.5 and its effective concentration was reduced. Zn (II) is the least toxic in all heavy metals discussed in this paper. It is, in fact, an essential element in plant nutrition. When Zn(II) in the culture media is below 30.0 μg/ml, it stimulates the growth of *C. roseus* to some extent. However, when Zn(II) is over 50.0 μg/ml, it

inhibits the growth of *C. roseus* and Zn(II) is 100 μg/ml, the cells hardly grow.

Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values for heavy metals can be estimated from plots of the dry biomass yield of the cultures for 17 days to the initial concentration of the heavy metals . In toxicology, LC_{50} is a lethal concentration required to kill 50% of the test animals. LC_{50} is resulted from acute toxicity test and mathematical statistics. In this plant - tissue cultures, it is inconvenient to evaluate LC_{50} by calculating died cells. Thus, the toxicities of heavy metals were campared and estimated by the percentage biomass yields of dry cells in the presence of respective metals. In this paper, IC_{50} is quoted in order to distinguish between it and LC_{50}. IC_{50} is the initial concentration of heavy metal in the culture media at half biomass. IC_{50} values for these metals can be estimated as MIC and MLC.

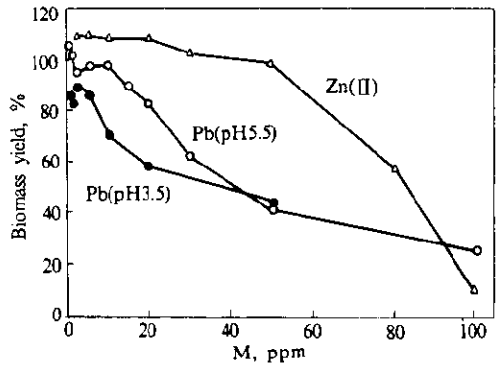

Fig. 3 Effects of Pb(II) and Zn(II) on growth of *C. roseus*

To sum up, when the initial concentration are blow MIC values, heavy metals have no impacts on the growth of *C. roseus*; the initial concentration are between MIC and MLC, the biomass yields of cell cultures are inversely proportional to the concentration of heavy metals. Hg (II), Cu(II) and Cr(VI) have great impacts on the growth of *C. roseus*. It is observed that the toxicities of heavy metals are related to their kinds and concentrations in the culture media. We use IC_{50} to judged the toxicities of heavy metals to the cultures of *C. roseus*. Thus, the toxic order is: Hg(II) > Cu(II) > Cd(II) > Cr(VI) > Cr(III) > Pb(II) > Zn(II) (Table 2).

3.2 The uptake of heavy metals by *Catharanthus roseus*

Some heavy metals, such as zinc, copper and chromium are essential in plant growth. However, mercury, lead and cadmium are not vital for plants. All elements may be uptaken and accumulated by plants. The uptakes of heavy metals by *C. roseus* are shown in Fig. 4, Fig. 5 and Fig. 6. The percentage uptakes are obtained by measuring the difference between the initial heavy metal concentration in the media and that remaining at the time of harvest. It is expressed as the percentage of the original heavy metals taken up from the media. The percentage uptakes of heavy metals by *C. roseus* decrease gradually with increasing the initial concentration of heavy metals in the culture media; the cells show higher uptakes at lower concentrations. However, the percentage uptakes of heavy metals may not give a clear indication of the actual amount incorporated into the cells . A lower percentage of uptake does not necessarily imply a smaller amount of heavy metals taken up by the cells than a higher uptake percentage. In fact, the metal contents in the cells are directly proportional to that in the culture media (Table 3). Heavy metals are absorbed and bioconcentrated by *C. roseus*. The bioconcentration factors may be stood for the capacity of uptaking heavy metal by *C. roseus*. These data are obtained by the concentrations of heavy metals in the dried cells divided by their initial concentrations in the culture media. Of course, the bioconcentration factors are interrelated to the initial concentration of heavy metals in

the culture media. It is indicated that the percentage uptake of Hg(II) by *C. roseus* is higher than that of Cu(II) or Cr(III); Cr(III) is more easily absorbed by the cells than Cr(VI). Heavy metals are bioconcentrated by *C. roseus* to varying degree.

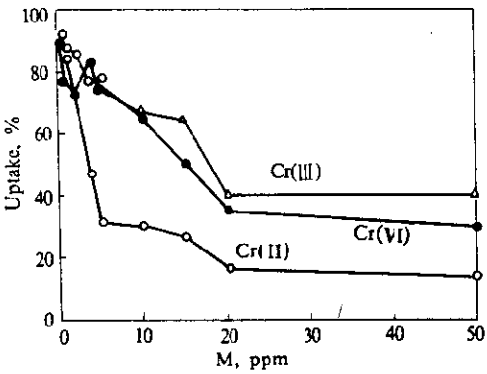

Fig. 4 Percentage uptake of Cd(II), Cr(III) and Cr(VI) by *C. roseus*

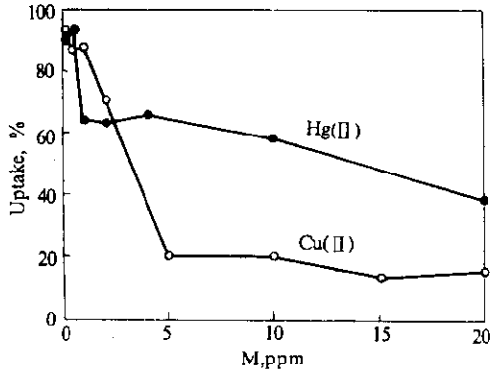

Fig. 5 Percentage uptake of Cu(II) and Hg(II) by *C. roseus*

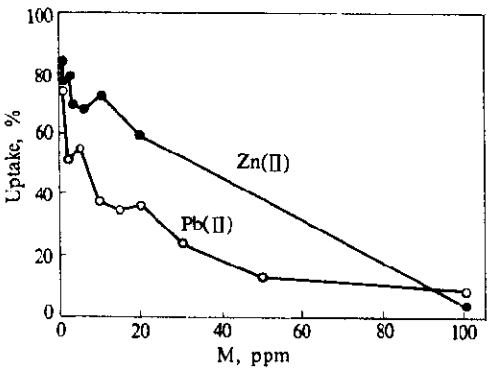

Fig. 6 Percentage uptake of Pb(II) and Zn(II) by *C. roseus*

Table 2 MIC and MLC and IC_{50} values of heavy metals

M	MIC, μg/ml	MLC, μg/ml	IC_{50}, μg/ml
Cd(II)	2.0	10.0	4.8
Cr(III)	2.0	—	15.0
Cr(VI)	0.75	20.0	10.0
Cu(II)	1.0	5.0	3.5
Hg(II)	0.50	1.0	0.75
Pb(II)	10.0	—	41.0
Zn(II)	50.0	100.0	79.0

Table 3 The contents of heavy metals in dried cells and maximum bioconcentration factors (MBF)

M	M, μg/ml in the media	M, mg/g in the cells	MBF
Cd(II)	0.10—50.0	0.009—14.10	680
Cr(III)	0.10—50.0	0.008—5.16	123
Cr(VI)	0.10—50.0	0.008—39.78	880
Cu(II)	0.10—100.0	0.030—10.30	440
Hg(II)	0.10—20.0	0.009—21.22	1870
Pb(II)	0.10—100.0	0.009—2.95	95
Zn(II)	0.10—100.0	0.058—4.27	86

3.3 Some factors influencing the toxicities of heavy metals

Effects of Pb(II) on the growth of C. *roseus* at initial pH 3.5 and pH 5.5 of the culture media are shown in Fig. 3. It is indicated that Pb(II) is below 50.0 μg/ml, its toxicity is higher at pH 3.5 than pH 5.5. IC_{50} of Pb(II) is pH 3.5, 33.0 μg/ml (1.59×10^{-4}); pH 5.5, 41.0 μg/ml (1.98×10^{-4}) respectively because of an higher effective concentration at a lower pH. Heavy metals easily form precipitations at higher pH, in this investigation, all media are adjusted to pH 5.5, thus the toxicities of heavy metals to *C. roseus* growth can be compared.

In addition, the toxicities of heavy metals are related to oxidation states. For example, Cr(VI) is more toxic than Cr(III) in cell cultures; IC_{50} is Cr(VI), 10.0 μg/ml (1.92×10^{-4} mol/L); Cr(III), 15.0 μg/ml (2.88×10^{-4} mol/L), respectively. Of course, the toxicity is also related to heavy metal species.

4 Conclusion

The toxicities of heavy metals in the cultures of *C. roseus* at pH 5.5 are: Hg(II) > Cu(II) > Cd(II) > Cr(VI) > Cr(III) > Pb(II) > Zn(II). When Zn(II) 30 μg/ml, it may stimulate the growth of *C. roseus*. These metals can be uptaken and bioconcentrated by *C. roseus*. The contents of heavy metals in the cells are directly proportional to that in the culture media. The toxicities of heavy metals are interrelated to their oxidation states, species and pH of the culture media.

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