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Environmental Contamination and Toxicology

Effects of Mercury (II) Species on Cell Suspension Cultures of *Catharanthus roseus*

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Mercury has received considerable attention because of its high toxicity. Widespread contamination with mercury poses severe environmental problems despite our extensive knowledge of its toxicity in living systems. It is generally accepted that the toxicity of mercury is related to its oxidation states and species, the organic forms being more toxic than the inorganic forms. In the aquatic environment, the toxicity of mercury depends on the aqueous speciation of the mercuric ion (Hg^{2+}). Because of the complex coordination chemistry of mercury in aqueous systems, the nature of the Hg^{2+} species present in aquatic environments is influenced greatly by water chemistry (e. g, pH, inorganic ion composition, and dissolved organics). Consequently, the influence of environmental factors on the aqueous speciation of mercury has been the focus of much attention (Bahich and Storzky 1980, Nuzzi 1972; Farrell et al. 1990). However, there is very little information available regarding the effects of the species and speciation on Hg (Ⅱ) toxicity in plant-tissue cultures.

Catharanthus roseus (C. roseus), commonly called the Madagascar Periwinkle, is a member of the alkaloid rich family Apocynaceae. The present investigation was concerned with the toxicity of mercury on the growth of C. roseus cell suspension cultures as influenced by mercury (Ⅱ) species and speciation. The specific objectives of the study were to (a) study the effects of mercury species on the growth of C. roseus cultures from the point of view of environmental biology and toxicology; (b) evaluate the effects of selenate, selenite and selected ligands such as chloride, l-cysteine in the media on the acute toxicity of mercuric oxide; (c) determine the impact of the initial pH of the culture media on the toxicities of mercuric compounds; (d) discuss the dependence of the toxicity on the chemical species and speciation of Hg(Ⅱ).

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MATERIALS AND METHODS

HgO, $Hg(Ac)_2$, and CH_3HgCl solution (1 mg/ml) were prepared by dissolving the corresponding mercuric compounds in distilled water. Chloride and cysteine solutions were made by dissolving potassium chloride and l-cysteine, respectively, in distilled water.

To each 500 ml erlenmeyer flask was added 100 ml of alkaloid production medium (Zhu and Cullen 1993) and distilled water. The medium was adjusted to pH 5.5. The flasks were autoclaved at 121℃ and 20 psi for 15 min. After cooling, the Hg^{2+} solution (filter-sterilized using 0.22 HA filter) was added. The total volume of the culture medium was 200 ml. The flasks were incubated with 15 ml of 10-day old C. roseus suspension cultured in l-B5 medium (Gamborg 1982). The cultures were inoculated at 25℃ in gyratory shakers at 135 rpm. Cell growth was monitored by determining the refractive index of the residual medium. Upon completion of the incubation (17 days), the suspension was vacuum-filtered through a Miracloth filter. The isolated cells were washed with distilled water. Wet weights and the dry weights after freeze-drying were recorded. The biomass yields in the presence of the various mercury(Ⅱ) concentrations were obtained. The experiment was done twice.

The effects of chloride and cysteine on the toxicity of mercuric oxide at pH 5.5 and the impacts of the initial pH of the culture medium on the toxicity were evaluated in a similar fashion.

Approximately 0.2 g of the dried material was placed into a 60-ml teflon digestion vessel with 5.0 ml concentrated HNO_3, and the vessel was capped. The cell samples were digested at 100% power for 2 min in a microwave oven. At the end of the heating process, a clear, light-yellow solution remained in the vessel. This solution was transferred to a volumetric flask and the volume was brought to 50 ml with distilled deionized water.

A Varian Techtron Model AA 1275 Atomic Absorption Spectrometer was used for analysis. Mercury(Ⅱ) in the residual medium or digested samples was determined by graphite furnace atomic absorption spectrometry; 0.4% Na_2S was used as a chemical modifier. The detection limit was 2.0 ng/ml. The recoveies for determining mercury were 94.0～100.6%.

RESULTS AND DISCUSSION

The impacts of various mercuric compounds on the growth of C. roseus cell suspension cultures at pH 5.5 were investigated. Dry biomass yields of C. roseus cultures were plotted against the initial total Hg(Ⅱ) concentrations (Fig. 1). IC_{50} (Initial Concentration of Hg^{2+} in the culture medium at half biomass) and MLC (Minimum Lethal Concentration) values for mercury(Ⅱ) compounds were estimated from Fig. 1 (Table 1). At lower Hg(Ⅱ) concentrations, cultures of C. roseus showed growth inhibition, and the biomass yields decreased as the mercuric concentration in the medium was increased. When the Hg(Ⅱ) concen-

tration was over the MLC, C. roseus did not grow. The toxicity of mercury (Ⅱ) species decreased in the order: $CH_3HgCl > HgCl_2 \text{--} Hg(Ac)_2 > HgO$. The mercuric toxicity results from its adsorption on cell surfaces, e. g, cell walls (Kuiper 1981) and also impacts many enzyme systems. Thus, methylmercuric chloride was more toxic than inorganic mercury since it was more easily adsorped on the cell walls of C. roseus. It was further indicated that toxicities of different mercuric compounds were influenced by the nature of the co-ion (Farrell et al. 1990). Therefore, ligands such as Cl^-, PO_4^{3-}, NO_3^- in the culture medium affected the toxicities of the mercuric compounds. The distribution of the Hg-ligand species in the culture medium may be the same whether Hg(Ⅱ) is added as mercuric chloride or as mercuric acetate; consequently, it is not surprising that there were no significant differences in the toxicities of the two mercuric compounds. However, mercuric oxide was less toxic to C. roseus cultures.

Table 1, IC_{50} and MIC values of mercuric compounds to C. roseus

Mercuric Compound	IC_{50}, ppm Hg	MLC, ppm Hg
HgO	1.40	2.0
$HgCl_2$	0.90	2.0
$Hg(Ac)_2$	0.90	2.0
CH_3HgCl	0.55	0.75

The mercuric uptake by C. roseus from the culture medium was studied as a function of the initial concentration. However, mercury content in the dry cells were directly proportional to that in the culture medium. Mercury was taken up and bioconcentrated by C. roseus (Table 2). Of course, factors were interrelated to the mercury concentration and its species or speciation in the culture medium. Methylmercuric chloride was accumulated more by C. roseus with the maximum bioconentration of 2500. Therefore, both toxicity and uptake of mercury(Ⅱ) were influenced by its species.

Table 2. Mercury contents in the dry cells and Maximum Bioconcentration Factors(MBF).

Mercuric Compound	Hg(ppm) in the media	Hg(ppm) in dry cell	MBF
HgO	0.10-5.0	0.0095-8.81	1760
$Hg(Ac)_2$	0.10-5.0	0.0092-8.72	1840
$HgCl_2$	0.10-5.0	0.0096-7.56	1702
CH_3HgCl	0.10-5.0	0.0078-7.69	2500

As previously mentioned, the nature of the Hg(Ⅱ) species present in aquatic environments is influenced greatly by water chemistry. In this investigation, the effects of chloride (as potassium chloride) and cysteine (l-cysteine) on the toxicity of mercuric oxide cell cultures were studied. Fig. 2 shows the effect of chloride alone on the growth of the C. roseus cell suspension culture. At lower chloride concentrations ($10^{-3} - 10^{-4}$ M), cultures of C. roseus appeared to be healthy, and biomass yields were comparable with those of control cultures

grown in the absence of chloride; however, at a higher chloride concentration (0.1M), there was a considerable aggregation of the cells.

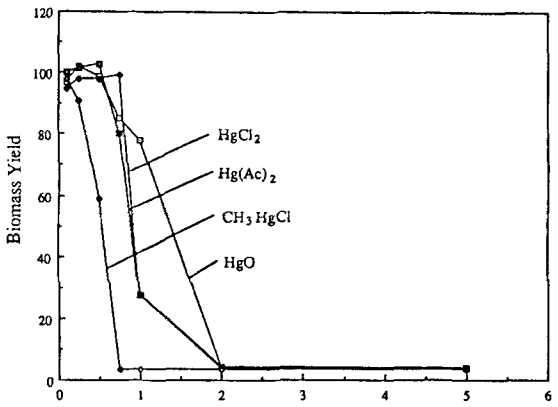

Figure 1. Effects of Hg(Ⅱ) on the growth of C. roseus at pH. 5.5.

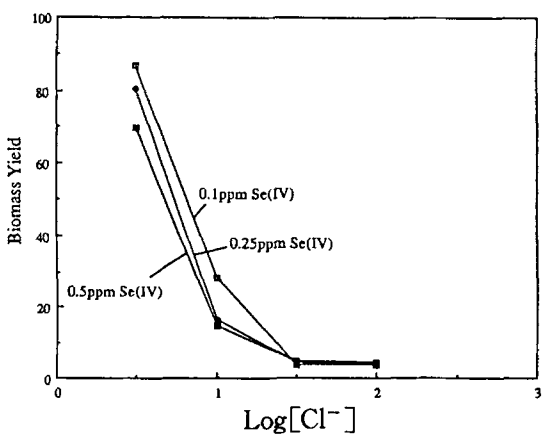

Figure 2. Effect of chloride on the growth of C. roseus at pH 5.5.

Bioassays were conducted to determine the effects of chloride on the acute toxicity of mercuric oxide at pH 5. Addition of lower concentrations (10^{-4}—10^{-2}M) of chloride had no significant effect on the toxicity of mercuric oxide. The effect of chloride on the toxicity of mercuric oxide is illustrated in Fig. 3. It was demonstrated that the toxicities of the Hg(Ⅱ) compounds were highly correlated to the molar fraction of the Hg-chloro complexes, regardless of the change of the complex (Farrell et al. 1990). As to the relative toxicities of the various Hg-chloro complexes, it was postulated that they should decrease in the order: $HgCl^+ > HgCl_2^0 > HgClOH^0 > HgCl_3^- > HgCl_4^{2-}$. Thus, chloride influenced Hg speciation in the medium resulted in different toxicities of mercuric oxide. However, chloride had no significant effects on the toxicity of mercuric oxide because chloride alone produced an impact on the growth of C. roseus. On the other hand, uptake of mercuric oxide by C. roseus were influenced by chloride in the medium because of the formation of Hg-chloro complexes. At lower concentrations (10^{-2}

-10^{-4}M), chloride appeared to have no marked effects on mercuric uptake by C. roseus. In the presence of 0.1 M chloride in the culture medium, the uptake of mercuric oxide by C. roseus decreased because a portion of the mercury(Ⅱ) formed $HgCl_3^-$, $HgCl_4^{2-}$ complexes.

Additions of cysteine to the culture medium caused significant decreases in the toxicity of mercuric oxide to C. roseus cultures at pH 5.5 (Fig. 4). Experiments with a set of cysteine controls (5×10^{-6}, 5×10^{-5}, 5×10^{-4}M, without Hg^{2+}) demonstrated that cysteine alone had no deleterious effect on the growth of C. roseus cell suspension culture. In the presence of cysteine, significant amounts of Hg(Ⅱ) was complexed as Hg $(CYS)_2^{2-}$. Because of the extreme affinity of mercury for sulfhydryl groups (the formation constants for the 1 : 2 Hg-cysteine complex is $10^{45.2}$), Hg(Ⅱ) was strongly bound to the added cysteine. Decreases in the toxicity of mercuric oxide were attributed to the formation of Hg $(CYS)_2^{2-}$ complexes. It was found that the toxicity of mercuric oxide decreased

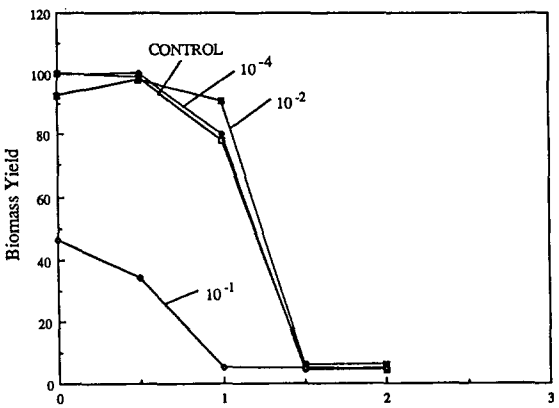

Figure 3. Effect of chloride on HgO toxicity in C. roseus culture at pH 5.5.

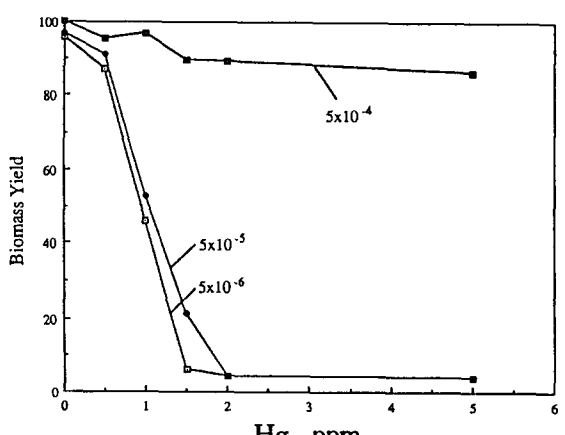

Figure 4. Effect of L-cysteine on HgO toxicity at pH 5.5.

gradually as cysteine concentration in the medium was increased. In the absence of cysteine, the growth of C. roseus was completely inhibited by 2.0 ppm Hg^{2+} (HgO). Nevertheless, the growth of C. roseus was inhibited slightly when the cysteine concentration of the medium was at 5×10^{-4}M. Even if Hg^{2+} was at 5.0 ppm, the biomass yield was 86.38 relative to the control in the presence of 5×10^{-4}M cysteine. It was also found that the growth of C. roseus was inhibited in the presence of 1.2 ppm Hg^{2+} (6×10^{-6}M), the biomass yield was only 35.36; when a cysteine concentration of 6×10^{-6}M (at cysteine : Hg molar ration of 200) was added, mercuric oxide did not appear to inhibit C. roseus growth. Therefore, cysteine at a higher concentration had an antagonistic effect on mercuric compounds. This effect was correlated to the cysteine concentration in the culture medium.

The nature of the Hg(Ⅱ) species present in the medium was also influenced greatly by pH. Impacts of initial pH of culture medium on mercuric toxicities are shown in Fig. 5. Hg^{2+} may exist in various species such as $Hg(OH)^{+}$, $Hg(OH)_2$ or HgClOH at different pH of the medium. On the other hand, the complex equilibrium of Hg-ligand is correlated to the pH of the aquatic system. The toxicities of mercuric compounds to C. roseus cultures were influenced by the pH of the medium. At initial pH 3.0, all mercuric compounds had the greatest inhibition to the growth of C. roseus. However, the cells were healthy at pH 3.0 in the absence of Hg. As the pH of the medium was increased, the toxicities of mercuric compounds to C. roseus cultures decreased. Methylmercuric chloride produced a similar toxicity to cell cultures at pH 4.0—7.0; at pH>7, its toxicity increased as the pH rose. The inhibition of mercuric chloride was weaker at pH 8.0. The toxicities of mercuric acetate and mercuric oxide had no marked differences at initial pH 4.0—9.0. In addition, mercuric uptake by C. roseus was influenced by the initial pH of the culture medium. C. roseus had the lowest mercuric uptake from the culture medium at pH 3.0. Between pH 4.0 and 9.0, the $Hg(Ac)_2$, $HgCl_2$, CH_3HgCl remained almost constant. However, at an initial pH of 9.0, HgO appeared to have a lower uptake by C. roseus.

Numerous laboratory studies, usually on birds or mammals, have demonstrated the antagonistic effect of selenium compounds on the toxicity of mercury (Davies and Russell 1988; Keoman and Peeters 1973; Siegel et al. 1991). The formation of granules of HgSe in the liver of marine mammals appears to act as part of a detoxification system. The phytotoxicity of Hg^{2+} was lessened by Group VIa oxyanions (Spencer and Siegel 1978). Pelletier (1985) reviewed mercury-selenium interactions in aquatic organisms, including the possibility of selenium affecting the accumulation of mercury by marine fish and shellfish. More extensive experimental studies have been carried out in freshwater ecosytems. Dissolved selenite reduced the rate of accumulation of inorganic mercury by a range of species. Selenium transferred throuhg the food web could control mercury accumulation by fishes in lakes contaminated by mercury. The wide range of aquatic organisms studied above mainly showed stabilized or reduced mercury concentrations in response to added selenite; this was in contradiction to much published laboratory work.

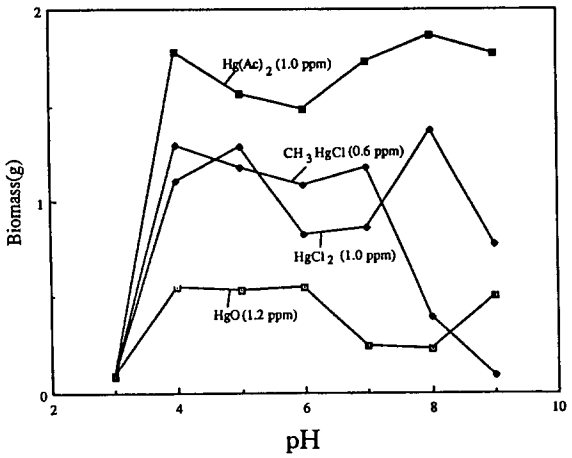

Figure 5. Effect of initial pH of medium on Hg(Ⅱ) toxicity.

In this investigation, the effects of selenate or selenite on the toxicity of mercuric oxide was studied. Figs. 6 and 7 show the effect of Hg and Se(IV) or Se(VI) on the growth of C. roseus at pH 5.5. In the presence of 0.10, 0.25, 0.50 ppm Se(IV) or Se(VI), the toxicity of mercuric oxide to C. roseus culture was not reduced, and the biomass yields were less than that in the presence of mercuric oxide alone. Selenate or selenite had no antagonistic action against mercuric oxide in C. roseus cultures. In general, selenate and selenite produced the antagonistic action against mercury because of Hg-Se bonding; thus, Se(VI) or Se (IV) were reduced to Se(Ⅱ) before forming the Hg-Se bond. In this plant-tissue, flasks with the medium were sterilized before addition of the C. roseus cultures. Selenate or selenite in the medium may not have been reduced to Se(Ⅱ) because of the absence of microorganisms, and the growth of C. roseus under aerobic condition. In addition, mercuric uptake did not decrease in the presence of 0.1—0.5 ppm Se. Therefore, in this cell culture, selenium compounds did not have any antagonistic action against mercuric oxide.

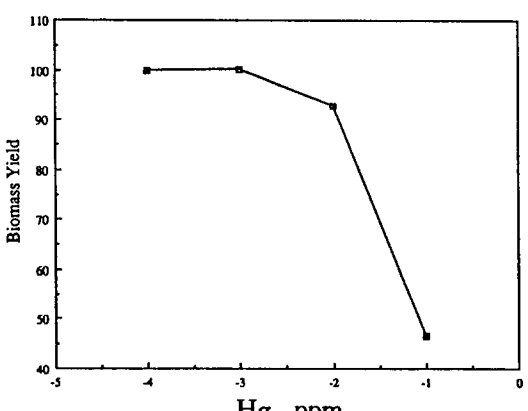

Figure 6. Effect of Hg(Ⅱ) and Se(Ⅳ) on the growth of C. roseus at pH 5.5.

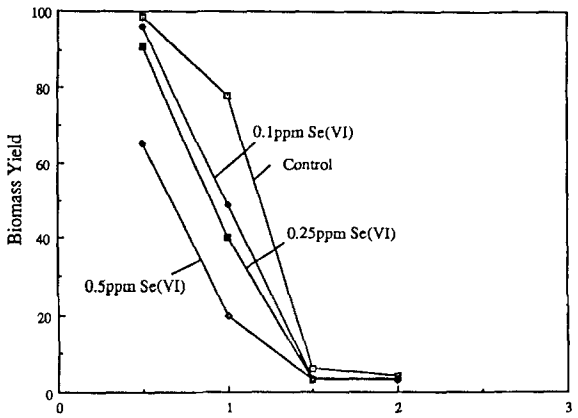

Figure 7. Effect of Hg(Ⅱ) and Se(Ⅵ) on the growth of C. roseus at pH 5.5.

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