Heavy Toxic Metal Resistance of Selected Rhizobium Strains

Juanita C. Mamaril, Lorele C. Trinidad, Lilia SP. Tolentino and Vilma T. Capuno
National Institutes of Biotechnology and
Applied Microbiology (BIOTECH)
University of the Philippines Los Baños
College, Laguna,

ABSTRACT

*Thirty-three **Rhizobium** strains isolated from different host plants and locations were screened for resistance to the heavy toxic metals mercury, copper and cadmium. Five strains, BJr 7 and 12 from **Vigna radiata** (mungbean) and BJL1 21, 23 and 30 from **Leucaena leucocephala** (ipil-ipil) were resistant to mercury up to 50 ppm but were sensitive to 40 ppm Cu and 10 ppm Cd. Strains from **Enterolobium saman** (acacia), BEs 10, 11, 15, 20, 24 and 30 and BJVr 2 and 4 could tolerate up to 40 ppm Cu but were sensitive to mercury. Strains tolerant to 10 ppm Cd were BL1 57, THA 201 and M5. The rest of the strains tested did not survive in 10 ppm Cd.*

The resistance of BL1 80 to mercury, copper and chromium was induced further by stepwise transfer of surviving culture grown at lower metal concentrations to culture media containing higher concentrations. By this technique, BL1 80 was able to tolerate concentrations of 50 ppm Hg, and 60 ppm Cr. Tolerance to copper was quite low. The results suggest that the resistance of microorganisms can further be developed to render a microbial culture that has a greater potential to detoxify heavy metals in the environment.

INTRODUCTION

Metals in the environment have undergone great changes in distribution and solubilization due to industrialization and modernization. Modern agriculture, mining and heavy to light industries in some way or another have introduced higher amounts of toxic metal to the environment which pose risks to the health and well-being of people, animals, plants and microorganisms. This imbalance of heavy metals imposes a burden on the regulatory systems of higher forms of organisms. The transport of metal ions through cell membranes that took billions of years to evolve is being challenged. However, microorganisms have a relatively shorter generation time and consequently their evolution rates are faster. This enables them to evolve within a short time, mechanisms to maintain low concentration of metals intracellularly.

This study deals with the survival of selected *Rhizobium* strains (isolated from different host plants and locations) in culture media containing varying concentrations of the heavy toxic metals mercury, copper and cadmium. The selection of the strains was based on their ability to produce large amounts of mucilaginous polysaccharides. The development of resistance to toxic metals by gradual exposure of a selected strain BL1 80 to increasing metal concentrations was also dealt with.

REVIEW OF LITERATURE

The ability of microorganisms to trap and concentrate metals has been reported for quite some time (**8**). Recently, Mamaril et al. (**7**) reported that tropical *Rhizobium* isolated from areas located near volcanic regions can tolerate high concentrations of lead and mercury. These *Rhizobium* strains sequester the metal on cell surfaces which are covered with a layer of mucilaginous polysaccharides.

It has also been reported that nodulated plants have lower metal content in their upper portion than non-nodulated plants. Douka and Xenoulis **(5)** have reported that soybean and other nodulated plants grown under field and laboratory conditions have significantly lowered heavy metal concentration in the upper portion of the plants. The concentrations of metallic elements such as Mo, Mn, Zn, Cu, Co and Sr in the plants were determined by X-ray fluorescence techniques. They also determined the radioactive content of a pasture field experiment after the Chernobyl accident. It was found that

the concentrations of Cs- 134, C-137 and (Ru-Rh)-105 were lower in the upper portion of the nodulated plants than in non-nodulated plants suggesting that these radioactive elements were concentrated more in the nodules or roots. These findings are important since the useful biomass of the plants are the upper portions which are eaten by man and animals.

Transmission electron micrographs of *Rhizobium* strains grown in culture media containing lead or mercury (**7**) showed that these metals were trapped on the cell surface as complexes or precipitates. Adsorption of heavy metals via extracellular traps minimizes the massive entry of these metals into the cell. A breakdown of the cell's defenses will cause an imbalance in the cell's metabolism and thus cause the death of the organism. Analyses of the supernatant after the cells were removed showed drastic reduction of lead and mercury concentrations.

The removal of heavy metals from solution by microorganisms can occur in several ways (**14**). One way is by altering the solubility of the heavy metal salts. Solubility of metal salts is affected by a number of factors such as pH, temperature, standard reduction potential, concentration of competing anions and cations and surface active substances. Surface active substances may include particulates and macromolecules (polysaccharides, proteins, etc.). The exposed negatively charged groups such as the hydroxyl, carboxyl, amino, amide, imidazole, sulfhydryl, thiol, phenolic and phosphate groups found on the cell surface are responsible for providing negatively charged surfaces to microorganisms. These charges could be neutralized or reversed by the presence of high concentrations of positively charged metallic ions (**4**). The negatively charged groups may act as extracellular ligands which can form stable complexes with the metals and thus prevent their cellular uptake.

The cell may also use the activities of surface bound enzymes for extracellular metal precipitation. The precipitation of insoluble metal complexes can occur through biosynthesis of membrane-associated sulfate reductases (**6**) or oxidizing agents (**14**). The reduction of sulfate to sulfide and the diffusion of oxygen or hydrogen peroxide can provide effective means of precipitation of metals. This metabolic activity of the cell is closely linked to the resistance of the microorganism to heavy metals.

Intracellular traps offer enzyme-mediated resistance to heavy toxic metalloids such as arsenic, antimony and cadmium (**1**). Toxic metals such as mercury and tin are removed via synthesis or organometals. Biomethylation gives certain

microorganisms advantages in eliminating heavy metals. The synthesis of less polar organometallic compounds from polar inorganic ions help regulate cellular elimination which involves diffusion controlled processes (**13**). Another means of controlling metal concentration is through the synthesis of ligands in the form of small molecules with high stability constants such as in the removal of iron by siderophores. These mechanisms require energy to pump the metal ion out of the cell. Enzyme-mediated activities are usually coded by DNA on bacterial plasmids or transposons and not by the normal chromosomal genes (**9**).

Microbial resistance to heavy metals has followed two distinct patterns. Microorganisms subjected to extreme environmental conditions as in active volcanic regions, which abound in hot springs, volcanic lakes and deep sea vents have evolved structures that enable them to adapt to high concentrations of metals (**3**). These evolutionary adaptations to the environment have been passed on to succeeding generations. The other pathway by which microorganisms acquire metal resistance is the acquisition of extachromosomal DNA called plasmids. The synthesis of these plasmids can be increased or decreased depending on the severity of environmental conditions. This mechanism requires inputs of cellular energy which involve nonequilibrium processes and are important considerations in determining rates of metal uptake by the cell.

Microorganisms which have resisted high metal concentrations due to extreme environmental condiditions or are genetically manipulated can be utilized to recover metals from industrial waste waters. Microbial biomass could effectively be used to decontaminate waste effluents from mines, refineries, nuclear fuel plants, battery and electroplating operations (**10**). Metals present in low concentrations in aqueous solutions can be concentrated up to several thousand times its concentration in the environment by a number of microbial species (**11**). The concentration of metals may be as much as 15-50% (w/w) of the cell dry weight. It has been reported that the adsorption of metals by microbial cell mass may be highly specific for one metal and exclude other metals (**2**). The adsorption capacities of some microbial biomass are found to be greater than some adsorbents available in the market (**12**).

The uptake of metal ions by microorganisms in general may occur in two stages. The first stage involves a rapid process occurring on the cell surface and does not involve metabolic processes or inputs or energy. The second stage occurs within the cell cytoplasm and is a slow process. The

processes involve cellular energy to transport ions across the cell membrane against a concentration gradient and are diffusion controlled. Transfer through membranes requires a combination of energized events with carrier molecules which may be specific small molecules or proteins.

MATERIALS AND METHODS

Rhizobium strains/isolates

Thirty-three *Rhizobium* strains which are heavy producers of mucilaginous polysaccharides were obtained from the BIOTECH Culture Collection Laboratory for screening for resistance to heavy metals.

Culture media and conditions

The *Rhizobium* strains were maintained on slopes of yeast extract mannitol agar (YEMA).

The culture medium for the growth experiments was yeast extract mannitol broth (YEMB) to which varying concentrations of metal were added. Control experiments were in YEMB without addition of metal salts. Metal concentration preparation ranged from 0 to 50 ppm Hg, 0 to 40 ppm Cu and 0 to 10 ppm Cd.

Ten ml of the prepared YEMB media were placed in 18 x 150 mm test tubes and inoculated with 0.2 ml of the precultured strain. The culture was shaken at 28°C and incubated for one week. All operations were done under aseptic conditions. A schematic diagram of the procedure is shown in Figure 1. Growth observations were made daily based on turbidity. Optical density readings were made at 570 nm after one week.

BL1 80 strain found to be tolerant to Hg in a previous study (**7**) was tested further for its tolerance to higher concentrations of Hg, Cu and Cr. Concentrations of 10, 20, 30, 40 and 50 ppm Hg, 10 and 20 ppm Cu and 30, 40, 50, 60, 70, 80 ppm Cr were prepared. Viable cell count was taken daily for four days for each of the tests. The surviving cells from a medium of lower metal concentration were again used as preculture for the test with a higher concentration. A stepwise increase of metal concentration was first cultured on low concentration of the metal and the surviving cells were used as pre-culture for the next higher concentration.

RESULTS AND DISCUSSION

The results of the screening test for the resistance of 33 selected *Rhizobium* strains to mercury, copper and cadmium are tabulated in Table 2. Based on the tabulated results, 13 strains were found to be tolerant to 40 ppm Hg. They are NGR 69, BL1 57, BL1 80, BJL1 21, 23, 30, BJVr 1, 5, 7 and 12 and TSU 357, CAI Jap 110 and CAI Tri 100. At higher concentrations of 50 ppm Hg, only five strains were resistant: BJL1 21, 23, 30, BJVr 7 and 12. These strains, however, were not resistant to 40 ppm Cu. A different set of strains was found resistant to copper. These were the strains isolated from acacia and mungbean. Strains tolerant to 15 ppm Cu were BEs 10, 15, 20, 24, 30, BJVr 1, 2, 3 and 4. The same strains were tolerant at 40 ppm Cu although growth was not as fast in this concentration. All these strains were sensitive to 10 ppm Hg except for BJVr 1 and 2 which showed moderate growth at this concentration. All the strains were able to tolerate 1 ppm Cd. Twelve strains were tolerant to 5 ppm Cd. Most of the strains tolerant to copper could withstand 5 ppm Cd. At 10 ppm Cd, only three strains survived: BL1 57, THA 201 and M5. However, these strains were sensitive to mercury except for BL1 57 which was moderately tolerant to this metal.

It is evident from these results that different *Rhizobium* strains differed in the physico-chemical nature of their cell surfaces. The cell surface may have differences in the functional groups of the proteins and polysaccharides that make up the mosaic of interspersed cationic and anionic exchange sites. The specificity of these sites plays a role in the resistance of a strain to a particular metal. For example, isolates from ipil- ipil were more resistant to mercury but quite sensitive to copper and cadmium while isolates from acacia were more resistant to copper but sensitive to mercury. Isolates from mungbean were variable in their tolerance to mercury, copper and cadmium. BJVr 7 and 12 were tolerant to mercury and 5 ppm Cd but sensitive to copper. BJVr 1 had a wider range of metal tolerance. It could tolerate 20 ppm Hg, 15 ppm Cu and 5 ppm Cd. On the other hand, BJVr 4 was sensitive to mercury but quite tolerant to copper and 5 ppm Cd. M5 was sensitive to both mercury and copper but tolerant to cadmium. The differences in the metal tolerance of these strains may be due to environmental and intrinsic factors which involve differences in cell surfaces and cellular uptake of the metals.

Resistance patterns of BL1 80 to mercury, chromium and copper

The survival and growth of BL1 80 cells in media containing increasing concentrations of metals are shown in Figures 2 and 3 for mercury, Figures 4 and 5 for chromium and Figure 6 for copper. The cell counts of BL1 80 at lower concentration of mercury showed increasing cell counts from initial inoculation up to the fourth day. The general trend showed that the cells could still survive at these lower mercury concentrations. At higher concentrations of 40 ppm Hg, the cell count after the second day of incubation was lower than the initial cell count, indicating a loss of inoculant cells which could not tolerate 40 ppm Hg. The cells that were able to survive may have evolved mechanisms to cope with the situation. Thus, growth was evident. The cell count after the fourth day was a bit higher than the initial count. At 50 ppm Hg, there was a gradual loss of surviving cells until the third day, indicating that most of the cells could not cope with 50 ppm Hg. Cell count on the fourth day increased which may mean that these surviving cells had adapted to their environment. However, the cell counts were lower than the initial cell count showing that the growth rate was very much affected by 50 ppm Hg.

Resistance to chromium was quite high. BL1 80 was very tolerant to 30 ppm Cr. At 40 and 50 ppm Cr, cell counts increased after the first day of incubation. After the second day, the surviving cells had adapted protective measures to enable them to cope with 40 and 50 ppm Cr. Cell counts after the fourth day were higher than the initial cell counts, indicating continued growth of the surviving cells. The behavior of BL1 80 cells at much higher concentration of 60, 70 and 80 ppm Cr was erratic. At these high concentrations of Cr, blue green precipitates were visible on the cell surfaces. The pre-culture made up of surviving cells in 50 ppm Cr continued to multiply in 60 ppm Cr.

Cell count of BL1 80 in 70 ppm Cr dropped on the first day but increased on the second and third day. A drop in cell counts, which was much lower than the initial count, was observed on the fourth day. Death of the cells may have been brought about by the massive entry of chromium ions into the cells, thereby breaking down some protective mechanisms. Cell count at 80 ppm Cr showed an initial increase on the second day but gradually decreased on the third and fourth days. Adaptation of the BL1 80 to 80 ppm of Cr was not adequate enough to insure growth of the surviving cells. Based on the figures, the tolerance of BL1 80 to chromium can be increased at 60 ppm. BL1 80 cells

start to become unstable at high concentrations of 70 and 80 ppm Cr.

Tolerance to BL1 80 to 10 and 20 ppm Cu was much lower than that for mercury and chromium. The trend of survival of BL1 80 cells at high concentrations of 40 and 50 ppm Hg was similar to that for Cu. Cell count dropped from the first day of incubation. More cells died at 20 ppm Cu than at 10 ppm Cu during the first and second days of incubation. On the fourth day, cell counts increased, an indication that the surviving cells evolved some strategies to cope with the high amount of copper. However, the cell count on the fourth day was still lower than the initial count, which meant that growth rates were adversely affected by the high concentration of copper.

The increased production of plasmids coding for the synthesis of proteins or enzymes responsible for detoxification of metals could be the mechanism adapted by BL1 80 cells to tolerate and resist high metal concentration.

SUMMARY AND CONCLUSION

Thirty-three *Rhizobium* strains observed to produce large amounts of mucilaginous polysaccharides were screened for their resistance or tolerance to mercury, copper and cadmium. These strains were isolated from different host plants (ipil-ipil, acacia, soybean, mungbean, peanut and clover) and places (Australia, Thailand, Japan and different provinces of the Philippines). In general, strains isolated from *Leucaena leucocephala* (ipil-ipil) were more resistant to mercury than to copper while those isolated from *Enterolobium saman* (acacia) were more tolerant to copper but quite sensitive to mercury. Isolates from *Vigna radiata* (mungbean) varied in their tolerance to mercury, copper and cadmium.

Strains resistant to 50 ppm Hg were BJV4 7 and 12 and BJL1 21, 23 and 30. These strains had lower resistance to copper. Strains resistant to 40 ppm Cu were BEs 10,11, 15, 20, 24 and 30 and BJVr 1, 2, 3 and 4. However, they were sensitive to mercury except for BJVr 1 which could tolerate 20 ppm Hg. All the strains could survive in 1 ppm Cd except for BJVr 3. At 5 ppm Cd, the tolerant strains were BL1 57, THA 2 and 201, CB 756, M5, BEs 10, 15, 20, 24 and 30 and BJVr 1, 2, 4 and 12. Moderately resistant to 5 ppm Cd were TSJ 357, A 702 KO2,

CAI Jap 110 and CAI Tri 100. Except for BL1 57, THA 201 and M5, no other strain survived at 10 ppm Cd. Some specificity by the strains to particular metals was evident in this study.

Resistance of BL1 80 to mercury, chromium and copper may be increased by stepwise transfer of surviving cells in a culture containing lower concentrations of the metal to a culture medium with higher concentrations of the metal. However, this acquired resistance can be increased to a certain degree only, after which the cells become unstable.

When the cells were transferred to a medium with higher concentrations of metal, there was a gradual loss of viable cells during the first and second day of incubation. On the third or fourth day, the surviving cells began to increase their growth rate. Maximum concentration of chromium that BL1 80 could tolerate under the conditions of this study was about 60 ppm; that for mercury may be increased above 50 ppm. Tolerance to 10 and 20 ppm Cu could not be determined since cell count on the fourth day was still below initial cell count. This may indicate that BL1 80 cells do not have adequate mechanisms for the detoxification of copper. However, this may be reinforced by this technique.

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Table 1. List of *Rhizobium* strains/isolates used in heavy metal tolerance tests

Accession No.	Strain Designation	Host Plant
1079	BL1 57	*Leucaena leucocephala* (ipil-ipil)
1086	TAL 600	*Leucaena leucocephala* (ipil-ipil)
1150	THA 2	Soybean (Bangkok)
1157	THA 201	Peanut
1159	NC 92	Peanut
1164	NGR 69	Ipil-ipil
1273	CB 756	(Australia)
1275	M5	Mungbean
1347	BL1 80	Ipil-ipil
1392	BEs 50	*Enterolobium saman* (*Samanea saman,* acacia)
1383	BEs 11	" " "
1395	BEs 15	" " "
1396	BEs 20	" " "
1397	BEs 24	" " "
1403	BEs 30	" " "
1431	BJL1 4	Ipil-ipil (Los Baños, Laguna)
1435	BJL1 14	Ipil-ipil (Taal, Batangas)
1437	BJL1 19	Ipil-ipil (Malolos, Bulacan)
1439	BJL1 27	Ipil-ipil (Mabalacat, Pampanga)
1442	BJVr 2	Mungbean (Calaca, Batangas)
1444	BJV4 4	Mungbean (Calaca, Batangas)
1445	BJVr 7	Mungbean (Calaca, Batangas)
1440	BJL1 5	Ipil-ipil (Los Baños, Laguna)
1456	BJL1 21	Ipil-ipil (Calumpit, Bulacan)
1458	BJL1 23	Ipil-ipil
1461	BJL1 30	Ipil-ipil
1462	BJVr 1	Mungbean (Calaca, Batangas)
1464	BJVr 3	Mungbean (Calaca, Batangas)
1464	BJVr 12	Mungbean (Davao City)
1638	TSJ 357	Mungbean
1639	A 70 3 KO2	
1641	CAI Jap 110	*R. trifolii*
1642	CAI Tri 100	*R. trifolii*

Table 2. Screening for tolerance/resistance of selected *Rhizobium* strains to mercury, copper, and cadmium

Strain	Metal Concentration, ppm							
	Mercury			Copper		Cadmium		
	30	40	50	15	40	1	5	10
BL1 57	+ +	+ +	-	+	-	+ + +	+ +	+
TA1 600	-	-	-	+	-	+ +	-	-
THA 2	-	-	-	+	-	+ +	+ +	-
THA 201	-	-	-	+	-	+ +	+ +	-
NC 92	-	-	-	+	-	+ +	-	-
NGR 69	+ + +	+ +	-	+	-	+ + +	-	-
CB 756	+ +	+	-	+	-	+	+	-
M5	+	+	-	+	-	+ +	+	+
BL1 80	+ +	+ +	-	+ +	-	+ +	-	-
BE2 10	+	-	-	+ +	+	+ +	+ +	-
BEs 11	-	-	-	+ +	+	+ +	-	-
BEs 15	-	-	-	+	+	+ +	+ +	-
BEs 20	-	-	-	+ +	+	+ +	+ +	-
BEs 24	-	-	-	+ +	+	+ +	+ +	-
BEs 30	-	-	-	+ +	+	+ +	+ +	-
BJL1 4	-	-	-	-	-	+ + +	-	-
BJL1 14	-	-	-	-	-	+ +	-	-
BJL1 19	+ +	-	-	-	-	+ +	-	-
BJL1 27	+ +	+	-	+	-	+	-	-
BJVr 2	+	-	-	+ +	+	+ +	+	-
BJVr 4	-	-	-	+ +	+	+ +	+	-
BJVr 7	+ +	+ +	+ +	+	-	+ +	+ +	-
BJL1 5	+ + +	+ +	-	-	-	+ +	+ +	-
BJL1 21	+ +	+ +	+ +	+	-	+ +	+	-
BJL1 23	+ + +	+ +	+ +	+	-	+ +	-	-
BJL1 30	+ +	+ +	+ +	+	-	+ +	+ +	-
BJVr 1	+ +	+ +	-	+ +	+	+ +	+ +	-
BJVr 3	-	-	-	+ +	+	-	-	-
BJVr 12	+ +	+ +	+ +	+	-	+ +	+ +	-
TSJ 357	+ +	+ +	-	-	-	+	+	-
A702 KO2	+ +	-	-	-	-	+ +	+	-
CAI JAP 110	+ +	+ +	-	-	-	+ +	+	-

Legend:	O.D.	
	0.00 - 0.10	negligible tolerance (-)
	0.11 - 0.35	fair tolerance (+)
	0.36 - 0.70	good tolerance (+ +)
	0.71 - 1.00	high tolerance (+ + +)

Optical density was measured at 570 nm

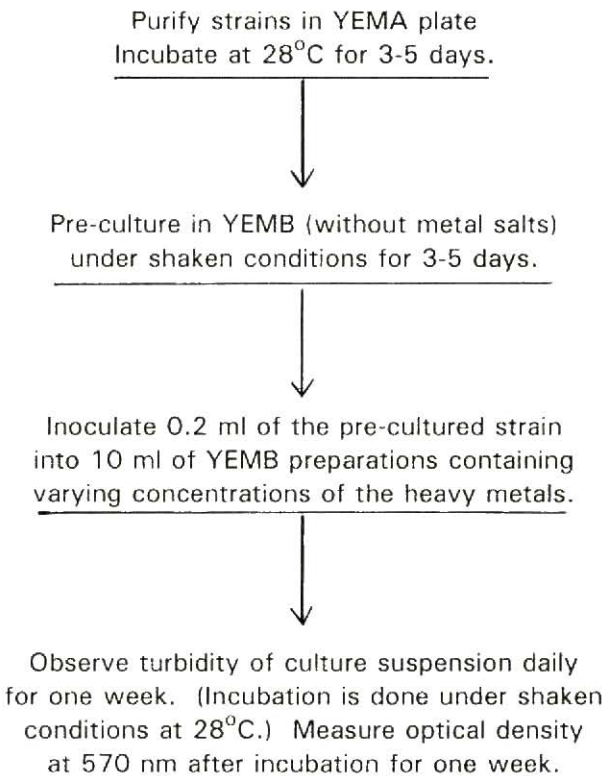

Figure 1. Schematic diagram for the determination of metal resistance by selected Rhizobium strains

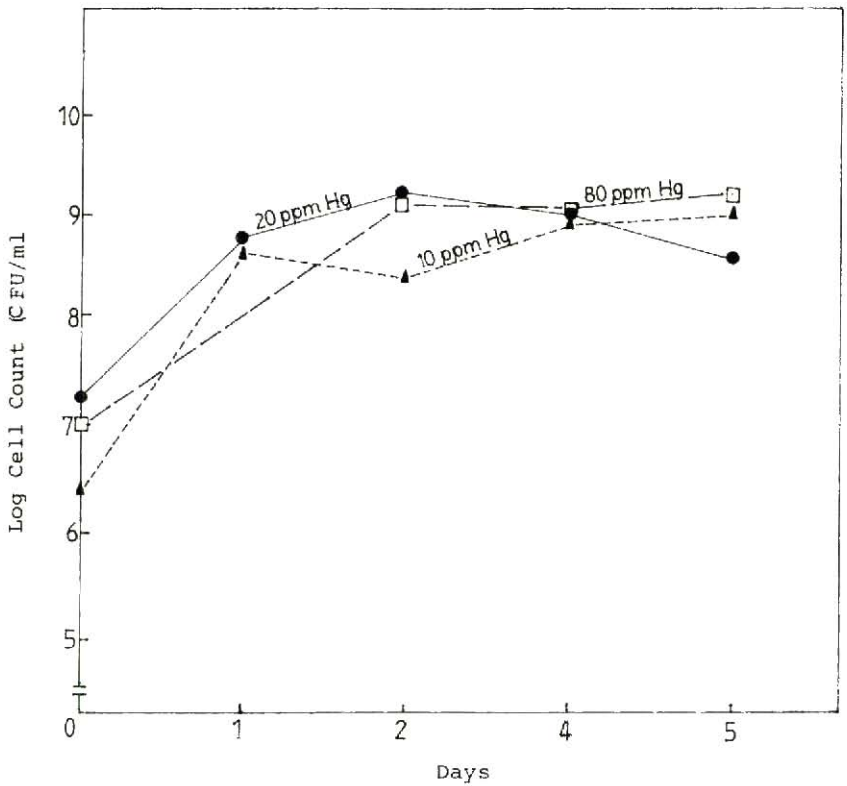

Figure 2. Growth of BL1 80 in YEM broth containing different concentrations of Hg^{2+}

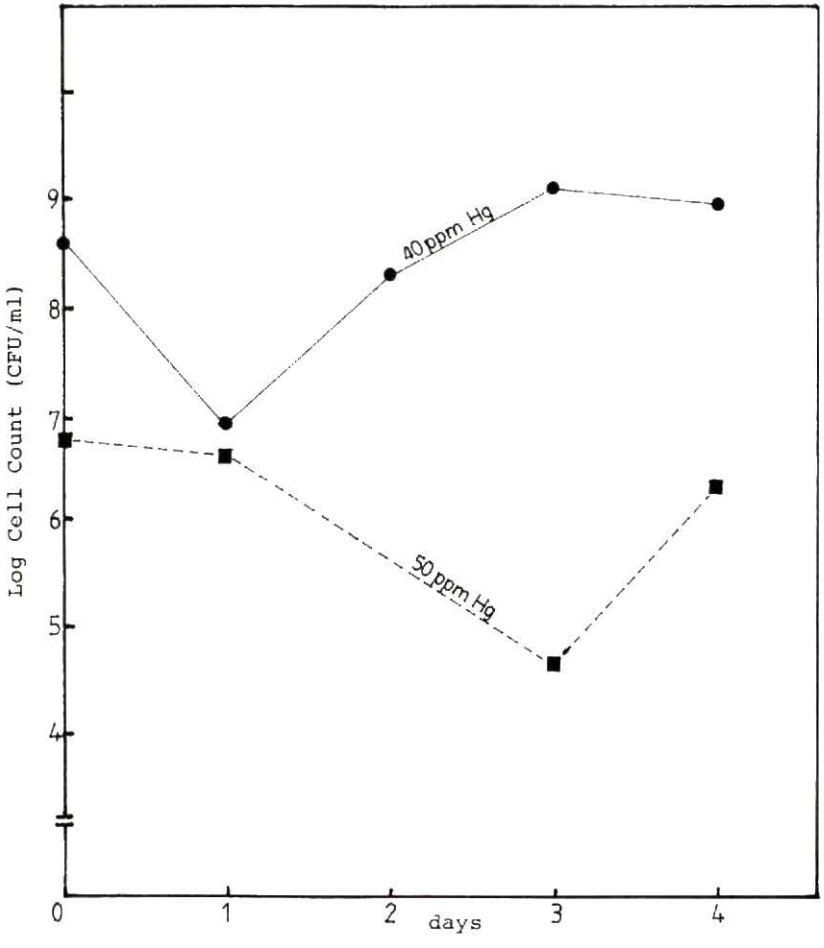

Figure 3. Growth of BL1 80 in YEM broth containing different concentrations of Hg^{2+}

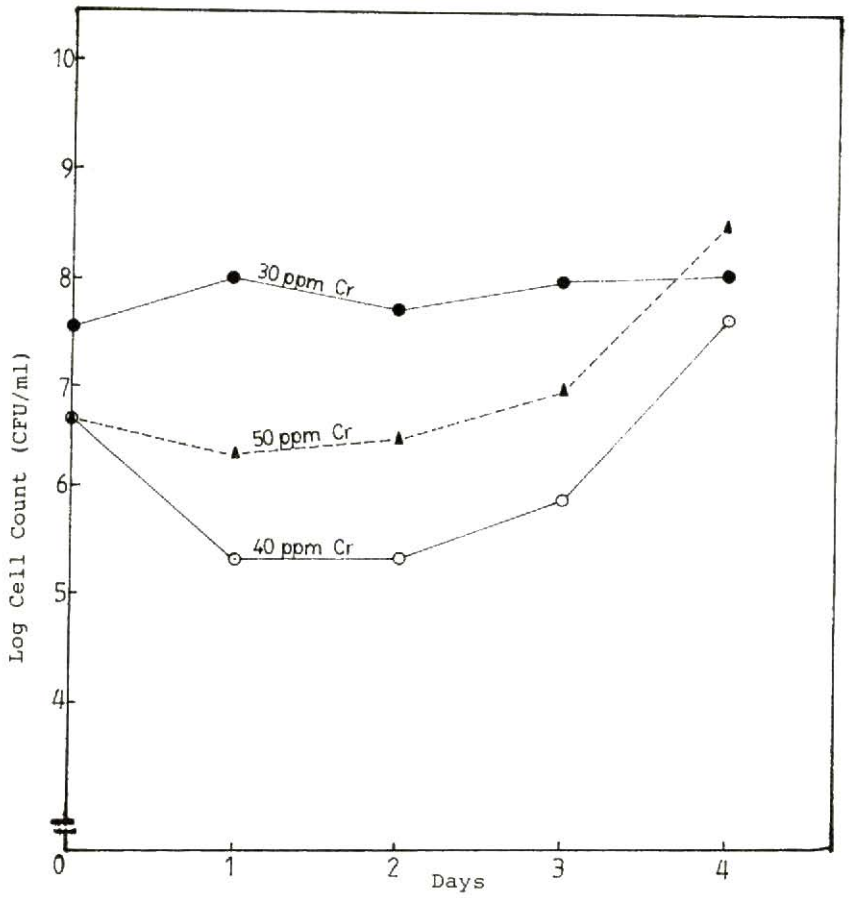

Figure 4. Growth of BL1 80 in YEM broth containing different concentrations of Cr^{2+}

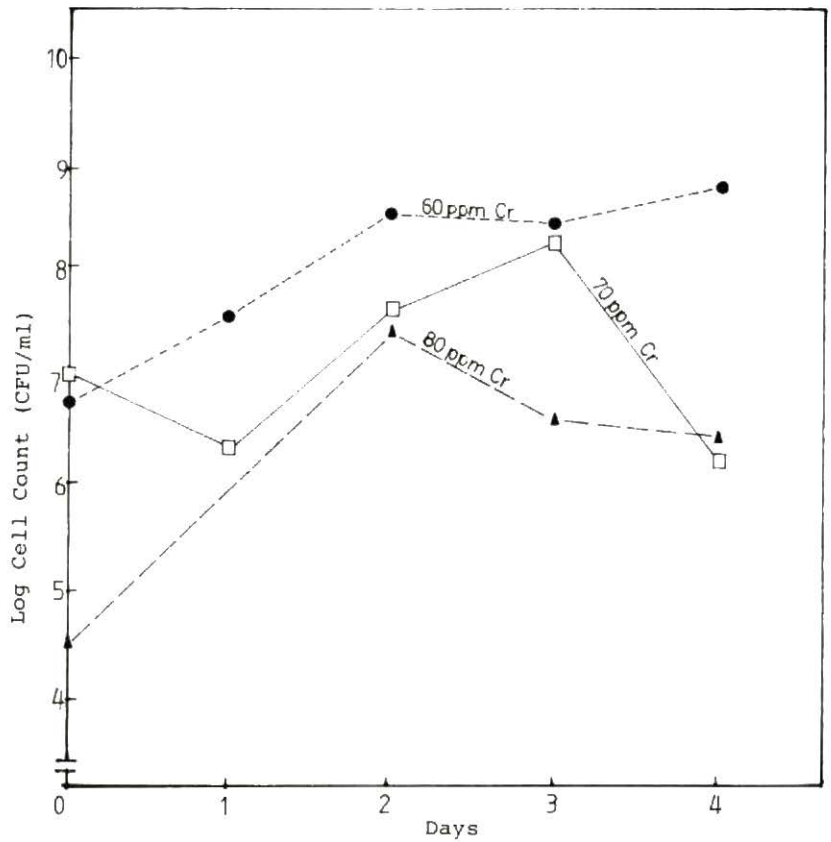

Figure 5. Growth of BL1 80 in YEM broth containing different concentrations of Cr^{2+}

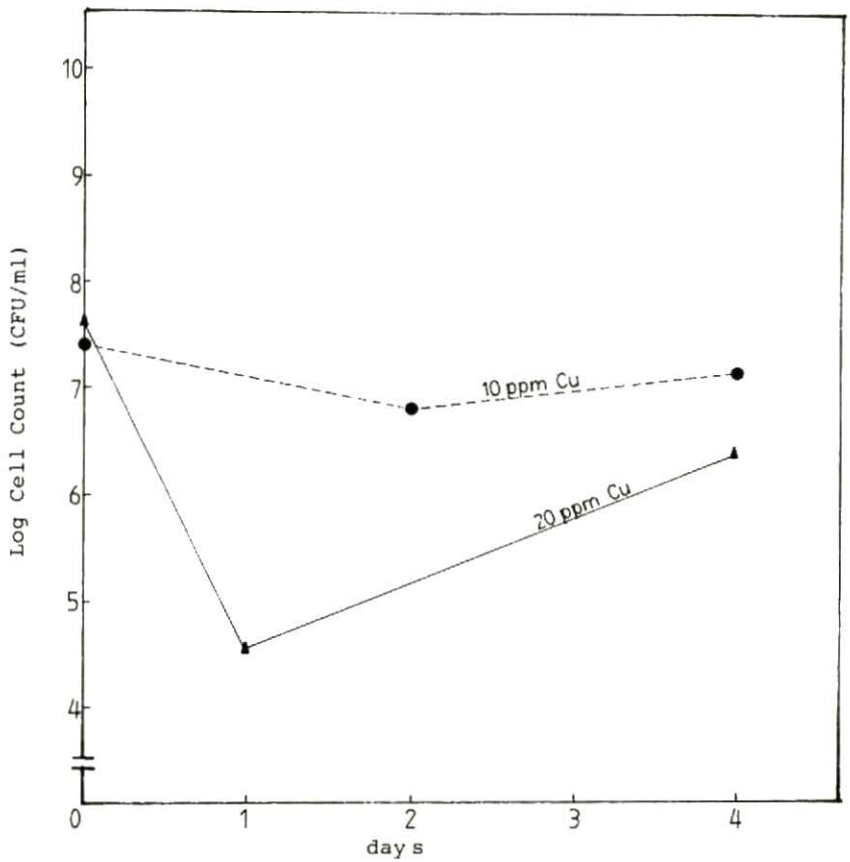

Figure 6. Growth of BL1 80 in YEM broth containing different concentrations of Cu^{2+}

LITERATURE CITED

1. **Albert, A.** 1973. Arsenicals, Antimonials and Momonmials selective toxicity. 5th ED. Chapman and Hall, London U.K.
2. **Baldry, M.G.C. and A.C.R. Dean.** 1980. Copper accumulation by bacteria, molds and yeasts. Microbios 29. 7-14.
3. **Brock, T.D.** 1978. Thermophilic microorganisms and life at high temperatures. Springer Verlag, Heidelberg, F.R.G.
4. **Davies, J.T., D.A. Haydon and E. Rideal.** 1956. Surface behaviour of bacterium *E. coli* I. The nature of the surface. Royal Soc. London. Proc. Ser. (B) 145. 375-383.
5. **Douka, C.E. and A.C. Xenoulis.** 1983. Beneficial effects of biological nitrogen fixation on the reduction of I. Metal toxicity of the biomass produced. II. Radio isotope uptake following the Chernobyl fall out in Nitrogen Fixation: Hundred Years After. H. Bothe, F.J. de Bruijn and W.E. Newton (Eds.). (Gustav Fischer, Stuttgart), 812.
6. **Galun, M., P. Keller, D. Malki, H. Feldstein, E. Galun, S.M. Seigal and B.Z. Seigal.** 1983. Removal of uranium (VI) from solution by fungal biomass and fungal wall-related polymers. Science 219, 285-286.
7. **Mamaril, J.C., E.T. Paner, V.T. Capuno and L.C. Trinidad.** 1989. Reduction of heavy metal concentration in liquids by *Rhizobium* polysaccharides. ASEAN Journal of Science and Technology. 6(2): 75-86.
8. **McCalla, T.M.** 1940. Cation adsorption by bacteria. J. Bacteriology 40. 23-32.
9. **Silver, S.** 1983. Biomineralization and Biological Metal Accumulation. P. Westbrook and E.W. de Jong. (Eds.). Reidel Publishing Co., Amsterdam, The Netherlands) 439.
10. **Tsezos, M. and D.M. Keller.** 1983. Adsorption of radium 226 by biological origin adsorbents. Biotechnol. Bioeng. 25. 201-215.
11. **Tuoviner, O.H. and D.M. Kelly.** 1974. Use of microorganisms for the recovery of metals. Int. Met. Rev. 19. 21-31.
12. **Volesky, R.** 1986. Biosorbent materials in Biotechnol Bioeng. Symposium No. 16. Enrlich, H.L. and Holmes D.S. (Eds.) (Wiley - Interscience) 121-126.
13. **Wood, J.M.** 1975. Biological cycles of elements in the environment. Naturwissenschaften 62. 357-364.
14. **Wood, J.M. and H. Wang.** 1983. Microbial resistance to heavy metals. Environ. Sci. Technol. No. 12. 17 582A-590A.