Heavy Metal uptake Potentials of *Pseudomonas aeruginosa* and *Micrococcus luteus*

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**Abstract**

Uptake of heavy metals, silver and cadmium by *Pseudomonas aeruginosa* (a Gram negative bacterium) and *Micrococcus luteus* (a Gram positive bacterium) was investigated in Cadmium and Silver stock solution using ion selective electrodes. Silver and cadmium uptake by the two organisms was described by Langmuir isotherms. Binding at one site type is indicated. It was observed that both organisms showed rapid uptake of metal mostly in the first minute. Uptake was complete by ten minutes. *Micrococcus luteus* concentrated more metal than *Pseudomonas aeruginosa*. *M. luteus* took up 200% more silver and 100% more cadmium than *P. aeruginosa*. We conclude that the two organisms, particularly *M. Luteus* have very high bioremediation potentials in heavy metals polluted environment.

**Keywords:** Heavy metals, *Pseudomonas*, Langmuir isotherms, Silver, Cadmium

**Introduction**

Man’s activities generate heavy metal wastes. For instance, the processes of industrial manufacturing (Ajmal and Khan, 1985; Gazsó, 2001) and metal mining (Denny and Welsh, 1979; Johnson and Eaton, 1980; Beyer et al., 1985) release large quantities of metals into the environment. Discharge of cadmium into natural waters may be through electroplating activities (Higgins and Desher, 1986), nickel-cadmium battery manufacture and/or smelter operations (Butterworth, et al., 1972). Silver is a waste generated by photographic and radiographic applications.

Industrial wastes that contain these metals find their way into sewage treatment works (Cheng et al., 1975; Tyagi et al., 1980; Beyer et al., 1985) release large quantities of metals into the environment. Discharge of cadmium into natural waters may be through electroplating activities (Higgins and Desher, 1986), nickel-cadmium battery manufacture and/or smelter operations (Butterworth, et al., 1972). Silver is a waste generated by photographic and radiographic applications.

There are three areas of interest in heavy metal uptake by microorganisms (a) these heavy metals find their way into the environment resulting in bioconcentration and biomagnification (Boda, 1972; Martin and Coughrey, 1975; Gipps and Biro, 1978; Eja et al., 2003). (b) Another area is in the economics of alternative methods of recovery of metals in low concentrations. Microbial extraction causes less pollution and energy use, and may be amenable to unique biological improvement techniques such as genetic engineering. (c) As more stringent effluent limitation standards have resulted in a need to remove or reduce heavy metal contaminants before they find their way into the environment, more sophisticated and costly treatment methods will be required, making biological treatment most attractive.

Therefore the aim of this study is to examine the metal uptake potentials of *P. aeruginosa* and *M. Luteus*, for their possible application in the bioremediation of heavy metal polluted environment.

**Materials and Methods**

**Cultures:** *Pseudomonas aeruginosa* (NCIB 950) and *Micrococcus luteus* (NCIB 8553) were obtained from The National Collections of Industrial and Marine Bacteria ltd. 23 Machar Drive, Aberdeen, Scotland.

**Metal solutions:** All solutions were made using deionised water. Sterilization was achieved by autoclaving at 121°C for 15 minutes.

**Cadmium and silver stock solution:** Cadmium stock solutions were made using cadmium nitrate (ANALAR BDH Chemicals Ltd Poole England) while Silver stock solutions were prepared using silver nitrate (ANALAR, Hopkin and Williams, Chadwell Heath Essex). To avoid photoreduction, solutions were stored in brown bottles.

**Ion selective electrodes**

Silver and cadmium: Silver was measured continuously using a silver/sulfide Electrode Model 94-16 ORION (Orion Research Incorporated Products Group), while cadmium was measured continuously using a cadmium Electrode Model 94-4489 Russell. Electrodes were calibrated before use.

**Silver and cadmium uptake over time:** Investigations were carried out using 20 ml of metal solution (20 mg/l) in 50 ml beakers that had previously been rinsed in distilled water. A suspension of the test organism (2.6mg dry weight in 2mls sterile distilled water) was added to the metal solution and decrease in free ion concentration was measured using an ion selective electrode. To ensure that any decrease in metal concentration was due to the test organism, controls were used which consisted of metal solution minus the test organism. The controls were continuously monitored using the same ion selective electrode. To allow for the slight increase in volume caused by introducing the test organism,
the controls had an identical amount of distilled water added to them. Adjustments were made where necessary based on the controls. The investigations were repeated three times but results were not pooled. The binding/uptake of silver and cadmium by the two organisms was described by Langmuir plots. The Langmuir isotherm was expressed as $\frac{[MF]}{[MB]} = \frac{1}{KB} + \frac{[MF]}{B}$ (Hughes & Poole, 1989), where a plot of $\frac{[MF]}{[MB]}$ against $[MF]$ should be linear. $[MF]$ = Free metal ion concentration, $[MB]$= Bound metal, $K$= Binding affinity, $B$ = binding capacity.

Results

Of the total silver taken up over a ten minute period Micrococcus accumulated 97% of its total uptake in the first minute, while Pseudomonas took up 87%. Micrococcus took up 200% more silver than Pseudomonas over the ten minute period (Fig. 1).

(Fig. 2). Cadmium and silver uptake by the two organisms can be described by Langmuir isotherms (Figs 3 - 6) which were straight line plots.

$$y = 0.007x - 0.600$$
$$R = 0.999$$

Discussion

In this study uptake of silver and cadmium by bacterial species P. aeruginosa and M. luteus, was compared. Metal removal by both the organisms studied here, showed the same general profile. That is, a rapid initial uptake, usually in the first one or two minutes, where the largest proportion of the removal takes place followed by a decrease in uptake, and ceasing or becoming negligible by about 10 minutes. Similar observations with microbial heavy metal uptake have been reported elsewhere (Khummongol et al., 1982; Costa and Leite, 1991; Arikpo and Eja, 2003; Gökşungur et al., 2003).

Of the two organisms studied Micrococcus took up a greater quantity of both silver and cadmium. The Gram positive Micrococcus took up far more metal than the Gram negative Pseudomonas.
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It has been mentioned that Gram-negative bacteria bind less metal than Gram-positive bacteria, sometimes up to ten times less (Hughes & Poole 1989). It may be suggested that Gram-positive bacteria have more porous walls, allowing metal to build up due to nucleation and precipitation of insoluble salts (Beveridge, 1988; 1989).

Cadmium and silver uptake by the two organisms is described by Langmuir isotherms which indicate binding at one site. On the other hand a biphasic curve may indicate the availability of more than one site type, while a sigmoidal curve, indicates that initial binding of the metal has brought about structural changes and an increase in the availability of binding sites (Hughes and Poole, 1989).

It was observed that the rates of uptake of silver and cadmium by the two organisms were measurable, with *M. luteus* taking up 200% more silver and 100% more cadmium that *P. aeruginosa*. We conclude that the two organisms, and particularly *M. luteus*, have very high metal uptake and bioaccumulation factor which underlie their potential application in bioremediation of heavy metal-polluted environment.

### References

Ajmal, M. and Khan, A.V. (1985). Effects of electroplating factory effluent on the germination and growth of Hyacinth bean and Mustard. *Environ. Res.* 38, 248-258.

Arikpo, G.E. and Eja, M.E., (2003) Silver uptake by the green alga *Chlorella emersonii*. *Afr. J. Environ. Pollut. Health* 2(1&2): 19-28.

Beveridge, T. J. (1988). The bacterial surface: general considerations towards design and function. *Can. J. Microbiol.* 34: 363-372.

Beveridge, T. J. (1989). Interactions of metal ions with components of bacterial cell walls and their biomineralization. In: Metal-Microbe interactions - Special Publications of the Society for General Microbiology. Vol. 26 IRL press.

Beyer, W.N., Pattee, O.H., Sileo, L., Hoffman, D.J. and Mulhern, M. (1985). Metal Contamination in Wildlife Living near two Zinc smelters. *Environmental Pollution* (Series A) 38, 63-86.

Broda E. (1972). The uptake of heavy cationic trace elements by micro-organisms. *Annu. Microbiol.* 22: 93-108.

Butterworth J, Lester P and Nickless G (1972). Distribution of Heavy Metals in the Severn Estuary. Marine Pollution Bulletin. 3: 72.

Cheng MH., Patterson JW and Minear RA (1975). Heavy metal uptake by activated sludge. *J. Wat. Poll. Cont. Fed.* 47(2): 362-367.

Costa, A.C.A. and Leite, S.G.F. (1991). Cadmium and Zinc Biosorption by *Chlorella homosphaera*. Biotechnology letters. 12 (12): 941-944.

Denny, P. and Welsh, P. (1979). Lead accumulation in Plankton Blooms From Ullswater, The English Lake District. *Environ. Pollut.* 18: 1-9.

Dressler RL, Storm GL, Tzikowski WM and Sopper WE (1986). Heavy metals in Cottontail Rabbits on mined lands treated with sewage sludge. *J. Environ. Qual.* 15(3): 278.

Eja, M.E., Ogri, O.R. and Arikpo, G.E., (2003). Bioconcentration of heavy metals in surface sediments from the Great Kwa River Estuary Calabar, South Eastern Nigeria. *J. Nig. Environ. Soc.* 2(2): 247-256.

Gazsó LG (2001). The key microbial processes in the removal of toxic metals and radionuclides from the environment *CEJOEM*. 7 (3-4): 178-185.

Gipps JF and Biro P (1978). The use of *Chlorella vulgaris* in a simple demonstration of heavy metal toxicity. *J. Biol. Ed.* 12: 207-214.

Göksu, Y., Oren, S. and Govenc, U. (2003). Biosorption of copper ions by caustic treated waste bakers yeast biomass. *Turk. J. Biol.* 27: 23-29.

Higgins, T.E. and Desher, D.P. (1986). Electroplating metal finishing and cyanide wastes. *J. Water Pollut. Cont.* 58: 586-589.

Hughes, M.N. and Poole, R.K. (1989). Metals and Micro-organisms. Chapman and Hall ltd. London. 400 pp.

Johnson, M.S. and Eaton, J.W. (1980). Environmental Contamination Through Residual Trace Metal Dispersal from a Derelict Lead-Zinc Mine. *J. Environ. Qual.* 9 (2) 175-179.
Khummnongkol, D., Canterford, G.A., and Fryer, C. (1982). Accumulation of Heavy Metals in Unicellular Algae. *Biotechnology and Bioengineering*, 24: 2643-2660.

Martin, M.H. and Coughtry, P.J. (1975) Preliminary observations on the levels of cadmium in a contaminated environment. *Chemosphere* 4: 155-60.

Schauer, P.S., Wright, W.R. and Pelchat, J. (1980). Sludge-borne Heavy Metal Availability and Uptake by Vegetable Crops under Field Conditions. *J. Environ. Qual.* 9: (1) 69-72.

Tyagi, R.D., Couillard, D. and Tran, F. (1988). Heavy Metals Removal from Anaerobically Digested Sludge by Chemical and Microbiological Methods. *Environ. Poll.* 50: 295-316.