Metal biosorption by *Helminthosporium solani* – a simple microbiological technique to remove metal from e-waste

Electronic waste (e-waste) or Waste from Electronic and Electrical Equipment (WEEE) as it is popularly called includes used, obsolete or damaged electronic and electrical equipments and other such scrap. These electronic goods range from personal computers, printed circuit boards, compact discs, mother boards, cables and toner cartridges to various house-hold appliances such as televisions, refrigerators, cell phones, light bulbs and tube lights, containing large amounts of metals like mercury and lead. The average computer monitor or television set holds non-recyclable compounds such as gold, platinum, cadmium, mercury, lead and brominated flame retardants. Globally around 20–50 million tonnes of e-waste are generated every year. Around 80% of it is generated in the US alone and is exported to recycling units in countries like India and China. E-wastes are fast accumulating, making up to 5% of all municipal solid wastes world-wide.

Recycling of WEEE has emerged as a lucrative business as these products are stripped down to obtain valuable metals like platinum, gold and copper. The rest of the waste is discarded in an unsafe manner which could cause ecotoxicity. E-waste is not completely recyclable, resulting in its accumulation in the environment. Conventional technologies being practised for the removal of heavy metals include precipitation, ion exchange and reverse osmosis.

The major anthropogenic sources of environmental manganese include municipal wastewater discharges, sewage sludge, mining and mineral (particularly nickel) processing emissions from alloy, steel and iron production, combustion of fossil fuels and, to a much lesser extent, emissions from the combustion of fuel additives. Our present study attempts to explain the biotreatment of soil, sediments and water bodies polluted by the manganese present in e-waste (as documented by E-Parisaraa Pvt Ltd, an e-waste recycling unit in Bangalore).

Manganese was chosen for the study of bioremediation as it shows 0% recycling efficiency. The metal has been characterized as a strategic metal for the purpose of bioremediation, meaning to say that manganese is not ecotoxic but is particularly significant from the recovery point of view. This study attempts to provide a method for: (i) isolation of suitable candidates for microbial bioremediation and (ii) study of metal uptake efficiency by *Helminthosporium solani* through the process of non-metabolism dependent biosorption under different environmental conditions of metal concentration, pH and dry biomass concentration.

Manganese sulphate heptahydrate ($MnSO_4 \cdot 7H_2O$), a highly water soluble form of manganese with a solubility constant for a wide pH range (2.0–9.0) was used for the experiment. Manganese is a trace element comprising 0.0315% of total weight of the e-waste, with 0% recycling efficiency (data provided by E-Parisaraa). For isolation of fungi, metal concentration from 5 to 10 mM of $MnSO_4 \cdot 7H_2O$ was used. Industrial effluent samples were collected from motor industry (Mico Bosch, Bangalore) and electroplating industry (Evershine Industries, Kanakapura Road, Bangalore); soil samples from storm water drain (Bull temple road, Bangalore) and sewage sample from Yediyur, Bangalore. The samples were serially diluted 10–10,000 fold in sterile distilled water and the suitable dilution (10^{-4}) was plated on Trypticase Soy Agar (Hi media) plates containing Mn in the concentrations 5, 8 and 10 mM. Stock solution (10 ml) of the metal was prepared for each concentration. 1 ml of the stock solution from each concentration was mixed with 100 ml of the medium separately. Trypticase Soy Agar (TSA) was prepared in two sets separately for pH 5.0 and pH 7.0 to isolate fungi. One ml each of the metal stock from the respective concentration was aseptically transferred to 100 ml of sterilized media prepared separately in different conical flasks. The fungal plates were incubated at 28°C for 7 days. Fungi were identified by tease mount method[1].

The first round of screening of the isolates for metal resistance was carried out by growing the fungi on Saboraud's Dextrose Agar (SDA) supplemented with different concentrations of $MnSO_4 \cdot 7H_2O$ (7–14 mM). Metal stock solutions of various concentrations were prepared in 10 ml, and 1 ml of the same was transferred into 100 ml of the media. The plates were incubated at room temperature (28°C) and the growth was observed.

During the second round of screening, the metal resistance was further confirmed by determining the MIC (minimum inhibitory concentration) of the metal using well diffusion method[2]. Fungal isolates grown for 72 h were maintained on SDA. Wells were punched using a cork borer at the centre of each petri plate under sterile condition. Metal solution of $MnSO_4 \cdot 7H_2O$ (60 μl) from the stock with concentrations ranging from 20 to 300 mM each was added directly into wells in separate plates. The plates were incubated and the metal resistance was further confirmed by measuring the diameter of the inhibition zone.

The fungal species showing resistance to manganese was inoculated into potato dextrose broth (PDB) and incubated at 28°C under static condition for 7 days. Biomass obtained was separated from the broth by filtration and dried at 60°C in a hot air oven for 3 days (72 h). The dried biomass was ground into a fine powder using pestle and mortar[3]. Aliquots (100 ml) of different concentrations of $MnSO_4 \cdot 7H_2O$ ranging from 250 to 300 mM were prepared in Milli-Q water. The biomass weight was fixed at 2 and 4 g/l in each case. The flasks were incubated on a shaker at 25°C for 18 h at 200 rpm and the system was allowed to come to equilibrium. The biomass was removed by filtration through a 4.5 μm Millipore membrane filter. The filtrates were analysed for metal concentration by titrimetry method. Flasks with different concentrations of $MnSO_4 \cdot 7H_2O$, without the biomass were used as controls[3]. Biomass concentrations of 2 and 4 g/l each were added to 250 ml flasks separately. Metal concentrations ranging from 250 to 300 mM were prepared in Milli-Q water and added to each flask. The flasks were incubated in a shaker at 180 rpm at 25°C for 18 h, and the metal concentration was analysed using titrimetry method[4].

Adsorption of metal ions by dried mycelial biomass was studied at pH values of 5.0, 6.0 and 7.0. A fixed biomass (4 or 2 g/l) was added to 100 ml of the metal

Table 1. Effect of pH, metal concentration and biomass concentration on metal (Mn)* adsorption in *Helminthosporium solani*

pH	Initial conc. of Mn (mM)	Biomass weight (g)	Final conc. (residual) of Mn (g)	Percentage uptake by adsorption
5.0	250	0.2	3.70	70.0
5.0	300	0.2	2.516	79.8
5.0	250	0.4	2.14	80.09
5.0	300	0.4	2.516	79.8
6.0	250	0.2	2.22	79.3
6.0	300	0.2	2.26	81.92
6.0	250	0.4	2.008	81.3
6.0	300	0.4	2.176	83.8
7.0	250	0.2	0.756	92.96
7.0	300	0.2	1.93	84.56
7.0	250	0.4	1.55	85.58
7.0	300	0.4	0.336	97.31

*Mn concentration analysed by Volhard's method.

solution with different concentrations dissolved in Milli-Q water[5]. The samples containing $MnSO_4 \cdot 7H_2O$ in different concentrations were titrated against standard $KMnO_4$ (0.127 M) solution. Using Volhard's method[4] the amount of manganese (in g) present in each of the diluted test and control samples was calculated and a dilution factor corresponding to 100,000 was calibrated with each of the values. Using these values, the manganese content in each of the original samples (100 ml) was calculated and recorded (Table 1). Percentage uptake of the metal (adsorption) by the biomass was calculated.

During the first round of screening, only four fungal isolates, i.e. *Aspergillus niger*, *Fusarium oxysporum*, *Cladosporium cladosporioides* and *H. solani*, out of the 32 fungi isolated, showed growth up to 13 mM of manganese and were selected for further study. The second round of screening by well-diffusion method at metal concentrations ranging from 20 to 50 mM resulted in the selection of fungi which showed no inhibition zone. The final round of screening by well diffusion method, to determine the MIC of $MnSO_4 \cdot 7H_2O$, was carried out with *H. solani* which showed no inhibition zone in the second round of screening. Two metal concentrations (250 and 300 mM) were selected. The respective zones of inhibition at these concentrations were studied indicating resistance to the metal. The MIC for *H. solani* was found to be 300 mM and hence was more resistant to the metal manganese.

The percentage uptake of manganese by *H. solani* was determined as a function of pH (5.0–7.0), metal concentration (250–300 mM) and biomass concentration (2 and 4 g/l). The maximum manganese adsorption was found to be 97% (3040 mg/g of biomass) at a pH of 7.0 (Table 1). The uptake of manganese from solution seemed to increase with the initial metal concentration and biomass concentration as in biosorption of manganese by *A. niger* and *Saccharomyces cerevisiae*[6]. The percentage uptake of manganese increased when the biomass concentration was increased from 2 to 4 g/l. The biomass seemed to adsorb manganese better at high initial metal concentration (300 mM) and at neutral pH (Table 1). It had been well documented that optimum pH values for biosorption are usually in the 4–7 range. Below this, increasing competition by H^+ for binding sites diminishes cation uptake levels[7,8]. The adsorption of manganese decreases at acidic pH due to competition with hydrogen ions for binding sites such as carboxyl, sulphydryl, amino and amido-functional groups present at the cell surface, which act as ion exchangers. The bioavailability of trace metal as a nutrient or as a toxicant is determined by its chemical speciation[9].

The present work is an effort to demonstrate the efficiency of certain fungi in removing metals from solution, commonly by adsorption. The fungus *H. solani* chosen for the present study showed a high adsorption (97.3%) capacity for the metal manganese, found as manganous ion (Mn^{2+}) in solution at a very high initial concentration (300 mM). This finding is significant because: (i) manganese, with a sparing solubility in water and poor bioavailability accounts for ecotoxic influences especially on humans (such as manganism, anemia); (ii) manganese is grouped as a strategic metal as it finds utility after recovery from the biomass; and (iii) manganese is a trace metal and forms a non-recyclable (0% recycling efficiency) component of e-waste.

The work communicated here is an effective ecofriendly approach for removal of manganese from e-waste by the fungus *H. solani*. Future research work would focus on the mechanism of uptake and also uptake of other metals present in e-waste by *H. solani*.

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