Biosorptive removal of contaminating heavy metals from plant extracts of medicinal value

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Granulated *Cladosporium cladosporioides* # 2 biosorbent removed lead and cadmium from aqueous extracts of *Nordostachys jatamansi* and *Vitis vinifera* with high efficiency. Different properties of the extracts such as pH, UV–visible spectra and total dissolved solids were unaltered after biosorption, indicating that none of the components of the extracts were removed and the biosorbent itself did not transfer its components to the extracts. These findings open up new avenues for the application of metal biosorption technology.

THE removal of metals from aqueous solutions using biosorption process has been an area of extensive research. Numerous authors have used metal biosorption for effluent treatment and metal recovery[1–5]. Applicability of this process in other important areas such as removal of metals from soils[6], waste photovoltaic cells[7], dyes[8] and treatment of metal-cyanides[9] has also been investigated.

Plant extracts of medicinal value have gained considerable importance in alternative medicine in recent times. A large number of Indian (Ayurvedic), Chinese, Tibetan and local traditional medicines containing plant extracts have an increasing demand all over the world. Although slow in action compared to modern chemotherapeutic agents, these medicines are popular on account of long-term effectiveness against many chronic disorders. They are also considered safe, relatively free from side effects and problems of overdose. However, there is an inherent health risk associated with many of these medicines, viz. presence of contaminating heavy metals. Presence of toxic metals in plant extracts can be attributed to (i) accumulation in plants through metal-contaminated soils[10], (ii) contamination during processing in industries and (iii) leaching of metals from metallic containers during storage.

In a recent sample survey carried out in India, over three thousand samples of a variety of plant extracts were analysed for their toxic metal content. It was observed that ~ 20% of the samples tested contained heavy metals such as lead, cadmium and chromium in concentrations far exceeding 1 mg kg^{-1} (unpublished data of Hi-Tech Bio Laboratories), the acceptable permissible limit for heavy metals in food and drugs[11].

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India is one of the major countries exporting plant extracts of medicinal value, with an estimated annual turnover exceeding US $ 2 million. These figures are likely to multiply several folds owing to increased demand and if the problem of contaminating heavy metals is not resolved, the exports will be adversely affected.

The present study explores the possibility of using metal biosorption for the removal of contaminating heavy metals from aqueous extracts of two most commonly used plants, viz. *Vitis vinifera* (raisin) and *Nordostachys jatamansi* (Jatamansi). The raisin extract is used in treatment of anaemia, bilious dyspepsia, haemorrhage, dysuria, heart disease, bronchitis, constipation, etc. Jatamansi extract is used as an antispasmodic, diuretic, nerve sedative as well as a cure for jaundice and kidney stone[12].

A non-pathogenic fungal culture, *Cladosporium cladosporioides* # 2 isolated from soil and possessing high cadmium, chromium and lead biosorption efficiency was used. Log phase culture (250 ml) was inoculated in 4 l Sabouraud's medium in a 5 l capacity fermentor (B. Braun, Germany). The growth parameters were: temperature, 30°C; pH, 5.7 ± 0.1; agitation, 100 rpm. Sterile air was sparged into the fermentor at a rate of 0.15 l min^{-1}. After 5 days incubation, biomass was harvested by vacuum filtration and washed. It was then granulated by admixing with a cheaply available polymer of natural origin using a proprietary process[13]. The resulting biosorbent beads had an average diameter of 2.3 mm and consisted of over 99% fungal biomass. The porous and mechanically strong beads were used in lead and cadmium biosorption experiments.

Aqueous extracts of *N. jatamansi* and *V. vinifera* were procured from Hi-Tech Bio Laboratories Pvt Ltd, Pune. *V. vinifera* extract was available in the form of concentrated viscous slurry. The slurry was diluted by adding 30 g of the concentrate to 100 ml of distilled water.

Samples were analysed for pH, total dissolved solids and ultraviolet–visible spectra. The pH measurements were made using a microprocessor-controlled pH meter (Labindia, India). Total dissolved solids (TDS) were estimated by using a refractometer (B. Braun, Germany). Ultraviolet–visible spectra (200 to 700 nm wavelength range) were recorded by using a double beam UV–Vis spectrophotometer (Chemito UV-2600, India). Heavy metal content was estimated by atomic absorption spectrophotometry (ATI-UNICAM, UK, model 929).

The samples contained lead and cadmium in variable amounts ranging from 1 mg l^{-1} to about 5 mg l^{-1}. In order to attain uniformity in experimental conditions and reduce errors, the samples that showed metal concentrations close to 5 mg l^{-1} were selected for experimental work. Table 1 gives characteristics of plant extracts used in the present study.

In order to optimize the period of contact, dried biosorbent beads (0.05 g) containing *C. cladosporioides* # 2 were added to 25 ml of plant extracts containing lead and cadmium (5 mg l^{-1}) and shaken at 100 rpm. At various

time intervals ranging from 10 to 80 min the reactions were stopped by separating metal solutions from the beads. Solutions were then analysed for residual metal content. The results were expressed in terms of per cent metal uptake efficiency using the following formula:

$$\text{Metal uptake efficiency (\%)} = C_i/C_{eq} \times 100,$$

where C_i is the initial metal concentration in solution and C_{eq} is the equilibrium metal concentration after contact with biosorbent beads.

To each sample (25 ml in duplicates) containing the metals, biosorbent beads (0.05 g) containing *C. cladosporioides* # 2 were added. The mixtures were agitated at 120 rpm for 70 min at 30°C. After the contact period was over, the sample solutions were separated from the beads and analysed for residual lead or cadmium, pH, TDS, ultraviolet–visible spectra and TLC data. Samples without addition of biosorbent beads served as controls.

Standardized thin layer chromatography techniques were used to determine whether chemical composition of the samples was altered after biosorption. Samples of *N. jatamansi* and *V. vinifera* extracts before and after contact with biosorbent beads were extracted in ethyl acetate and filtered through Whatman no. 1 filter paper. TLC plates were loaded with these extracts and placed in chromatography chambers. The solvent system used was 4% and 3% ethyl acetate for *N. jatamansi* and *V. vinifera* extracts, respectively. The chromatograms were developed by iodine vapour.

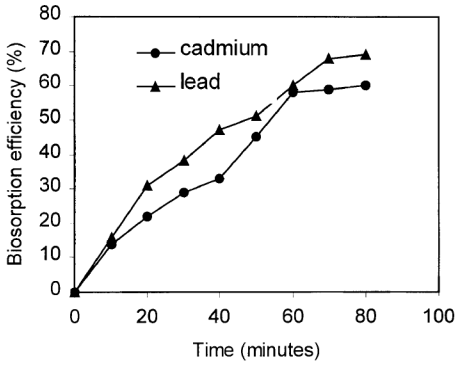

Figure 1. Rate of uptake of lead and cadmium by *C. cladosporioides* # 2.

N. jatamansi and *V. vinifera* extracts (50 ml) containing added lead and cadmium (5 mg l^{-1} final concentration of each metal) were passed in upward flow mode through biosorbent columns (height 5 cm, internal diameter 0.8 cm), each containing 2 g beads of *C. cladosporioides* # 2. The bed volume of the reactors was 3 ml. The retention time was adjusted to 20 min using a programmable peristaltic pump, after the beads were completely soaked. The effluents coming out of the columns were analysed at regular intervals for residual metal content.

It was observed that metal removal was dependent upon the period of contact between biosorbent and metal ions in solution (Figure 1). Maximum uptake of metals occurred when the beads were in contact with metal solutions for 70–80 min.

Table 2 shows that residual metal content of *N. jatamansi* and *V. vinifera* extracts were significantly reduced (about 60% reduction) after they were treated with the biosorbent.

An analysis of pH and TDS of samples after contacting with biosorbent beads showed that there was no alteration in any of these properties. When the samples of *N. jatamansi* and *V. vinifera* extracts (before and after contact with biosorbent) were scanned in the ultraviolet–visible range (200–700 nm), the spectral patterns were identical (Figures 2 and 3).

Thin layer chromatograms of plant extracts before and after biosorption showed that contacting with biosorbent beads was identical (Table 2).

It was observed that 18 bed volumes each of lead and cadmium containing *N. jatamansi* extract could be passed through the biosorbent columns. The lead content in the extract was brought down to 0.06 mg l^{-1}, while the cadmium content was lowered to 0.07 mg l^{-1} (Figure 4 *a*). In the case of *V. vinifera* extract, 15 bed volumes of the extract could be passed and the residual metal contents were 0.09 mg l^{-1} and 0.11 mg l^{-1} for lead and cadmium, respectively (Figure 4 *b*).

The main objective of the present work was to find out whether metal biosorption could be used in tackling a major problem related to the pharmaceutical industry. India is one of the major countries exporting plant extracts of medicinal value, with an estimated annual turnover exceeding US $ 2 million. However, these medicinal preparations may contain contaminating heavy metals at concentrations

Table 1. Analysis of herbal extracts before and after contact with biosorbent

	Lead						Cadmium					
	Metal content (mg/l)		pH		TDS (%)		Metal content (mg/l)		pH		TDS (%)	
Sample of herbal extract	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)
Nordostachys jatamansi extract	5.0	2.7	6.7	6.7	n.d.	n.d.	5.1	2.8	6.7	6.7	n.d.	n.d.
Vitis vinifera extract	5.1	2.9	4.8	4.8	15	15	5.0	3.1	4.8	4.9	15	15

(a), Before contact with biosorbent; (b), After contact with biosorbent; n.d., Not detectable.

Table 2. Thin layer chromatography of Jatamansi extract and raisin concentrate showing experimental conditions and R_f values of sample constituents before and after biosorption of lead and cadmium

Plant extract	Solvent system	Identification reagent	Number of spots	R_f values before sorption	R_f values after sorption
Nordostachys jatamansi	4% ethyl acetate	Iodine vapour	3	0.02 0.41 0.46	0.02 0.41 0.46
Vitis vinifera	3% ethyl acetate	Iodine vapour	4	0.24 0.44 0.87 0.96	0.24 0.44 0.87 0.96

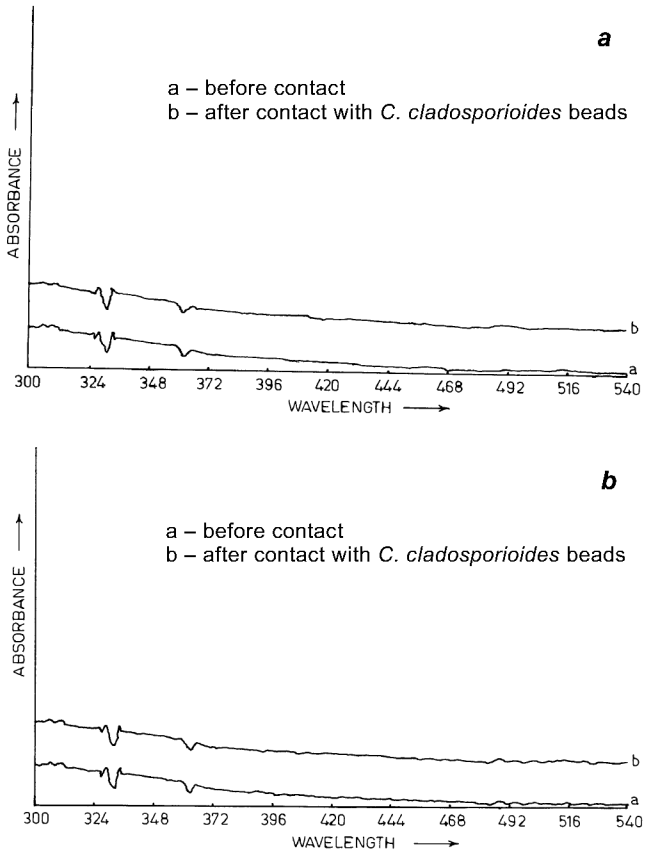

Figure 2. UV spectra of *N. jatamansi* extract containing (***a***) lead and (***b***) cadmium.

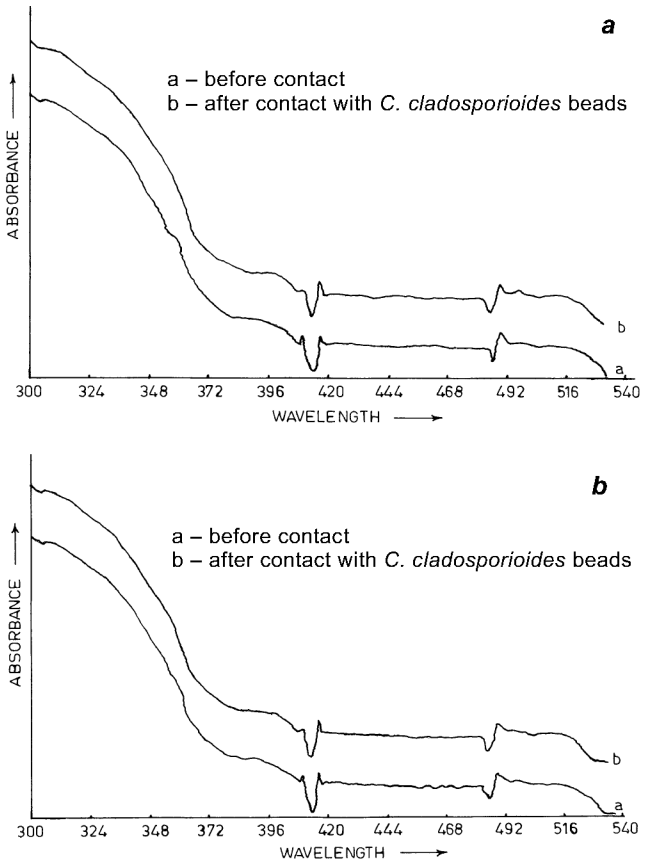

Figure 3. UV spectra of raisin extract containing (***a***) lead and (***b***) cadmium.

that are unacceptable to statutory bodies abroad. Therefore, there is as an urgent need to develop suitable methods for removal of metals from plant extracts. In the present study, metal biosorption was successfully used for removal of contaminating heavy metals from plant extracts. This is a novel use of metal biosorption process, which may open up new avenues for technology in the food and pharmaceutical industries. Granulated biomass of *C. cladosporioides* # 2 that was selected as a highly efficient metal biosorbent in an earlier screening programme was used in these studies.

The optimum period of contact between biosorbent and metal ions in solution was determined. It showed that the biosorptive process was fast. Maximum amounts of both lead and cadmium were removed in 70–80 min. Hence in all further experiments, the biosorbent beads were allowed to be in contact with metal solutions for 70 min. Both lead and cadmium were effectively removed from jatamansi and raisin extracts. However, it was important to study the effect of contacting the biosorbent with the medicinal extracts. An ideal metal biosorbent should not remove the essential components of the extracts that confer medicinal properties. In addition, the biosorbent should not transfer its own components into the extract. Therefore, physical and chemical properties of the extracts were studied before and after contact with biosorbent material. Since

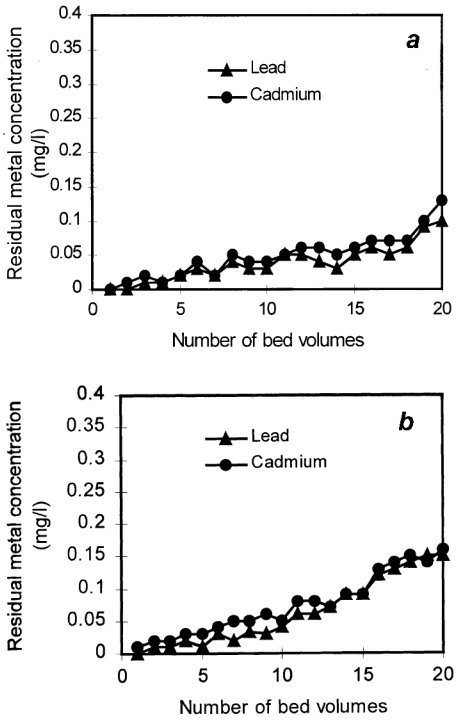

Figure 4. Removal of lead and cadmium from (***a***) *N. jatamansi* extract and (***b***) *V. vinifera* extract using a packed bed reactor containing beads of *C. cladosporioides* # 2.

there were no alterations in pH, UV–visible spectra and TDS and also no changes in number, size and position of spots appearing in thin layer chromatograms, it could be said that medicinal properties of the extracts remained unchanged after biosorption.

The results of column experiments clearly indicate that the toxic metals from herbal extracts could be brought down to below permissible limits. Large-scale trials are however required to be performed to test the commercial applicability of the process.

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First record of the Ranid frog *Paa annandalii* (Boulenger 1920) from north-eastern region (Arunachal Pradesh) of India with a note on its larval stages

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Arunachal Pradesh has remained underexplored for its amphibian faunal wealth. *Paa annandalii* adults and tadpoles were collected from a snowfed stream in Tawang, Tawang district of Arunachal Pradesh. In India this frog was first recorded by Annandale[1] from Darjeeling district in West Bengal. This species was included in the genus *Rana*. Dubois[5] later included this species in the genus *Paa*. Detailed taxonomic description of the adults and tadpoles, and food habits of the tadpoles are presented in this paper.

ARUNACHAL Pradesh is a part of the Eastern Himalayan region, a hotspot of biodiversity. Report of amphibian fauna of this region is very fragmentary[1–4]. The present collection of *Paa annandalii* (Boulenger, 1920) is from the north-eastern region of India. This frog was originally known as *Rana annandalii* and was collected by Annandale[1] from Sureil, Darjeeling district (1800 m amsl), West Bengal. Later this species was included in the subgenus Paa[5] of the genus *Rana*. Subsequently the species has been included in the genus *Paa* and in *Paa liebegii*

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