

BIOSORPTION OF SELECTED TOXIC HEAVY METALS USING ALGAL SPECIES *ACANTHOPORA SPICEFERA*

Narayanaswamy Tamilselvan, Kumar Saurav, Krishnan Kannabiran*

Biomolecules and Genetics Division, School of Biosciences and Technology

VIT University, Vellore, Tamil Nadu, India.

Summary

Occupational exposure of heavy metals and toxins has been shown to produce adverse health effects on humans. Biosorption has emerged as a cost-effective and efficient alternative technology for removal of heavy metals. In the present study the biosorption of heavy metals by an algal species, *Acanthophora spicifera* was evaluated. Acid digestion method and batch biosorption method was used to evaluate the efficiency of biosorption by *A. spicifera*. The effect of biomass dosage of *A. spicifera* (1-30g/L) on biosorption of metal ions Cr (VI), Cr (III), Pb (II), Cd (II) and Hg (II) was also studied. Acid digestion method showed maximum biosorption of Pb (II) (93.61%) whereas the batch biosorption method showed maximum biosorption of Cr (VI) (96.36%) followed by Hg (II) (96.20%), Cd (II) (92.68%), Pb (II) (51.84%), and Cr (III) (50.29%) in the order of (Cr (VI) >Hg (II) >Cd (II) >Pb (II) >Cr (III)). The biosorption was biomass dosage dependent and increase of biomass increased the biosorption process. The maximum biosorption of the metal ions was attained at a biomass dosage of 25g/L. Fourier transform infrared absorption spectra indicated the chemical interactions between the hydrogen atoms of carboxyl (-COOH), hydroxyl (-CHOH) and amine (-NH₂) groups of biomass with metal ions. Scanning electron microscopy revealed the enlargement size and surface modification of biomass. The results of our study suggest that seaweed biomass can be used efficiently for biosorption of heavy metals.

Keywords: Heavy metals, Biosorption, *Acanthophora spicifera*, FT-IR, Scanning electron microscopy.

**Corresponding Author*

Dr. K. KANNABIRAN,
Senior Professor & Division Head
Biomolecules and Genetics Division
School of Bio-Sciences and Technology
VIT University, Vellore-632014, Tamil Nadu, India
E -mail: kkb@vit.ac.in

Introduction

Among the toxic heavy metals Cr(VI) is an acute carcinogen and more mobile and toxic than Cr(III) is an acute carcinogen and more mobile and toxic than Cr(III). Human activities are responsible for contamination of drinking water by Cr(VI). Lead poisoning is considered to be a great threat to young childrens and is the number one environmental disease among children in developing countries. Long term exposure of cadmium (Cd) during fetal life has been reported to be particularly dangerous for the central nervous system. Toxic effects of mercury (Hg (II)) include damage to the brain, kidney, and lungs. Humans have more chances of exposure these toxic heavy metals by several ways. Hence there is an urgent need for effective removal of heavy metals from various environmental sources thereby it is possible to bring control over exposure of these heavy metals to humans. Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material (1). Heavy metal pollution is the greatest problem today all are facing. Various industries discharge heavy metals into environment such as mining, leaching, surface finishing industry, energy and fuel production, fertilizer and pesticide industry, metallurgy, iron and steel, electroplating, electrolysis, electro-osmosis, leatherworking, photography, electrical appliance manufacturing. Thus, metal as a kind of resource is becoming shortage and also brings about serious environmental pollution, threatening human health and ecosystem (2). Biosorption has emerged as a cost-effective and efficient alternative treatment technology for removal of heavy metals.

Different types of biomass in non-living form have been studied for their heavy metal uptake capacities. Bacteria, fungi, algae, plant leaves and root tissues were used as biosorbents for recovery of metals from industrial discharges (3,4). Among these different types of biomass, seaweeds are extensively used for metal biosorption due to their high uptake capacities. The metal biosorption potentials of various marine microorganisms, brown, green and red seaweeds have been evaluated by many investigators (5-7). In general, brown seaweeds always performed well irrespective of metal ions employed. This is due to the presence of alginate, which is present in a gel form in their cell walls (8). On the other hand, green algae are typically comprised of xylans and mannans; whereas red algae contain sulphate esters of xylans and galactans in their cell walls (9).

In the present study, seaweed was employed as a unique adsorbent for heavy metal removal in aqueous solutions. The effects of different experimental parameters were investigated. In addition, the experimental data were correlated and evaluated by scanning electron microscopy for their morphological changes. *A. spicifera* is a Rhodophycean alga with wide distribution throughout the tropics and subtropics. It occurs on a wide variety of substrata, from hard bottom, as an epiphyte on other algae, or as a free living drift alga. It is often a large component of drift algae biomass. Color is highly variable, and can be shades of red, purple, or brown. *A. spicifera* grows upright to approximately 25cm. In present study the biosorption potential of heavy metals by *A. spicifera* was investigated under *in vitro* conditions.

Materials and methods

Algal samples

Samples were collected from the Mandapam (9°16'47"N 79°7'12"E) region of Tamil Nadu, India. Scissors were used to cut lower stem (50cm) of the algae and the root was not disturbed to protect the seashore vegetation environment. The collected algal species was identified with the help of botanist as *A. spicifera* and subjected to heavy metal biosorption studies.

Sample processing and biomass preparation

Seaweeds were washed with distilled water to remove wastes and salt debris and shade dried at room temperature for a month. After shade drying the samples were dried in hot air oven at 60°C for 2 to 3 days. After complete drying the samples were processed for biomass preparation. The samples were ground in mixer and blender and sieved achieve particle with size ranging from 0.5 to 0.85mm. The heavy metal standard solutions Cr (III) and Cr (VI), Hg (II), Pb (II) and Cd (II) were prepared with the metal salt available using procedure of Saurav and Kannabiran (10).

Acid digestion

The biomass was added with 100ml of metal solutions (100ppm) of Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) in conical flask, sealed and kept in rotary shaker with agitation of 200rpm for 24h. After shaking the samples were subjected to centrifugation at 7000rpm for 15min and filtered using Whatmann no.1 filter paper. After filtering the biomass was subjected to drying in hot air oven at 70° C for 4h. After drying the biomass was treated with a mixture of acids such as sulphuric acid, perchloric acid and hydrochloric acid in the ratio of 3: 5: 11.5. The mixture was then kept on hot plate at 80°C until the appearance of brown colour. Double distilled water was added and filtered through filter paper and the filtrate was analyzed for metal concentration using flame atomic absorption spectrophotometer (AAS).

Batch biosorption procedure

General uptake procedure experiment was carried out in 250 ml conical flask containing 100ml of 100ppm metal solutions such as Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II). To the metal solution, 1g of biomass was introduced into conical flask. The flask was sealed with rubber cork. Then the flask was kept in rotary shaker with an agitation rate of 200rpm for 24h. After shaking the biomass and the metal solution mixture was subjected to centrifugation at 7000 rpm for 15min. Then it was filtered with the help of Whatmann no.1 filter paper. Filtered sample was analyzed for concentration of heavy metal in atomic absorption spectroscopy. The percent biosorption of metal ion was calculated as follows:

$$\text{Biosorption (\%)} = \frac{(C_i - C_f)}{C_i} \times 100$$

C_i – initial concentration, C_f – final concentration

FTIR analysis

Fourier Transform Infrared Spectroscopic (FTIR) spectra were taken to know the chemical bonding or molecular structure of organic or inorganic materials. Samples for FTIR analysis were prepared with the biomass loaded with and without metal solutions such as Cr (VI), Cr (III), Hg (II), Cd (II) and Pb (II). Spectra was taken using FT/IR-AVATAR 330 in order to investigate the functional groups and the possible metal binding sites present in the seaweeds samples.

SEM Analysis

Samples for Scanning Electron Microscopic (SEM, Leo Electron Microscopy Ltd., UK at 20 Kv) studies were prepared with biomass treated with and without metal solution. SEM analysis was used to study the morphological changes of seaweeds. Morphological changes confirm that the seaweeds have absorbed metal solution.

Results and discussion

A. spicifera is one of the most abundant red algal species found on reef flats (11). It has a wide distribution in both tropical and subtropical habitats, occurring primarily in the tidal and subtidal zones. It is found extensively on shallow reef flats throughout Florida, the Virgin Islands and Puerto Rico to depths of 22 meters, although it typically inhabits more shallow waters from 1 - 8 meters in depth (12,13). In the Indian River Lagoon, *A. spicifera* is commonly found attached to rocks and oyster rubble. Dead or dying specimens can be found smothering *Thalassia testudinum* beds.

The algal biomass prepared from *A. spicifera* showed significant biosorption of heavy metals by both acid digestion and batch biosorption method. In acid digestion method *A. spicifera* showed maximum biosorption of heavy metal Pb (II) when compared to other metals. The biosorption (%) for different metals found to be for Pb (II) (93.61%), Cr (III) (86.52%), Cr (VI) (75.34%), Hg (II) (39.3%), and Cd (36.63%). The biosorption of heavy metals was in the order of (Pb (II) >Cr (III) >Cr (VI) >Cd (II) >Hg (II). In batch biosorption method *A. spicifera* showed maximum biosorption of Cr (VI) (96.36%), Hg (II) (96.20%), Cd (II) (92.68%), Pb (II) (51.84%), and Cr (III) (50.29%) and the biosorption of heavy metals was in the order of (Cr (VI) >Hg (II) >Cd (II) >Pb (II) >Cr (III).

The biosorption of heavy metals by sea weeds was studied by several researchers and they reported the biosorption heavy metals by different sea weeds. The chromium bioaccumulation potential of microalgal strains was already been reported (14). Similarly Cu²⁺ and Zn²⁺ biosorption by dried marine green algae *Chaetomorpha linumi* from aqueous solutions was reported (15). Biosorption of Cr (III) by raw and acid treated green alga (*Oedogonium hatei*) (16), detoxification of chromium hexavalent metal by seaweeds (17), biosorption of Cd²⁺, Cu²⁺, Ni²⁺ and Zn²⁺ with the help of brown algae in aqueous solutions (18), selenium biosorption in aqueous solution by seaweeds (19), and the removal of lead in aqueous solution by seaweeds (20) have already been reported.

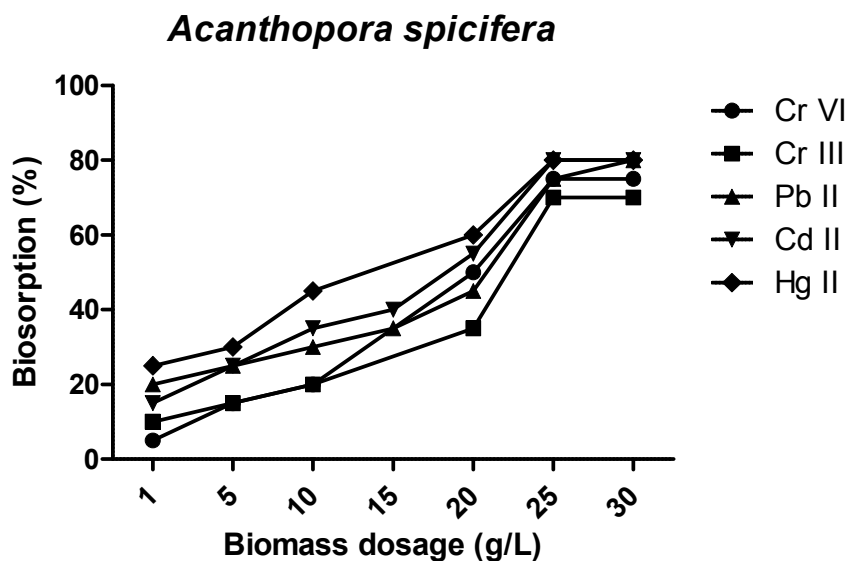


Fig.1. Effect of *Acanthopora spicifera* biomass dosage on biosorption of heavy metals

The effect of biomass dosage of *A. spicifera* (1-30g/L) on biosorption of metal ions Cr (VI), Cr (III), Pb (II), Cd (II) and Hg (II) is shown in Fig.1. Results showed that the biosorption efficiency is highly dependent on the increase in biomass dosage of the solution. The maximum biosorption of the metal ions was attained at a biomass dosage of 25g/L and it was almost same at higher dosages. This trend could be explained as a consequence of a partial aggregation of biomass at higher biomass concentration, which results in a decrease in effective surface area for the biosorption.

The FT-IR spectroscopy method was used to obtain information on the nature of possible interactions between the functional groups of seaweeds sp. biomass and the metal ions. The FTIR spectra of dried unloaded biomass (Fig. 3A), Cr (VI), Cr (III), Hg (II), Cd (II) and Pb (II)-loaded biomass are shown in Fig. 3 (B, C, D & E). Seaweeds cell wall are structurally and chemically more complex and heterogenous when compared to land plants. Cell wall is known to be rich in sulfated and branched polysaccharides which are associated with proteins and various bound ions like calcium and magnesium (21). These polymers form abundant source of metal binding ligands. These compounds may contain several functional groups such as amino, carboxyl, sulphate, hydroxyl, etc. which could play an important role in the biosorption metal ions. The biosorption of metal ions by algal biomass has several advantages over other conventional methods used to remove the heavy metals. It was reported that metabolism-independent metal binding to the cell walls and external surfaces is the

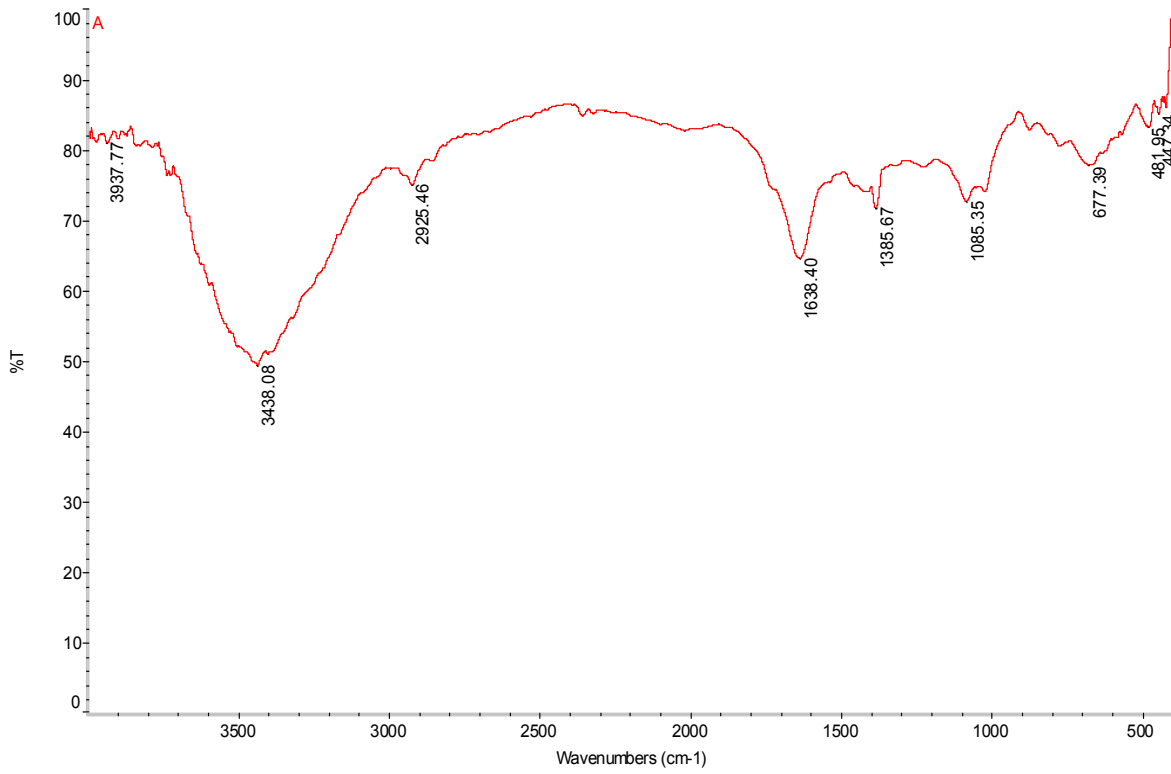


Fig. 2. (A) FT-IR spectra of metal unloaded biomass sample of *Acanthopora spicifera*

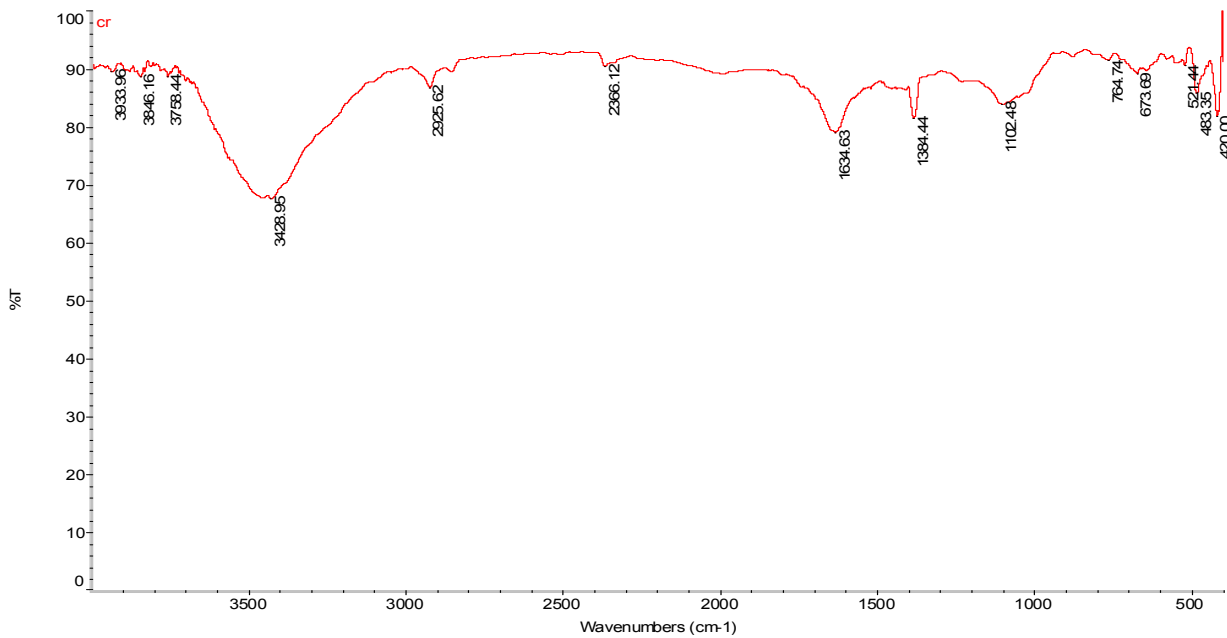


Fig.2. (B) FT-IR spectra of Cr metal loaded with biomass sample of *Acanthopora spicifera*

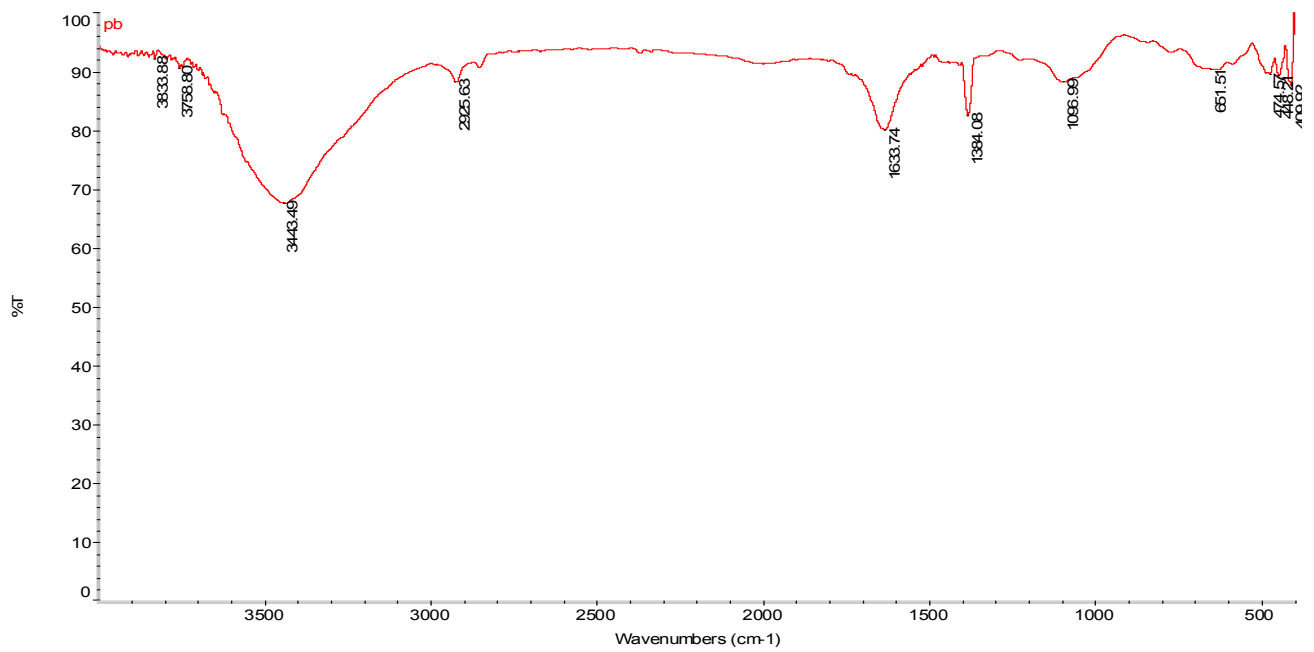


Fig. 2 (C) FT-IR spectra of Pb metal loaded with biomass sample of *Acanthopora spicifera*

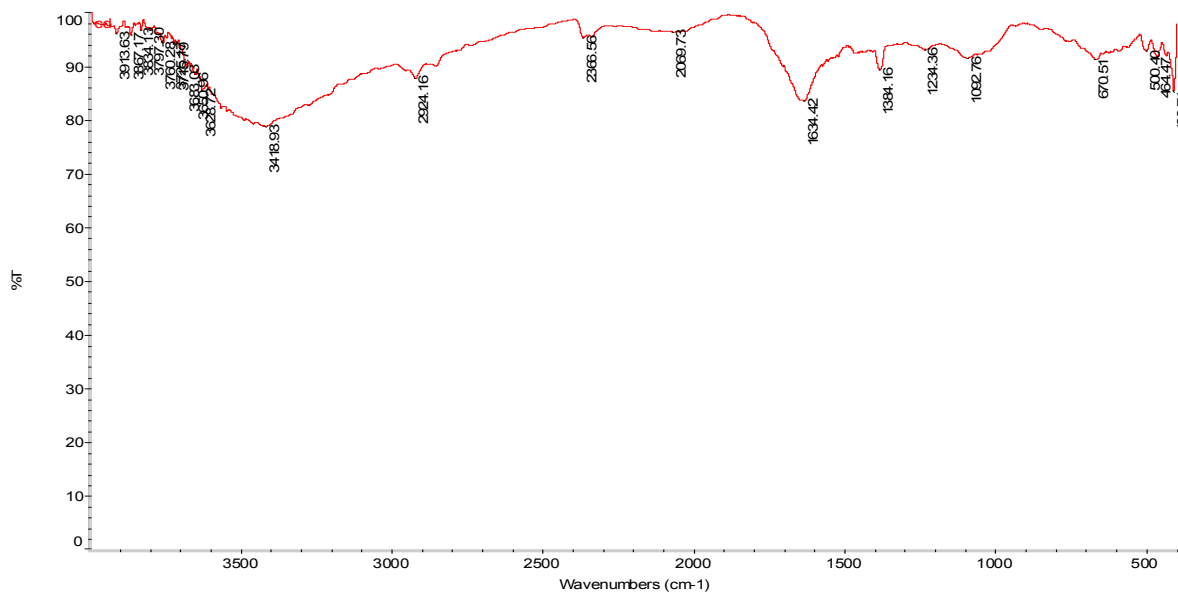


Fig. 2. (D) FT-IR spectra of Cd metal loaded with biomass sample of *Acanthopora spicifera*

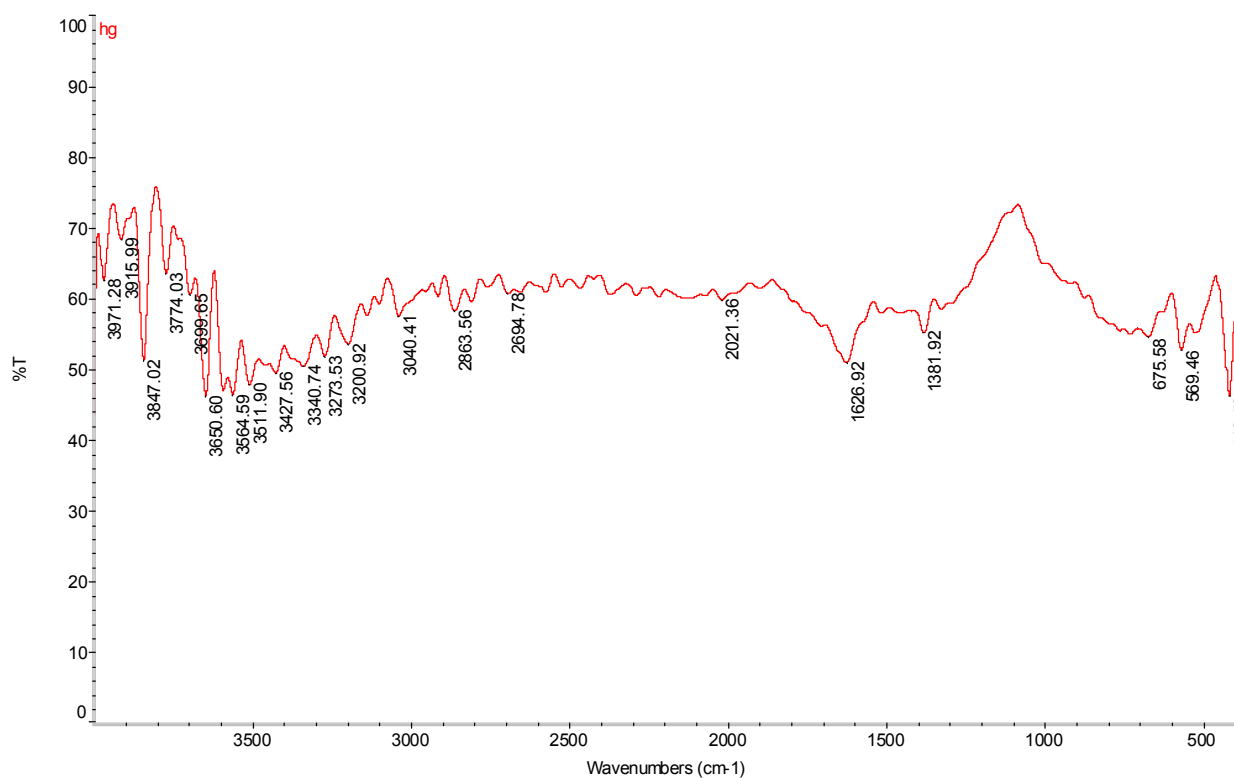


Fig. 2 (E) FT-IR spectra of Hg metal loaded with biomass sample of *Acanthopora spicifera*

only mechanism present in the case of non-living biomass (22). Metabolism-independent uptake essentially involves adsorption process such as ionic, chemical and physical adsorption. Metal ions could be adsorbed by complexing with negatively charged reaction sites on the cell surface (23). The relative importance of each functional group in biosorption is often difficult to resolve (24). Determination of the exact mechanism is further complicated by complex solution chemistry of the metals and the inability to determine the precise metal complex present in the solution which is not readily amenable to instrumental analysis (25). Differences in affinities between elements and their ionic species may exist for various ligands encountered in biological system. FTIR spectroscopy was also more often used to study the biosorption of metal ions (26). The removal of chromium, cadmium and copper from dilute aqueous solution using dead polysaccharide producing *Ochrobactrum anthropi* isolated from activated sludge was already been reported (27).

Unloaded native sample shows the peak at 3438.08 cm^{-1} stretching and was observed in the entire metal loaded sample also, which rule out the role of C=O functional group in metal complex formation Fig. 3 (A). A new stretching peak at 2366.12 cm^{-1} observed with Cr loaded sample and gives possibility of the role of acid group involvement in the formation of the complex Fig. 3 (B). In case of Pb (II) loading there is no change in vibration at 3428.96 cm^{-1} , 2925.62 cm^{-1} , 1634.63 cm^{-1} , 1384.44 cm^{-1} and 1095.99 cm^{-1} . In case of Cd (II) loading there is new vibration at 1234 cm^{-1} is due to the C-O aromatic ethers Fig. 3 (D), then there is no change in remaining functional group. In case of Hg (II) loading there is a peak in 3847.02 cm^{-1} due to

the N-H amines, 3650.60 cm^{-1} due to the O-H group, 3040.41 cm^{-1} is due to the high concentration of O-H groups, 2863.56 cm^{-1} is due to alkyl group Fig. 3 (E), remaining groups are same.

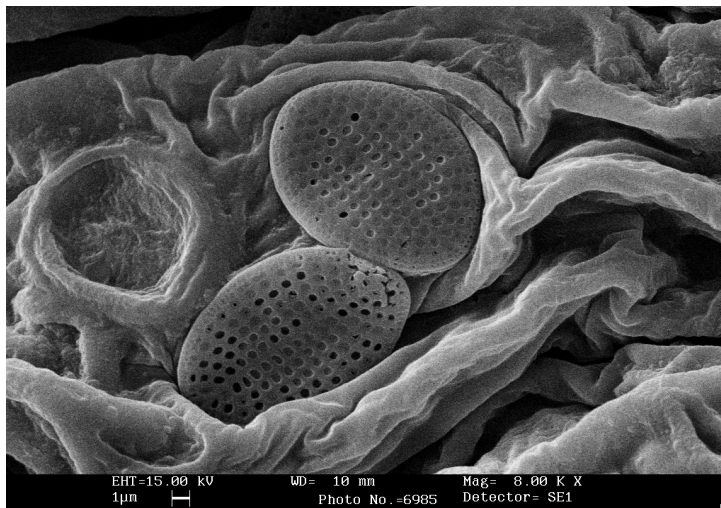


Fig. 4. SEM surface morphology of *Acanthopora spicifera* biomass loaded with Cr (VI) showing size enlargement and surface modification. Bar represents $1\mu\text{m}$.

SEM analysis revealed the change in surface morphology of *A. spicifera* biomass treated with metal ion. The surface morphology of Cr metal treated *A. spicifera* is shown in Fig. 4. The change in morphological characterization of seaweeds after interaction with chromium showed size enlargement and surface modification. It was found that the substratum pebbles and stones supported maximum biomass.

Conclusion

Based on the results it can be concluded that *A. spicifera* is a potential algal species for effective removal of heavy metals such as Cr (VI), Cr (III), Hg (II), Cd (II) and Pb (II) from environmental sources.

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