



Document heading

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## Biosorption of heavy metals from aqueous solution by *Gracilaria corticata varcartecala* and *Grateloupia lithophila*

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### PEER REVIEW

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#### Comments

This is a valuable research work in environmental field. Authors have demonstrated the *G. corticata varcartecala* and *G. lithophila* as the potential sources for the removal of toxic heavy metals. Biosorption and acid digestion method were carried out to prove great potent of seaweeds in removal process. Optimization parameters were carried out for the effective removal of heavy metals.

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### ABSTRACT

**Objective:** To study the biosorption of heavy metals viz., Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) using *Gracilaria corticata varcartecala* (*G. corticata varcartecala*) and *Grateloupia lithophila* (*G. lithophila*) biomass.

**Methods:** Batch biosorption and acid digestion methods were used. Different physical and chemical parameters were optimized for biosorption.

**Results:** Both seaweeds absorb Hg (II) upto 99.9% and 98.2% in batch biosorption method; whereas in acid digestion method, Cr (III) absorbed upto 96.49% by *G. corticata varcartecala* and Pb (II) absorbed upto 93.71% by *G. lithophila*. FT-IR analysis was used to know the involvement of different functional groups in the biosorption process. Scanning electron microscopy was carried out to study the morphological cell surface changes due to biosorption.

**Conclusions:** It can be concluded that *G. corticata varcartecala* and *G. lithophila* are potential algal species for effective removal of heavy metals namely Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) from environmental sources.

### KEYWORDS

Biosorption, Heavy metals; *Gracilaria corticata varcartecala*, *Grateloupia lithophila*, SEM

## 1. Introduction

The heavy metal contamination in aqueous streams, arising from the discharge of untreated metal containing effluent into water bodies, is one of the most important environmental issues[1]. Their presence in aquatic ecosystem causes harmful effect to living organisms[2]. Biosorption can be defined as the removal of metal or metalloid species, compounds and particulates from solution by biological material[3]. Heavy metal pollution is an alarming problem today in developing countries. 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 and electrical appliance manufacturing industries. Thus, metal as a kind of resource is being depleted and also brings about serious environmental pollution, threatening human health and ecosystem[4]. Three kinds of heavy metals are of great concern, which include toxic metals namely Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc., precious metals namely Pd, Pt,

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Ag, Au, Ru etc., and radio nuclides namely U, Th, Ra, Am etc[5].

Large quantities of bio-materials have been investigated as biosorbents for the removal of metals or organics extensively. Marine macro algae otherwise known as seaweeds are extremely efficient biosorbents with the ability to bind various metals from aqueous effluents because of their cosmopolitan distribution both in fresh and salt waters, relative high surface area and binding affinity[6]. Numerous chemical groups may be responsible for metal biosorption by seaweeds (e.g. carboxyl, sulphonate, hydroxyl and amino groups)[7].

In our present study, various seaweeds were collected from Mandapam coast of Tamilnadu and identified as *Caulerpa racemosa* (Chlorophyta), *Sargassum wightii* (Phaeophyta), *Gracilaria corticata* var. *corticata*, *Grateloupia lithophila* (Rhodophyta) (*G. lithophila*) and *Acanthopora* sp. (Rhodophyta). However, two species viz. *Gracilaria corticata varcartecala* (*G. corticata varcartecala*), and *G. lithophila* were selected for the biosorption studies on the basis of the preliminary efficiency.

## 2. Materials and methods

### 2.1. Algal collection and processing

Seaweeds were collected from Mandapam coast near Vethalai, Ramanathapuram district, Tamil Nadu, India. Sterile scissors were used to cut the lower stem (50 cm) of the seaweeds and the root was not disturbed to protect the seashore vegetation environment. Seaweeds were packed in sterile polythene bag before transferred to the laboratory. Seaweeds were identified as per the manual of previous reported identification key[8]. The material was washed with deionized water to remove wastes, salt debris and unwanted present in it. After washing, seaweeds were subjected to shade drying for a month. After that, seaweeds were processed for hot drying in hot air oven at 80 °C for 48 h, then samples were grinded and sieved at the pore size of 0.5 to 1 mm.

### 2.2. Heavy metal stock solution preparation

The heavy metal standard solutions Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) were prepared with the metal salt available in the laboratory. Salt forms of the above mentioned heavy metals namely chromium trioxide, potassium dichromate, mercuric chloride; lead acetate and cadmium chloride were used. Primarily 1000 mg/L solution was prepared, which was then further serially diluted with deionized water to get varying concentrations from 1000–100 mg/L. The standard solutions were sterilized separately for 15 min at 110 °C at 21 lbs.

### 2.3. Batch biosorption procedure

Biosorption experiment were carried out in 250 mL Erlenmeyer flask, 50 mL of metal solutions Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) with 100 mg/L concentration were

taken, and 1 g of biomass was inoculated and kept in rotary shaker at 200 r/min for 24 h. After incubation, the biomass sample was centrifuged at 7000 r/min for 15 min, and filtered with Whatman No. 2 filter paper. Filtered samples were subjected to atomic absorption spectroscopy (flame) for heavy metal concentration[9].

The percentage biosorption of metal ions was calculated as follows:

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

$C_i$  and  $C_f$  are the initial and final metal ion concentrations, respectively.

### 2.4. Acid digestion treatment

Biomass after centrifugation was subjected to acid digestion treatment with nitric acid, hydrochloric acid, sodium per chloric acid in the ratio of 3:11.5:5. The mixture was kept in hot plate, heated at 80 °C until brown colour appears. Distilled water was added and filtered through Whatman No. 2 filter paper and the filtrate was analyzed for metal concentration using atomic absorption spectrophotometer Varian SpectrAA 240.

### 2.5. Effect of pH

Biosorption experiments were carried out at different pH ranging from 5–9. Then the metal solutions of Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) of 100 mL (100 mg/L) was taken in 250 mL conical flask, then the pH was adjusted to our desired level by adding 1 mol/L NaOH and 1 mol/L HCl; then 1 g biomass was added. The flask was sealed with rubber cork. Then the flask was kept in rotary shaker with the agitation rate of 200 r/min for 24h. After shaking the biomass with metal solution, it was subjected to centrifugation at 7000 r/min for 15min. Then it was filtered with the help of Whatman No. 2 filter paper. Filtered sample was analyzed for concentration of heavy metal with the help of atomic absorption spectroscopy.

### 2.6. Effect of biomass dosage

Biosorption experiment was carried out for different concentration of biomass ranging from 5–35 g. An initial metal solution with different concentration of Cr (VI), Cr (III), Hg (II), Pb (II), and Cd (II) was taken. The flask was sealed with rubber cork. Then the flask was kept in rotary shaker with the agitation rate of 200 r/min for 24 h. After shaking the biomass with metal solution, it was subjected to centrifugation at 7000 r/min for 15 min. Then it was filtered with the help of Whatman No. 1 filter paper. Filtered sample was analyzed for heavy metal concentration by atomic absorption spectroscopy.

### 2.7. Effect of time

An initial metal solution of Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) was taken. The flask was sealed with rubber cork. Then the flask was kept in rotary shaker with the agitation

rate of 200 r/min at different time interval (12, 24, 36, 48 and 60 h). After shaking the biomass with metal solution, it was subjected to centrifugation at 7000 r/min for 15min. Then it was filtered with the help of Whatman No. 1 filter paper. Filtered sample was analyzed for heavy metal concentration by atomic absorption spectroscopy.

### 2.8. SEM analysis

Samples for scanning electron microscopic studies were prepared with biomass treated with both metal solution and solution devoid of metal (SEM, Leo Electron Microscopy Ltd., UK at 15 Kv). Sample preparation for SEM analysis followed were fixation, washing, post-fixation, washing, dehydration, critical point drying, sample mounting, metal coating, viewing specimens in SEM and image analysis<sup>[10]</sup>.

## 3. Results

### 3.1. Batch biosorption

In batch biosorption process, *G. corticata varcartecala* showed maximum biosorption of Hg (II) 99.9% compared to Pb (II) 99.89%, Cd (II) 99.18%, Cr (VI) 92.59% and Cr (III) 90.72%. Whereas *G. lithophila* recorded as Hg (II) 98.2%, the maximum biosorption compared to other heavy metals such as Cr (III) 95.45%, Cd (II) 82.01%, Cr (VI) 73.45% and Pb (II) 41.65%. Both *G. corticata varcartecala* and *G. lithophila* effectively involved in biosorption of Hg (II). The results are shown in Table 1.

**Table 1**

Chromium, mercury, lead and cadmium uptake of *G. corticata varcartecala* and *G. lithophila* by batch biosorption process.

Seaweeds	Uptake (%)				
	Cr (VI)	Cr (III)	Hg (II)	Pb (II)	Cd (II)
<i>G. corticata varcartecala</i>	92.59±0.94	90.72±1.52	99.90±0.02	99.89±0.03	99.18±0.07
<i>G. lithophila</i>	73.45±1.68	95.45±1.29	98.20±0.74	41.65±1.91	82.01±2.01

Values expressed in mean±SD of three replicates. Heavy metals expressed in mg/mL.

### 3.2. Acid digestion

*G. corticata varcartecala* revealed elevated heavy metal absorption of Cr (III) 96.49% and Pb (II) 90.85%, whereas other metals recorded minimal amount of metal absorption. *G. lithophila* revealed the maximum biosorption of Pb (II) 93.71%, Cr (VI) 89.34% and Cr (III) 87.79%, whereas other metals recorded minimum level of biosorption. The results are shown in Table 2.

**Table 2**

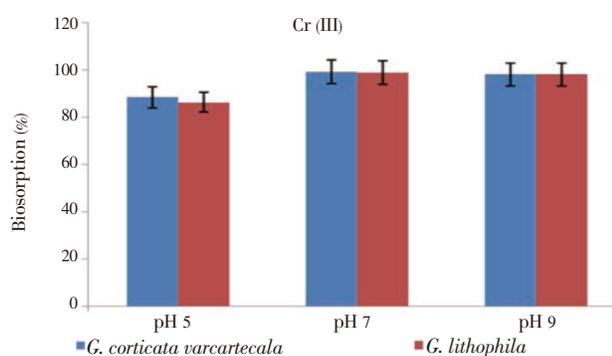
Chromium, mercury, lead and cadmium uptake of *G. corticata varcartecala* and *G. lithophila* by acid digestion method.

Seaweeds	Uptake (%)				
	Cr (VI)	Cr (III)	Hg (II)	Pb (II)	Cd (II)
<i>G. corticata varcartecala</i>	79.79±1.41	96.49±1.14	9.3±1.90	90.85±1.70	4.82±2.01
<i>G. lithophila</i>	89.34±0.86	87.79±1.43	19.6±1.15	93.71±0.66	35.28±1.70

Values expressed in mean±SD of three replicates. Heavy metals expressed in mg/mL.

### 3.3. Effect of pH

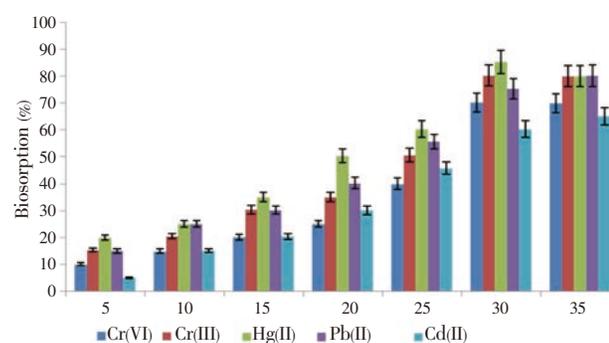
The effect of pH on the biosorption of metal ions Cr (VI), Cr (III), Pb (II) Cd (II) and Hg (II) by *G. corticata varcartecala* and *G. lithophila* biomass was studied at pH range 5–9. The optimized pH founded to be pH 7 for *G. corticata varcartecala*, was found to be 99.66% for Cr (III), 99.98% for Cd (II). The maximum biosorption for *G. lithophila* was found to be 78.19% for Cr (VI), 99.97% for Cr (III) at pH 7, hence optimized pH was found to be pH 7 (Figure 1). In our present studies, pH played a significant role in biosorption of heavy metals. *G. corticata varcartecala* absorbed Cd (II) and Cr (III) maximally at pH 7, Pb (II) at pH 5 and Hg (II), Cr (VI) at pH 9. *G. lithophila* absorbed Cr (III), Cr (VI) and Hg (II) at pH 7, Cd (II) at pH 9 and Pb (II) at pH 5. At higher pH values, the biosorption was dramatically decreased when compared to that of lower pH.



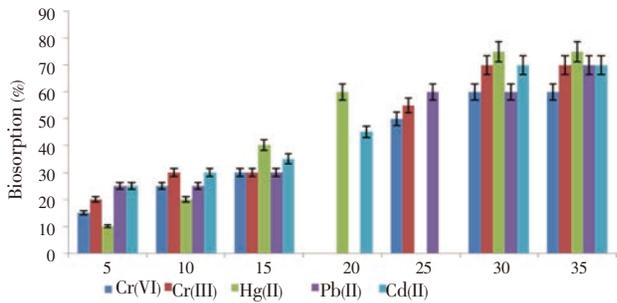
**Figure 1.** Effect of optimized pH on biosorption of Cr (III). Maximum biosorption takes at pH 7.

### 3.4. Effect of biomass dosage

The effect of biomass dosage of *G. corticata varcartecala* (5–35 g/L) on biosorption of metal ions Cr (VI), Cr (III), Pb (II), Cd (II) and Hg (II) was studied (Figure 2). The maximum biosorption of metal ions was observed at biomass dosage of 30 g/L. The effect of biomass dosage of *G. lithophila* (5–35 g/L) on Cr (VI), Cr (III), Pb (II), Cd (II) and Hg (II) on biosorption was recorded (Figure 3). The maximum biosorption of metal ions was obtained at dosage of 30 g/L. The studies revealed that biomass was directly proportional to biosorption process. An increased level of biosorption was recorded at 30 g/L.



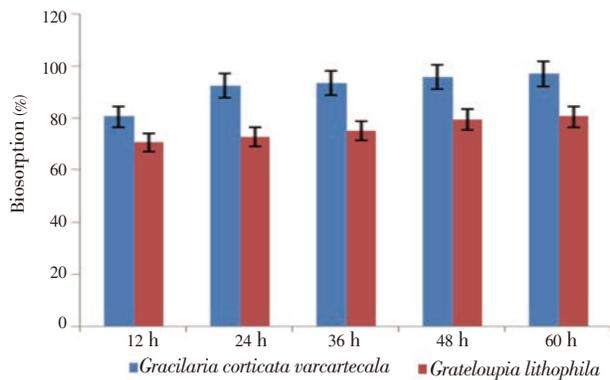
**Figure 2.** Effect of biomass dosage on biosorption of *G. corticata varcartecala* on different heavy metals.



**Figure 3.** Effect of biomass dosage on biosorption of *G. lithophila* on different heavy metals.

### 3.5. Effect of time

The effect of time on the biosorption of metal ions Cr (VI), Cr (III), Pb (II) Cd (II) and Hg (II) by *G. corticata varcartecala* and *G. lithophila* biomass was studied at different time intervals of 12, 24, 36, 48 and 60 h (Figure 4).



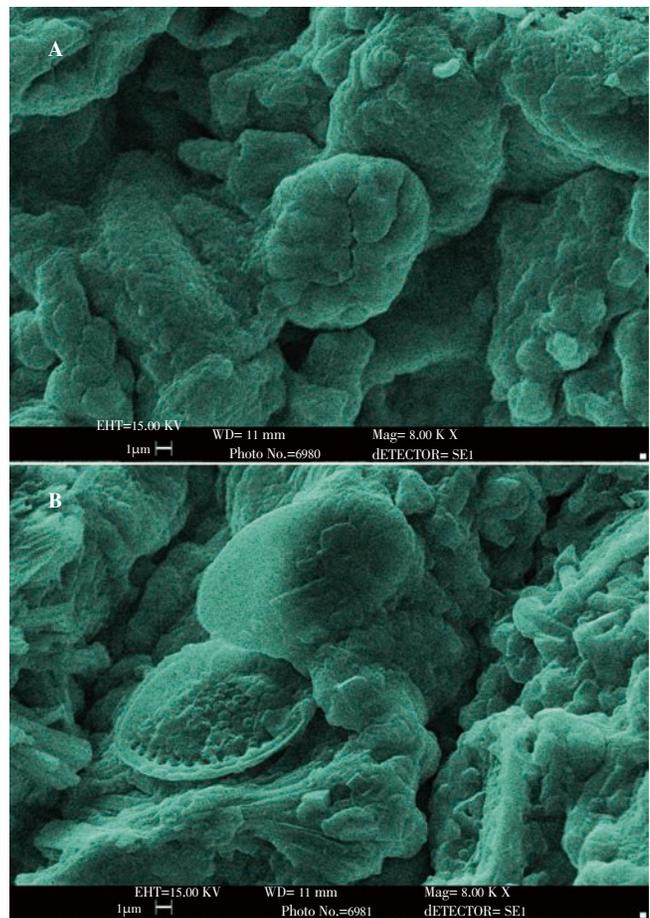
**Figure 4.** Effect of optimized time on biosorption Cr (VI). Maximum biosorption takes at 60 h.

The maximum biosorption by *G. corticata varcartecala* for Cr (VI) and Cr (III) metal ions was at the time interval of 60 h, for Hg (II), Pb (II) and Cd (II) metal ions was recorded at the time interval of 24 h. The maximum biosorption by *Grateloupia* sp. for Cr (VI), Cr (III), Pb (II) and Cd (II) metal ion at the time interval of 60 h, for Hg (II) metal ion maximum biosorption was obtained at 24 h of time interval. Contact time played a great role in biosorption of heavy metals in our studies.

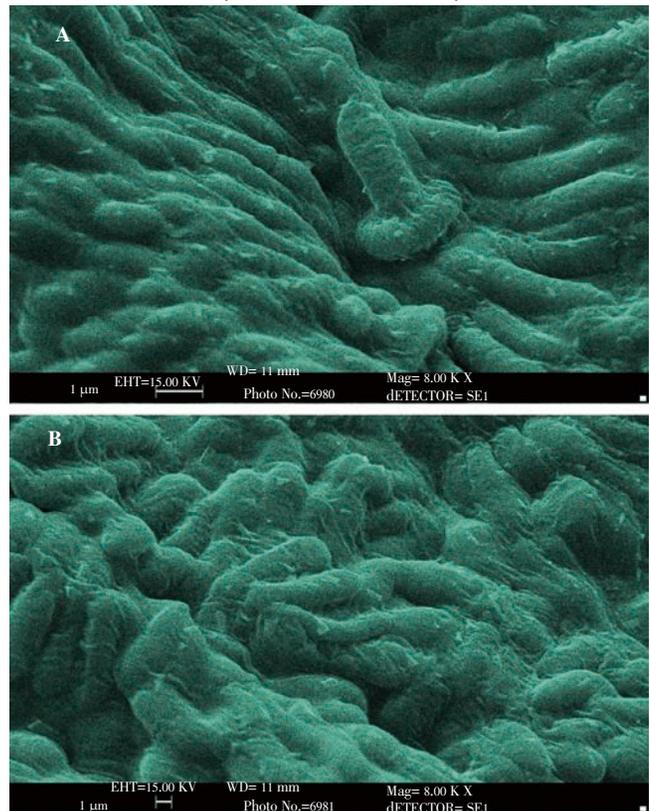
### 3.6. SEM analysis

SEM analysis revealed the change in surface morphology of *G. corticata varcartecala* and *G. lithophila* biomass treated with metal ions. The surface morphology of metal unloaded and metal loaded *G. lithophila* was shown in Figure 5. This revealed the morphology change which might be due to the binding of metals in the surface of the biomass.

The surface morphology of metal unloaded and metal loaded *G. corticata varcartecala* was shown in Figure 6. This revealed the morphology change which might be due to the binding of metals in the surface of the *G. corticata varcartecala*.



**Figure 5.** The surface morphology of metal unloaded and metal loaded *G. lithophila*. (A) Unloaded with heavy metals; (B) Loaded heavy metals.



**Figure 6.** The surface morphology of metal unloaded and metal loaded *G. corticata varcartecala*. (A) Unloaded with heavy metals; (B) Loaded with heavy metals.

## 4. Discussion

Earlier studies shown that seaweeds were effectively involved in the biosorption of heavy metals. *Cystoseira indica*, *Sargassum glaucescens*, *Nizimuddiniana zanardini* and *Padina australis* involved in biosorption of Cd (II), Ni (II) and Pb (II)[11]. *Sargassum* sp. involved in biosorption of Hg (II) and Fe (III)[6], Samarium (III), Praseodymium (III) and Uranium[12,13]. *Chaetomorpha linum* involved in biosorption of Cu (II) and Zn (II)[14]. *Turbinaria conoides* involved in Au (0) from Au (III) biosorption[15]. *Cystoseira indica* involved biosorption of hexavalent chromium[16]. *Maugeotia genuflexa* involved in As (III) biosorption[17]. *Cladophora hutchinsiae* involved in Se (IV) biosorption[18]. *Cystoseira baccata* involved aluminium biosorption[19]. Due to the effective removal of heavy metals, researchers mostly concentrated on seaweeds for the removal of heavy metals in the environment.

The increased biosorption may be due to more of negatively charged biomass surface binds with positively charged metal ions. Decrease in biosorption at higher pH might be due to the formation of soluble hydroxylated complexes of the metal ions and their competition with the active sites, and as a consequence, the retention would decrease. Algal biomasses contain high content of carboxyl groups from mannuronic and guluronic acids on the cell wall polysaccharides, which suggests that the biosorption process could be affected by pH changes in the solution[20].

The increase in the percentage of the metal ions removal with increase in adsorbent dose is due to the greater availability of the exchangeable sites or surface area at higher concentration of the adsorbent. After a certain dose of adsorbent, the maximum adsorption is attained and hence the amount of ions bound to the adsorbent and the amount of free ions remains constant even with further addition of the dose of adsorbent[21]. For fixed adsorbent dose, the total available adsorption sites are limited thereby adsorbing almost the same amount of sorbate thus resulting in a decrease in percentage removal of the adsorbate corresponding to an increase initial sorbate concentration[22].

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[23]. Results showed that the biosorption efficiency is highly dependent on the increase in biomass dosage of the solution. Trivalent chromium (Cr (III)) is the most prevalent form found in natural water. Almost all hexavalent chromium (Cr (VI)) contaminant is from human activities. Cr (VI) is an acute carcinogen and more mobile and toxic than Cr (III). Hence, Cr (VI) is more important than Cr (III) in water pollution control. Brown algae are one of the most commonly used biosorbents[19]. It has been observed that a significantly high amount of organic substances are dissolved from seaweed-based biosorbents during the biosorption[13]. The presence of several functional groups such as hydroxyl, carbonyl amino, ester, sulfhydryl and phosphate groups are found; and several classes of organic

compounds which includes alcohol, aldehydes, organic acids, fatty acids, lipids, polysaccharides, carbohydrates and nucleic acids are presented[24]. These functionalities act as binding sites for metals with their relative importance depending on factors such as the quantity of sites, their accessibility and the affinity between site and metal. The main metal binding mechanisms include physical adsorption, ion-exchange and complex formation[25].

Earlier studies revealed that time plays an effective role in biosorption process. *Aspergillus foetidus* showed the maximum biosorption at the time range of 92 h for chromium[26]. At 36 h *Aspergillus oryzae* showed the maximum biosorption for chromium[27]. *Micrococcus* species showed the maximum biosorption at 18 h interval of time for chromium[28].

In our study, it reveals that Algal biomass that efficiently absorbs heavy metals. Here pH, biomass dosage, contacts time and temperature play roles in the effective biosorption of heavy metals. Optimized pH for the effective biosorption of both seaweeds was found to be pH 9. Biomass dosage of 30 g/L shows the maximum level of biosorption. SEM analysis shows the morphological changes of seaweeds reveals metal biosorption. Based on the results it concluded that *G. corticata varcartecala* and *G. lithophila* is a potential algal species for effective removal of heavy metals namely Cr (VI), Cr (III), Hg (II), Pb (II) and Cd (II) from environmental sources.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## Comments

### Background

This research paper is fully concentrated on current problem related to environment pollution. This paper could be more useful for environmentalist and scientific companies. Seaweeds are used in industrial areas and pharmaceutical companies'. It plays an important role in the detoxification of chemicals and drugs. Biosorption of heavy metals by seaweeds is effective. The health benefits of a safe and effective heavy metal and environmental toxin removal system.

### Research frontiers

The present research work revealed bioremediation of heavy metals from aqueous solution by two sea weeds. Various parameters were carried out to prove the efficacy of biosorption of heavy metals.

### Related reports

Batch biosorption and acid digestion method were followed to prove the biosorption capacity. pH, dosage and time optimization were carried out.

### Innovations and breakthroughs

Seaweeds were used for the removal of heavy metals. In the present study, authors used *G. corticata varcartecala* and *G. lithophila* with the biosorption process and acid digestion method to removal heavy metals.

### Applications

From this study it has been found that *G. corticata varcartecala* and *G. lithophila* are safe to removal environmental pollution and toxic heavy metals.

### Peer review

This is a valuable research work in environmental field. Authors have demonstrated the *G. corticata varcartecala* and *G. lithophila* as the potential sources for the removal of toxic heavy metals. Biosorption and acid digestion method were carried out to prove great potent of seaweeds in removal process. Optimization parameters were carried out for the effective removal of heavy metals.

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