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Short communication

# Phycoremediation of heavy metals by the three-color forms of *Kappaphycus alvarezii*

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**Abstract**

In the present investigation, three living color forms (brown, green and pale yellow) of *Kappaphycus alvarezii* were examined for their biosorption ability in the laboratory. The brown color form proved to be an excellent metal biosorbent, i.e. it could adsorb good amount of cadmium 3.064 mg/100 g f.wt. and cobalt 3.365 mg/100 g f.wt. It also removed 2.799 mg/100 g f.wt. of chromium. The green color form absorbed 2.684, 3.43 and 2.692 mg/100 g f.wt. of cadmium, cobalt and chromium, respectively. In contrast, the pale yellow form removed almost equal proportion of cadmium 0.961 mg/100 g f.wt. and chromium 0.942 mg/100 g f.wt. It also removed 1.403 mg/100 g f.wt. cobalt. Thus, the living color forms of this seaweed could form an effective biosorbent material for removal of heavy metals.


*Keywords:* Biosorption; Color forms; Heavy metals; *Kappaphycus alvarezii*; Marine

## 1. Introduction

Metal sorption involves binding of metals onto the cell surface and to intracellular ligands [1]. Metal remediation through common physico-chemical techniques is expensive and not eco-friendly. Hence, biotechnological approaches have received a great deal of attention as an alternative tool in the recent years. Biosorption, the process of passive cation binding by dead or living biomass, provides a potentially cost-effective way of removing toxic metals from industrial wastewaters [2], and it could be employed most effectively in a concentration range below 100 mg $l^{-1}$, where other techniques are ineffective or costly [3,4]. Metal ion binding during biosorption processes has been found to involve complex mechanism, such as ion-exchange, complexation, electrostatic attraction and microprecipitation [5]. There have been some indications that ion-exchange plays an important role in metal sorption by algal biomass [6]. Applicability of growing bacterial/fungal/algal cells for metal removal and the efforts directed towards cell/process development to make this option technically and economically viable for the treatment of metal rich effluents have been reviewed by Malik [7].

Marine algae (seaweeds) are readily available in large quantities for the development of highly effective biosorbent materials. However, considering the large number of macro-algal species identified so far, only a few have been studied for their heavy metal uptake properties. Most of these studies are limited to the *Ascophyllum* and *Sargassum* sp. [8]. The non-living biomass of marine algae *Sargassum* sp., *Padina* sp., *Ulva* sp., and *Gracilaria* sp., have been investigated for their biosorption performance in the removal of lead, copper, cadmium, zinc, and nickel from dilute aqueous solutions. It was also found that the biosorption capacities were significantly affected by solution pH, with higher pH favoring higher metal-ion removal [9]. A number of workers investigated the feasibility of using cheaply available marine or fresh water algae for heavy metal removal [10,11]. The passive removal of toxic heavy metals by brown marine algae via biosorption was reported by Davis et al. [12,13], who attributed this property to cell wall polysaccharides like alginate and fucoidan. On the other hand, high adsorption capacities of various low cost adsorbents, e.g. chitosan (815, 273, 250, 222, 75 mg/g of $Hg^{2+}$, $Cr^{6+}$, $Cd^{2+}$, $Cu^{2+}$, and $Zn^{2+}$, respectively), zeolites (175 and 137 mg/g of $Pb^{2+}$ and $Cd^{2+}$, respectively), waste slurry (1030, 560, 640 mg/g of $Pb^{2+}$, $Hg^{2+}$, and $Cr^{6+}$,

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respectively), and lignin (1865 and 95 mg/g of $Pb^{2+}$ and $Zn^{2+}$, respectively) have been reported [14]. Removal of zinc from aqueous solutions using bagasse fly ash, a waste from sugar cane industry as a low cost adsorbent has also been studied by Gupta and Sharma [15]. Fresh algal biomass of *Spirogyra* species was used as biosorbent for the removal of Cr(VI) from aqueous solutions [16].

*Kappaphycus alvarezii* [17] is a promising carrageenophyte and three-color forms (brown, red and green) have been reported in this alga from Philippines [18]. In India, three-color forms (brown, green and pale yellow) were also obtained during the cultivation of this alga at Port Okha, northwest coast of India. This study is aimed at using these living color forms of this seaweed in the laboratory for biosorption of toxic metals like Cd, Co and Cr.

## 2. Materials and methods

### 2.1. Collection of samples

Three-color forms (brown, green and pale yellow) of *Kappaphycus alvarezii* were obtained at Port Okha (22°28′N and 69°05′E) Northwest coast of India during cultivation (April 2006). The collected fresh algae were thoroughly washed with sterilized seawater to eliminate the adhering foreign materials, such as sand and debris, and materials used for heavy metal uptake studies.

### 2.2. Heavy metal uptake

The following sets of experiments for biosorption studies were designed. Three 500 ml flat bottom flask with spout for air circulation were used containing 400 ml of sterilized seawater to which 25 mg $l^{-1}$ each of cadmium, cobalt and chromium, as cadmium sulfate, cobalt sulfate and potassium dichromate (analytical grade) was added (pH 7.7). To avoid heavy metal contamination, the glassware was soaked in 10% $HNO_3$ for 24 h, rinsed with deionised water and oven dried prior to use.

Ten grams of each living algal color form (a single thallus fragment) was added aseptically to the three respective flasks containing mixture of heavy metals. One set without heavy metal was also inoculated with each color form that served as control. Experiments were conducted in duplicate.

The living color forms were maintained at 25 °C ± 1, in a clean environment where aeration was continuously provided. After 5 days of incubation, the algae were removed and shade dried at room temperature followed by drying in an oven at 80 °C for 1 h in a porcelain crucible. These samples were ashed at 550 °C in muffle furnace for 2 h. The ash was cooled at room temperature, moistened with 10 drops of distilled water and carefully dissolved in 3 ml $HNO_3$ (1:1 v/v). The crucible was then heated on a hot plate at 110 °C till the acid solution totally evaporated. The crucible was returned to muffle furnace and ashed again for 1 h at 550 °C and cooled. Subsequently the ash was dissolved in 10 ml of HCl (1:1 v/v), and the solution was filtered through millipore filter paper (0.25 μ) into a 50 ml volumetric flask and 2 ml 0.1N $HNO_3$ was added to the filtrate and the final volume was made up to 50 ml using distilled water [19]. This was subjected to heavy metal analysis.

### 2.3. Instrumentation

Analysis of heavy metals cadmium (Cd), cobalt (Co) and chromim (Cr) was carried out using inductively coupled plasma atomic emission spectroscopy, ICP-AES (Perkin-Elmer, Optima 2000). The mean value of the results obtained here was considered and the heavy metal uptake was calculated as mg/100 g f.wt.

## 3. Results and discussion

The three-color forms of *Kappaphycus alvarezii* were compared for their metal biosorption efficiency, as well as survival under stressed condition (i.e. presence of different heavy metal) after 5 days of incubation. It was observed that all the three-color forms could survive in this stress environment. This could be considered as a positive indication for this seaweed to be used for phycoremediation.

Terry and Stone [20] reported that living algae are known to adsorb more heavy metals due to metabolic uptake and continuous growth, e.g. cadmium and copper biosorption by *Scenedesmus abundans*. Sloof et al. [21] found that cadmium uptake by living *Selenastrum capricornutum* was rapid in the first adsorption stage, and then continued more slowly in the physiological metabolic stage. Resistance, accumulation and allocation of zinc in two ecotypes of the green alga *Stigeoclonium tenue* Kütz coming from different habitats with different heavy metal concentrations has been reported [22].

As seen in Table 1, heavy metal treated brown color form of *Kappaphycus alvarezii* could sorb maximum metals, i.e. cadmium, cobalt and chromium 3.064, 3.365 and 2.799 mg/100 fresh weight (f.wt.) respectively. The pale yellow color form treated with metal could take up only 0.961, 1.403 and 0.942 mg/100 g f.wt. of cadmium, cobalt and chromium respectively. Similarly, the green color form showed a biosorption of 2.684, 3.430 and 2.692 mg/100 g f.wt. of cadmium, cobalt and chromium respectively. However, the brown color form is the superior one in biosorption of cadmium and cobalt followed by

Table 1
Biosorption (mg/100 g f.wt.) of heavy metals by three-color forms of *Kappaphycus alvarezii*

| Color form | Metal | Control | Biosorption |
|---|---|---|---|
| Brown | Cd | 0.0225 | 3.064 |
| | Co | 0.0060 | 3.365 |
| | Cr | 0.0040 | 2.799 |
| Green | Cd | 0.0050 | 2.684 |
| | Co | 0.0040 | 3.430 |
| | Cr | 0.0045 | 2.692 |
| Pale yellow | Cd | 0.0310 | 0.961 |
| | Co | 0.0160 | 1.403 |
| | Cr | 0.0230 | 0.942 |

green and pale yellow color forms of this seaweed. Biosorption of cobalt in the brown color form is slightly less than the green one. The control contained very little amount of heavy metal reflecting that these are absorbed from the surrounding environment (seawater) where they are cultivated.

It is observed that all the three color forms of *Kappaphycus alvarezii* exhibited high cadmium, cobalt and chromium adsorption capacities when tested as fresh material. This is of extreme significance, as most algae do not survive in stressed environment containing heavy metals. The algae could not only survive up to a period of 5 days but also retained its unique ability to remove heavy metal ions. High adsorption efficiency of the algae, low biomass cost (mainly transportation cost), less labor input and high yields of biomass under cultivation makes this process of biosorption an effective, cheap and alternative technique for treatment of metal-bearing polluted marine environment.

Biosorption of cadmium and copper contaminated water by *Scenedesmus* abundans revealed that living algae could reduce cadmium from 10 to 0.10 mg/l in 36 h [20]. In the present study the brown color form of *Kappaphycus alvarezii* proved competent enough as it could adsorb 3.064 mg/100 g f.wt. cadmium.

Biosorption efficiency of brown algae, *Macrocystis pyrifera*, *Kjellmaniella crassiforia* and *Undaria pinnatifida* have been exploited for the recovery of lead and cadmium ions [23]. It has been reported that alkali-pretreated *Ulva* biomass showed the sorption capacity ($q_m$) from 60 to 90 mg $g^{-1}$ and the sorption affinity from 0.03 to 0.04 l $mg^{-1}$ at pH 7.8 while studying the uptake of cadmium, copper and zinc [24].

Studies on time dependent heavy metal uptake (by increasing or decreasing the contact time) could be taken up based on the positive results obtained here. Moreover, heavy metal uptake by biomass of these three-color forms would surely prove the efficiency of this seaweed as an effective biosorbent.

Cultivation and use of this seaweed as a biosorbent in different parts of the world could be embarked upon as a greener (environment friendly) and profitable approach leading to employment generation for coastal living people on one hand and cleaning the environment on the other hand. This is an eco-friendly option for remediation of various coastal waters and would help develop a new scope of research. Employing this seaweed for biosorption studies fulfils the parameters like eco-friendliness and economic feasibility as suggested by Mehta and Gaur [1].

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