



**Characterization of seaweeds collected from Baixada Santista littoral, and their potential uses as biosorbents of heavy metal cations**

**Caracterización de algas recolectadas en la Baixada Santista littoral y sus posibles usos como biosorbentes de cationes de metales pesados**

Y.J.O. Asencios<sup>1,2\*</sup>, L.M. Parreira<sup>2</sup>, E.A. Perpetuo<sup>2</sup>, A.L. Rotta<sup>2</sup>

<sup>1</sup>Center for Natural Sciences and Humanities, Federal University of ABC (UFABC), Av. dos Estados, 5001, 09210-580, Santo André, SP, Brazil.

<sup>2</sup>Institute of Marine Sciences, Federal University of São Paulo (UNIFESP), Rua. Dona Maria Máximo, 168, 11.030-100, Santos, SP, Brazil.

Received: September 9, 2021; Accepted: February 8, 2022

**Abstract**

This work aims to characterize biosorbent materials based on seaweed present on the littoral of Baixada Santista (São Paulo state, Brazil), and test their adsorption properties to remove heavy metal cations ( $\text{Co}^{+2}$ ,  $\text{Pb}^{+2}$ , and  $\text{Cd}^{+2}$ ) in water, aiming its future application *in situ* remediations degraded ecosystems. Three types of natural seaweed were studied: *Sargassum sp.*, *Ulva lactuca*, and *Padina sp.* These biosorbents were collected, pretreated, and modified by two chemical treatments: 1) Protonation with HCl, and, 2) Oxidation with  $\text{KMnO}_4$ . The preliminary results showed that the *Ulva lactuca* is promising for the adsorption of heavy metal cations in water, which is also very attractive due to its great availability in the ecosystem, and it is renewable. The biosorbents (natural and modified by chemical treatments) were characterized by Fourier Transform Infrared Spectroscopy (FTIR), and by Scanning Electron Microscopy (SEM). The best heavy metal removal values for the best biosorbent *Ulva lactuca* (after chemical treatment) were:  $65 \text{ mg.g}^{-1}$  for Co (II),  $71 \text{ mg.g}^{-1}$  for Cd(II), and  $78 \text{ mg.g}^{-1}$  for Pb(II).

**Keywords:** biosorption, Co (II), Pb(II), Cd (II); *Sargassum sp.*; *Ulva lactuca*; *Padina sp.*

**Resumen**

Este trabajo tiene como objetivo caracterizar materiales biosorbentes a partir de algas presentes en las playas de Baixada Santista (estado de São Paulo, Brasil) y probar sus propiedades de adsorción para eliminar cationes de metales pesados ( $\text{Co}^{+2}$ ,  $\text{Pb}^{+2}$  y  $\text{Cd}^{+2}$ ) en agua, con miras a su futura aplicación en remediaciones *in situ* de ecosistemas degradados. Se estudiaron tres tipos de algas naturales: *Sargassum sp.*, *Ulva lactuca* y *Padina sp.* Estos biosorbentes fueron recolectados, pretratados y modificados mediante dos tratamientos químicos: 1) Protonación con HCl, y 2) Oxidación con  $\text{KMnO}_4$ . Los resultados preliminares mostraron que la *Ulva lactuca* es prometedora para la adsorción de cationes de metales pesados en el agua, lo que también es muy atractivo por su gran disponibilidad en el ecosistema, y es renovable. Los biosorbentes (naturales y modificados por tratamientos químicos) se caracterizaron por Espectroscopía Infrarroja por Transformada de Fourier (FTIR) y Microscopía Electrónica de Barrido (SEM). Los mejores valores de remoción de metales pesados para el mejor biosorbente *Ulva lactuca* (después del tratamiento químico) fueron:  $65 \text{ mg.g}^{-1}$  para Co (II),  $71 \text{ mg.g}^{-1}$  para Cd(II) y  $78 \text{ mg.g}^{-1}$  para Pb(II).

**Palabras clave:** biosorción, Co (II), Pb (II), Cd (II); *Sargassum sp.*; *Ulva lactuca*; *Padina sp.*

\* Corresponding author. E-mail: yvan.jesus@unifesp.br

<https://doi.org/10.24275/rmiq/IA2600>

ISSN:1665-2738, issn-e: 2395-8472

## 1 Introduction

---

The advance of industrialization has resulted in several environmental problems, among them the presence of heavy metals in terrestrial and coastal environments (Lavado-Meza *et al.* 2021). Particularly, effluents containing heavy metal cations are a big challenge, since they are considered toxic to the environment and humans, also, they are difficult to diminish by conventional water treatment processes. Heavy metals are persistent in the environment as they cannot be degraded and, they accumulate through the food chain offering risks to the health of the population. Excess metals in organisms can cause changes in growth, disturbances in behavior and reproduction, unwanted cell mutation, etc. (Azevedo *et al.*, 2009).

The wastewater of the cadmium-nickel batteries, the metal plating industry, and mining, are principal sources of cadmium in water. Lead accumulates in the brain, bones, and kidney, therefore it is hazardous even in small amounts. Battery manufacture is the major source of lead pollution. According to the Agency for Toxic Substances and Disease Registry (ATSDR) of the U.S. Department of Health and Human Services, the permissible limits in drinking water are 0.005 mg.L<sup>-1</sup> for Cd (ATSDR, Cadmium), 5 mg L<sup>-1</sup> for Co (ATSDR, Cobalt) and 0 mg.L<sup>-1</sup> for Pb (ATSDR, Lead).

Baixada Santista is a region of the south coast of São Paulo state in Brazil; Santos city (part of Baixada Santista region) has the largest port in Latin America, and their neighboring cities have chemical and petrochemical industries, with growing oil production. In this context, finding technologies to diminish problems caused by activities of local factories (which favor the deterioration of water quality) is very important and necessary.

Biosorption is a process of metallic cation uptake where cations are adsorbed on the surface of biological solid material. Such solids can be for example seaweeds, lignocellulosic materials, among other macrophytes, they are usually used in their immobilized form (or commonly known as a dead plant). Biosorbents are characterized by functional groups (such as -OH, CO-OH, C=O, among others) present in the cell wall of the plant, they act as binding sites for the adsorption of cations, with a relatively uniform distribution. The biosorbents derived from seaweed are very attractive because they are environmentally friendly, not toxic,

abundant (some species, in some regions of Brazil), biodegradable, and renewable (Bulgariu *et al.* 2012ab; Lima and Asencios, 2021).

Ibrahim *et al.* (2016) reported the use of dead *Ulva lactuca* and its respective carbon, reaching values of heavy metal adsorption of: 62.5 mg.g<sup>-1</sup> (dead biomass) and 84.61 mg.g<sup>-1</sup> (carbon) for Cd(II), 68.92 mg.g<sup>-1</sup> (dead biomass) and 83.34 mg.g<sup>-1</sup> (carbon) for Pb (II). Asnaoui *et al.* (2015) reported the use of dead *Ulva lactuca*, they treated the dead biomass with an aqueous solution of nitric acid, they reported the biosorption capacity of *Ulva lactuca* for Cd(II) of 3.02 mg/g. Similarly, Ghoneim *et al.* (2014) used dead *Ulva lactuca* for Cd(II) removal, they reported the maximum removal value of 29.1 mg.g<sup>-1</sup>.

Despite there are various works that reported the use of dead *Ulva lactuca*, there is no consensus regarding the adsorption capacity (mg.g<sup>-1</sup>) for heavy metal removal. Even though the biomass can be classified as the same species, as reported by other authors, the uptake of heavy metal capacity may not be the same because it also depends on the chemical composition of the seaweeds, the concentration of functional groups presents on their cell wall, the close vicinity where seaweed is collected, the degree of pollution of its environment, among other factors.

In this work, some types of seaweed: *Sargassum sp.*, *Ulva lactuca*, and *Padina sp.*, were collected from the coast of Baixada Santista. *Ulva lactuca* and *Sargassum sp* are very abundant in this region. The seaweeds were treated aiming to improve their adsorption properties; therefore, two types of chemical treatments were studied: oxidation with KMnO<sub>4</sub> solution (to increase the number of carboxylic groups), and protonation with HCl (to increase the ion exchange capacity of the functional groups). The adsorption capacity of biosorbents was tested using aqueous solutions containing heavy metal cations (Cd<sup>2+</sup>, Co<sup>2+</sup>, and Pb<sup>2+</sup>). The biosorbents were characterized by Fourier Transformed Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). It is expected that the present paper may be useful for the scientific community and serve as a preliminary study for scale-up studies.

## 2 Materials and methods

### 2.1 Preparation of biosorbents

#### 2.1.1 Collection and pre-treatment of seaweeds

Seaweeds were collected from Pernambuco beach, near Mar Casado Island (in Guarujá city, São Paulo, Brazil). The samples were washed several times with clean water and with distilled water to remove visible contaminants as well as other marine organisms, shell remains, and other debris. The clean seaweeds were subsequently dried at 80°C (for 10 hours), macerated, and then sieved in N°40 mesh (0.42 mm <).

#### 2.1.2 Chemical Treatment 1: Oxidation with Potassium Permanganate (KMnO<sub>4</sub>)

In this procedure, 1 g of seaweed (obtained in the previous procedure, a)) was mixed with 5 ml of KMnO<sub>4</sub> (10 mM) at 25 °C (for 30 minutes, under continuous magnetic stirring of 500 rpm). After such treatment, the treated seaweed was filtered, washed (until reach neutral pH) with distilled water, and dried at 80 °C (for 8 hours).

#### 2.1.3 Chemical Treatment 2: Protonation with hydrochloric acid (HCl)

In this procedure, 1 g of seaweed (obtained in the previous procedure, a)) was mixed with 120mL of HCl (60 mM) at 25°C (for 30 minutes, under continuous magnetic stirring of 500 rpm). After such treatment, the treated seaweed was filtered, washed (until reach neutral pH) with distilled water, and dried at 80 °C (for 8 hours).

### 2.2 Preparation of effluents

*Synthetic effluent containing cobalt (Co<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>)*

Artificial effluents containing each heavy metal cation (Co<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>), 100 mg.L<sup>-1</sup>, separately, were prepared. Their respective soluble salts were used (Co(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O; Cd(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O; Pb(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O, respectively), and in distilled water.

### 2.3 Characterization of biosorbents

The biosorbents were characterized by Fourier Transform Infrared Spectroscopy (FTIR) analysis, and

by Scanning Electron Microscopy (SEM) analysis. Before this analysis, the biosorbents were dried at 60°C for 24h. The analyses were performed using a Varian-Agilent 640-IR FT-IR spectrometer in ATR mode. The SEM analyzes were performed in an FEI Quanta 250 Microscope using a current of 10kV.

### 2.4 Biosorption tests

These tests were carried out in beakers glass containing 25 ml of each effluent (previously prepared in 2.2)) and 0.0250 g of biosorbent (treated and untreated), this relation leads to a proportion of 1 gram of biosorbent per liter of effluent. Each suspension was stirred (at 500 rpm) for 3 hours at room temperature (25°C). Subsequently, the solution was filtered using a quantitative filter paper and stored for later analysis. The equilibrium time was predetermined as 1 hour. The pH of the effluent was that natural inherent of the solution (5.5-6).

The quantification of heavy metal cations in solution was performed by Optical Emission Spectrometry with ICP-OES plasma (Varian), using an analytical standard and a calibration curve. This analysis determined the amount of heavy metal present in the solution before and after the tests. The percentage of heavy metal removed by biosorbent (X%) was calculated as follows:  $X\% = ((M_0 - M_f)/M_0) \times 100$ ; where  $M_0$  and  $M_f$  are the concentrations (mg.L<sup>-1</sup>) of each metal at the beginning and the end of each experiment, respectively.

**Freundlich isotherm:** is based on the assumption that the surface of the adsorbent is energetically heterogeneous (composed of adsorption sites of differing energies). The linearized equation is:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (1)$$

where  $q_e$  (mg.g<sup>-1</sup>) means the amount of adsorbate (in mg) retained per gram of adsorbent.  $C_e$  is the concentration (mg.L<sup>-1</sup>) of adsorbate at equilibrium time (previously determined).  $K_f$  (related to the adsorption capacity) and  $n$  (related to adsorption intensity) are the Freundlich constants.

**Langmuir isotherm:** is based on the assumption that all active sites of the adsorbent are equivalent. Furthermore, this implies that the ability of a molecule to interact with the referred active site (on the surface) is independent of whether neighboring active sites are occupied or not. In this case, adsorption is restricted to

a monolayer. There are no lateral interactions between adsorbed molecules. The linearized equation is:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} * b} + \frac{1}{q_{\max}} C_e \quad (2)$$

where  $C_e$  and  $q_e$  were defined previously,  $q_{\max}$  ( $\text{mg.g}^{-1}$ , related to the maximum adsorption capacity) and  $b$  ( $\text{L.mg}^{-1}$ , related to adsorption energy) are Langmuir constants.

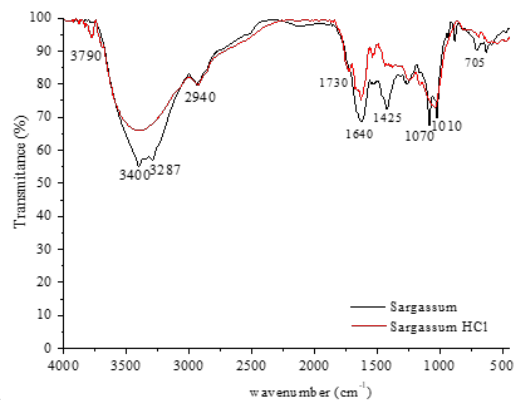
### 3 Results and discussion

#### 3.1 Characterization of biosorbents by Fourier Transform Infrared Spectroscopy (FTIR)

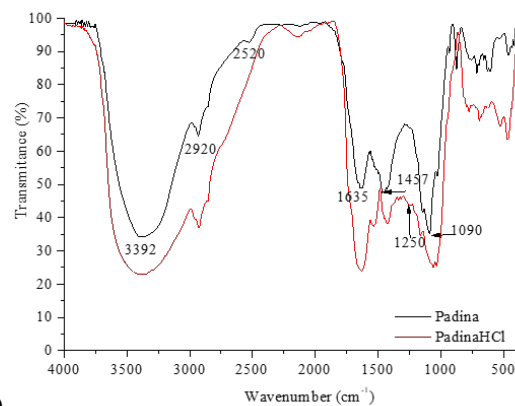
##### 3.1.1 Treatment with HCl

This treatment aimed to remove alkaline metal cations such as Na, Ca, K, and Mg, present in the polymeric structure of seaweed (Yang *et al.* (1999); Hayashi (2001)). The protonation is expected after this treatment, as  $\text{H}^+$  ions may substitute the alkaline metal cations that are bound to the functional groups present in the seaweed. The FTIR spectra of seaweed before and after HCl treatment are presented in Figures 1a-d.

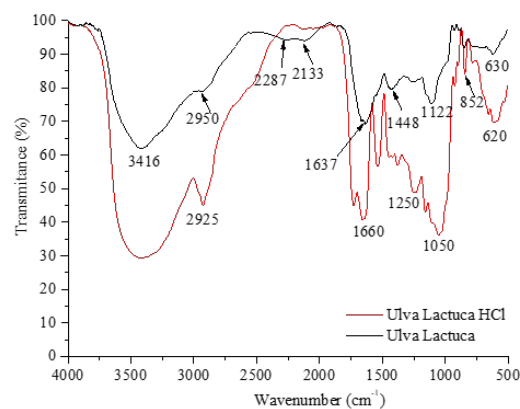
The FTIR spectrum of natural *Sargassum sp.* is shown in Fig. 1a. This spectrum shows a principal band around  $3400 \text{ cm}^{-1}$  that is assigned to -OH and -NH groups (Yang and Chen, 2008). This principal band is composed of two well-defined peaks, suggesting that these functional groups have some degree of organization among polymeric chains of seaweed. Fig. 1a also shows that the FTIR spectrum of *Sargassum sp.* is slightly different from *Sargassum sp.* treated with HCl. The main band located around  $3400 \text{ cm}^{-1}$  of the spectrum for this latter one is amorphous, which may indicate that the acid treatment may have modified the chemical environment of -OH and -NH groups, as a result of the removal of alkali cations by HCl, such cations are bond to the referred groups, and are present between the polymer chains of seaweed (Chen and Yang 2005).



a)



b)



c)

Figure 1. a) FTIR results of *Sargassum sp.* seaweed, before and after HCl treatment. b) FTIR results of HCl-treated and natural *Padina sp.* seaweed. c) FTIR results of natural and HCl-treated *Ulva lactuca* seaweed.

The peak at  $2940 \text{ cm}^{-1}$  is related to the -CH group (Yang and Chen, 2008), this peak is present in natural and in HCl-treated *Sargassum sp.* spectra. The chemical treatment did not change this peak's shape.

The peaks at  $1640 \text{ cm}^{-1}$  and  $1425 \text{ cm}^{-1}$  in the natural *Sargassum sp.* spectrum correspond to the

carboxylic acid group in coordination with metal ions (COO-M bonds, where M represents metal cations such as Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup>, which naturally exist in *Sargassum sp*) (Yang and Chen 2008; Chen and Yang 2005). The HCl treatment modified these peaks shape, which is coherent because the HCl treatment aimed to remove the Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> cations from this seaweed, thus demonstrating that the HCl treatment reached such purpose. It is expected that HCl treatment enhances the adsorption properties of seaweed to remove heavy metal cations.

In the spectrum of natural seaweed, the band starting from 1070 cm<sup>-1</sup> to 1010 cm<sup>-1</sup> is due to tension vibration of -CO of alcohol groups and carboxylic acids (Sheng *et al.* 2004; Yang and Chen, 2008). The acid treatment also slightly affected the intensity of these bands.

Figure 1b shows the Infrared spectrum (FTIR) of the HCl-treated and untreated *Padina sp* seaweed. In the spectrum of untreated seaweed, bands at 3392 and 1635 cm<sup>-1</sup> correspond to hydroxyl and carboxylic groups, respectively (Silva *et al.* 2005, Sari and Tuzen, 2009). The strong band at 3392 cm<sup>-1</sup> can also be attributed to the flexion vibrations of the groups -OH and -NH. In the spectrum of untreated *Padina sp* seaweed, the band at 1090 cm<sup>-1</sup>, was attributed to the CO bond of alcohol groups and carboxylic acids (tension vibration) (Sheng *et al.* 2004; Yang and Chen, 2008). The HCl treatment modified the intensity peaks located around 1090, 1635, and 3392 cm<sup>-1</sup>; probably caused by the lixiviation of the light metal cations, caused by the acidic treatment.

The band at 2920 cm<sup>-1</sup> can be attributed to vibrations of the -CH chains of the seaweed. The band at 1635 cm<sup>-1</sup> corresponds to the asymmetric tension vibration of the carboxylic group, and the symmetrical tension vibration band at 1457 cm<sup>-1</sup>. These bands suffered changes after the acidic treatment, as well. The spectra of untreated *Padina sp*. present a very small band around 1250 cm<sup>-1</sup>, which is characteristic of the group -SO<sub>3</sub> in tension vibration, this group is characteristic of polysaccharide sulfonic acid such as fucoidan. After HCl treatment, the band around 1250 cm<sup>-1</sup> became more intense, probably due to such acidic treatment exposing such a -SO<sub>3</sub> group.

In Figure 1c, the broadband at 3416 cm<sup>-1</sup> shown in the FTIR spectrum of *Ulva lactuca* is due to the O-H bond of hydroxyl groups (the prolongation of the vibration from intermolecular and intramolecular hydrogen bond). After HCl-treatment this band was widened, meaning that the treatment affected these groups, as expected.

The weak band located at 2950 cm<sup>-1</sup> results from stretching of C-H groups, after HCl treatment, this peak became a stronger one at 2925 cm<sup>-1</sup>. The band at 1637cm<sup>-1</sup> is attributed to the presence of uronic acids and the vibration of asymmetric stretching of the carboxyl groups; this band was intensified by HCl-treatment and detected at 1660 cm<sup>-1</sup>.

The band located at 1448 cm<sup>-1</sup> is due to the stretching of the carboxyl, ketones, and aldehydes groups. The band at 1122 cm<sup>-1</sup> is attributed to the sulfate groups and the glycosidic bonds, these bands were intensified a lot after HCl-treatment (band located at 1050 cm<sup>-1</sup>). The spectra show the band at 852 cm<sup>-1</sup>, which would correspond to C-O-S flexion vibrations in the axial or equatorial positions (C-2 or C-3, respectively), which was intensified after HCl-treatment.

Similar to the observed in the *Padina sp* seaweed, the spectrum of treated *Ulva lactuca* seaweed presents a discrete band at 1250 cm<sup>-1</sup>, which is characteristic of the group -SO<sub>3</sub> in tension vibration, this group is characteristic of the polysaccharide sulfonic acid, such as fucoidan. This band became more intense after HCl treatment.

### 3.1.2 FTIR Analysis of biosorbents treated with KMnO<sub>4</sub>

The FTIR spectra of seaweed before and after KMnO<sub>4</sub> treatment are shown in Figures 2a-c. In resume, the long band between 3500-3300 cm<sup>-1</sup>, common for all seaweed profiles, indicates the presence of the -OH group of the corresponding C-O-H bond. All seaweed profiles showed a strong signal, especially *Padina sp* seaweed, at intervals between 1300-1000 cm<sup>-1</sup> and, another around 1650 cm<sup>-1</sup>, which are attributed to CO bond (Basha *et al.* 2008). These peaks are related to the presence of functional groups such as alcohols, carboxylic acids, and alginic acids; the latter being related to brown algae. These groups play an important role in the adsorption of the cations in water. All seaweed also showed a small peak around 2900 cm<sup>-1</sup> which corresponds to the vibration of the asymmetric CH<sub>2</sub> bond.

Fig. 2a shows the FTIR spectra of *Sargassum sp.* before and after KMnO<sub>4</sub> treatment. As described in the previous section, the spectrum of the untreated *Sargassum sp.* shows bands around 3400 cm<sup>-1</sup> that are assigned to -OH (and -NH groups) (Yang and Chen, 2008). Additionally, the peaks at 1640 cm<sup>-1</sup> and 1425-1467 cm<sup>-1</sup> in the natural *Sargassum sp.* correspond to the coordination of the carboxylic acid group with a)

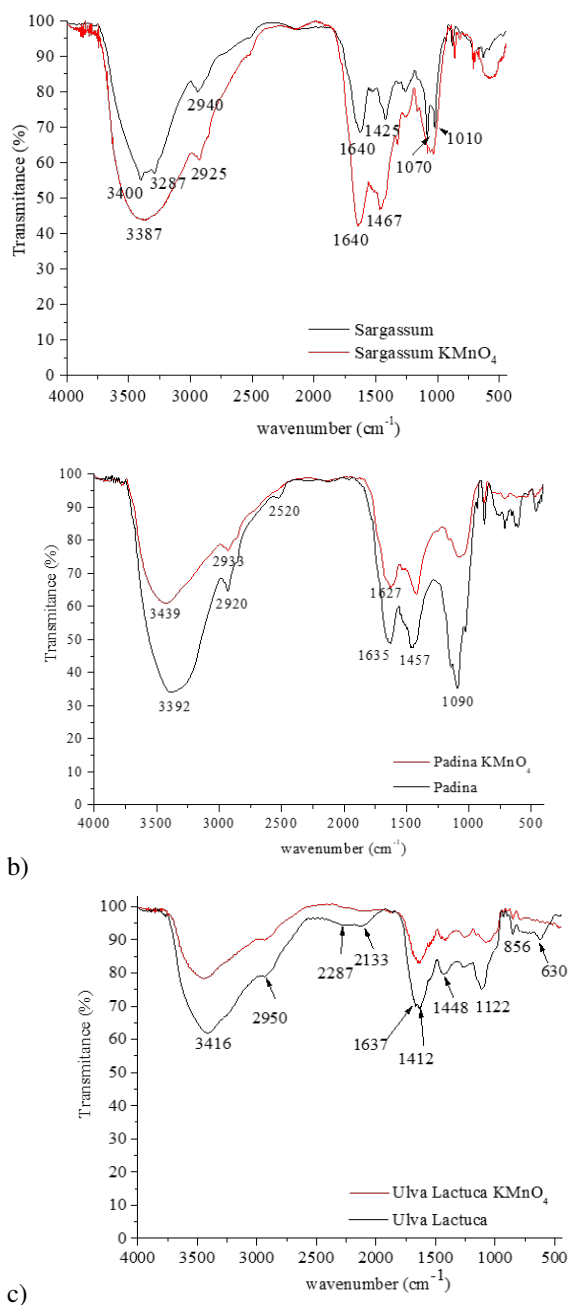


Figure 2. a) FTIR results of seaweed: a) *Sargassum sp.* before and after  $\text{KMnO}_4$  treatment. b) *Padina sp.* before and after  $\text{KMnO}_4$  treatment. c) *Ulva lactuca* before and after  $\text{KMnO}_4$  treatment.

light metal cations (such as  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ ) which naturally exist in seaweeds (Yang and Chen 2008; Chen and Yang 2005)). The bands detected from  $1070\text{ cm}^{-1}$  to  $1010\text{ cm}^{-1}$  are due to tension vibration of  $-\text{CO}$  of alcohol groups and carboxylic acids ( $-\text{COOH}$ ); in the spectrum of natural seaweed (Sheng

et al. 2004; Yang and Chen, 2008). After treatment with  $\text{KMnO}_4$ , the shape of these bands changed, thus indicating that the basic treatment influenced the chemical environment of these groups ( $-\text{COOH}$ ,  $-\text{OH}$ , and  $-\text{NH}$ ) (for example by lixiviation of metal cations or by oxidation of these groups).

The peak at  $2940\text{ cm}^{-1}$  is related to the  $-\text{CH}$  group (Yang and Chen, 2008), the shape of this peak also changed in *Sargassum sp.* after  $\text{KMnO}_4$ -treatment.

Figure 2b shows the Infrared spectrum (FTIR) of the  $\text{KMnO}_4$ -treated and untreated *Padina sp.* As previously explained, in the spectrum of untreated seaweed, the bands at  $3392$  and  $1635\text{ cm}^{-1}$  correspond to hydroxyl and carboxylic groups, respectively (Silva et al. 2005; Sari and Tuzen, 2009). The band at  $1635\text{ cm}^{-1}$  corresponds to the asymmetric tension vibration of the carboxylic group ( $-\text{COOH}$ ), and the symmetrical tension vibration band is detected at  $1457\text{ cm}^{-1}$ . The wide and intense band at  $3392\text{ cm}^{-1}$  can also be attributed to the flexion vibrations of the groups  $-\text{OH}$  and  $-\text{NH}$ . The band at  $1090\text{ cm}^{-1}$ , was attributed to the  $\text{C}=\text{O}$  bond of alcohol groups ( $\text{C}-\text{OH}$ ) and carboxylic acids ( $-\text{COOH}$ ) (tension vibration) (Sheng et al. 2004; Yang and Chen, 2008). The band at  $2920\text{ cm}^{-1}$  can be attributed to vibrations of the  $-\text{CH}$  chains of the seaweed. The  $\text{KMnO}_4$  treatment modified the intensity (and the shape) of all these peaks (located around  $1090$ ,  $1635$ ,  $2920$ , and  $3392\text{ cm}^{-1}$ ). This is probably, due to  $\text{KMnO}_4$  treatment causing oxidation reactions, as well as lixiviation of the light metal cations present in the natural seaweed.

The FTIR profiles of seaweeds suffer some changes after treatment with  $\text{KMnO}_4$ . In the samples of *Ulva lactuca* and *Padina sp.* the FTIR profiles showed a narrowing of the signals. According to the reported by Jeon et al. (2002) who treated alginic acid with  $\text{KMnO}_4$ , the difference of the signal around  $1700\text{ cm}^{-1}$ , which indicates the presence of the  $\text{CO}$  bond (from the carboxylic group) that can be associated with the increase of this group due to oxidation reaction. The difference observed in the band at  $2850\text{--}3000\text{ cm}^{-1}$ , which indicates the existence of the  $\text{CH}$  bond, suggests that there was oxidation of the hydroxyl group, increasing the concentration of  $\text{CH}$  bonds. The seaweed *Sargassum sp.* showed the stronger intensity of these bands after treatment.

The spectrum of untreated *Ulva lactuca* and *Padina sp.* presents a very small band at  $1250\text{ cm}^{-1}$ , which is characteristic of the group  $-\text{SO}_3$  in tension vibration, this group is characteristic of the polysaccharide sulfonic acid, such as fucoidan.

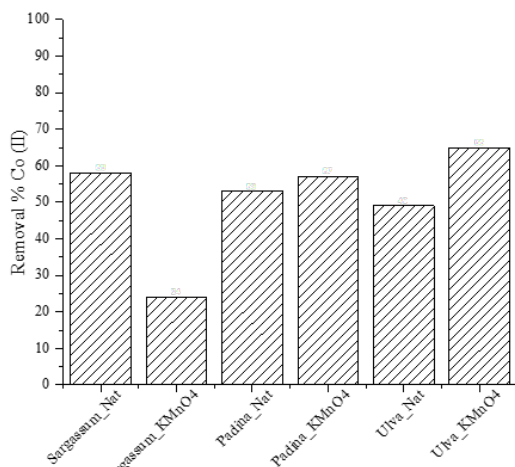


Figure 3. Results of Co (II) removal by KMnO<sub>4</sub>-treated and natural seaweeds (untreated).

### 3.2 Biosorption tests

#### 3.2.1 Biosorption tests over seaweed treated with KMnO<sub>4</sub>

The chemical treatments applied to the seaweeds aimed to improve their adsorption properties, moreover, more stable adsorbents are expected (Mohammad *et al.* 2011). The results for the removal of Co(II) from water by the seaweeds are shown in Figure 3. According to these results, the natural samples of seaweeds showed the capacity to remove cobalt cations from the aqueous solution.

The chemical treatment with KMnO<sub>4</sub> caused different effects on every seaweed that influenced the removal of Co(II) capacity. For instance, KMnO<sub>4</sub> treated-*Sargassum sp.* removed 24 % of Co(II), while the removal reported for KMnO<sub>4</sub> treated-*Padina sp.* and KMnO<sub>4</sub> treated-*Ulva lactuca* was 57% and 65%, respectively. These values showed clearly that the treatment of seaweed with KMnO<sub>4</sub> increased the

removal capacity of Co(II) of *Padina sp.* and *Ulva lactuca*, but *Sargassum sp.* This treatment was more beneficial for *Ulva lactuca*, this seaweed seems to be more promising for this application.

Some studies have shown that the treatment of seaweeds with KMnO<sub>4</sub> can increase the adsorption capacity due to the increase of the carboxylic groups, which is one of the main groups involved in the biosorption process, in this case in the removal of Co (II) from water (Basha *et al.*, 2008). According to the FTIR spectra (Figure 2), the carboxyl groups are present in the seaweeds and the treatment with KMnO<sub>4</sub> affected the corresponding signals in the spectrum. This fact may be the reason for the higher removal percent obtained by *Ulva lactuca* and *Padina sp.*

Despite the FTIR spectrum of KMnO<sub>4</sub>-treated *Sargassum sp.* showing strong peaks related to carboxyl groups, the chemical treatment was not beneficial. Probably the KMnO<sub>4</sub> solution employed in the treatment of *Sargassum sp.* was in excess, which results in oversaturating the number of adsorption sites, thus decreasing the removal % of Co (II).

Henriques *et al.* (2019) found that the biosorption process over *Ulva lactuca* was efficient in removing various toxic metals (Hg, Cd, Pb, Cr, Ni, As, Cu, Mn) from water in concentrations of 0.05 to 2 mg.L<sup>-1</sup>. Their best results were reported with a proportion of biosorbent of 6 g.L<sup>-1</sup>. The proportion of biosorbent used in the present research was 1 g.L<sup>-1</sup> (see in Section 2.1 of Materials and Methods). The results for *Ulva lactuca* shown in Figure 3 can be considered more efficient than those reported by Henriques *et al.* (2019), furthermore, the Cobalt removal (%) reached 65% with an initial solution of 100 mg.L<sup>-1</sup> of Co (II) over only 1 g.L<sup>-1</sup> of biosorbent. In our study, only one dosage of adsorbent was tested, the optimization of the dosage of biosorbent should be carried out in further studies.

Table 1. Langmuir and Freundlich parameters for the adsorption of Co(II) on seaweeds.

Adsorption Co(II)	Langmuir Parameters (Co)			Freundlich Parameters (Co)		
	$q_m$ (mg/g)	$b$ (L/mg)	$R^2$	$K_f$	$n$	$R^2$
<i>Padina sp.</i>	56.8182	0.2973	0.9937	13.6962	3.0349	0.9519
<i>Sargassum sp.</i>	59.8802	0.7952	0.9951	23.1100	3.9809	0.9818
<i>Ulva lactuca</i>	51.8135	0.2830	0.9931	11.2642	2.7196	0.9851
Padina_KMnO <sub>4</sub>	60.9756	0.3527	0.9922	17.2306	3.0572	0.9681
Sargassum_KMnO <sub>4</sub>	24.5700	0.5306	0.9967	13.3721	7.3046	0.9937
Ulva_KMnO <sub>4</sub>	65.3595	2.0400	0.9995	26.9712	3.4578	0.9099

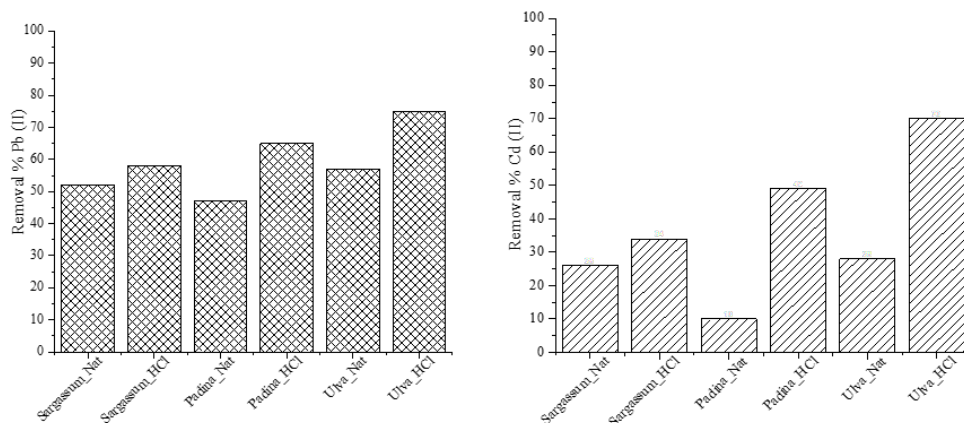


Figure 4. Results for Cd(II) and Pb(II) removal by HCl-treated and natural seaweed (untreated).

Table 1 shows the correlation of the biosorption of Co(II) results with Freundlich and Langmuir models (equations 1 and 2). According to this table, the Langmuir model fits the data best (highest correlation coefficient  $R^2$ ). According to the Langmuir model, the adsorption process using seaweeds takes place by the formation of a monolayer of adsorbate ( $Co^{2+}$ ) on the surface of the adsorbent (which is reasonable since the chemisorption is involved in this process, as will be discussed in further paragraphs) (Asencios *et al.* 2012). *Ulva\_KMnO<sub>4</sub>* is the best biosorbent for Co(II) removal among all materials tested in this work.

### 3.2.2 Biosorption tests over seaweed treated with HCl

Figure 4 shows the removal % of Cd(II) from an aqueous solution using seaweeds. According to these values, the treatment of seaweeds with HCl increased the adsorption capacity of seaweeds to remove Cd(II) from water. *Padina sp* and *Ulva lactuca* recorded the best removal values. *Ulva lactuca* is the most promising biosorbent for such an application.

Figure 4 also shows the removal % of Pb(II) from an aqueous solution using seaweeds. According to these results, all the natural seaweeds presents good adsorption properties to remove Pb(II). The chemical treatment with HCl affected the adsorption of Pb(II) by the seaweeds. The HCl treatment was more beneficial for *Ulva lactuca*, as the chemical treatment, in this case, led to the best removal Pb(II) values.

Comparing the results of removal % of Cd(II) and Pb(II) using seaweeds (treated and untreated), it is clear that seaweeds have more affinity for Pb(II) than for Cd(II). This affinity may be due to factors such as the ionic radius such as 1.19 Å for  $Pb^{2+}$  which

is greater than 0.95 Å for  $Cd^{2+}$ , and the degree of hydration of aqua-ions which is lower for  $Pb^{2+}$  than for  $Cd^{2+}$  that would be favoring the adsorption of Pb(II) on the functional groups present on the surface of the seaweeds (Asencios *et al.* 2012).

The chemical treatments of biosorbents influence their adsorption capacities by modifying the functional groups responsible for the adsorption of heavy metal cations. The effect of chemical treatment on the adsorption capacity of biosorbents is not clear yet, because in some cases it increases or decreases adsorption properties. In the present study the chemical treatment with  $KMnO_4$  improved the adsorption capacity of *Padina sp* and *Ulva lactuca* (probably due to the increase in the carboxyl groups in the cell wall (Basha *et al.* 2008)), but not *Sargassum sp* (see Fig. 3). Similarly, the chemical treatment using diluted HCl was beneficial for the adsorption capacity of all seaweeds of this study (probably such treatment caused the protonation of the biomass by releasing the pre-existing  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$ , and  $Mg^{2+}$  at the binding sites (Davis *et al.* 2003)).

In general, the results described in the present article indicated that *Ulva lactuca* is promising for the biosorption of heavy metal cations (Cd(II), Pb(II)) from water. It is important to mention that such seaweed is found in great abundance, compared to the other seaweeds. Furthermore, *Ulva lactuca* is also an indicator of pollution (in the collection area), and its abundance would facilitate its possible application on a large scale in future water treatment studies. *Ulva lactuca* besides its high growth potential, *Ulva lactuca* offers an excellent capacity for nutrient assimilation, especially regarding high rates of ammonium ( $NH_4^+$ ) assimilation, and grows well in eutrophic waters



Table 2. Langmuir and Freundlich parameters for the adsorption of Cd on seaweeds.

Adsorption Cd(II)	Langmuir Parameters (Cd)			Freundlich Parameters (Cd)		
	$q_m$ (mg/g)	$b$ (L/mg)	$R^2$	$K_f$	$n$	$R^2$
<i>Padina sp</i>	10.4932	0.3148	0.9759	7.3892	18.3688	0.6707
<i>Sargassum sp</i>	26.2467	0.6447	0.9989	13.4679	6.5445	0.9951
<i>Ulva lactuca</i>	28.4091	0.4156	0.9948	10.2967	4.1929	0.9691
Padina_HCl	10.7527	2.3134	0.9950	16.7109	3.3422	0.9635
Sargassum_HCl	34.7222	0.2595	0.9839	14.7197	5.6850	0.9711
Ulva_HCl	71.9424	1.0859	0.9982	26.0315	3.0656	0.9907

Table 3. Langmuir and Freundlich parameters for the adsorption of Pb on seaweeds.

Adsorption Pb(II)	Langmuir Parameters (Pb)			Freundlich Parameters (Pb)		
	$q_m$ (mg/g)	$b$ (L/mg)	$R^2$	$K_f$	$n$	$R^2$
<i>Padina sp</i>	51.0204	0.2068	0.9886	13.0828	3.2140	0.9705
<i>Sargassum sp</i>	55.2486	0.3627	0.9916	18.5481	3.9936	0.9716
<i>Ulva lactuca</i>	61.3497	0.3228	0.9933	13.5738	2.4582	0.9727
Padina_HCl	69.9301	0.3115	0.9852	19.2975	3.0102	0.9749
Sargassum_HCl	58.8235	0.3778	0.9861	19.9986	3.8417	0.9754
Ulva_HCl	78.1250	0.8421	0.9941	27.6439	3.0544	0.9889

(which is an advantage for bioremediation purposes) (Nielsen *et al.* 2012).

Tables 2 and 3 show the correlation of the biosorption of Cd(II) and Pb(II) results with Freundlich and Langmuir models (equations 1 and 2). According to this table, the Langmuir model fits the data best (highest correlation coefficient  $R^2$ ). According to the Langmuir model, the adsorption process using seaweeds takes place by the formation of a monolayer of adsorbate ( $Cd^{2+}$  and  $Pb^{2+}$ , in each case) on the surface of the adsorbent (which is reasonable since the chemisorption is involved in this process, as will be discussed in further paragraphs) (Asencios *et al.* 2012). Among the natural biosorbents *Ulva lactuca* is superior to *Padina sp* and *Sargassum sp* to remove Cd(II) and Pb(II), whereas, among chemically modified biosorbents Ulva\_HCl is superior to the others biosorbents.

### 3.2.3 Possible mechanisms of biosorption of cations of heavy metals on seaweeds

Seaweeds have good properties to remove heavy metal cations given the structure of their cell wall, which has functional groups that act through mechanisms based on electrostatic attraction and complexation to chemisorb metallic ions in water. Some seaweeds have large amounts of polysaccharides (as in the case of the Red-algae), and alginate (as in the case of the Brown-

algae), which enhances the biosorption process (Davis *et al.*, 2003).

Metallic cations chemisorb on functional groups such as carboxylic (-CO-OH), amine (-NH<sub>3</sub>), hydroxyl (-OH), phosphate (-PO<sub>3</sub>), among other groups present on the cell wall of the biosorbents (Montazer-Rahmati *et al.* 2011). The biosorption capacity of seaweeds is directly related to the amount of these functional groups. The cell wall is composed, among various constituents, of alginate and fucoidan which content the referred functional groups in their structure (Davis *et al.* 2003). The carboxylic groups are one of the most important active sites (Crist *et al.*, 1981).

Gardea-Torresdey *et al.* (1990) conducted experiments in which the carboxylic groups were esterified with methanoic acid (which blocks the action of the carboxyl groups present in the biomass). This treatment decreased the biosorption of Cu and Al by biomass. Such results led the authors to conclude that the carboxylic group is the main responsible for the removal of these metals from water.

Cid *et al.* (2015) studied *Durvillaea antarctica*, and used methanol in acidic media to block the carboxyl groups present in the biomass. They found that Cu(II) biosorption decreased 43.3% of the initial value. Fourest *et al.* (1996) studied *Sargassum fluitans*, they neutralized the carboxyl acid groups of the biomass by treatment with propylene oxide, and they reported a 50% of reduction of the biosorption capacity of Cd(II)

and Pb(II) ions.

Similar results were reported by Romero-González *et al.* (2001), who studied the nature of the functional group responsible for Cd(II) adsorption on seaweeds. In this case, the carboxyl groups were esterified using acidic methanol solution. The results of adsorption experiments showed that the biosorption of Cd(II) was reduced from 95% to 17%, suggesting that Cd (II) binds principally to carboxyl groups. Furthermore, the residual sorption of Cd(II) after esterification indicated the presence of other cadmium binding sites; as seaweed contains poly-uronates (alginates).

The carboxylic groups are generally the most abundant acidic functional group in the brown algae (ex. *Sargassum*); they constitute typically greater than 70% of active sites, in dried brown biomass. The adsorption capacity of the seaweed is directly related to the presence of these sites on the alginate polymer. Alginate polymer comprises up to 40% of the weight of the dried seaweed biomass. (Davis *et al.* 2003)

The role of carboxylic groups (-COOH) in the adsorption process has been demonstrated by Fourest *et al.* (1996)ab in their studies of removal of Cd (II) and Pb (II) over dried *Sargassum* seaweed. According to the report, Fourier-transformed infrared (FTIR) spectral analyses showed that cadmium biosorption on *Sargassum* arises from bridging or bidentate complex formation with the carboxylate groups of the alginate, this model was consistent with the “egg-box” model proposed previously by Rees *et al.* (1982) and Thom *et al.* (1982). Sulfonic acid groups play a secondary role (except when adsorption takes place at low pH); on the other hand, despite hydroxyl groups are less abundant, they are present in all polysaccharides, and become negatively charged at pH>10, therefore, playing a secondary role in metal binding at low pH, as well (Davis *et al.* 2003).

Although there is not a consensus about the mechanism of adsorption over biosorbents (seaweeds in this case), it is known that metal biosorption follows complex mechanisms including ion exchange (in which metallic cations compete with protons for negatively charged sites) and complexation with oxidation/reduction reactions. Owing to the complex composition of seaweed, more than one of these mechanisms are involved, these mechanisms are influenced by the type of seaweed, the type of metal ion, and the pH of the aqueous solution (Naja *et al.* 2010).

Studies reported by Romera *et al.*(2007) and Bulgariu *et al.* (2012)a,b about the adsorption process of heavy metal cations suggested that the dissociation

degree of carboxylic acid present in the cell wall is strongly affected by the pH of the aqueous solution. Furthermore, pH affects the speciation and solubility of metal ions. Low pH decreases the adsorption capacity because at this condition a large amount of H<sup>+</sup> ions in the solution compete with the cation metal (present in the solution) for binding sites (Sheng *et al.*, 2004). In the case of the present manuscript, all the experiments were carried out under the natural pH of the effluent, in the range of 5.5-6 (as described in Section 2.3 of Materials and Methods), and it was observed during our experiments that the number of hydrogen ions in the solution increased at the final of the adsorption experiments because a slight decrease of the pH of the solution was observed. This decrease may be related to the ion metal removal, and probably indicated that the proton exchanges mechanism occurred.

Bulgariu *et al.* (2012) studied the biosorption of Cd(II) using *Ulva lactuca*. An FTIR analysis of the biosorbent before and after adsorption tests with Cd(II) (in aqueous solution) showed that the bands correspond to the carboxylic (-COOH), hydroxyl (-C-OH) and amino (-NH<sub>3</sub>) groups suffered changes. These analyzes indicate that these groups have a role in the adsorption mechanism. Similar to the other reports cited above, the carboxylic group was found to be the main responsible for the biosorption process.

Given all these studies referred, we can suggest that the same effects occurred during the biosorption of our seaweeds (*Padina sp.*, *Ulva lactuca*, and *Sargassum sp.*). The purpose of washing the seaweeds with diluted HCl was to produce the displacement of light metals by protons on the biomass surface. Moreover, the acid treatment can dissolve polysaccharide compounds of the external cell wall and create additional bonding sites of alginate, as was seen in the seaweeds studied in this article (*Padina sp.*, *Ulva lactuca*, and *Sargassum sp.*) the HCl treatment (under our experimental conditions) is a benefit.

In the case of KMnO<sub>4</sub> treatment, such a process benefits the biosorption properties of *Ulva lactuca* and *Padina sp.* However, the contrary effect was observed for *Sargassum*, as it seemed to lost active sites after contact with KMnO<sub>4</sub>, which was similar to the found by Figueira *et al.* (2000) when the treatment was with NaOH. This is probably owing to the treatment with an excess of oxidant. Therefore, to a large extent, the polysaccharide compounds may have been dissolved off the external cell wall. In agreement with the reported by Romera *et al.* (2006; 2007), the pretreatment of the biomass does not always improve

adsorption properties.

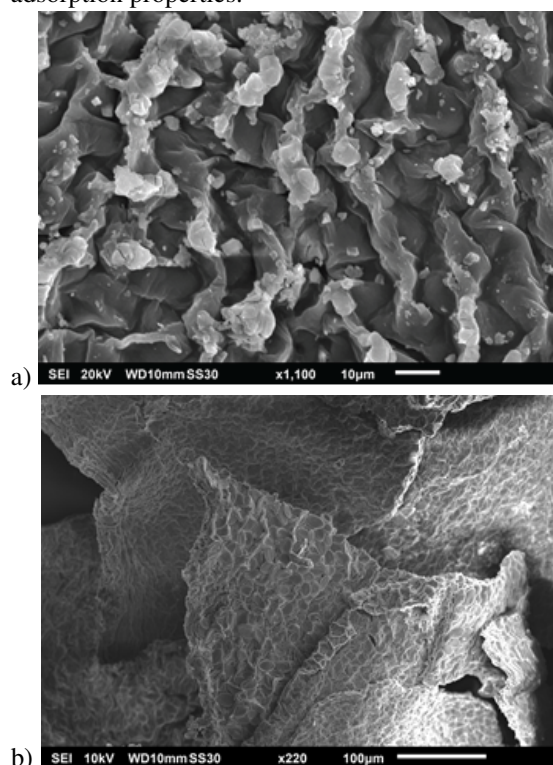


Figure 5. a) Scanning electron microscopy (SEM) image of natural *Ulva lactuca* seaweed. b) Scanning electron microscopy (SEM) image of *Ulva\_HCl*.

In some cases, the adsorption capacity decreases by the solubilization of different components of the biomass.

### 3.3 Characterization of biosorbents by Fourier Transform Infrared Spectroscopy (FTIR)

Figures 5a and 5b show the SEM images of *Ulva lactuca* and *Ulva\_HCl* seaweeds. This seaweed was chosen because it registered the best performance in the biosorption tests. The image of natural *Ulva lactuca* seaweed showed the morphology of continuous and regular sheets covered by fine and solid blocks (distributed in the form of wires). According to EDX analysis in this region, solid blocks correspond to MgO.

The HCl treatment removed these solid blocks, as can be observed in Figure 5b, the morphology of *Ulva lactuca* is clean, and uniform spread and continuous sheets can be observed.

## Conclusions

- The natural seaweeds collected from the littoral of Baixada Santista (*Ulva lactuca*, *Sargassum sp.*, and *Padina sp.*) were characterized by Infrared Spectroscopy (FTIR), showing the main signals of -OH, COOH, C-OH that are characteristic for each type of seaweed. The chemical treatment of these seaweeds with HCl and  $\text{KMnO}_4$  influenced the chemical environment of these functional groups. This was detected through FTIR signals.
- The treatment of seaweeds with HCl improved the adsorption capacity to remove heavy metal cations (Cd(II) and Pb(II)) of all the seaweeds collected. The most promissory seaweed for this process under HCl treatment was *Ulva lactuca*. The seaweeds have more preference to remove Pb(II) than Cd(II) from water.
- The treatment of seaweeds with  $\text{KMnO}_4$  slightly improved the removal of heavy metal cations (Cd(II)). This treatment was not beneficial for *Sargassum sp.* Under these conditions, *Ulva\_KMnO4* was the best sample for this process.
- The results of these preliminary tests demonstrated that biosorption technologies based on seaweed on *Ulva lactuca* (natural or chemically modified) are very attractive, as it is environmentally friendly, it is renewable, and it is abundant in the collection site.
- The chemical treatments appointed in this report have a cost that may not be attractive from an economic point of view (special at a large scale). The improvements of the adsorption capacity of seaweed must be very significant to think about in the implementation of chemical treatment of seaweed for wastewater treatment. Additionally, the chemical treatment should be considered in case of the improvement of the physical stability of seaweed granules is needed, in special for column tests.

### Acknowledgements

The authors thank the Brazilian National Council for Scientific Development (CNPq, Grant N° 407097/20) and to Multiuser Center of Federal University of ABC (CEM-UFABC).

## References

---

- Asencios Y.J.O. , Sun-Kou M. R. (2012). Synthesis of high-surface-area  $\gamma$ -Al<sub>2</sub>O<sub>3</sub> from aluminum scrap and its use for the adsorption of metals: Pb(II), Cd(II) and Zn(II). *Applied Surface Science* 258 (24), 10002-10011 <https://doi.org/10.1016/j.apsusc.2012.06.063>
- Azevedo, J.S., Fernandez W.S., Farias L.A., Fávoro, D.T.I. , Braga E.S. (2009). Use of *Cathorops spixii* as bioindicator of pollution of trace metals in the Santos Bay, Brazil. *Ecotoxicology* 18 (5), 577-586. <https://doi.org/10.1007/s10646-009-0315-4>
- Asnaoui, H., Laaziri, A., Khalis, M. (2015). Study of the kinetics and the adsorption isotherm of cadmium(II) from aqueous solution using green algae (*Ulva lactuca*) biomass. *Water Science and Technology* 72 (9), 1505-1515. <https://doi.org/10.2166/wst.2015.359>
- ATSDR (Lead) [https://www.atsdr.cdc.gov/csem/leadtoxicity/safety\\_standards.html](https://www.atsdr.cdc.gov/csem/leadtoxicity/safety_standards.html)
- ATSDR (Cadmium) <https://www.atsdr.cdc.gov/csem/cadmium/Safety-Standards.html>
- ATSDR(Cobalt) <https://wwwn.cdc.gov/TSP/ToxProfiles/ToxProfiles.aspx?id=373&tid=64#bookmark01>
- Basha, S., Murthy, Z., Jha, B. (2008). Biosorption of hexavalent chromium by chemically modified seaweed, *Cystoseira indica*. *Chemical Engineering Journal* 137 (3), 480-488. <https://doi.org/10.1016/j.cej.2007.04.038>
- Bulgariu, D., Bulgariu, L. (2012). Equilibrium and kinetics studies of heavy metal ions biosorption on green algae waste biomass. *Bioresource Technology* 103, 1, 489-493, 2012. <https://doi.org/10.1016/j.biortech.2011.10.016>
- Bulgariu, L., Lupea, M., Macoveanu, M. (2012). Biosorption of Cd(II) from aqueous solution on marine green algae biomass. *Environmental Engineering and Management Journal* 11 (3), 607-615. <https://doi.org/10.30638/eemj.2012.076>
- Chen P. J., Yang Lei (2005). Chemical modification of *Sargassum sp.* for prevention of organic leaching and enhancement of uptake during metal biosorption. *Industrial Engineering and Chemistry Research* 44, 9931-9942 <https://doi.org/10.1021/ie050678t>
- Cid H., Ortiz C., Pizarro J., Barros D., Castillo X., Giraldo L., Moreno-Piraján J.C. (2015). Characterization of copper (II) biosorption by brown algae *Durvillaea antarctica* dead biomass. *Adsorption*. 21(8), 645-658. <https://doi.org/10.1007/s10450-015-9715-3>
- Crist R. H., Oberholser K., Shank N., Nguyen M. (1981). Nature of bonding between metallic ions and algal cell walls. *Environmental Science & Technology* 15 (10), 1212-1217. <https://doi.org/10.1021/es00092a010>
- Davis, T., Volesky, B., Mucci, A. (2003). A review of the biochemistry of heavy metal biosorption by brown algae. *Water Research* 37, 4311-4330. [https://doi.org/10.1016/S0043-1354\(03\)00293-8](https://doi.org/10.1016/S0043-1354(03)00293-8)
- Figueira, M. M., Volesky, B., Ciminelli, V. S. T., and Roddick, F. A. (2000). Biosorption of metals in brown seaweed biomass. *Water Research* 34, 196-204. [https://doi.org/10.1016/S0043-1354\(99\)00120-7](https://doi.org/10.1016/S0043-1354(99)00120-7)
- Fourest E., Volesky B. (1996). Contribution of sulfonate groups and alginate to heavy metal biosorption by the dry biomass of *Sargassum fluitans*. *Environmental Science & Technology* 30 (1), 277-282. <https://doi.org/10.1021/es950315s>
- Fourest, E., Serre, A., Roux, J. C. (1996). Contribution of carboxyl groups to heavy metal binding sites in fungal wall. *Toxicological & Environmental Chemistry* 54, 1-4, 1-10. <https://doi.org/10.1080/02772249609358291>
- Gardea-Torresdey, J. L., Becker-Hapak M. K., Hosea J. M., Darnall D. W. (1990). Effect of chemical modification of algal carboxyl groups on metal ion binding. *Environmental Science & Technology* 24 (9), 1372-1378. <https://doi.org/10.1021/es00079a011>
- Ghoneim, M. M., El-Desoky, H. S., El-Moselhy, K. M., Amer, A., El-Naga, E. A., Mohamedein, L. I., Al-Prol, A. E. (2014). Removal of cadmium from aqueous solution using marine green algae,

- Ulva lactuca*. *The Egyptian Journal of Aquatic Research* 40, 235-242. <https://doi.org/10.1016/j.ejar.2014.08.005>
- Hayashi, M. A. (2001). Remoção de Cromo hexavalente através de processos de bioadsorção em algas marinhas. Tese de Doutorado em Engenharia Química, Universidade Estadual de Campinas, Campinas, Brasil. <http://repositorio.unicamp.br/jspui/handle/REPOSIP/266215>
- Henriques B., Teixeira A., Figueira P., Reis A.T., Almeida J., Vale C, Pereira E. (2019). Simultaneous removal of trace elements from contaminated waters by living *Ulva lactuca*. *Science of the Total Environment* 652, 880-888. <https://doi.org/10.1016/j.scitotenv.2018.10.282>
- Ibrahim, W. M., Hassan, A. F., Azab, Y. A. (2016). Biosorption of toxic heavy metals from aqueous solution by *Ulva lactuca* activated carbon. *Egyptian Journal of Basic and Applied Sciences* 3, 241-249. <https://doi.org/10.1016/j.ejbas.2016.07.005>
- Jeon, C., Park, J. Y., Yoo, Y. J. (2002). Characteristics of metal removal using carboxylated alginic acid. *Water Research* 36, 7, 1814-1824. [https://doi.org/10.1016/S0043-1354\(01\)00389-X](https://doi.org/10.1016/S0043-1354(01)00389-X)
- Lavado-Meza, C., Asencios, Y.J.O., Cisneros-Santos, G., Unchupaico-Payano, I. (2021). Removal of methylene blue dye using nostoc commune biomass: kinetic, equilibrium and thermodynamic study. *Revista Mexicana de Ingeniería Química* 20, 2, 941-954. <https://doi.org/10.24275/rmiq/IA2291>
- Lima, H.d., Asencios, Y.J.O. (2021) Eichhornia crassipes (Mart.) Solms (natural or carbonized) as biosorbent to remove pollutants in water. *SN Applied Sciences* 3, 750. <https://doi.org/10.1007/s42452-021-04736-9>
- Montazer-Rahmati, M.M., Rabbani, P., Abdolali A., Keshtkar A.R. (2011). Kinetics and equilibrium studies on biosorption of cadmium, lead, and nickel ions from aqueous solutions by intact and chemically modified brown algae. *Journal of Hazardous Materials* 185, 1, 401-407. <https://doi.org/10.1016/j.jhazmat.2010.09.047>
- Naja, G. M., Murphy, V., Volesky B. (2010). Biosorption metals. *Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology*, John Wiley & Sons. <https://doi.org/10.1002/9780470054581.eib166>
- Nielsen M. M., A. Bruhn, M. B. Rasmussen, B. O. Martin, M. Larsen, H. B. Møller. (2012) Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. *Journal of Applied Phycology* 24, 449-458. <https://doi.org/10.1007/s10811-011-9767-z>
- Rees D.A., Morris E.R., Thom D., Madden J.K. (2010). Shapes and interactions of carbohydrate chains. *Molecular Biology: An International Series of Monographs and Textbooks*; Aspinall GO, editor. *The Polysaccharides*, vol. I. New York: Academic Press; (1982). 195-290.
- Romera E., González F., Ballester A., Blázquez M. L., Muñoz J. A. (2006). Biosorption with Algae: A statistical review. *Critical Reviews in Biotechnology* 26, 223-235. <https://doi.org/10.1080/07388550600972153>
- Romera E., González F., Ballester A., Blázquez M.L., Muñoz J.A. (2007) Comparative study of biosorption of heavy metals using different types of algae. *Bioresource Technology* 98, 17, 3344-3353. <https://doi.org/10.1016/j.biortech.2006.09.026>
- Romero-González M. E., Williams C. J., Gardiner P. H. E. (2001). Study of the mechanisms of cadmium biosorption by dealginated seaweed waste. *Environmental Science and Technology* 35, 14, 3025-3030. <https://doi.org/10.1021/es991133r>
- Sari A. Tuzen, M. (2009). Equilibrium, thermodynamic and kinetic studies on aluminum biosorption from aqueous solution by brown algae (*Padina pavonica*) biomass. *Journal of Hazardous Materials* 171, 973-979. <https://doi.org/10.1016/j.jhazmat.2009.06.101>
- Sheng, P.X., Ting Y.P, Chen P.J., Hong L. (2004). Sorption of lead, copper, cadmium, zinc, and nickel by marine algal biomass: characterization of biosorptive capacity and investigation of mechanisms. *Journal of Colloid and Interface*

*Science* 275, 131-141. <https://doi.org/10.1016/j.jcis.2004.01.036>

Silva T.M.A., Alves L. G., Queiroz K.C.S., Santos M.G.L., Marques C.T., Chavante S.F., Rocha H.A.O., Leite E.L. (2005). Partial characterization and anticoagulant activity of a heterofucan from the brown seaweed *Padina gymnospora*. *Brazilian Journal of Medical Biological Research* 38, 523-533. <https://doi.org/10.1590/S0100-879X2005000400005>

Thom D., Grant G. T., Morris E. R., Rees D. A. (1982) Characterisation of cation binding and gelation of polyuronates by circular dichroism.

*Carbohydrate Research* 100, 29-42. [https://doi.org/10.1016/S0008-6215\(00\)81023-X](https://doi.org/10.1016/S0008-6215(00)81023-X)

Yang J., Volesky B. (1999). Modeling uranium-proton ion exchange in biosorption. *Environmental Science and Technology* 33, 4079-4085. <https://doi.org/10.1021/es990435q>

Yang L., Chen P. J. (2008). Biosorption of hexavalent chromium onto raw and chemically modified *Sargassum* sp. *Bioresource Technology* 99, 297-307. <https://doi.org/10.1016/j.biortech.2006.12.021>