

# An Investigation on Zinc Biosorption with Agar Extraction Waste from *Gracilaria tenuistipitata*

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## ABSTRACT

This study determined the biosorption capacity of algal waste, obtained through agar extraction from *Gracilaria tenuistipitata*, a common red seaweed in the Philippines and potential biosorbent, on  $Zn^{2+}$  ions. Agar was extracted from the seaweed and characterized through Fourier-transform infrared spectroscopy (FT-IR). The algal waste was then subjected to a biosorption study involving various  $Zn^{2+}$  standards. Atomic absorption spectroscopy (AAS) and FT-IR were employed to evaluate sorption processes. Biosorption data was analyzed using Langmuir, Freundlich, and Dubinin-Radushkevich (D-R) isotherms which yielded  $R^2$  values of 0.9680, 0.8103, and 0.5254, respectively. The Langmuir ( $K_L$ ), Freundlich ( $K_F$ ), and D-R ( $B$ ) constants garnered were 0.0231 L/mg, 0.9829 mg/g, and  $2.159 \times 10^{-6} \text{ mol}^2 \text{ J}^{-2}$  respectively, qualifying the adsorption of  $Zn^{2+}$  ions onto the algal waste as a favorable and physical process. FT-IR spectra analysis suggests that carboxylic acid and amide groups are involved in biosorption, possibly an ion-exchange process taking place for the uptake of  $Zn^{2+}$  ions. It was concluded that *G. tenuistipitata* waste is a promising biosorbent for  $Zn^{2+}$  ions. Performing the biosorption procedure in triplicates and the inclusion of kinetic studies are recommended to improve and ensure the accuracy in determining biosorption capacity and optimization in future studies.

**Keywords:** *Gracilaria tenuistipitata*; biosorption; zinc; agar extraction; waste

## INTRODUCTION

Due to the accumulation of factories' effluents, water pollution has become a great concern. Around 22% of water usage goes to industries and a bulk of this water is released as wastewater (Zeraatkar et al., 2016). Water pollution is primarily caused by the accumulation of toxic heavy metals and the removal of such in different bodies of water, especially those near industries, is a priority in maintaining water quality standards (Teimouri et al., 2016). Zinc, as  $Zn^{2+}$ , is a common heavy metal found in industrial effluents (Ngabura et al., 2018; Sedlakova-Kadukova et al., 2019). Though zinc is an essential micronutrient for all forms of life, an excess amount of this renders it

toxic to aquatic life and can lead to a wide variety of illnesses in humans (Paduraru et al., 2015; Ngabura et al., 2018; Sedlakova-Kadukova et al., 2019). The World Health Organization (WHO) suggested that the maximum contamination levels of  $Zn^{2+}$  for surface water, groundwater, and drinking water be 0.01, 0.05, and 3 mg/L, respectively (Ngabura et al., 2018). Many companies have used heavy metal removal methods such as chemical precipitation, solvent extraction, filtration, and coagulation, etc. However, most of these yielded high costs and insufficient regulation (Teimouri et al., 2016; Volesky, 2001; Zeraatkar et al., 2016). Consequently, the search for more efficient and cost-effective forms of heavy metal removal arose.

Biosorption has emerged as one of the most promising and suitable methods because it involves the uptake of metals by certain chemical sites that remain present and functional even when its biomass of origin is dead (Volesky, 2001). The search for the right biosorbent has revolved around the potential of waste and abundant biomass types such as seaweeds (Volesky, 2001). Algae have proven their worth due to their affinity to heavy metals and their affordability (Teimouri et al., 2016; Volesky, 2001). In addition, their reusability and abundance in saltwater and freshwater make them cost-effective for industries. According to statistics on biosorption, algae has been used 15.3% more than any other biomass and 84.6% more than fungi and bacteria (Anastopoulos & Kyzas, 2015). Though studies concentrated on brown algae over the past two decades, focus has shifted towards the use of green and red algae, and even biopolymers derived from various biomaterials (He & Chen, 2014).

*Gracilaria* seaweeds, known locally in the Philippines as *gulaman* (Caces-Borja, 1977), as the second largest genus amongst the red algae, the phylum *Rhodophyta*, are distributed worldwide and cultivated for hydrocolloids in various countries including China, Vietnam, the Philippines, Indonesia and the Republic of Korea (McHugh, 2003). Particularly, *Gracilaria tenuistipitata* (Figure 1) has been widely exploited in the Philippines for its products that are economically valuable, like agar (Trono, 1997). Considerable sources of the seaweed in the Philippines include the Manila Bay area, Zamboanga, Sulu, and Cagayan (Caces-Borja, 1977).

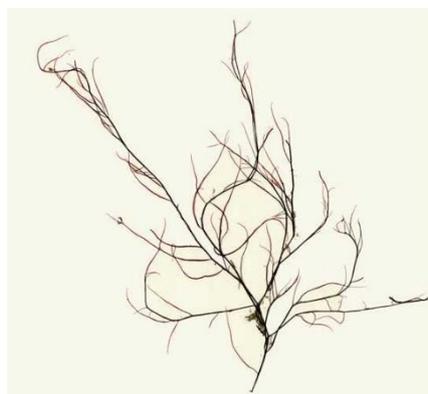


Figure 1. *Gracilaria tenuistipitata* var. *luisi*. (Lin, n.d.)

Species belonging to *Rhodophyta* are unique from all other plants in terms of polysaccharide composition of their cell walls, and their intercellular matrices. Most of these polysaccharides come in the form of sulfated galactans and are grouped into two main classes, carrageenans and agars. Agar, a major component for *Gracilaria* species, has a linear backbone chain consisting of alternating 3-linked  $\beta$ -D-galactopyranose and 4-linked  $\alpha$ -L-galactopyranose residues uniquely present in agars (Figure 2), in contrast to the D-configuration of the latter present in carrageenans. These 4-linked galactopyranose residues may also be present as 3,6-anhydro derivatives. The formation of this derivative is a result *in vivo* of the elimination of a sulfate group and proton from

a hydroxyl group, where a similar result can be achieved with the alkaline treatment of the polysaccharide (Usov, 2011).

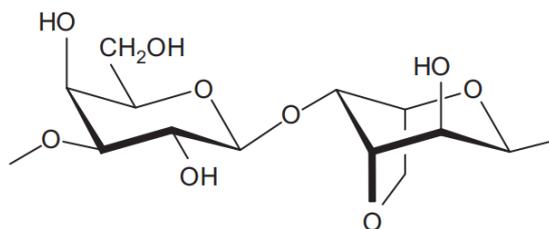


Figure 2. Structural agarose monomer unit (Usov, 2011).

Important and relevant uses for agar are mainly centered around its gelling, synergetic, and hysteric properties (McHugh, 2003). Among these, its high relative hysteric property works well for applications involving food, including maintaining gels at warmer temperatures and reducing the need for refrigeration. In terms of agar extraction, previous works generally outline the procedure starting with washed and cleaned seaweed, treated with base, and then subjected to hot water extraction (McHugh, 2003). This extract is left to form a gel-like substance, mainly made of water and with low concentrations of agar. Water is expelled from the set gel through different methods, including the freeze-thaw and syneresis methods, finishing in a final oven drying process.

The seaweed family *Gracilariaceae* has been the focus of several biosorption studies, where *Gracilaria spp.* have also been made known to be effective biofiltration agents (Trono, 1999). Among the vast seaweed genera that can be utilized for bioremediation, *Gracilaria* predominates over all other types of red seaweeds (Neveux et. al., 2018). Since *Gracilaria* is a genus of red algae, its adsorptive properties are mainly associated with the presence of sulfated polysaccharides made of galactans (Anastopoulos & Kyzas, 2015). Recent biosorption studies have frequently made use of *G. corticata* and *G. verrucosa* and investigated the adsorption of heavy metals such as cadmium, lead, copper, zinc, and chromium (Teimouri et al., 2016; Hannachi et. al., 2013; Nasab et. al. 2017; Ata et. al. 2012). The factors that were studied to determine the conditions for maximum biosorption for a certain seaweed species were mainly pH of the solution, biomass quantity, competing ions, contact time between absorbent and absorbate, and initial heavy metal ion concentration.

Besides utilizing non-treated seaweeds, studies were also done on the adsorptive properties of algal waste, such as those after certain extraction procedures, to test if there would be any variation in biosorption. In line with this, Nasab et. al. (2017) conducted a study on the biosorption capacity of Cd(II) ions by not only *G. corticata*, but also its algal waste produced after agar extraction. This study concluded that the alga *G. corticata* was more effective and had higher results than the algal waste. However, there was no significant difference between the two; indicating that algal waste could also be utilized for biosorption processes. With this, algal waste produced from industrial agar extraction procedures still holds the potential to be repurposed for other uses. As biosorption is an up and coming phenomenon that has the potential to provide a means for toxic metal removal from wastewater, numerous species of seaweed have been tagged as potential biosorbent materials. Studies have been performed on other *Gracilaria* species, but this possibility remains unexplored for *G. tenuistipitata*, hence this study.

The objectives of this study include the following: 1) to extract agar from *Gracilaria tenuistipitata* through a modified process involving a pre-alkali treatment; 2) to partially characterize the extracted product through Fourier-transform infrared spectroscopy (FT-IR); and 3) to determine the Zn<sup>2+</sup> biosorption capability and mechanism of produced agar extraction waste through isotherm modelling and FT-IR analysis.

## METHODS

**Materials and Equipment.** Freeze-dried seaweed samples were initially stored in small plastic containers. Prepared solvents were stored in sealable bottles and flasks. When not used, samples, extracts, and filtrates involved were temporarily stored in the refrigerator while excess seaweed were stored in the freezer. FT-IR measurements were performed with a Thermo Scientific Nicolet 6700 FT-IR Spectrometer. Atomic absorption spectrometry (AAS) experiments were carried out on a Shimadzu AA-6300 Atomic Absorption Spectrophotometer. The following chemicals and reagents were used for the study: technical grade hydrochloric acid (HCl) and sodium hydroxide (NaOH), 1000 mg Zn standard (1000 mg  $ZnCl_2$  in 0.06% HCl, Lot No. HC259714, Titrisol), Agarose Type I-A standard (Sigma, Lot No. 043K1179), sodium carbonate buffer for pH meter, and KBr powder for pelletization for IR analyses.

**Seaweed Collection, Sample Preparation, and Alkali Treatment.** *Gracilaria tenuistipitata* was purchased from a public market in Bacoor Bay, Cavite, Philippines during December 2018, known locally as *gulaman*. The identity and taxonomy of the purchased seaweed was verified through a certification from the Botany Division of the National Museum in Manila, Philippines. The seaweeds were cleaned with running tap water to remove solid impurities. A batch of 100 g fresh weight of washed, cleaned seaweed was then subjected to a strong pre-alkali treatment involving 2 L of approximately 6.0% NaOH. The seaweeds were soaked in the NaOH solution at room temperature for 24 h. The seaweeds were then removed from the NaOH and washed with 3 batches of 1 L distilled water. The washed seaweeds were then submerged in another batch of distilled water and the pH was obtained. The solution was made neutral by adding 0.01% HCl, washed, and pH measured again. This was repeated until observed pH = 7. It was noted that the red color of the seaweed changed to green with the pre-alkali treatment (Figure 3).

**Agar Extraction.** Around 2 L of distilled water was placed into a metal pot and heated until it reached 80 °C. The seaweeds were boiled for 1.5 h with occasional stirring. After boiling, the seaweeds were immediately filtered from the extract. The residue (seaweed, now algal waste) was temporarily stored in a refrigerator for use in the succeeding biosorption procedure while the filtrate (extract) was kept in containers and was left to set for 4 h at room temperature. The semi-solid gels that formed were frozen overnight and were thawed at room temperature the next day. Water was drained from the containers and the gels left were transferred to a baking tray lined with parchment paper. The gels and algal waste biomass were placed in an oven to dry at 60 °C, with the dried algal waste ground and sieved through a 0.5 mm sieve, shown in Figure 3.

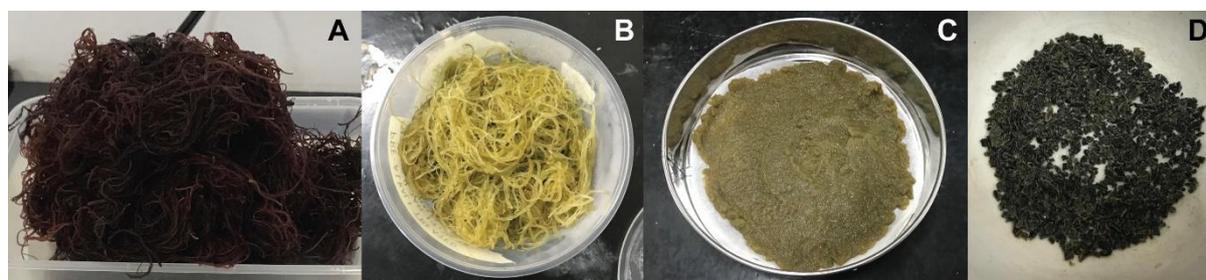


Figure 3. *Gracilaria tenuistipitata* seaweed as treated through the methods of the study. A) Seaweed before pre-alkali treatment; B) Pre-alkali treated seaweed after three dilute acid washes; C) Agar extraction waste (residue) biomass before drying; D) Agar extraction waste (residue) biomass after drying, grinding, and sieving.

**Batch Zinc Biosorption.** Two batches of standard solutions of concentrations of 5, 10, 20, 40, 80, 160, 280, 350, and 400 mg/L were prepared for AAS experiments and biosorption experiments. The biosorption procedure was obtained from a study done by Hannachi et al. (2013) and was performed at pH = 5. The ground algal waste generated from the previous agar extraction procedure was used as the “biosorbent” for this procedure. In a 25-mL Erlenmeyer flask, 0.1 g of the corresponding biosorbent was added. Twenty-five mL of the Zn standard solutions were first poured into separate beakers for a biosorbent dosage of 4.0 mg/L. The Zn standards were poured in simultaneously to the Erlenmeyer flasks and were constantly shaken for 1 h, the optimum time duration for the process stated in Hannachi et al. (2013), and was carried out at 25°C or 298 K. Afterwards, seven simple filtration set-ups were prepared and each contained a beaker paired with a funnel fitted with filter paper. The filtrates were covered with parafilm and stored in a refrigerator for AAS analysis.

**AAS Analysis and Adsorption Isotherms.** To create a calibration curve, separate standard zinc solutions of 5, 10, 20, 40, 80, 160, 280, 350, 400, and 1000 mg/L were first run through AAS to obtain their initial concentrations and absorbance values. The standard calibration curve method was then used for each of the seven filtrates and seven pre-biosorption solutions to determine their respective initial and final concentration and absorbance values. Two calibration curves were constructed based on the appropriate ranges of concentrations detectable by the AAS: the first calibration curve was acquired at the 213.9 nm Zn line, corresponding to an optimum working range of 0.01-2 mg/L; the second calibration curve was acquired at the 307.6 nm Zn line, corresponding to an optimum working range of 100 to 14000 mg/L. AAS parameters included a slit width of 0.7 nm, through an air-acetylene flame, and no background correction performed. Calculated concentrations before ( $C_0$ ) and after biosorption ( $C_e$ ) were used to calculate for efficiency and were appropriately fitted on three adsorption isotherm models: Langmuir, Freundlich, and Dubinin-Radushkevich to calculate relevant parameters.

**FT-IR Analyses of Samples.** The extracted gel samples, along with the agarose standard, were further subjected to Fourier-transform infrared spectroscopy (FT-IR) to confirm the presence of agar in the extract and to determine if contaminants could have interfered with the analyses and resulting spectra. In addition, the algal waste biomass was dried at 60°C and subjected to FT-IR analysis before and after biosorption. Peaks resulting from treated biomass were taken both before and after biosorption to determine functional groups responsible for the Zn uptake. Dried samples, including extracted gels, dried non-treated seaweed, and pre- and post-biosorption biomass were pelletized with KBr in preparation for analysis. The FT-IR analyses were performed with 16 sample scans with a resolution of 4.000 with a range from 400 to 4000  $\text{cm}^{-1}$ , and a Happ-Genzel apodization.

## RESULTS AND DISCUSSION

**Biosorption Efficiency.** The efficiency of the biosorption of  $\text{Zn}^{2+}$  ions from *G. tenuistipitata* algal waste was quantified by calculating the metal uptake capacity ( $Q_e$ , mg/g) and the metal percentage removal (%) as given by the following equations (Zaib et al., 2016):

$$Q_e = \frac{C_0 - C_e}{W} \times V \quad (1)$$

$$\% = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

where  $C_0$  and  $C_e$  are the initial and final metal concentrations (mg/L), respectively, V is the volume of metal solution (L) and W is the biosorbent amount (g).

The calculated metal uptake capacities ( $Q_e$ ) and removal percentages (%) are summarized in Table 1. As seen from the calculated values, the amount of  $Zn^{2+}$  adsorbed increased from 1.2631 to 15.0611 mg/g with an increase in initial metal ion concentration. However, the percentage removal decreased from approximately 69% to 16%. This decrease in percentage ion removal can be explained by the lack of binding sites available on the biosorbent as the initial concentration increases (Zaib et al., 2016). At high  $C_0$ , an increase in metal ions competing for binding sites likely indicates saturation (Li et al., 2017). At low  $C_0$ , the metal ions readily bind to the biosorbent, resulting in high removal efficiency. The opposite is observed in  $Q_e$ , which increases as initial ion concentration increases as well. The increase in initial ion concentration led to an increase in the  $Zn^{2+}$  concentration gradient between the liquid (aqueous solution) phase and the solid (biosorbent) phase.

**Table 1. Biosorption efficiency of  $Zn^{2+}$  ions onto *G. tenuistipitata* algal waste.**

$C_0$ (mg/L)	$C_e$ (mg/L)	$Q_e$ (mg/g)	Removal percentage (%)
7.6721	2.3875	1.2631	68.8812
15.5382	6.0624	2.2497	60.9841
28.8915	16.7227	2.8809	42.1189
56.8551	17.8092	9.3233	68.6762
392.6502	330.1767	15.0611	15.9107

**Adsorption Isotherms.** Adsorption isotherms, which correlate the uptake of ions and the equilibrium solute concentration in the solution, are often used to evaluate a biosorbent's performance (Abdi & Kazemi, 2015). The Langmuir, Freundlich, and Dubinin-Radushkevich (D-R) isotherm models are commonly used in describing such.

*Langmuir Adsorption Isotherm.* The Langmuir adsorption isotherm assumes that sorption takes place at homogeneous sites within the biosorbent and focuses on the formation of a monolayer of heavy metal ions on the outer surface of the biosorbent (Hannachi et al., 2013), and that the ability of a species to be adsorbed on a given site is independent from its neighboring site's occupancy (Paduraru et al., 2015). The Langmuir isotherm model can be represented as (Hannachi et al, 2013):

$$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{Q_m K_L} \quad (3)$$

Where:

$C_e$  is the equilibrium concentration of adsorbate (mg/L),

$Q_e$  is the amount of metal adsorbed per gram of the adsorbent at equilibrium (mg/g),

$Q_m$  is the maximum monolayer coverage capacity (mg/g),

$K_L$  is the Langmuir isotherm constant (L/mg).

Values of  $Q_m$  and  $K_L$  are directly proportional to the adsorption percentage (Nethaji et al., 2013). Calculations yielded  $K_L$  to be 0.0231 L/mg with a  $Q_m$  of 17.637 mg/g. The  $K_L$  constant is related to the affinity between the biosorbent and the metal ions and also indicates whether strong or weak bonds exist; the higher the value, the stronger the bond (Paduraru et al., 2015). From the value, it can be concluded that the bonds existing between the biosorbent and the  $Zn^{2+}$  ions are not that strong.

The Langmuir constant is essential for determining another parameter of the Langmuir isotherm: the separation factor or equilibrium parameter,  $R_L$ , which is represented by the equation (Meroufel et al., 2013):

$$R_L = \frac{1}{1 + K_L C_o} \quad (4)$$

Where:

$C_o$  is the initial metal ion concentration (mg/L),

$K_L$  is the Langmuir isotherm constant (L/mg).

The separation factor describes the shape of the isotherm, indicating if the biosorption process is unfavorable (chemisorption dominant), linear, favorable (physisorption dominant), or irreversible (nonreversible chemisorption) (Meroufel et al., 2013; Ibrahim & Jimoh, 2012).

Using the Langmuir constant, the separation factor was calculated for using Eqn. 4 with each value for initial concentration. The values are summarized in Table 2. Since the values range from approximately 0.099 to 0.850,  $0 < R_L < 1$ , and this indicates that the adsorption of  $Zn^{2+}$  onto the algal waste is favorable and that physisorption is the predominant process. Looking back at the Langmuir constant, its relatively low value supports the idea that physisorption, a reversible process, occurs since the bonds existing between the biosorbent and  $Zn^{2+}$  ions are relatively weak.

**Table 2. Separation factor values from the Langmuir isotherm.**

Initial Concentration ( $C_o$ )	Separation factor ( $R_L$ ) value
7.67206	0.84966
15.5382	0.73618
28.8915	0.60012
56.8551	0.43266
392.650	0.09945

Despite obtaining high values for  $R^2$  (Table 3), it is noted that there are underlying violations to the assumptions of the Langmuir isotherm. Davis et al. (2003) pointed out that in biosorption processes, more than one type of functional group contributes to the biosorption, violating binding site homogeneity. Also, these groups have different affinities for sorption given a metal. Despite these, the Langmuir isotherm is still frequently applied for fitting experimental data from biosorption studies and is seen in multiple reviews on macroalgal biosorption (Santos, 2018). Here, the Langmuir constant becomes a fitting parameter, since biosorption processes cannot completely adhere to the assumptions of the Langmuir theory. It may be described that the biosorption of  $Zn^{2+}$  ions to the biomass follows Langmuir behavior despite violating some of its assumptions.

*Freundlich Adsorption Isotherm.* Freundlich's isotherm describes adsorption that occurs on heterogeneous sites on the biosorbent surface that contains varied affinities, with stronger binding sites occupied first and binding strength decreasing with higher site occupation (Zaib et al., 2016). In addition, the Freundlich isotherm is not limited to the formation of a monolayer and postulates a multilayer adsorption process (Hannachi et al., 2103; Shikuku et al., 2018). The Freundlich equation is denoted as (Hannachi et al., 2013):

$$\log Q_e = \log K_F + \frac{1}{n} \log C_e \quad (5)$$

where  $K_F$  and  $\frac{1}{n}$  are Freundlich isotherm constants related to adsorption capacity (mg/g) and sorption intensity, respectively. The constant  $K_F$  is denoted as the adsorption distribution coefficient and describes the relative adsorption capacity of the adsorbent based on the bonding energy (Ibrahim & Jimoh, 2012). The constant  $\frac{1}{n}$ , where  $n$  is also known as the heterogeneity factor, measures the adsorption intensity or surface heterogeneity wherein the surface is more

heterogeneous as its value approaches zero (Guerrero-Coronilla et al., 2015; Li et al., 2017). On calculation,  $\frac{1}{n}$  was found to be 0.5060, indicating a favorable adsorption, as  $0 < \frac{1}{n} < 1$  (Bordoloi et al., 2017). This yielded an  $n$  value equals to 1.9763. Shikuku et al. (2018) stated that values of  $n$  between 1 and 2 have moderately difficult adsorptive potential. In addition, Ibrahim & Jimoh (2012) mentioned that if  $n < 1$ , the adsorption process is chemical, and if  $n > 1$ , the adsorption is a favorable physical process. With the results in this current work, the biosorption is a favorable physical process, having moderately difficult adsorptive potential.

*Dubinin-Radushkevich (D-R) Adsorption Isotherm.* The D-R isotherm is more general than Langmuir and does not assume a homogeneous surface or a constant biosorption potential for all binding sites (Hannachi et al., 2013; Zaib et al., 2016). This model also assumes that adsorption follows a pore filling mechanism, which has a multilayer character involving van der Waals forces, usually applicable for physical adsorption processes (Ayawei et al., 2017). This isotherm is also temperature-dependent (Dada et al., 2012). The D-R isotherm can be represented by the following linearized form (Bordoloi et al., 2017):

$$\ln Q_e = \ln Q_m - B\varepsilon^2 \quad (6)$$

where  $\varepsilon$  is the Polanyi potential and  $B$  is the Dubinin-Radushkevich isotherm constant ( $\text{mol}^2\text{J}^{-2}$ ). The Polanyi potential equates to  $RT \ln \left(1 + \frac{1}{c_e}\right)$ , where  $R$  is the gas constant,  $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ , and  $T$  is temperature in K. The D-R isotherm focuses on whether chemisorption or physisorption of the metal ions occurs and is determined with the adsorption mean energy ( $E$ ,  $\text{J mol}^{-1}$ ) per molecule of adsorbate, given by (Bordoloi et al., 2017):

$$E = \frac{1}{\sqrt{2B}} \quad (7)$$

If the adsorption mean energy lies between  $8\text{-}16 \text{ kJ mol}^{-1}$ , the adsorption process takes place chemically while if  $E < 8 \text{ kJ mol}^{-1}$ , it proceeds physically (Hannachi et al., 2013). From the plotted data on the D-R isotherm,  $B$  was found to be  $2.159 \times 10^{-6} \text{ mol}^2\text{J}^{-2}$  and the adsorption mean energy ( $E$ ), was found to be  $0.481 \text{ kJ mol}^{-1}$ . Given that the value of  $E$  is less than  $8 \text{ kJ mol}^{-1}$ , the idea that physical adsorption of  $\text{Zn}^{2+}$  on *G. tenuistipitata* waste occurs rather than chemical adsorption is further supported since not much energy is needed to remove the  $\text{Zn}^{2+}$  ion from the biosorbent surface.

The calculated isotherm constants of the above-mentioned models, along with their corresponding  $R^2$  values, are summarized in Table 3. The parameters and constants obtained from the Langmuir, Freundlich, and D-R isotherms all suggest that the biosorption of  $\text{Zn}^{2+}$  ions by *Gracilaria tenuistipitata* algal waste is a favorable process wherein physical adsorption predominates, caused by van der Waals forces of attraction originating from electric dipoles (Tripathi & Ranjan, 2015).

**Table 3. Results of analysis of Zn<sup>2+</sup> biosorption onto *G. tenuistipitata* algal waste based on two-parameter isotherm models.**

Isotherm model	Equation (linearized form)	Isotherm significance	Parameter values
Langmuir	$\frac{C_e}{Q_e} = \frac{C_e}{Q_m} + \frac{1}{Q_m K_L}$	$K_L$ [L/mg]	0.0231 L/mg
		$Q_m$ [mg/g]	17.637 mg/g
		$R_L$	0.0995 to 0.8497
		$R^2$	0.9680
Freundlich	$\log Q_e = \log K_F + \frac{1}{n} \log C_e$	$K_F$ [mg/g]	0.9829 mg/g
		$n$	1.9763
		$1/n$	0.5060
		$R^2$	0.8103
Dubinin-Radushkevich	$\ln Q_e = \ln Q_m - B\varepsilon^2$	$Q_m$ [mg/g]	6.397 mg/g
		$B$ [mol <sup>2</sup> J <sup>-2</sup> ]	$2.159 \times 10^{-6}$ mol <sup>2</sup> J <sup>-2</sup>
		$E$ [kJ/mol]	0.4812 kJ/mol
		$R^2$	0.5254

**Relative Biosorption Capability of Red Seaweeds.** From biosorption data presented, particularly with Langmuir biosorption parameters, the capability of the biomass to sequester Zn<sup>2+</sup> ions are relatively lower compared to other possible biosorbents in literature, particularly brown seaweeds. However, it remains important in the field of biosorption to study red seaweeds, especially with their abundance in Southeast Asian regions. Santos (2018) reported that multiple studies show the order of heavy metal uptake capacity decreases from brown to green to red seaweeds. Additionally, the affinity for polysaccharide functional groups for heavy-metal ions decrease from Pb to Cu to Cd and to Zn. However, despite maximum adsorption capacities of red algae being relatively lower than those for brown seaweeds, red seaweeds can exhibit affinities and sequester amounts even higher than brown seaweeds (Murphy et al., 2008), implying the potential of red seaweeds for heavy-metal biosorption.

**FT-IR Analysis for Agar Extraction.** After extraction and drying, the gel extract was subjected to FT-IR analysis to determine functional groups present and confirm the extract's identity as agar. Peaks from the spectra of the extracted gel were compared with those of an agarose Type I standard, and are shown in Table 4 with peak assignments determined from agreement with a previous study on extracted agar characterization by Rocha et al. (2019). A comparison of the two spectra, stacked together are shown in Figure 4.

In addition to the peaks described, absence of peaks at 805, and 830 cm<sup>-1</sup> were observed, which indicates the absence of sulfation at C-2 in LA (4-linked α-L-galactose) and G (3-linked β-D-galactose) moieties, and 820 cm<sup>-1</sup> for low sulfation at C-6 of G moieties, notably a result of the pre-alkali treatment leading to desulfation of the said moieties. The extracted gel's spectra also notably lacked a shoulder at 2850 cm<sup>-1</sup> described in the Rocha et al. (2019) study, which was assigned to a high degree of agar methylation. This contrasts the shoulder near 2899 cm<sup>-1</sup> on the agarose standard's spectra. This shoulder peak may have been buried in the very broad absorption region 3800 to 2600 cm<sup>-1</sup>, which is attributable to O-H functionalities found in carboxylic acids due to their known characteristic as hydrogen-bonded dimers (Smith, 2011). The data suggest the identity of the extracted gel being that of agar, with differences in spectra being associated with the diversity of chemical modifications and residue arrangement that may occur within polysaccharides, which are realistic deviations from the ideal agarose structure in the addition of chemical groups as in methylation or sulfation. Such diversity can yield considerable differences in spectra, but still reveal similar patterns of peaks due to a common set of functionalities.

Table 4. FT-IR spectral characterization for extracted gel and agarose standard.

IR peak from gel extract (cm <sup>-1</sup> )	IR peak from agarose standard (cm <sup>-1</sup> )	Peak assignment in agreement with Rocha et al. (2019)
3400.87	3397.05	
2632.45		
	2899.69	Agar methylation
2333.10		
2168.90	2146.89	
1654.83	1644.46	C=O stretch
1414.15		
1384.32	1376.28	S=O stretch
	1308.75	
1273.98	1250.56	Ester sulfates
1115.23	1157.25	Polysaccharide, C-O-H bending, CO stretch, C-C stretch
	1081.26	
968.54	967.25	
931.97	931.09	C=O vibration of LA (4-linked $\alpha$ -l-galactose) residues
889.05	890.04	C-H anomeric bending in G (3-linked $\beta$ -D-galactose) residues
744.22	773.76	Galactose skeletal ring bending
	741.13	
	714.58	
618.73	623.29	
	600.61	
577.60	577.97	
550.04	538.06	
	517.26	

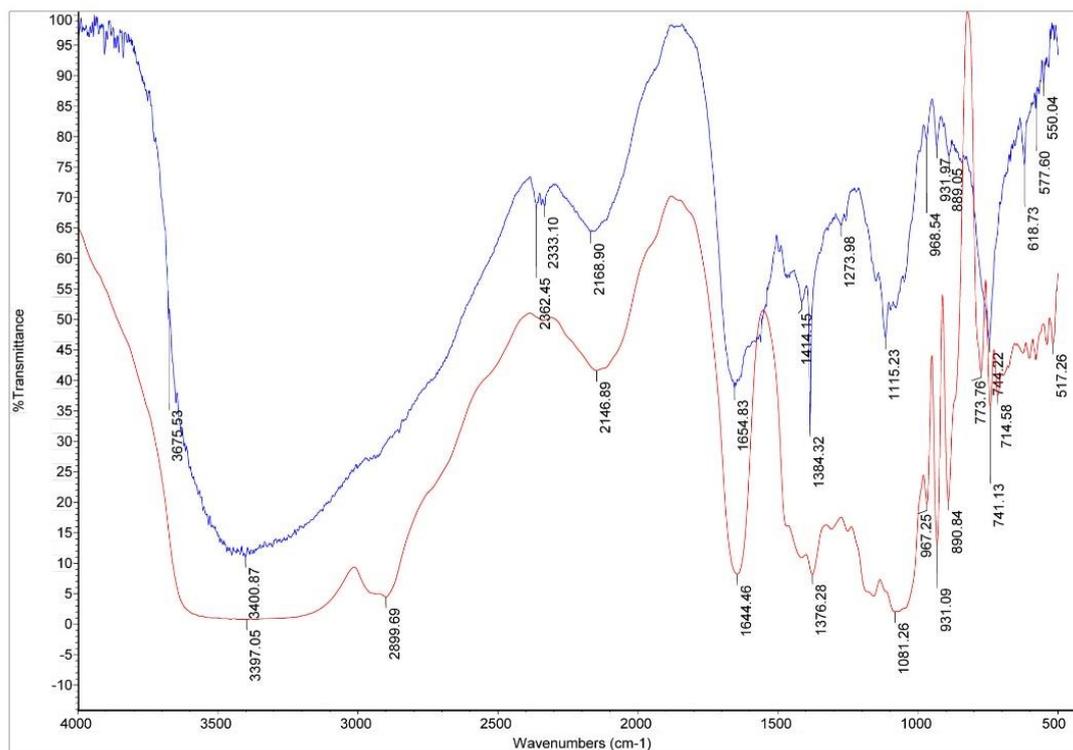


Figure 4. IR spectra for extracted gel (blue) and agarose Type I standard (red).

**FT-IR Analysis for Unloaded and Loaded Biomass.** After gel extraction, the seaweed extraction waste biomass was dried and subjected to FT-IR analysis before and after metal biosorption to determine functional groups which may be involved in the sequestration of Zn<sup>2+</sup> ions. Shifts in peaks were noted for the spectra before and after biosorption. Peaks from the spectra of the unloaded biomass (before biosorption) and loaded biomass (after biosorption), are compared in Table 5 with peak assignments determined from related literature. A comparison of the two spectra, stacked together are also shown in Figure 5.

**Table 5. FT-IR spectral characterization of waste biomass before and after biosorption.**

IR peak before biosorption (cm <sup>-1</sup> )	IR peak after biosorption (cm <sup>-1</sup> )	Associated peak characteristic <sup>1</sup>
3410.48	3405.22	OH, NH stretch
2960.09	2957.37	C-H symmetric stretch
2921.15	2920.47	C-H asymmetric stretch
2852.75	2851.39	C-H asymmetric stretch
2166.01	2163.50	Alkyne stretch, variable strength
1655.24	1654.87	C=O stretch
1563.18	1563.34	
1466.62	1466.74	
1411.42	1409.33	Deformation of phenolic OH, C=O stretch of carboxylate, or aromatic C=C
1384.29	1384.40	
1258.49	1253.32	Amide III or phosphate
1156.73	1157.02	
1070.28	1068.41	C-OH bend (protein); C-O stretch alcohols and carboxylic; Ether and OH for polysaccharides
743.91	743.92	

<sup>1</sup> Sedlakova-Kadukova et al., 2019; Chairat & Bremmer, 2016; Hannachi et al., 2013; Yang et al., 2010; Ngabura et al., 2018

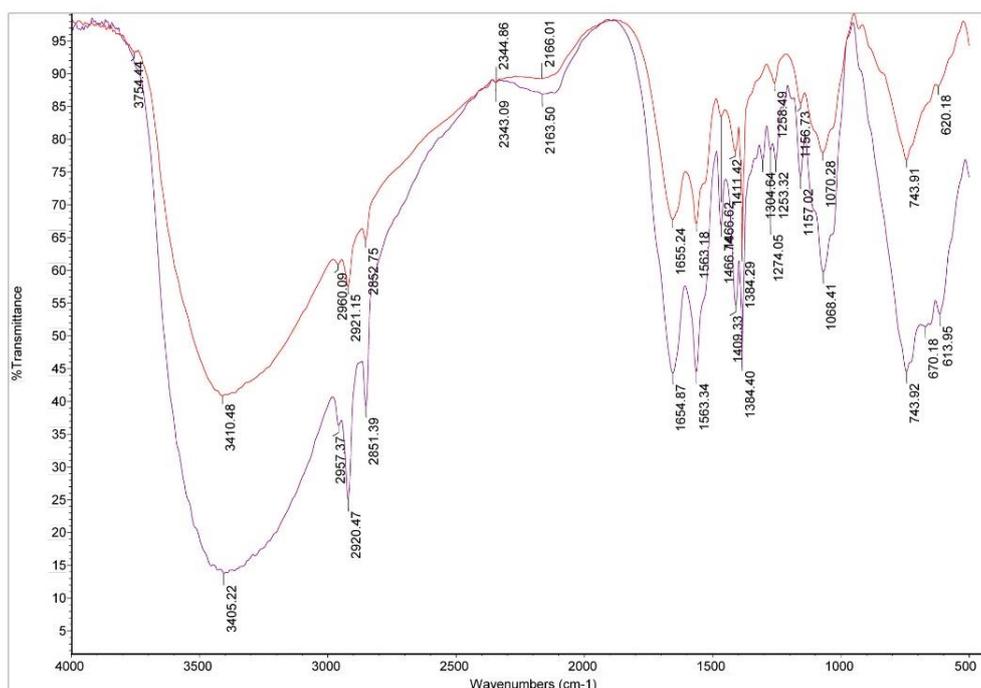


Figure 5. IR spectra for waste biomass before (red) and after (purple) biosorption.

Peaks that were observed to have shifted before and after biosorption were evaluated and associated with functional groups based on recent related zinc biosorption studies (Sedlakova-Kadukova et al., 2018; Chairat & Bremmer, 2016; Hannachi et al., 2013; Yang et al., 2010; Ngabura et al., 2018). The data suggest the involvement of O-H, N-H, aliphatic, C=O, amide, and phosphate groups in  $Zn^{2+}$  sequestration.

**Possible Mechanism for Biosorption.** Correlating FT-IR with the D-R isotherm data and the Langmuir isotherm data through the  $R_L$ , the physical nature of the  $Zn^{2+}$  sorption process indicates a probable mode of interaction of the functional groups as an ion-exchange process, where electrostatic interactions are involved. Davis et al. (2003) discussed this interaction particularly in algal biomass, where untreated biomass generally contains lighter metal ions, like  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$ . The biomass is protonated with a strong acid, where the proton displaces the light metal ion from its binding site, which may be a carboxylic or sulfonic group. This explains for the requirement of an optimal pH being very important in biosorption in maintaining electroneutrality during the ion-exchange process (Kumar & Jain, 2013). An ion leaving the functional group during the exchange and crosses the boundary between the biomass and the solution, i.e. an interphase boundary, produces an electric potential and must be compensated for by the movement of the metal ion in the opposite direction toward the functional group. In this manner, protonated acid functional groups then exchange for the binding metals, and release the  $H^+$  to the solution, as shown in Figure 6.

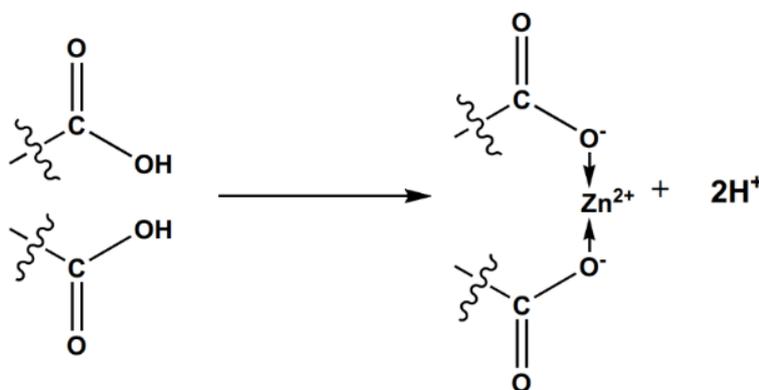


Figure 6. Possible uptake of  $Zn^{2+}$  ions by carboxylic acid groups.

Through shifts from the FT-IR data, the functional groups that were associated with the  $Zn^{2+}$  uptake can be associated with the agaropeptin fractions left behind in the biomass after agar extraction. Specifically, agaropeptin polymers are known to contain both D-glucuronic acid and pyruvate ketal groups, mainly on C-5 and C-6 of monosaccharide residues (Matsushashi, 1990), which both contain carboxylic acid functionalities. Santos (2018) also reported on the optimal pH of 5 observed in many algal biosorption studies being consistent with the  $pK_a$  value of carboxylic acids, which is around 5. Such mechanisms are manifested in the agar extraction and biosorption procedures performed in this study, with some modifications. Agars are generally made of two fractions: the neutral, gelling polysaccharide agarose, and the non-gelling, charged polysaccharide agaropeptin (Figure 7). The seaweed pre-alkali treatment with NaOH assures that the carboxylic acid groups present within the seaweed are deprotonated and are replaced with the light  $Na^+$  ion. As the agar extraction is performed, the polysaccharide fractions within the cell wall that have been desulfated, and eventually converted to the gelling agarose are removed from the seaweed, resulting in a biomass that would contain a relatively higher amount of agaropeptin. As the biomass is treated during the biosorption process in an acidic environment, the light  $Na^+$  ion is once again replaced with an  $H^+$  ion, and the exchange with  $Zn^{2+}$  for binding proceeds, completing its sequestration.

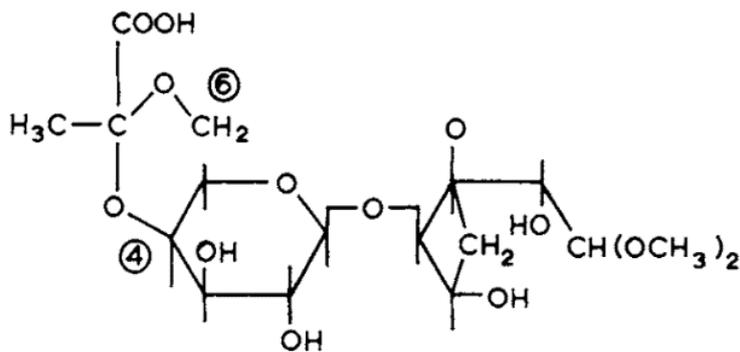


Figure 7. One of the units of agarose structure, noting the presence of the pyruvic acid residue on the sites marked 4 and 6 (Matsushashi, 1990).

In addition, the shifts in the amide and amine group observed may also be attributed to proteins present in the algal cells, and hexosamines and *N*-acetylhexosamine residues present in polysaccharides. Here, a potential mechanism may involve complexation occurring on the cell surface between the metal ion and the functional groups (Bulgariu & Gavrilescu, 2015). In this type of interaction, however, both electrostatic and coordinate covalent interactions may be involved. In context of the data yielded by the Dubinin-Radushkevich isotherm, this would most likely occur to a lesser extent, associating the adsorption to a physical one, considering that the interaction between the functional groups and the metal ion should be more stable in a chemical interaction than in the physical.

## CONCLUSION

Agar was successfully extracted from *Gracilaria tenuistipitata* and its identity was verified through Fourier-transform infrared spectroscopy. A biosorption study was also conducted to test the adsorption capacity of the algal extraction waste for  $Zn^{2+}$  ions. Among the three different two-parameter isotherm models used, the Langmuir model yielded the highest  $R^2$  value (0.9680), indicating that it fits and describes the biosorption procedure best and suggests the monolayer coverage of  $Zn^{2+}$  on the algal waste. In addition, all three isotherms indicate that a favorable physical adsorption occurs between the metal ions and biosorbent. Thus, it can be concluded that *G. tenuistipitata* waste is a promising and effective biosorbent for  $Zn^{2+}$  ions in aqueous solution.

To further improve this research, it is recommended to perform the biosorption procedure using additional samples to ensure the accuracy of the garnered data. It is also recommended to use finer biosorbent particles to increase the surface area of the biosorbent, further increasing biosorption capacity. In addition, the inclusion of other metal ions or the use of actual effluents in the study could further verify the effectivity of *G. tenuistipitata* waste as a biosorbent. Further kinetic studies, resorption studies, and statistical analysis may also be applied on the gathered data to provide significant support on the claims being made.

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