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Biosorption is the most favourable technique for the treatment of heavy metals as it is fast, powerful, and low cost, it takes place in a wide range of temperatures as well as it can be used for almost all types of heavy metals. In this study, the biosorption technique adsorbs Cu^{2+} and Zn^{2+} on the dried macroalgae (*Halimeda opuntia* and *Turbinaria turbinata*) in a batch system. Experimental parameters affecting the biosorption process are initial metal ion concentrations (5, 10, 15 and 25 mg/L), pH between (4.5 and 5.2), biomass dosage (1 gm) and agitation speed 150 rpm applied at contact time (30, 60 and 120 min). The significant-high average removals of Cu^{2+} by *H.opuntia* (> 96%) were recorded in concentrations of 10, 15 and 25 ppm at 120 min and the highest average removals by *T.turbinata* (81.07%, 78.32% and 74.7%) were recorded in concentrations of 5, 10 and 15 ppm at 120 min. The lowest average removal of Cu^{2+} 89.22% was recorded by *H.opuntia* and 49.9% was recorded by *T.turbinata* in a concentration of 25 ppm at 30 min. In the same way, significant-high average removals (>94%) were recorded in a concentration of 10 ppm at 120 min for *H.opuntia* and in a concentration of 5 ppm by at 60 min for *T.turbinata.* In conclusion, the dead biomass of marine algae can provide a promising and low-cost technique for removing heavy metal pollutants in medical industries.

Keywords: Biosorption, copper, zinc, *Halimeda opuntia* and *Turbinaria turbinata*.

Introduction

Recently, metal pollution has become a major issue all over the world, where metal ions in potable water and wastewater exceed, in many cases, the permissible sanitary levels. Many industrial activities contain dissolved heavy metals in their aqueous effluents; if these discharges are emitted without treatment, they may have adverse effects on the environment (Lu et al., 2017; Mir-Tutusaus et al., 2018). Trace amounts of heavy metals are required by living organisms, but when they exceed the permissible levels, they cause various diseases and disorders as well as deleterious ecological effects, as they are toxic and non-degradable (Mustapha & Halimoon, 2015).

Despite the importance of zinc and copper, as important minerals for living organisms, zinc at a concentration of more than 2 mg/L in wastewater causes irritation, stomach cramps, and lung disorders. Long-term exposure to high doses of copper may cause copper toxicity, which is distinguished by nausea, fever, passing out, vomiting, abdominal cramps, diarrhoea and over time causes liver damage, kidney diseases, brain damage and heart failure (Jewell, 2019). Dissolved metals cannot be removed from the natural environment, so conventional methods for their removal have been studying extensively. These methods include chemical precipitation, ion exchange/chelation, adsorption on activated carbon, and membrane processes (Sharma, 2015; Morin-Crini & Crini, 2017). However, application of these methods is often restricted because they are ineffective and costly. Consequently, biosorption has emerged as an alternative, inexpensive and effective technology (Nadeem et al., 2016).

Biosorption, the passive uptakes process to bind heavy metals on the cellular structure of biological mass, has emerged as an attractive technology due to its simplicity, high efficiency, flexibility of operation, and low cost (Espinosa et al., 2016). Other features of the biosorption technique are easy regeneration of the biosorbent and recuperation of the sorbate. The biosorption process involves absorption of heavy metals in solution on the surface of a cell wall through interaction with functional groups (i.e., carboxylate, amine, amide, imidazole, phosphate, hydroxyl, and other groups) found in cell walls of biopolymers. Many parameters affect the biosorption process efficiency such as biosorbent characteristics (i.e., permeability, surface territory), metal ion features (i.e., molecular weight, oxidation state, ionic radius), in addition to other parameters such as pH, temperature, contact time, biosorbent and sorbate concentration etc. (Escudero et al., 2019).

For years, bacteria, yeast, fungi and algae have been widely used as biosorbents for metal ions (Rahman et al., 2019). Among the biological materials, algae are the most efficient and modest biosorbents because of their slight nutrient requirement. In view of the statistical analysis on algae efficiency in the biosorption process, it has been accounted that algae retain 15.3%–84.6% percentage higher than other microbial biosorbents (Mustapha & Halimoon, 2015). The mechanism of the macroalgae biomass absorption process can be explained simply that macroalgae contain chemical groups attached to the cell wall polysaccharides and proteins such as amino, carboxyl and hydroxyl groups that react with metal ions through many reactions, i.e., complexation, coordination, microprecipitation and ion exchange (Ali Redha, 2020).

The aim of the current study was to evaluate the biosorption potential of dried macroalgal biomass for removing and recovering heavy metals ions (Cu and Zn) from a synthetic aqueous solution and to specify the effects of various factors, i.e., initial metal ion concentration and contact time at pH (4.5 to 5.2) and biosorbent dose (1 gm) for the Cu and Zn ion removal.

Material and Method

Preparation of biosorbents

One kilogram of *H.opuntia* and *T.turbinata* was collected from Red Sea coast in front of NIOF region, the collected samples then were washed with tap water followed by distilled water to remove any agglutinated materials as the epiphytes, sand or mud particles. The algae were shade dried, and then oven dried at 60°C for 48 h to remove excess water and moisture. The dried algae

biomass was cut and ground in a mechanical grinder to be a ground fine powder, subsequently sieved and the particles with an average size of 0.5 mm were used as a biosorbent (Christobel & Lipton, 2015).

Preparation of standard solutions

Cu $(No_3)_2$. $3H_2O$ and Zn $(NO_3)_2$. $6H_2O$ were used as the source of metal in solutions. Copper and zinc solutions were prepared in concentration levels at 5, 10, 15 and 25 ppm by diluting the stock solution (1000 ppm) with distilled water.

Experimental procedure

The experiment on the effects of varying initial metal ion concentrations (5, 10, 15 and 25 ppm) and contact time (30, 60 and 120 min) at pH between 4.5 to 5.2 and adsorbent dose 1gm was carried out by adding 1 g dry weight of *H.opuntia* and *T.turbinata* to a series of flasks containing 100 mL of solution of varying initial metal concentrations. The flasks were shaken at 150 rpm for 30, 60 and 120 min in the dark in a temperature-controlled shaking incubator (at 20°C) to allow the metal uptakes process (Hashim et al., 2004). After that, the samples were filtered through a GF/C filter and the filtrates were analyzed for residual metal concentration by flame atomic absorption spectrophotometer (AAS, GBC-932). We made triplicate samples to assess the accuracy and the mean values that were used for the concentration calculations. The results were expressed in terms of percentage removal of Cu and Zn ions, as given below by the equation:

$$\% \text{ Removal} = ((Co-Ce)/Co)* 100 \tag{1}$$

Where Co is the initial metal ion concentration in ppm, Ce is the concentration levels of Cu and Zn ions after adsorption (ppm). (Mamatha et al., 2012).

Statistical analysis

Statistical analysis was carried out by using Minitab (version 19) to indicate a significance of treatment. One-way analyses of variance (ANOVA) followed by Fisher's grouping test were used to indicate a significant difference between copper and zinc adsorption at different concentrations and contact times.

Results and Discussion

SEM analyses

The change in morphology of using macroalgae species after the biosorption process was predicted by using SEM spectra at 15.0 kV x 2000 resolutions for *H.opuntia* and 15.0 kV x 500 resolutions for *T.turbinata* and was shown in Plates 1 and 2. Plate (1A) and (2A) showed the raw form of using *H.opuntia* and *T.turbinata* before the biosorption process; meanwhile, plates 1 (B, C) and 2 (B, C), it showed the same species after biosorption of

Plate 1. *SEM spectrum of H.opuntia before (1A) and after adsorption of Cu (1B) and Zn (1C) at 150 KV x100 and 150KV x2000*
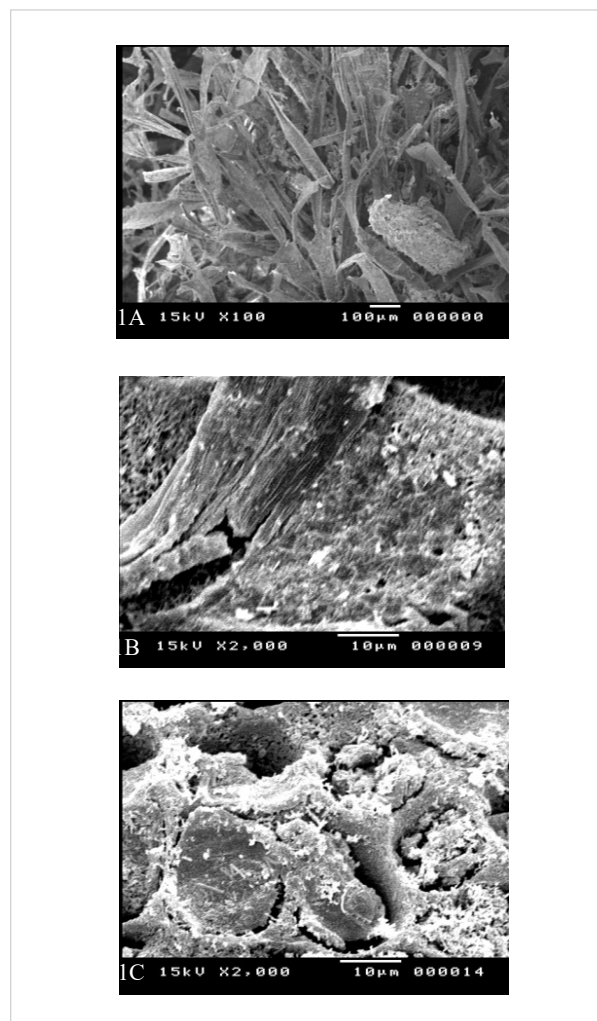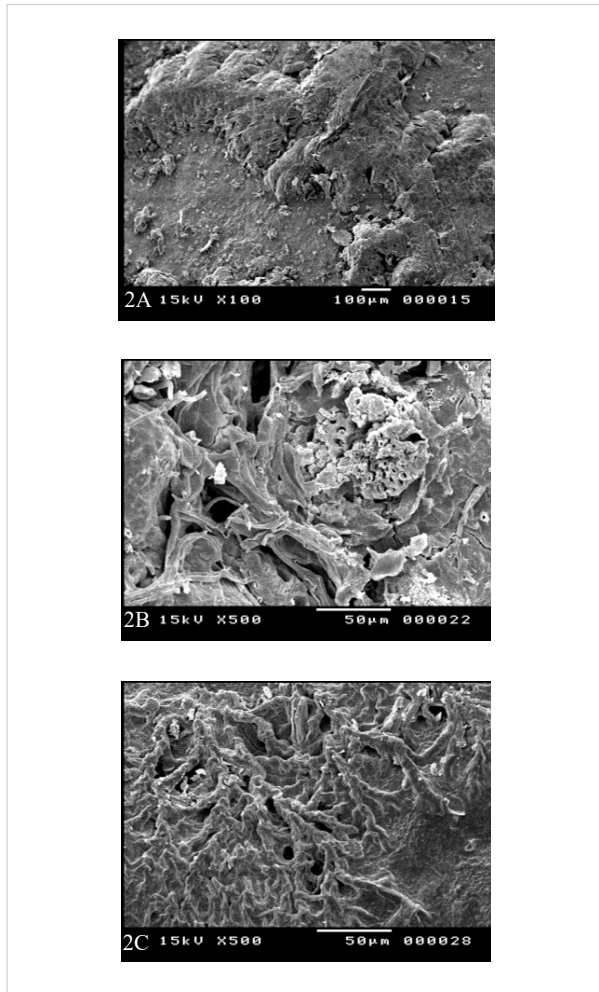
Plate 2. *SEM spectrum of T.turbinata before (2A) and after adsorption of Cu (2B) and Zn (2C) at 150 KV x100 and 150KV x500*

Cu and Zn ions. These plates illustrated that the pores, surfaces, small channels and hollow cylindrical shaped pores were covered and filled with Cu and Zn ions. This is evidence of the change in the initial structure of *H.opuntia* and *T.turbinata* after the biosorption process.

Effect of initial ion concentration

Ashraf et al. (2011) found that the metal biosorption capacity increases when the concentration of initial metal increases until a certain concentration. Initial metal concentration has a similar effect as the contact time. When low metal concentration is used, most of the binding sites of the biosorbent may not be occupied, so the true maximum biosorption capacity of the biosorbent would not unveil. With increasing metal concentration to the extent that the binding sites of biosorbent become fully saturated with the metal, the increase in metal concentration will not be effective. Initial ion concentration plays an important role in the biosorption process. *H.opuntia* was recording Cu^{2+} removal efficiency between 89.22% in 25 ppm concentration and 96.80% in 15 ppm concentration, while *T.turbinata* was recording removal efficiency between 49.90% in 25 ppm concentration and 81.07% in 5 ppm concentration, respectively. A slight decrease in the biosorption efficiency was observed to occur as the concentration of Cu^{2+} ions increased from 10 to 25 ppm with *T.turbinata*; meanwhile, the biosorption efficiency values were close to each other at 10 and 15 ppm and slightly decreased at 25 ppm with *H.opuntia* (Fig. 1). The estimated results indicated that *H.opuntia* was more efficient than *T.turbinata* in the removal of Cu.

Fig. 1. *The effect of initial ion concentration on Cu and Zn removal efficiency by H.opuntia and T.turbinata*
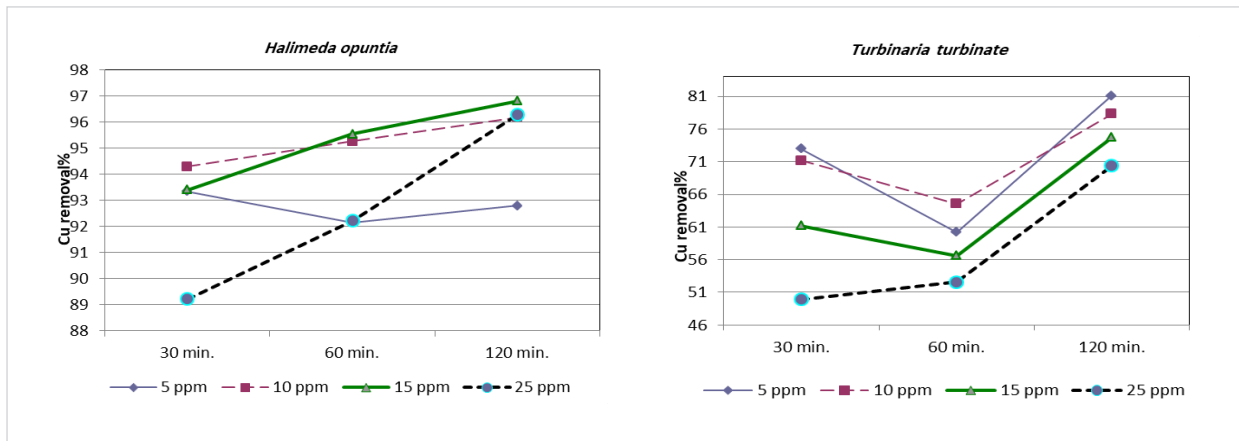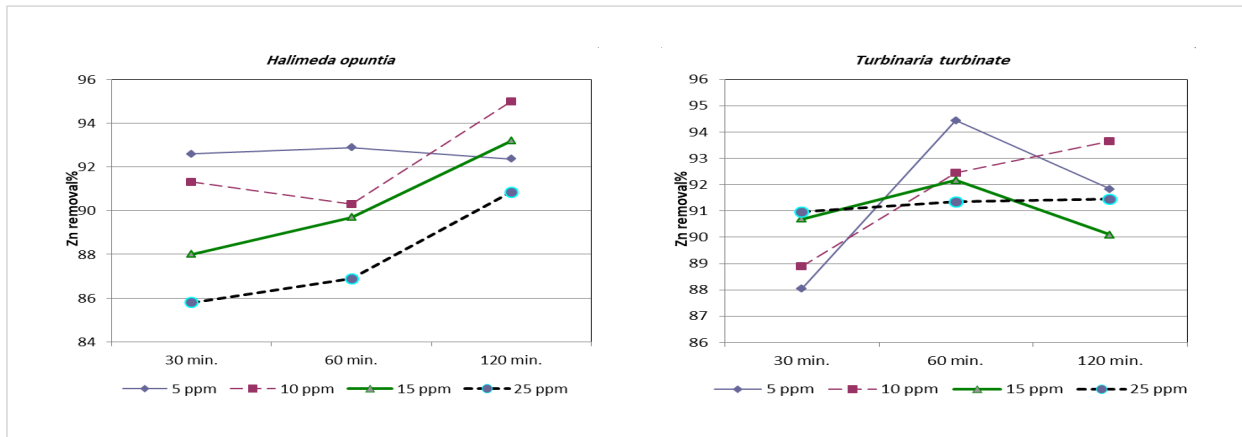
Fig. 2. *The effect of contact time on Cu^{+2} and Zn^{+2} removal efficiency by H.opuntia and T.turbinata*

In case of Zn^{2+}, *H.opuntia* was recorded to show the optimum adsorption efficiency (94.98%) in 10 ppm ion concentration. Contrary to the case of Cu^{2+}, there was a prominent decrease in biosorption efficiency when the concentration of Zn ions was increased from 10 to 25 ppm. On the other hand, the maximum removal efficiency of *T.turbinata* (94.44%) was observed in 5 ppm concentration. It was noticed that the removal efficiencies of Zn^{2+} with *H.opuntia* and *T.turbinata* were almost equal (Table 2). It is clear that increasing Zn^{2+} concentrations from 5 to 25 ppm showed a significant decrease in the removal efficiency. These illustrations indicated that the dry marine algae of both species had the highest efficient removal property in the low Zn^{2+} concentration solutions and were more effective in Cu^{2+} solutions with all concentrations. This illustration is in complete agreement with Ibrahim et al.'s (2016) study, where they report that the adsorption process is more effective at low concentrations because all active sites on the algal surface are vacant. Inversely, at the higher metal levels in aqueous solutions, the binding sites of the algal surfaces become consumed and finally the biosorbent surfaces become saturated and uptakes rates decrease (Al-Qodah, 2006; Tsai & Chen, 2010; Yang et al., 2010).

Effect of contact time

Contact time refers to the required time for the biosorption process to carry out. The contact time was one of the vital factors affecting the biosorption process efficiency. To specify experimental conditions, increasing contact time would allow the biosorbent material to detect the maximum biosorption amplitude. As the biosorbent reaches its maximum biosorption capacity, the binding sites become fully saturated and an increase in the contact time becomes worthless. Fig. 2 summarizes the biosorption efficiency of Cu and Zn ions by *H.opuntia* and *T.turbinata* as a function of contact time at different concentration levels. The adsorption behaviour shows variation among the studied metal ions. The rate of Cu ion removal was fast, as 89.2% to 94.30% and 49.9% to 73.00% of the metal was removed at 30 min within *H.opuntia* and *T.turbinata*, respectively. During the initial stage, a larger surface area of algae is available; therefore, the adsorption quickly occurs at the active biding sites (El-Moselhy et al., 2017). It was noticed that within 60 min contact time, removal efficiency decreased when *T.turbinata* was used and raised again at 120 min. In case of zinc, within 30 min, 85.8% to 92.59% and 88.05% to 90.96% were removed by *H.opuntia* and *T.turbinata*, respectively. Within *H.opuntia* concentrations of 15 and 25 ppm, the removal efficiency increased gradually with time until 120 min. Within *T.turbinata*, concentration of 5, 10, 15, and 25 ppm, the removal efficiency increased within an increase of time from 30 to 60 min, then a slight decrease occured with 5 and 15 mg/L at 120 min. Percentages of Zn^{2+} and copper removal at the definite conditions of different types of macroalgae used in previous studies are listed in Table 1.

Table 1. *Comparison between the obtained biosorption capacity of Cu²⁺ and Zn²⁺ within* **Halimeda opuntia** *and* **Turbinaria turbinata** *and some pervious studied macroalgal species*

Seaweed	Type	Treatment conditions	Heavy Metals	Adsorption capacity	References
Halimeda opuntia	Green seaweed	Cu^{2+} t =120 min T = 20°C Biomass = 1 g pH = 4.5–5.2 rpm = 150 Ions concentration = 10, 15 and 25 mg/L Zn^{2+} t =120 min T = 20°C Biomass = 1 g pH = 4.5–5.2 rpm = 150 Ions concentration = 10 mg/L	Cu^{2+} and Zn^{2+}	Cu^{2+} > 96% Zn^{2+} > 94%	Present study
Turbinaria turbinata	Brown seaweed	Cu^{2+} t = 120m T = 20°C Biomass = 1 g pH = 4.5–5.2 rpm = 150 Ions concentration =5 and 10 mg/L Zn^{2+} t = 60 min T = 20°C Biomass = 1 g pH = 4.5–5.2 rpm = 150 Ions concentration = 5 mg/L	Cu2+ and Zn2+	Cu^{2+} (74.7–81.07%) Zn^{2+} > 94%	Present study
Sargassum sp.	Brown seaweed	t = 60 min T = 25°C Biomass = 1 g pH = 3 rpm = 200 Ions concentration = 5 mg/L	Zn^{2+}	Zn^{2+} = 90.3% (acid treated)	(Mahmood et al., 2017)
Sargassum sp.	Brown seaweed	t = 6 h T = 30°C Biomass = 0.1 g pH = 5 rpm = 150 Ion concentration 0–7 mmol/L	Cu^{2+}	Cu^{2+} = 1.483 mmol/g	(Barquilha et al., 2017)
Sargassum filipendula	Brown seaweed	t = 24 h T = 25°C Biomass = 2 g/L pH = 3.5 rpm = 180 Ion concentration = 1 mmol/L	Cu^{2+} and Zn^{2+}	Cu^{2+} = 69.05% Zn^{2+} = 44.21%	(Cardoso et al., 2017)

Table 1 (continued)

Seaweed	Type	Treatment conditions	Heavy Metals	Adsorption capacity	References
Osmundea pinnatifida	Red seaweed	t = 180 and 60 min T = 20 to 23°C Biomass = 20 g/L pH = 5 rpm = 500 Ion concentration=100 and 50 mg/L	Cu^{2+}	Cu^{2+} = 50.89% to 71.64%	(El Hassouni et al., 2014)
Fucus vesiculou	Brown algae	t = 2h T = 25°C Biomass = 0.25 g/L pH = 5 Ion concentration=10–150 mg/L		0.97 mg/g	(Brinza et al., 2007)
Gracilaria fisheri	Red algae	t = 30m T = 22°C Biomass = 2 g/L pH 4 rpm = 100 Ion concentration = 1 mM	Cu^{2+}	90% (treated with NaoH) 80% (native)	(Chaisuksant, 2003)
Ulva sp	Green algae	t = 2 h T = 22°C Biomass = 8 g/L pH = 5.5 Ion concentration = 10 to 220mg/L	Zn^{2+}	29.63 mg/g	(Badescu et al., 2017)
Ulva fasciata and Sargassum sp.	Green algae Brown algae	t = 30 m T =22°C Biomass = 0.1 to 5 g/L pH 5.5 rpm = 150 Ion concentration = 10 to 220 mg/L	Cu^{2+}	73.5 mg/g for *U. fasciata* and 72.5 mg/g for *Sargassum sp*	(Karthikeyan et al., 2007)

Data analysis

The ANOVA result indicates highly significant differences between two tested seaweeds in the removal of copper and zinc at different concentrations (P = 0.001, Tables 2, 3). In addition, Fisher's grouping test indicates a significant difference between copper and zinc at different concentrations and times. The highest removal of copper was 96.8%, 96.27%, and 96.19% at concentrations of 15, 25, and 10 ppm, respectively, by *H.opuntia* at 120 min, while the lowest removal of copper was 49.9%

Table 2. *ANOVA and Fisher's grouping test of removal (%) of Cu at different concentration (ppm) and time (min)*

	ANOVA		Time (min)	Fisher's test for different concentrations (ppm) grouping			
	F	P		5	10	15	25
H.opuntia	373.1	0.001	30	93.34 ± 0.25[B]	94.30 ± 0.13[A]	93.40 ± 1.49[B]	89.22 ± 0.22[C]
	140.3	0.001	60	92.14 ± 0.247[B]	95.26 ± 0.24[A]	95.55 ± 0.322[A]	92.21 ± 0.29[B]
	312.9	0.001	120	92.8 ± 0.16[C]	96.19 ± 0.09[B]	96.8 ± 0.21[A]	96.27 ± 0.21[B]
T.turbinata	437.2	0.001	30	73 ± 0.06[A]	71.2 ± 0.1[B]	61.22 ± 011[C]	49.9 ± 0.06[D]
	191.9	0.001	60	60.22 ± 0.07[B]	64.52 ± 0.08[A]	56.59 ± 0.04[C]	52.6 ± 0.05[D]
	521.3	0.001	120	81.07 ± 0.02[A]	78.32 ± 0.05[B]	74.7 ± 0.02[C]	70.4 ± 0.01[D]

Table 3. *ANOVA and Fisher's grouping test of removal (%) of Zn at different concentration (ppm) and time (min)*

	ANOVA		Time (min.)	Fisher's test for different concentrations (ppm) grouping			
	F	P		5	10	15	25
H.opuntia	902.3	0.001	30	92.59 ± 0.04^A	91.3 ± 0.05^B	88 ± 0.08^C	85.8 ± 0.01^D
	197.8	0.001	60	92.88 ± 0.01^A	90.29 ± 0.03^B	89.7 ± 0.03^C	86.88 ± 0.02^D
	177.6	0.001	120	92.37 ± 0.09^A	94.98 ± 0.09^B	93.19 ± 0.01^C	90.84 ± 0.04^D
T.turbinata	182.6	0.001	30	88.05 ± 0.08^A	88.89 ± 0.02^B	90.69 ± 0.01^C	90.96 ± 0.07^D
	805.2	0.001	60	94.44 ± 0.05^A	92.44 ± 0.1^B	92.16 ± 0.04^C	91.34 ± 0.09^D
	628.2	0.001	120	91.83 ± 0.05^B	93.65 ± 0.05^A	90.09 ± 0.07^D	91.45 ± 0.11^C

at concentrations 25 ppm by *Turbinaria turbinata* at 30 min. As for zinc, the highest removal was 94.9% at concentrations of 10 ppm by *H.opuntia* at 120 min, followed by 94.4% at a concentration of 5 ppm by *T.turbinata* at 60 min, while the lowest removal was 85.8% at a concentration 25 ppm by *H. opuntia* at 30 min.

Conclusion

Based on the obtained results, it can be concluded that the studied species (*H.opuntia* and *T.turbinata)* have high abilities for copper and zinc removal at low concentrations. Both species are able to remove significant amounts of copper and zinc, hence supporting their potential application in the treatment of wastewater polluted by the metals. *H.opuntia* has higher efficiency for copper removal in comparison to *T.turbinata*, where more than 96% of copper is removed at 120 min within *H.opuntia*, while *T.turbinata* removes 81% of Cu ions at 120 min. In case of zinc, the two-used macroalgae have close competencies; they remove more than 85% within 30 min.

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