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Bioremoval of Mercury (Hg^{+2}), Copper (Cu^{+2}), and Lead (Pb^{+2}) from Water by macroalgae; *Enteromorpha intestinales*

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Abstract—Bioremoval is an economically and successful technique to take away heavy metals by macroalgae. *Enteromorpha intestinales* is one of the main species of green macroalgae in the water Arabian Gulf, Saudi Arabia. It is tested as bioremoving for the three heavy metals; Hg, Cu, and Pb from water. The concentrations solutions of heavy metals have been used in five replicates; Cu (2,4,6,8,10) ppm, Hg (1,2,4,6,8) ppm, and Pb (0.5,1,1.5) ppm with live *E. intestinalis*. The results were demonstrated that the relative growth was little affected in the three heavy metals concentrations. The removal % and the sorption capacity were more significant with Hg and Cu and lower with Pb. The concentration of heavy metals in dry E. intestinalis was increased with increased concentration of metals in solutions. The bioconcentration; BCF was taken a decreased trend with increased metals. From this study, we can use E. *intestinalis* for removing the Hg Cu and pb at low concentrations in water pollution.

Keywords— Macroalgae - Enteromorpha intestinales – Heavy metals – Bioremoval – Hg, Cu, and Pb

I. INTRODUCTION

Heavy metals like as Pb, Cu, and Hg are no degradable pollution in open water. They are toxic and a little important for living organisms. Those pollutants have wonderful bad effects in the environment, due to their extreme toxicity [1]. Bioremoval is a useful solution for water contaminating at low concentrations of heavy metals [2]. Algae gather heavy metals from their aquatic environment. The use of living algae as a bioremoval will be fine. They can be divided, colonized, and grown easily, and made biomass production. They need simple requirements for growth; they have rapid growth rates and create regenerating material for removing heavy metals, [3]. They are the capability to grow both heterotrophic and autotrophic, large surface, area/volume ratios [4]. Green macroalgae have been used as bioindicators pollution in several parts of the world as Enteromorpha, [5]. *Enteromorpha intestinales* is one of the main species of green macroalgae in the water Arabian Gulf, Saudi Arabia [6]. The strategies as precipitation by chemical compounds, adsorption, ion exchange, and purification by membrane were used for removing heavy metals from aquatic environments [7] and [8]. Most of those strategies are highly-priced, and incompletely get rid of heavy metals, require excessive electricity also are lower than biosorption techniques [9] and [10]. Bioremoval is an economically and successful technique to take away heavy metals by algae. Many algae have tolerances mechanisms as phytochelatins and metallothioneins, they can form complex compounds with heavy metals and rest them into vacuoles [11]. They contain functional groups that can be active as binding sites for metals as carboxyl, hydroxyl, amino, and sulfate [12]. Pb, Cu, Zn Ag, Hg, and Cd were detoxification mechanisms by metallothionein and phytochelatins [13]. Algae can do that by cell wall components.

II. RELATED WORK

The green macroalgae as *Enteromorpha intestinales* were the dominant species which can be used as a bioremoval filter for removing the heavy metals like Cu, Hg, and pb at low concentrations in the Gulf water, marine aquaculture water. The use of macroalgae is an economically and successful technique to get rid of heavy metals. We can examine other heavy metals and other species of green macroalgae that dominance in the water environments.

III. METHODOLOGY

Seaweed Samples collection

Green seaweed was freshly collected by hand from the nearshore the water coast of the Arabian Gulf at Al-Qateif, Saudi Arabia (The surface Gulf water temperature 21.2°C, salinity 37.9). They washed with Gulf water to get rid of the foreign particles, and epiphytes. They were stored in an icebox and at once transported to the lab. They washed by tap and distilled water to remove the salts on the surface of the samples. They were identified by species based on morphology **[14].** Macroalgae*; Enteromorpha intestines*, was incubated in Gulf water after dilution by distilled water at condition (salinity 30 g/l, temperature 25°C, and 100 µmol photons $m^{-2\ s-1}$ with photoperiod 12 hrs light and 12 hrs dark) for 14 days for acclimatization.

The removal experiment design

The bioaccumulation experiments have been carried out in Erlenmeyer flasks (250 mL) that washed with dilute nitric acid ($HNO_{3)}$ to dispose of metals after that washed with deionized water. Every flask plant 2.0 g of the fresh thalli *E. intestinales* and 200 mL of heavy metals solution of preference concentration. Two controls (one without algae and other with algae however without HMs) had been included to confirm metallic pollution and outcomes of environmental situations/elements at the explosion of algae at a similar point of the test. The heavy metals solution concentrations (ppm) have been Cu (2,4,6,8,10), Hg (1,2,4,6,8) and Pb (0.5,1,1.5) . The solution of the heavy metal was prepared as Cu from copper sulfate, Hg from mercuric chloride, and Pb from lead nitrate all chemicals compounds from Loba Chemie Pvt. Ltd. - Mumbai, India. All treatments have been organized in 5 replicates and the tested period was one week under light/dark period 12:12h at temperature 25^{o}C. The algae collected and washed with deionized water and dried by paper tissue for the later determined of heavy metals and measured the heavy metals also in solution after one week.

The heavy metals measurement

Dried fresh thalli *E. intestinales* was digests with 8 ml HNO_3 (65%) in microwave mineralization (microwave Milestone Ethos one). The heavy metals (Cu and Pb) were determined by the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Varian 720-ES) and they were quantified in ppm for *E. intestinales* and solution of heavy metals

Mercury analysis in all samples solution of heavy metals and E**.** *intestinales* thalli has been realized using the Direct Mercury Analyser (DMA-80, Milestone).

Pigment concentrations analysis in *E. intestinales*

Chlorophyll a & b, and cartonidoids as µg/g was determined by spectrometric measurements. 0.5 g cleaned *E. intestinales* thalli was homogenated and extracted in 90% acetone. cartonide was determined according to [15] and Chlorophyll according to [16]

Statistical analysis

The mean of five replicates ± Str obtained as (Standard Error). Besides the mean values of each analysis were subjected to a one-way ANOVA test at p<0.05 using the SPSS Inc. program version 22 to detect significant differences among the target.

IV. RESULTS AND DISCUSSION

Results

1- Hg metal

The results are tabulated in Table 1 and Figure 1&2 indicate that the relative growth of *E. intestinales* exposed to different concentration of Hg 1,2,4,6,8 µg/ml (ppm) was hardly ever significant between them (0.8007 b ± 0.04187, 0.8236 b ± 0.02113, 1.0403 a ± 0.10699, 0.935 ab ± 0.04775.9817 ab ± 0.02203) at (p<0.05), the treatment of concentration 4 µg/ml was more significant (1.0403 a ± 0.10699) than all treatments and no significant between the treatments concentration 1 and 2 µg/ml Hg and also between treatments 6 and 8 µg/ml. The sorption capacity was recorded more significantly increased between

Table 1: Means ± Std. Error of Means of Relative growth, Sorption Capacity, Removal %, Bioconcentration Factor, and heavy metals concentration g/kg in dry tissue of E. intestinalis at different heavy metals concentration (Hg, Cu, and pb) ppm in water.

Heavy metals	Conc. in water ppm	Relative growth	Sorption Capacity	Removal %	Bioconcentration Factor	Conc. in dry tissue g/kg
Hg	1.0	0.8007 b ±.04187	65.1837 e ±3.75829	68.500 c ±3.51888	0.3150 a ±.03519	0.3150 d ±.03519
	2.0	.8236 b ±0.02113	123.399 d ±4.59079	64.825 c ±1.50000	0.3518 a ±.01500	0.7035 c ±.03000
	4.0	1.0403 a ±0.10699	289.06 c ±9.93704	75.812 b ±.35444	0.2419 b ±.00354	0.9675 b ±.01418
	6.0	0.935 ab ±.04775	478.65 b ±11.90154	83.775 a ±.20000	0.1623 c ±.00200	0.9735 b ±.01200
	8.0	.9817 ab ±.02203	633.72a ±18.07939	85.025 a ±.37894	0.1498 c ±.00379	1.1980 a ±.03032
Cu	2.0	0.9433 a ±0.09821	202.003 d ±7.47002	63.910 b ±2.11335	1.0067 a ±0.16756	2.0133 d ±0.32987
	4.0	1.0233 a ±0.13346	433.513 c ±47.45364	73.773 b ±1.03345	0.8733 a ±0.07839	3.4900 c ±0.31565
	6.0	1.0900 a ±0.03512	790.766 b ±23.45712	87.386 a ±0.79075	.9467 a ±0.02848	5.6833 b ±0.16677
	8.0	1.1633 a ±.05364	1115.89 a ±42.18434	87.856 a ±1.59179	0.9733 a ±.05667	7.8033 a ±.46667

Pb	0.50	0.823 a ±.08253	11.220 b ± 0.53201	28.893 b ±4.15412	0.540 a ± 0.057	0.270 c ± 0.035
	1.0	0.8500 a ±.05508	20.893 a ± 0.67817	28.823 b ±1.05302	0.408 a ± 0.087	0.408 b ±0.0213
	1.50	0.8100 a ±0.07000	27.266 a ±3.55784	38.983 a ±0.64957	0.412 a ±0.0537	0.618 a ±0.0172

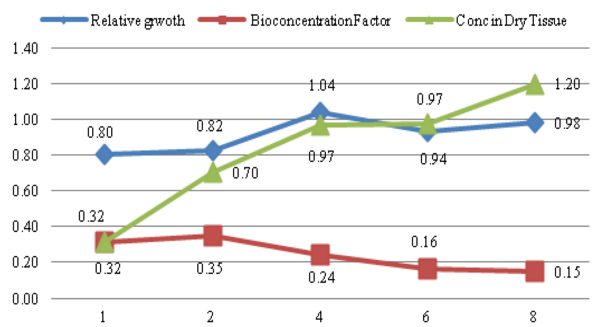

Fig 1 : The consequence of various concentrations of Hg (ppm) on relative growth, bioconcentration factor and concentration of Hg in dry tissue of E. intestinalis

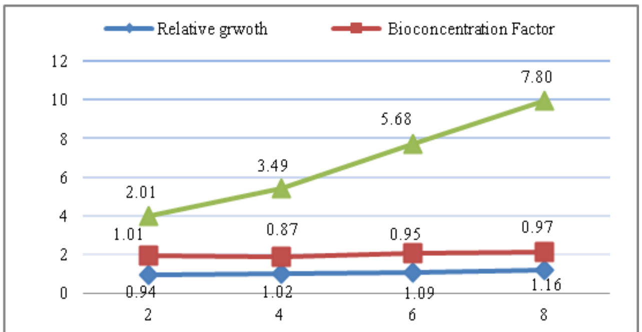

Fig 3: The consequence of various concentrations of Cu (ppm) on relative growth, bioconcentration factor and concentration Cu in dry tissue of E. intestinalis

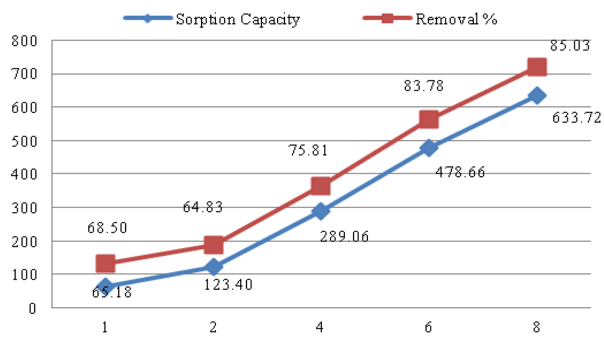

Fig 2 : The concequence of various concentrations of Hg (ppm) on sorption capacity and removel % of E. intestinalis

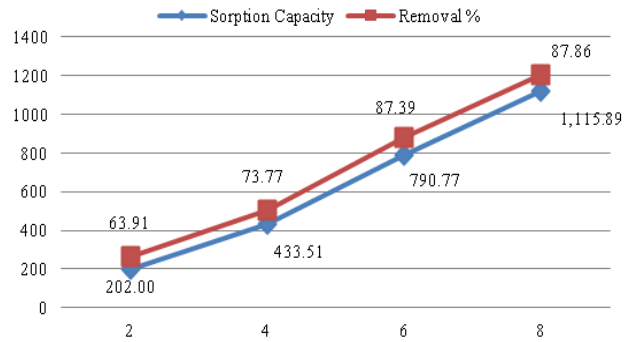

Fig 4 : The consequence of various concentrations of Cu (ppm) on sorption capacity and removal % of E. intestinalis

treatments, (65.1837 e ± 3.75829, 123.399 d ± 4.59079, 289.06 c ± 9.93704, 478.65 b ± 11.90154, 633.72a ± 18.07939), it was increased with increased the concentrations of Hg in the medium solution .The removal percentage of Hg metals was recorded increased significantly with increased the concentration (68.500 c ± 3.51888, 64.825 c ± 1.50000, 75.812 b ± 0.35444) but notice that no significant between the two concentrations 1 & 2, and 6 & 8 µg/ml Hg (83.775 a ± 0.20000, 85.025 a ± 0.37894). The bioconcentration factor of Hg metals was decreased significant with increased the concentration of treatments and no significant different between the concentration 1 & 2 µg/ml (0.3150 a ± 0.03519, 0.3518 a ± 0.01500) and concentration 6 & 8 µg/ml, (0.1623 c ± 0.00200, 0.1498 c ± 0.00379). The concentration of Hg metal in dry ***E. intestinales*** tissues was recorded increased with the increased the concentrations of Hg in medium solution (0.3150 d ± 0.03519, 0.7035 c ± 0.03000, 0.9675 b ± 0.01418, 0.9735 b ± 0.01200, 1.1980 a ± 0.03032) and no significant between 4 & 6 µg/ml of Hg treatments.

2- Cu metal

The results of the study of Cu metal removal by *E. intestinales* were shown in Table 1 and Figures 3 & 4. The relative growth values (0.9433 a ± 0.09821, 1.0233 a ± 0.13346, 1.0900 a ± 0.03512, 1.1633 a ± .05364) and bioconcentration factor values (1.0067 a ± 0.16756, 0.8733 a ± 0.07839, 0.9467 a ± 0.02848, 0.9733 a ± 0.05667) were recorded no significant different at $p<0.05$ between all treatments of Cu concentration in medium 2,4,6,8, µg/ml. On another hand, the sorption capacity values (202.003 d ± 7.47002, 433.513 c ± 47.45364, 790.766 b ± 23.45712, 1115.89 a ± 42.18434) and concentration of Cu in dry tissues of macroalgae values (2.0133 d ± 0.32987, 3.4900 c ± 0.31565, 5.6833 b ± 0.16677, 7.8033 a ± 0.46667) were recorded more significantly increased with the increased the Cu concentrations in the medium. The removal percentage values (63.910 b ± 2.11335, 73.773 b ± 1.03345, 87.386 a ± 0.79075, 87.856 a ± 1.59179) were recorded increased significantly with increased the concentration of Cu treatments, although nonsignificant differences between 2 & 4 µg/ml and also 6 & 8 µg/ml Cu concentration in the medium was shown.

3- Pb metal

With regard the results of removal Pb metals ; the relative growth values (0.823 a ± 0.08253, 0.8500 a ± 0.05508, 0.8100 a ± 0.07000) and bioconcentration factor values (0.540 a ± 0.057, 0.408 a ± 0.087, 0.412 a ± 0.0537) were nonsignificant different between the all treatments concentration of pb µg/ml. The sorption capacity values (11.220 b ± 0.53201, 20.893 a ± 0.67817, 27.266 a ± 3.55784), the removal percentage values (28.893 b ± 4.15412, 28.823 b ± 1.05302, 38.983 a ± 0.64957) and concentration of pb metal in dry *E. intestinales* values (0.270 c ± 0.035, 0.408 b ± 0.0213, 0.618 a ± 0.0172) were increased significant with increased the pb concentration in the medium. Also noticing that no significant between 0.5 &1.0 pb µg/ml for removal % and not significant for 1.0 & 1.5 µg/ml pb for sorption capacity. The bioconcentration factor decreased with increased concentrating pb and nonsignificant was recorded between the treatments; those results were illustrated in table 1 and figure 5 & 6.

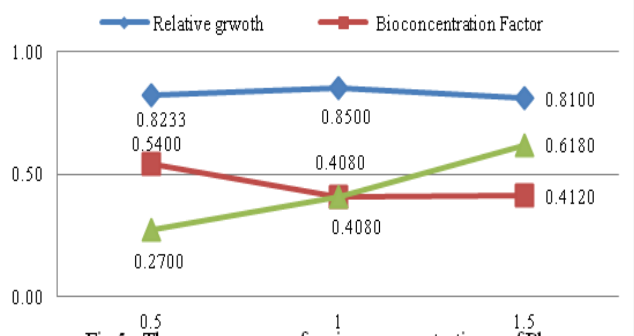

Fig 5 : The consequence of various concentrations of Pb (ppm) on relative growth, bioconcentration factor and concentration of Pb in dry tissue of E. intestinalis

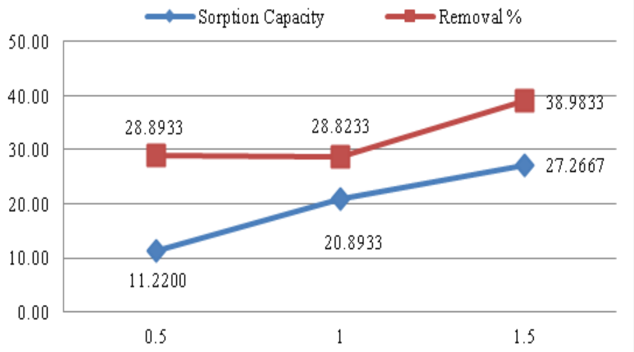

Fig 6 : The consequence of various concentrations of Pb (ppm) on sorption capacity and removal% of E. intesinalis

The consequence of various concentrations of metals (Hg, Cu, Pb) in medium on pigments of macroalgae *E. intestinalis* ; chlorophyll a, chlorophyll b, and cartonid are shown in Figures 7, 8 & 9. There were significantly decreased at (P<0.05) of all pigments with increased concentration of the metals in a medium.

Discussion

In this study, the relative growth of *E. intestinales* exposed to heavy metals (Hg, Cu, and Pb) was hardly ever significant, and the sorption capacity and concentration of metals in dry algae tissues were amplified with amplified the concentration of the metals in the water because the concentration of metals was low and little effect on the growth algae

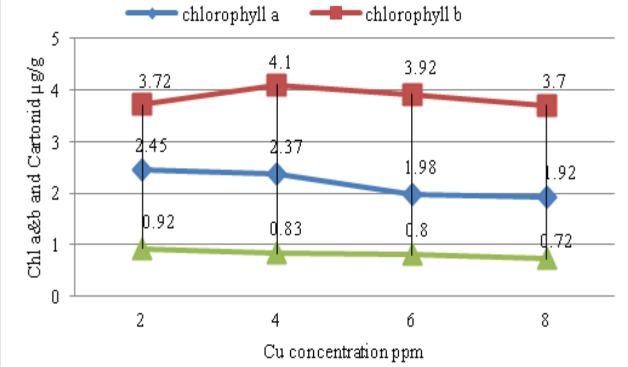

Fig 8: The consequence of various concentrations of Cu (ppm) on chlorophyll a , b and cartonid in E. intestinalis

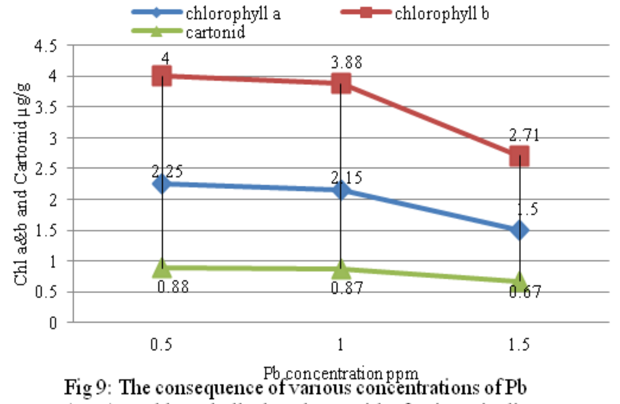

Fig 9: The consequence of various concentrations of Pb (ppm) on chlorophyll a,b and cartonid of E. intestinalis

where the metals can be accumulated in algae tissues to concentrations above their concentrations in seawater, giving get higher to concentration factors up 10^3 [17]. It has appeared no effect with Cu metals concentrations in growth; the Cu element is attributed to the fact that it is important as micronutrients for macroalgae metabolic functions. It plays a function in metabolism and essential for enzymes (amino oxidase, and cytochrome) and electrons transport components in photosynthesis [1] and [18].

BCF is a helpful factor to estimate the possibility of macroalgae for gathering metals . In this work, the BCF values of macroalgae in all treatments of Hg, Cu, and Pb were lower significant with the higher concentration of metals in the medium, The BCF for Cu was higher than Hg and Pb; this is demonstrated that algae uptake of Cu was greater than Hg and Pb. There was a regular decrease in the Pb and Hg uptake possible with an increased concentration in the medium. The metal concentration in water is the central point affecting the metal take-up effectiveness. The overabundance of Pb and Hg causes restraint of development mitosis, photosynthesis, and water assimilation, enzymes action, and upsets mineral nutrition. [19] Hg and Pb are nonessential elements for macroalgae metabolism and their toxicity effects normally metabolic functions of macroalgae and linked with amplified ROS production with a decreased capacity of cellular antioxidant [20].

The high concentration ions of heavy metals destroyed chloroplasts of macroalgae. It is notable that heavy metals can cause confusion of chloroplasts leading a decrease of the photosynthetic pigments [21]. Both Cd and Pb were reported to inhibit chlorophyll biosynthesis and lowered chlorophyll content [22]. They found that the decreased in chlorophyll content might be caused by a decrease in the synthesis of chlorophyll, likely by increasing chlorophylls activity by disorderliness of chloroplast membrane and by inactivation of electron transportation in photosystem [23] and [24]

V. CONCLUSION AND FUTURE SCOPE

The resulted of this work demonstrated that *E. intestinales* can be used as a bioremoval filter for some of the heavy metals like Cu, Hg, and Pb at low concentrations in polluted natural marine water, this method is very cheap and may apply in marine aquaculture before drainage water to Gulf, or Seawater. We can test the removal of other heavy metals with *E. intestinales* or other heavy metals with other species of macroalgae in the future.

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