Pharmacophore

ISSN-2229-5402

Journal home page: http://www.pharmacophorejournal.com

REMOVAL OF DANGEROUS HEAVY METAL AND SOME HUMAN PATHOGENS BY DRIED GREEN ALGAE COLLECTED FROM JEDDAH COAST

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ARTICLE INFO

Received:
03th Nov 2018
Received in revised form:
14th April 2019
Accepted:
21th April 2019
Available online:
28th Jun 2019

Keywords: *Ulva lactuca, Jeddah Coast, green algae, human pathogens, lead, Heavy metal, MIC, pathogens.*

ABSTRACT

Contamination with heavy metals in industrial wastewater of many tanneries and metal industries is increasing and dangerous problem. Red sea was habited by diverse groups of marine macroalgae with different economic importance, as feed, fodder and medicine. The biosorption ability of some red sea algae for lead ions was poorly studied. This study aimed to study the removal of dangerous heavy metal and some human pathogens by dried macroalgae collected from Jeddah Coast. The tested algal species were belonging to Chlorophyta (two species named *Halimeda opuntia* and *Ulva lactuca*), Phaeophyta (two species named *Dictyota dichotoma* and *Sargassum muticum*), and Rhodophyta (*Digenea simplex*). *Ulva* and *Sargassum* were the most dominant genera in the collecton area of the red sea. The dead algal mass of all tested macroalgae have the ability to absorb lead from solutions which is one of the most dangerous heavy metals. The green algal species, *Ulva lactuca* showed the best removal level of lead followed by the red algal species, *Digenea simplex.* The removal percentages of lead by *Halimeda opuntia, Dictyota dichotoma* and *Sargassum muticum* were nearly the same. Thus, *Ulva lactuca* was selected for detailed studies. Metal sorption included binding on the algal cell surface and to intracellular ligands. Various pretreatments enhance metal sorption capacity of algae. Maximum adsorption of lead by *Ulva* powder was obtained at 45°C and pH 4. Percentage of lead adsorption was about 100% by *Ulva* dry mass, suspended in lead solution (1000 mg/l) at pH 4 or pH 5, while the percentage was decreased to 40% and 10% at pH 4 and pH 5 at 5000 mg/l, respectively, which means at pH 5, high lead concentrations, the removal process is decreased. Maximum removal of lead by *Ulva* dry mass was obtained after 6 hrs of incubation at static conditions. Increasing shaking rate decreased biosorption process. It is clear that, in the biosorption process, increasing the weight of the used *Ulva* dry mass, enhanced removal process and maximum removal percentage was obtained using 1 g dry mass/l, while the lowest was obtained for 0.12 g/l. The antimicrobial activities of the tested algal extract against different microorganisms were determined. Maximum growth inhibition was recorded by aqueous extract of *Ulva* for both *Escherichia coli* and *Aspergillus niger,* thus they were used as control test organisms for both bacteria and fungi. It was clear that, the effect of *Ulva* extract was excellent on gram-negative bacteria compared to gram-positive bacteria with MIC ranged from 30-40 µg/ml for gram-negative bacteria and 60-65 µg/ml for gram-positive bacteria. Moderate antifungal activities were recorded against *Candida albicans* and *Alternaria alternata* compared to *Aspergillus niger.* In conclusion, using *Ulva* dry mass to remove lead from contaminated solutions is effective and low-cost method. Moreover, *Ulva* extract inhibited certain bacterial and fungal pathogens, thus, *Ulva* dry mass can be used to clean wastewater from heavy metals and some pathogens.

To Cite This Article: Reda H. Amasha, Magda M. Aly, (2019), "*Removal of Dangerous Heavy Metal and Some Human Pathogens by Dried Green Algae Collected from Jeddah Coast*", ***Pharmacophore, 10(3),*** *5-13.*

Introduction

Increasing concentration of different heavy metals in soil and water is one of the difficult problems. Bioremediation or removal of dangerous heavy metals by living or dead cells of algae have not been studied extensively. This process have low cost, high adsorbing capacity and causes no secondary pollution [1]. Red sea contain many macroalgal isolates which can be used to treat heavy metals in industrial waste water. Many factors are affecting the removal process (temperature, pH, type of heavy metal, the heavy metal concentration, and organism used). The adsorption processes are mainly rapid adsorption followed by chemical adsorption (a complex formation between functional groups on/in the surface structure of the biological material, and heavy metal ions) [2]. Bacteria, fungi and different species of algae have different adsorption capacity to heavy metal ions, thus their dry masses can be used successfully as a new method for refining treatment of wastewater. Screening for the best biological adsorbent is of great interest in wastewater treatment [3, 4].

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Removal of many heavy metals from wastewater using microalgae, cyanobacteria, bacteria, yeast and higher plants in immobilized or free cells is excellent technique with many advantages over the other removal techniques including chemical treatment, ion exchange, membrane filtration, reverse osmosis and activated carbon adsorption [1, 5, 6]. Srinath *et al*., [7] reported the advantages of biosorption process which are the reusability of metals, low cost, improved selectivity for specific metals, needing short operation time and no production of toxic secondary compounds. Biosorbents of biological origin for heavy metal removal in shallow water (1-5 m) with metal concentrations of about 1 to 20 mg/l was carried out [8]. The previous technique has emerged in the last decade as one of the most promising alternatives to traditional heavy metal management strategies [9, 10]. Heavy metals have many adverse effects on living organisms and these effects depend upon duration of exposure, dose and type of metal used [11, 12]. The attention has been focused on non-living dry algal biomass e. g. *Spirogira* [13], fungi including *Aspergillus niger*, *Saccharomyces* and other microorganisms for metal removal [10, 14].

Biosorption of heavy metals including Pb^{+2}, Ni^{+2} and Cr^{+2} ions by inactive *Saccharomyces cerevisiae* was affected by temperature, initial pH of the solution, and initial heavy metal concentration [14]. Sorption of Hg^{++} and Pb^{++} from heavy metal containing solutions using dried *Aspergillus niger* biomass was studied by [9], while [6] used *Mucor rouxii* biomass immobilized in a polysulfone matrix for the biosorption of metal ions such as Pb^{+2}, Cd^{+2}, Ni^{+2} and Zn^{+2}. However, green algal species such as *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Chlorella homosphaera*, have also been studied apart from marine algae for their biosorption capacities [10, 15, 16].

The Red sea separate the Arabian Peninsula from northeastern Africa and extends northwest from the strait of Bab el Mandeb to Suez, Egypt, for a distance of 1,900 km. Its maximum depth and width are 3,040 m and 350 km, respectively. Jeddah in Saudi Arabia, Mukalla of Yemen and Suez of Egypt present its ports. Jeddah coast is the richest source of micro- and macroalgae including cyanobacteria, green, red and brown algae. Aleem *et al*. [17] recorded 16 species of blue green algae and 27 species of chlorophyta in the Red sea at the area of Obhour, Jeddah. Furthermore, they recorded new algal species like *Chrocoocus turgidus*, *Microcoleus chihonuplastes*, *Symplca muscorum*, *Tydemania expeditionis*, *Padina boyryana*, *Dictyopteris delicatula* and *Asterocystis ramose*. Moreover**,** phytoplankton succession in relation to some physicochemical characters of some water bodies at Jeddah Coast was studied [18]. Collection of some algal species from the coast of Jeddah and using these materials for heavy metal removal are of considerable interest. This study aimed to isolate some marine algal species from local environment and selection of the most active ones in lead removal. Factors affecting removal process and inhibitory activity against some pathogens were studied.

Materials and Methods

Algal samples collections and preparation

Some algal isolates were collected during spring season (April, 2017) from marine water, Jeddah corniche, 21.5899° N, 39.1067° E, Jeddah, Saudi Arabia (Figure 1A). The algal isolates were separated and washed with tap water followed by washing with double distilled water thoroughly to eliminate sand, debris and any adhering foreign particles [19]. The washed biomass was identified up to species level at Biology department, Faculty of Science, King Abdulaziz University. Each algal type was spread on filter papers for 2 days until air dried, followed by drying at 60°C in an oven until constant weight. The dried algal species were powdered using electrical grinder and sieved using 0.1-0.2 mm mesh size sieve (Figure 1B). The sieved dried algal materials were preserved at 4°C until used as adsorbents.

Metal ion solutions

Stock solution (5000 mg/l) of lead acetate (analytical grade) was prepared in deionized water. The working solution was prepared by diluting stock solution to appropriate concentration. Algal powder was suspended in the tested heavy metal solution (Figure 1C) and removal percentage was calculated.

Heavy metal removal using dried algal powder

In 250 ml Erlenmeyer glass flasks, 0.1 gram of each different algal species was added to 100 ml of lead acetate (2000 mg /l). The mixtures were agitated on a rotary shaker at 30°C and 50 rpm. After 4 hr, percentage of lead removal was calculated as follows:

Lead removal (%) = 100(Ci − Cf) Ci [20]

Ci is the concentration of initial metal ions (mg/l), and Cf is the final concentration of metal ions (mg/l).

Biosorption capacity (Q), the amount of metal adsorbed per gram of biosorbent, can be calculated in mg/g as follows:

Q = (Ci − Cf) x Volume/ m

Ci is the initial concentration of metal ions (mg/L), Cf is the final concentration of metal ions (mg/l), V is the volume of solution in litter, and m is the mass of algal material (g).

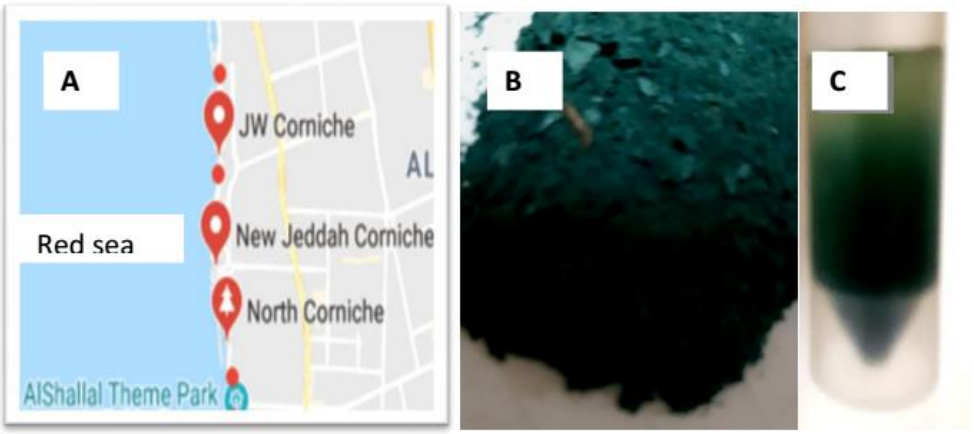

Figure 1. Collection and preparation of the algal sample, A: Google map showing collection side, B: Dried and powdered *Ulva* sample, and C: Adsorption of heavy metal on the algal powder.

Effect of some factors on lead removal and Bio-sorption process

In 250 ml Erlenmeyer glass flasks, 0.1 of each *Ulva* dry mass was add to 100 ml of lead acetate (2000 mg /l). The mixtures were agitated on a rotary shaker at 20, 30, 37, 45 and 50°C and 50 rpm. After 4 hr of agitation, the solutions were centrifuged at 3000 rpm for 15 min to separate the algal biomass. The percentages of lead removal in the clear solutions were calculated [20]. Similarly, flasks with 100 ml of lead acetate solutions were prepared as before and pH of the solutions were adjusting to pH 3, 4, 5,7, 9 and 11 before adding the dry powder of *Ulva*. All flasks were incubated at 30°C and 50 rpm for 4 hrs, and the percentages of lead removal in the clear solutions were calculated. Removal percentages of lead at different concentrations of lead (200-5000 mg/l) were studied at two different pH values (pH 4 and pH 5) and % of lead removal in the solutions were calculated. Using shaking incubators, the effect of different shaking rate, 50,100,120 and 150 rpm and incubation time (2, 4, 6, 8 and 10 hrs) were studied. Controls flask without shaking was prepared as control. Moreover, effect of different concentrations of the adsorbent material (*Ulva* powder) from 0.12- 1.0 g/l was studied in flasks containing lead solution. At the end of the experiment, biosorption efficiency was calculated for each concentration of the dry matter. All the experiments were carried out in triplicates and the mean value was calculated.

Heavy metal analysis

After centrifugation, heavy metal concentration in the filtrates was quantified as described by [21]. The amount of metal adsorbed at equilibrium, Q (mg/g), which represents the heavy metal uptake was calculated from the difference in metal concentration in the aqueous phase before and after adsorption according [20].

Preparation of algal extracts

The aqueous extract of *Ulva* was prepared as described by [22]. About 5 g of the dried material was suspended in 100 ml of boiling water and in shaking water bath for 2 hrs. and the mixture was filtered using filter paper (No. one). The obtained extract was filtered sterilized using 0.45µm bacterial filter.

Antimicrobial activity

Antibacterial activities on Mueller Hinton agar (Sigma-Aldrich) of the tested extract against some bacteria were recorded using paper disc diffusion method [23]. *Enterococcus feacalis*, *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Salmonella enterica*, *Escherichia coli* and *Klebsiella pneumonia* were kindly obtained from King Faisal Hospital, Jeddah, KSA and used as test organisms. Antifungal activities against different fungi, including *Candida albicans*, *C. tropicals*, *Alternaria alternata*, *Aspergillus niger*, *Fusarium latenicum*, *Geotrichum candidum* and *Mucor hiemalis*, obtained from Microbiology lab., Faculty of Science, KAU, were detected on PDA medium. All plates were incubated for 4 days at 37°C for bacteria and *Candida* species and for 5 days at 25°C for filamentous fungi. The minimum inhibitory concentrations (MICs) were determined using broth microdilution method [24].

Results and Discussion

The Red sea contain hundreds of different marine algae and there is considerable potential to use these marine algae to remove heavy metals from contaminated water. Five algal samples, *Halimeda opuntia*, *Ulva lactuca*, *Dictyota dichotoma*, *Sargassum muticum*, and *Digenea simplex* were collected, identified, dried and powdered. Similarly, *Ulva lactuca*, *Digenea simplex* and *Sargassum crassifolia* from green, red and brown algae were collected and chemically analyzed [25]. Sixteen genera of the three major classes, Chlorophyta , Phaeophyta and Rhodophyta of macroalgae were collected from Red sea of Jizan, Saudi Arabia. The obtained genera were *Cladophora*, *Derbesia*, *Ulva*, *Fucus*, *Hormophysa*, *Laminaria*, *Macrocystis*, *Padina*, *Sargassum*, *Turbinaria*, *Rhodophyta*, *Acanthophora*, *Galaxaura*, *Hypnea*, *Laurentia*, *Gelidium*, and *Microcladia*. These collected algae have economic values and can be used in a range of ways to improve the human health and environments [26].

All over the world, heavy metal pollution is one of the most dangerous problems especially for wastewater. Lead (Pb) is a heavy metal, among the most hazardous environmental pollutants. Pollution of air, water and agricultural soil by lead is common and has impact on plants, animals and human health [27, 28]. It has adverse effects on both plants and animals. In plants, it affectes photosynthesis, plant growth, seed germination and mineral contents [27, 29]. For heavy metal removals, preparation and characterization of different chemical materials were carried out [30, 31]. Moreover, algal dry biomass was used for Pb(II) and Cd(II) biosorption from solutions using isotherm model and the needed free energies were 10.4 and 9.6 kJ/mol for chemisorption process of Pb(II) and Cd(II), respectively, which were pseudo-second-order kinetic process [32]. Thus, this study aimed for lead removal from solutions using dried algal powder. After selection and identification of the most active algal powder with high metal sorption capacity, optimization of work conditions for maximum removal of lead was carried out. Percentage of lead removal and biosorption capacity (Q) were calculated for each algal type (Figures 2 and 3). The green algal species, *Ulva* showed the best removal level of lead followed by the red algal species, *Digenea simplex*. The difference in removal percentages of lead between *Halimeda opuntia*, *Dictyota dichotoma* and *Sargassum muticum* was not significant. Thus, *Ulva lactuca* was selected for detailed studies. Metal sorption includes binding on the algal cell surface and to intracellular ligand. The adsorbed quantity of the heavy metal may be several times greater than intracellular metal. Carboxyl group mat have a role in metal sorption and binding. Dead cells of algae can more effectively remove metals from multi-metal solutions or wastewaters than live cells and can be used many times for removal cycles [19, 33]. The dried algae have many characters that make them suitable for the selective removal of heavy metals. These algae are considered highly tolerant to heavy metals and can grow auto-tropically with large surface area/volume ratios [34]. The growth and sorption or desorption of heavy metals from sewage and industrial wastewater effluents by cyanobacteria such as *Anabaena subcylindrica*, *Oscillatoria anguistissima*, *Spirulina platensis* and *Nostoc muscorum* were studied [35, 36]. Various factors can improve metal removal by algae e.g. temperature, pH value, time, metal concentration and type, shaking rate, age, and weight of the used algae. In this study, maximum adsorption of lead by *Ulva* powder was obtained at 45^{o}C and pH 4 (Figures 4 and 5). The percentage of lead adsorption was about 100% by *Ulva* dry mass, suspended in lead solution (1000 mg/l) at pH 4 and pH 5, while at 5000 mg/l, the percentages were 40 and 10% at pH 4 and 5, respectively (Figure 6). Temperature changed biosorption efficiency which increased at optimum temperature due to increased functional groups, found on algal surface and enhanced travel rate of ions to the surface to be adsorbed, while at high temperature, a decrease insorption efficiency was obtained. This may be due to increase in mobility of the metals from the algal surface. Similarly, [37, 38] studied the effect of temperature on adsorption of heavy metals by natural materials and noticed similar results. Santhi and Jagadeeswari [39] used dead biomass of *Aspergillus niger* for biosorption of lead and the optimal temperature for adsorption and biosorption were 40^{o}C. Moreover, pH affected biosorption process. In contrast to our results, biosorbtion of Cd^{++} by *A. niger* was at pH 6 due to negative charge of microbial surfaces and ionization of the functional groups, thereby contributing to metal binding. The pH of the biosorption medium affects the solubility of the metal ions and the ionization state of the functional groups. Fungal surfaces have a negative charge in the pH range of 2-6. It is clear that pH value is important parameter in the biosorption processes by bacteria, algae, and fungi, whereas biosorption process was maximum at low pH values (4-6) but reduced at very low pH values [40, 41]. At pH 2, minimum biosorption rate was noticed while the uptake increased with increasing pH from 3.0 to 5.0 [42]. The effect of incubation period and shaking rate were summarized in Figures 4 and 5. Maximum removal of lead by *Ulva* dry mass was obtained after 6 hrs of incubation at static conditions (Figures 7 and 8). Contact time affect biosorption process whereas Abbas *et al.* [42] reviewed that the contact time was ranged from 0.33-8.0 hrs for different biosorbent materials. It is clear that, in the biosorption process, increasing the weight of the used *Ulva* dry mass enhanced removal process and maximum removal percentage was obtained using 1 g dry mass/l while the lowest was obtained for 0.12 g/l. In this study, 100% of lead was removed by 0.75-1.0 g/l where maximum Q value was obtained (Figure 9). It was found that 0.7 g/l of dry biomass removed 84% of cadmium ions [43] and 0.5 g/l removed 80% of cadmium ions [42]. Increasing shaking rate decreased biosorption process. Our results were confirmed by [19] who compared adsorption of heavy metal ions by *Ulva lactuca* and its activated carbon, and found that optimum adsorption by activated carbon was at pH 5.0 after 60 min of using 0.8 g/l of algal powder. They added that activated carbon of algal powder, especially green algal powder, can be used for heavy metal removals (Cu^{+2}, Cd^{+2}, Cr^{+3}, and Pb^{+2}) from polluted water. The abilities of HCl-treated biomass of some algae (three brown and one red) for Cr^{+++}, Co^{++} , Ni^{++}, Cu^{++}, and Cd^{++} ions adsorption were depended on the biosorbent type, pH, time and algal weight. Uptake of different metals was high in the first 2 hr, and then slightly increased [44]. Mixed biomass of marine algae has been used for removal of lead and equilibrium was noticed after 2 hrs at pH 4 [45].

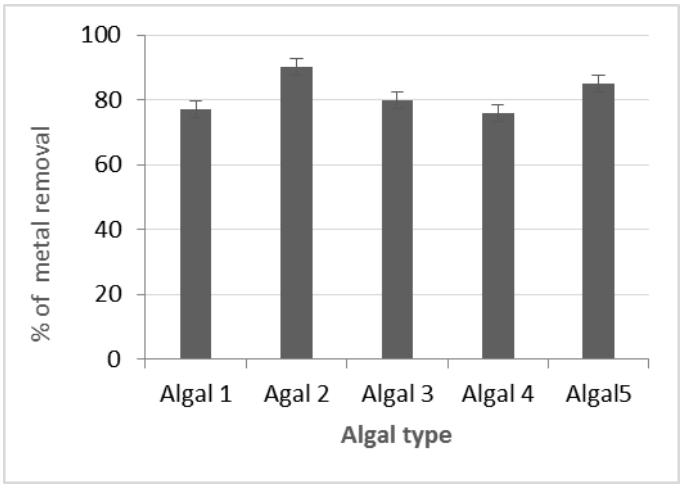

Figure 2. Percentage of heavy metal (Pb^{++}) removal by 5 different algal species, collected from the red sea; 1: *Halimeda opuntia*, 2: *Ulva lactuca*, 3: *Dictyota dichotoma*, 4: *Sargassum muticum*, 5: *Digenea simplex*.

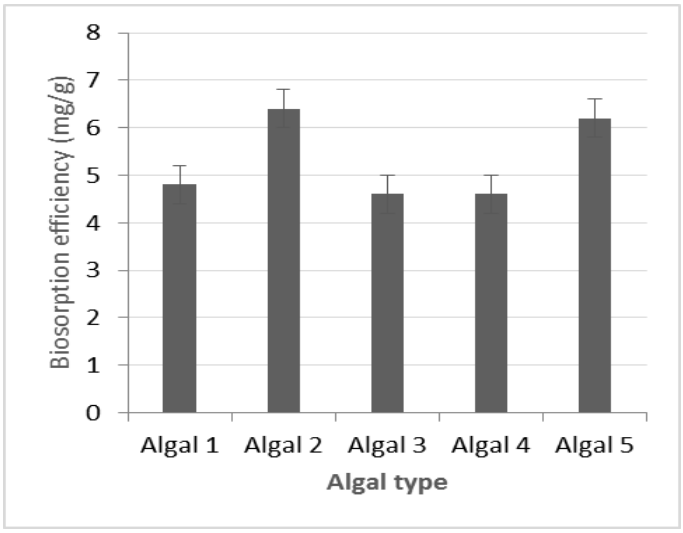

Figure 3. Biosorption efficiency (mg/g) of lead in 5 different algal species, collected from the red sea; 1: *Halimeda opuntia*, 2: *Ulva lactuca*, 3: *Dictyota dichotoma*, 4: *Sargassum muticum*, 5: *Digenea simplex.*

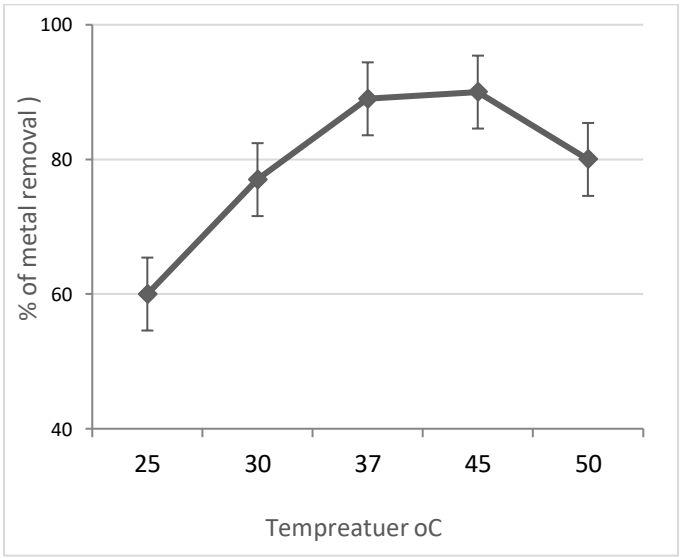

Figure 4. Percentage of heavy metal (Pb^{++}) removal by *Ulva* dry mass at different temperatures.

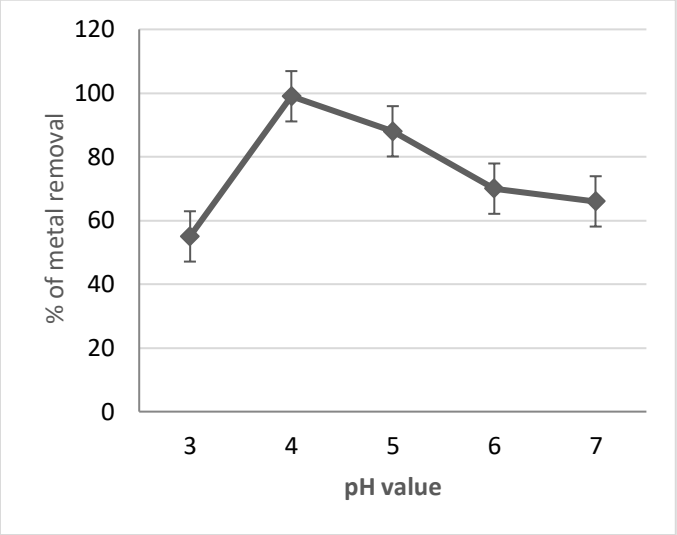

Figure 5. Percentage of heavy metal (Pb^{++}) removal by *Ulva* dry mass at different pH values.

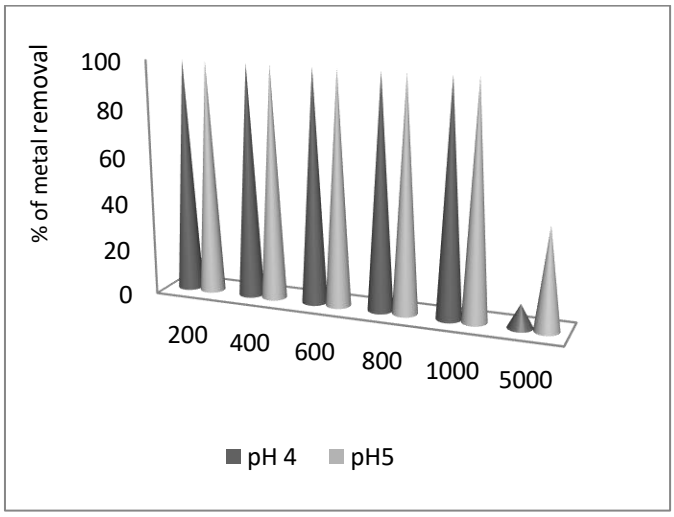

Figure 6. Percentages of heavy metal (Pb^{++}) removal by *Ulva* dry mass suspended in different concentrations of heavy metal at pH 7 and pH 9.

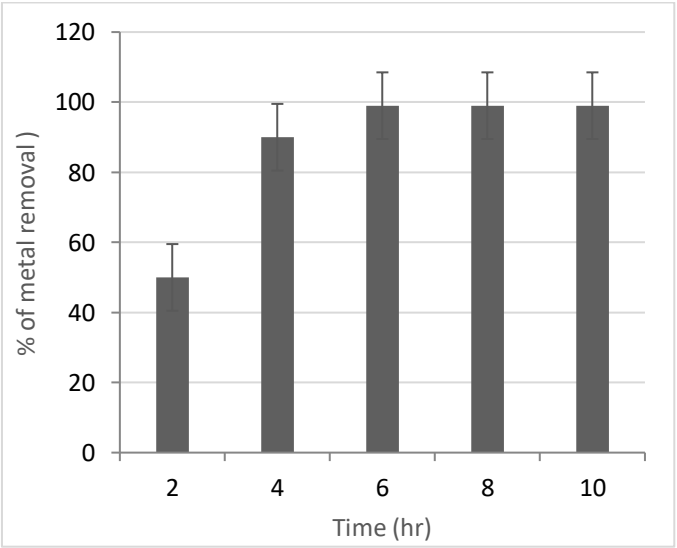

Figure 7. Percentage of heavy metal (Pb^{++}) removal by *Ulva* dry mass after different incubation periods.

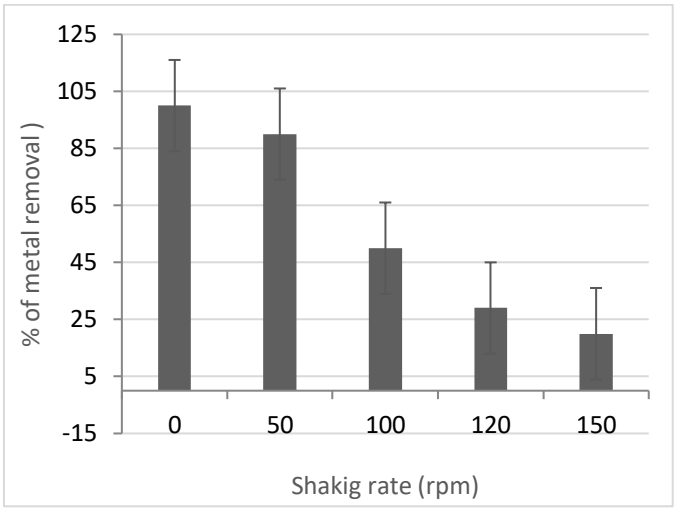

Figure 8. Percentage of heavy metal (Pb^{++}) removal by *Ulva* dry mass after 6 hours of incubation at different shaking rates.

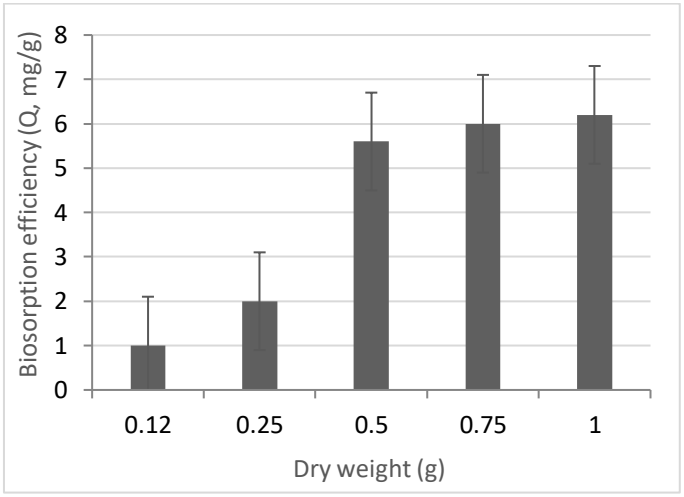

Figure 9. Biosorption efficiency (mg/g) of lead using different dry mass weights of *Ulva* powder.

The antimicrobial activities of the tested algal extract against different microorganisms were determined (Table 1). Ampicillin & Amphotericin B were used as positive controls for bacteria and fungi, respectively, while sterile water was used as negative control for both bacteria and fungi. Maximum growth inhibition was recorded by aqueous extract of *Ulva* for both *E. coli* and *A. niger*, thus they were used as control test organisms for both bacteria and fungi. It was clear that, the effect of *Ulva* extract was excellent on gram-negative bacteria compared to gram-positive bacteria with MIC ranged from 30-40 µg/ml for gram-negative bacteria and ranged from 60-65 µg/ml for gram-positive bacteria (Table 1). Moreover, moderate antifungal activities were recorded against *Candida albicans* and *Alternaria alternata* compared to *Aspergillus niger*. The major pathogens, *Staphylococcus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella* sp. were inhibited by two algal extracts [46]. Similarly, *Dunaliella salina* extract inhibited *E. coli*, *P. aeruginosa*, *S. aureus*, *Candida albicans*, and *Aspergillus niger* [47-49]. Deveau *et al.* [50] reported that the extract of marine macroalgae, *Ulva* inhibited antibiotic-resistant bacteria, but the mode of action is poorly understood and future work is needed.

In conclusion, the dried algal biomass showed efficient potential for lead removal from solutions, the optimal conditions enhanced heavy metal removal percentages. Using *Ulva* powder (1g/l) at 45°C and at pH 4 after 6 hrs at static conditions gave maximum removal of lead from solution. It was noted that biosorption increased with the increase in temperature. The acidic pH played a role in biosorption of lead ions where the negative charges of the algal biomass are needed for attraction process of positive ions of heavy metals. Thus, the maximum benefits of *Ulva* powder can be obtained during wastewater treatment, thereby helping to increase water supply for irrigation of plants in water poor and developing countries.

Table 1. The antimicrobial activities of the tested algal extract against different microorganisms and compared to control

Tested bacteria	Inhibition zone (mm)	**MIC (µg/ml)**	Tested fungi	Inhibition zone (mm)	**MIC**
E. coli (Control)	14.2±0.59	40 ±5.0	*Candida albicans*	11.2±2.2*	50 ±2.5*
Klebsiella pneumoniae	14.2±0.72	40 ±2.5	*Candida tropicals*	8.5±1.6*	ND
Salmonella enterica	15.2±0.29	30 ±2.5	*Alternaria alternata*	11.1±1.00*	50 ±7.5*
Pseudomonas aeruginosa	15.4±1.06	40±1.2	*A. niger* (Control)	17.1±2.4	40 ±2.5

Proteus mirabilis	15.1±1.03	40 ±2.5	*Fusarium latenicum*	7.5±1. 5*	ND
Enterococcus faecalis	12.5±0.87*	60±1.2*	*Geotrichum candidum*	9.0±1.5*	ND
Staphylococcus aureus	11.5±0.80*	65±0.2*	*Mucor hiemalis*	9.1±1.4*	ND

*: significant results compared to control test organisms, ND: Not detected

Acknowledgements

This Project was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, under grant No (J1436- 130- 609). The authors, therefore, acknowledges with thanks DSR technical and financial support

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