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Removal of Heavy Metals from Aqueous Solutions Using Multi-Metals and Antibiotics Resistant Bacterium Isolated from the Red Sea, Egypt

Mohamed T. Shaaban[1], Hassan A.H. Ibrahim[2], Ahmed S. Abouhend[3,*], Khalid M. El-Moselhy[3]

[1]Botany Department, Faculty of Science, Menoufia University, Egypt
[2]Marine Microbiology Laboratory, National Institute of Oceanography and Fisheries, Egypt
[3]Marine Pollution Laboratory, National Institute of Oceanography and Fisheries, Egypt
*Corresponding author: aabouhend@umass.edu, ahmed_salah7257@yahoo.com

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Abstract This investigation was incorporate screening for the highest multiple metal and antibiotics resistant marine bacteria at the Northern Red Sea. The two selected bacterial isolates were identified on the basis of phenotypic and genotypic characterization through 16S rDNA gene technique as *Alteromonas macleodii* and *Nitratireductor basaltis*. *A. macleodii* revealed high efficiency in the removal of heavy metals from aqueous solution. Different factors influenced the removal of heavy metals from aqueous solution by *A. macleodii* such salinity, pH, temperature, biomass and contact time were optimized. The metal removal was greater at the lowest initial metal concentration (50 mg l^{-1}) and decreased with increase in the metal concentration. *A. macleodii* showed high efficiency in biosorption of different metals in single and multiple metal solution systems. Removal percentage of different metals by *A. macleadii* in a single metal system at the highest tested metal concentrations (200 mg l^{-1}) reached Pb, 73.8%; Mn, 66%; Fe, 65%; Cu, 64%; Zn, 62%; Ni, 54%; and Cd, 53%. In multiple metal systems containing 30 mg l^{-1} of different metals, biosorption percentage was Pb, 93%; Fe, 89%; Zn, 55%; Cd, 50%; Cu, 44.5%; Mn, 40% and Ni, 36%. These findings suggest the possibility of using these bacterial isolates for bioremediation of heavy metals from heavy metal contaminated ecosystem.

Keywords: *biosorption, heavy metals, antibiotics, marine bacterium*

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1. Introduction

Marine environments provide a vital sink for many heavy metals and their compounds. There is a growing concern that the natural cycling rates of many metals are being disturbed by anthropogenic activities, especially the release from industrial, domestic and urban effluents of increasing amounts of Pb, Zn, Cd, Hg and Cu [1]. Chemical pollution in the Red Sea is limited to the vicinity of industrial zones and facilities, which usually discharge their effluents directly into the sea.

Toxic effects of heavy metals in the marine environment has been a major concern since they constitute a potential risk to a number of flora and fauna species, including humans, through food chains. Furthermore, there is increasing evidence that presence of heavy metals is linked to the exacerbation of some microbial diseases in aquatic organisms. It is important to prevent water pollution by heavy metals. Research has now been focused to develop suitable technologies either to prevent heavy metal pollution or to reduce it to very low level. This can be achieved either by decreasing the afflux of heavy metals to the receiving bodies (oceans, rivers, sewer and lakes...etc.) or by their removal from contaminated environments. There are many conventional methods available for the removal of metal ions from effluents. These technologies are divided into two categories, chemical and physical. The chemical technologies are very expensive and have disposal problems. Although these methods include efficient for treatment of water contaminated with heavy metals, they are very costly and commercially unattractive. The high electrical energy demand and consumption of chemical reagents are common problems. While, physical methods are membrane filtration and adsorption on activated carbon. Membrane filtration processes are nanofiltration, reverse osmosis, electrodialysis…etc. The major disadvantage of this membrane filtration is limited life time before membrane fouling occurs [2].

There are some non conventional remediation technologies for heavy metals such as adsorptions. Adsorption has been found to be superior compared to the other techniques for water reuse in terms of initial cost, flexibility and simplicity of design, ease of operation and

insensitivity to toxic pollutants and does not result in formation of harmful substances [3]. Disadvantage of this method is that it requires large area and is constrained by sensitivity toward diurnal variation as well as toxicity of some chemicals and less flexibility in design and operation [4,5]. Biosorption of heavy metals can be defined as the ability of biological materials to accumulate metals from wastewater through metabolically mediated or physico-chemical pathways of uptake from aqueous solution. The advantages of biosorption are low cost, high efficiency and regeneration and possible metal recovery. Biosorption of metals not based on only one mechanism, it consists of several mechanisms that quantitatively and qualitatively differ according to the species used, the origin of the biomass and its processing. There are several chemical groups that could attract and sequester the metals in microbial biomass such as, acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, sulfhydryl and carboxyl groups in proteins and hydroxyls in polysaccharides. However, it should be stressed that the presence of some functional groups does not guarantee their accessibility for sorption, perhaps due to steric, conformational, or other barriers [6]. Bacterial biomass as a biosorbent has become popular because of their small size, ability to grow under controlled conditions, and their resilience to a wide range of environmental situations; furthermore, inexpensive nutrient sources are readily available [2].

2. Material and Methods

2.1. Selected Sites for Bacterial Isolation

Red Sea is a deep semi-enclosed and narrow basin, lies between 12 - 30° N and 32 - 44° E, it is about 1930 km long with an average width of 280 km [7]. It has a maximum depth of 2211 m in the central median trench and an average depth of 490 m. It is connected to the Indian Ocean through Bab El-Mandab strait and extends north word to Sinai Peninsula, which divides it into the shallow Gulf of Suez and deep Aqaba Gulf [8,9]. The Red Sea is the habitat of more than 1000 invertebrate species, 200 soft and hard corals (3.8% of the world's coral reefs) and over 1200 fish species (Ormond and Edwards, 1987).

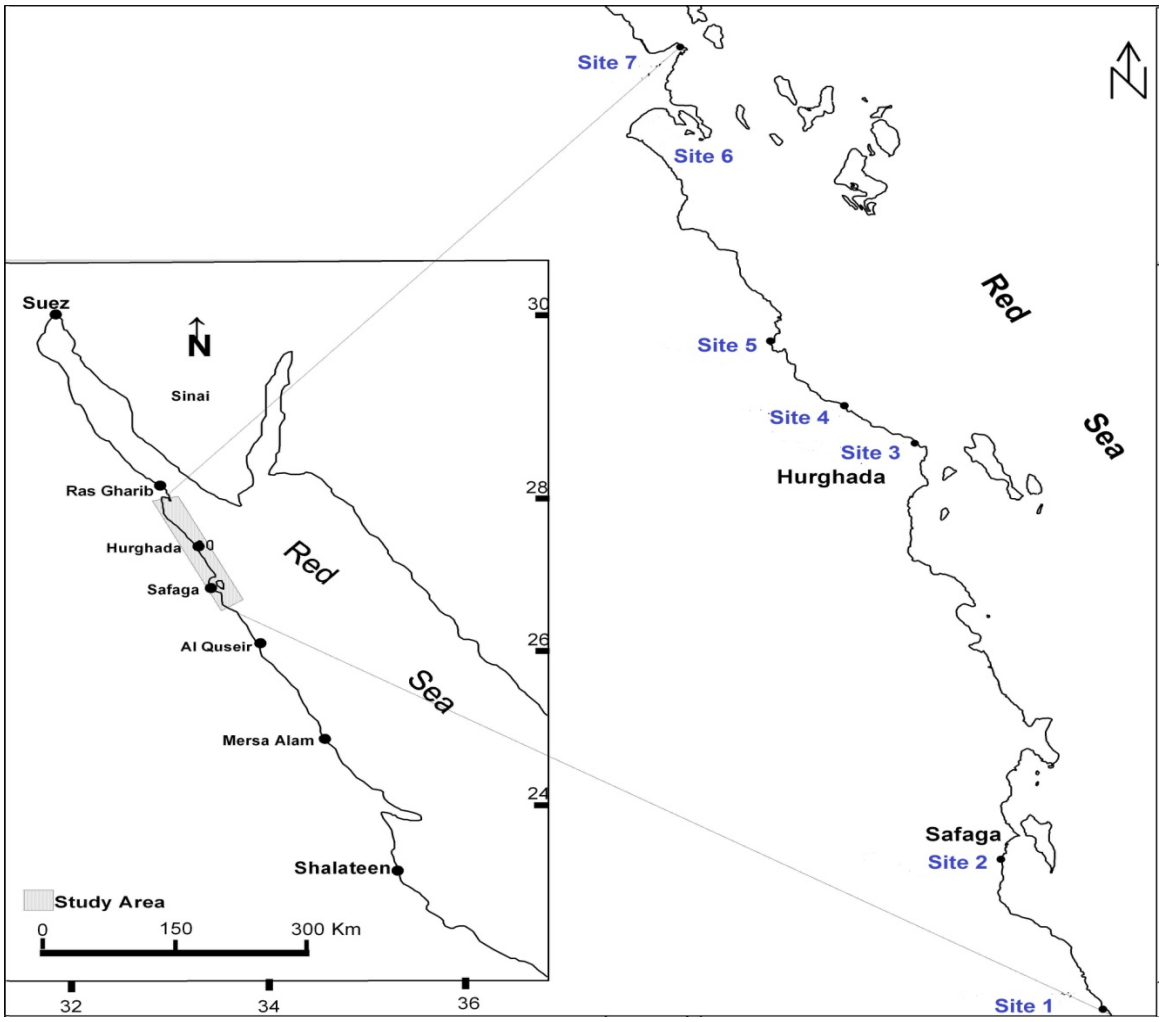

Figure 1. Map of the Red Sea showing the study area and the different sites

In the present investigation, the study area (Figure 1, Figure 2) included seven sites along the northern coast of the Red Sea from the Mangrove area (77 km south Hurghada City) to Ras El-behar (80 km north Hurghada City). **Site I (Mangrove area)**, it is located at the end of Safaga bay, about 77 km to the south of Hurghada City (26°36\59\\N and 34°00\ 41\\E). **Site II (Phosphate harbor)**, it is located out of Safaga City at about 60 km south of Hurghada (26°41\ 46\\ N and 33° 56\ 20\\ E). **Site III (Hurghada harbor)**, it is located at the center of

Hurghada City (27° 13\ 48\\N and 33° 50\37\\E), which represents the main shipyard of Hurghada City. **Site IV (NIOF)**,it is located in front of the National Institute of Oceanography and Fisheries (NIOF), 5 km north to Hurghada, (27° 17\ 3\\ N and 33° 46\ 21\\E). **Site V (El-Gona resort)**,it is located about 22 km north of Hurghada (27° 22\ 39\\N and 33° 41\ 00\\ E), the investigated area included the touristic harbor at which construct safari, touristic diving ships and yachts are found. **Site VI (Gemsha Bay**), it is located in the northern part of the Red Sea, about 60 km north to Hurghada (27° 39\ 11\\N and 33° 35\ 37\\E). **Site VII (Ras Elbehar)**,it is located about 80 km north to Hurghada City (27°43\ 43\\ N and 33°32\ 56\\ E).

Figure 2. Field pictures for the different sites of the study area

2.2. Chemicals and Glassware

All Chemicals used in the present investigation were analytical grade, purchased from Oxford, Fluka and Sigma Aldrich. Growth media and antibiotic discs were purchased from Oxoid. Chemicals and growth media were previously tested for heavy metals contamination. Glassware was previously washed by acid then by distilled water before usage.

2.3. Physical and Chemical Properties of Seawater

The pH, temperature and salinity values were determined in water by taking three readings at each site using the multiparameter instrument (Hanna Instruments, HI 9829). Dissolved oxygen was determined by Winkler's method [10]. Water samples for Biological oxygen Demand (BOD) were collected and determined according APHA method [11].

2.4. Chemical Analysis of Seawater for Nutrients

About one liter of the seawater was collected seasonally from each site in previously acid-washed polyethylene bottles (except for inorganic phosphate which should be stored in hard glass bottle) and transferred immediately to icebox and transported to the laboratory [12].

Ammonia determination method was adopted after Koroleff [13] and the blue formed color was measured using a spectrophotometer at 630 nm. Dissolved nitrite was determined according Grasshoff *et al* [12]. The azo dye color was completely developed in 20 - 30 min, its intensity was measured using a spectrophotometer at 540 nm. The Dissolved nitrate method employed was based on the reduction of nitrate to nitrite using cadmium column. The Dissolved phosphate method employed was suggested by Grasshoff *et al*. [12]. The absorbance of the blue phosphorus complex should be measured within 20-30 minutes using a wavelength of 880 nm. Dissolved silicate was determined according Grasshoff *et al*., [12]. The color reached its maximum after 30 minand absorbance was then measured at 810 nm. All nutrients values are expressed in µM l^{-1}.

2.5. Determination of Heavy Metals

Two liters of sub-surface water samples were collected from the different sites in previously acid-washed polyethylene bottles. Samples were then transported immediately in icebox to the laboratory where the pH of the samples was adjusted to 3 - 4 [14]. Sediment samples (500 g) were collected from the same sites of water samples using a Van-Veen grab [15]. The samples were kept in self-sealed pre-cleaned plastic bags. The samples were deep-frozen until analysis. Water samples were filtered as soon as possible after collection through 0.45 µm membrane filter paper and checked for their pH value (3 - 4). Metals in the filtered seawater were pre-concentrated by complexing the metals with ammonium pyrrolidine dithiocarbmate (APDC), the complexed compound was extracted into methyl isobutyl ketone (MIBK) and back-extracted into an acidic aqueous solution [16].

Sediment samples were air dried at room temperature. 0.5 g of each sample was weighed into a screw capped teflon beaker and digested with 10 ml of a mixture of concentrated HNO_3, $HClO_4$ and HF acids (3: 2: 1) and left overnight (12 h). The samples were then digested at 120°C for 1 - 2 h on a hot plate [17]. Concentrations of heavy metals were measured using a flame atomic absorption spectrophotometer and expressed in µg l^{-1} for water and in µg g^{-1} for sediments.

2.6. Bacteriological Studies

2.6.1. Sampling

Water and sediment samples were collected from the selected sites during the summer 2012. Sub-surface (0 - 20 cm) water samples were collected using 250 ml sterile bottles [11]. Sediment samples were collected from the upper layer (0 - 5 cm depth) and stored in 250 ml sterile bags. Water and sediment samples were brought to the laboratory in an ice box and processed within 4 h of collection.

2.6.2. Stock Solutions of Metals

The stock solutions of 1000 mgl^{-1} concentration of Cd^{+2}, Cu^{+2},Ni^{+2},Zn^{+2}, Pb^{+2}, Mn^{+2} and Fe^{+2} were prepared by dissolving cadmium nitrate, copper sulfate, nickel nitrate, zinc sulfate, lead nitrate, manganous sulfate and ferrous sulfate in deionized water. All solutions were checked for their concentration using atomic absorption spectrophotometer and used in further experiments.

2.6.3. Isolation of Heavy Metal Resistant Bacteria

Bacteria were isolated from the seawater and sediments using the spread plate technique on seawater nutrient agar plates supplemented with 50 mgl^{-1} concentration of each metal. One milliliters of water sample was serially diluted in sterile saline water and plated. One gram of the sediments was homogenized in 9 ml sterile saline water, serially diluted from 10^{-2} to 10^{-6} and plated on the agar plates supplemented by differing metals. Plates were incubated at 30°C for 48 h and the growing separated colonies were transferred to the higher metal concentration. Growing colonies differed in morphological characteristics were selected and used for further studies [18].

2.6.4. Optimization of the Bacterial Growth Conditions

The optimal growth conditions for bacterial isolates with reference to pH, salinity and temperature were determined in normal broth culture and in presence of 50 mgl^{-1} of Cd. The optical densities of the growing cultures after 24 h were measured at 550 nm as a criterion for growth [18].

2.6.5. Screening for Multiple Metal Resistance and Determination of the Minimum Inhibitory Concentrations (MICs)

Bacterial isolates resistant to a single metal were tested for their resistance to the other metals concentrations. Using seawater agar medium amended with different concentrations of different metals, and the pH of the medium was adjusted to 7. After inoculation, the plates were incubated for 48 h at 30°C; the highest resistant isolates were selected.

Maximum resistance of the selected isolates against increasing concentrations of different metals on seawater nutrient broth media was evaluated until the strains were able to grow and give suitable optical density at 550 nm. The bacterial grown cultures were subsequently transferred to the next concentration. MICs of the metal was considered to be the minimum concentration at which no bacterial growth occurred at 30°C after 48 h incubation period [19].

2.6.6. Optimization of Heavy Metals Biosorption and Determination of the Biosorptive Capacity of the Isolate

The factors affected the biosorption process were optimized on cadmium such, Temperature (25, 30 and 35°C), pH (5, 6, 7 and 8), biomass (50, 100, 150, 200 and 250 mg of wet biomass), contact time (2, 4, 5, 6, 8 and 10 h) and initial metal concentrations (25, 50, 100, 150 and 200 mgl^{-1}).

After the bacterial growth at broth media for 24 h, the cells were harvested by centrifugation (5000 rpm for 15 min) then the pellet was washed twice by saline water. The optimum wet weight of the bacterial biomass was suspended in 25 ml of a metal solution containing different metal concentrations and the solution was continuously stirred on a shaker (200 rpm) at 30°C for the optimum contact time [20]. After the contact time, the supernatant was collected by centrifugation at 5000 rpm for 15 min, filtered through 0.2 µm filter membranes and analyzed for metal ions using flam atomic absorption spectrophotometer. All the biosorption experiments were repeated twice to confirm the results. Also blank (without biomass) experiments were conducted to ensure that no adsorption had taken place on the walls of the apparatus used.

The amount of metal sorbed to the bacterial biomass was calculated by the following equation: $Q = V (C_i - C_f) / m$. Where: Q = metal uptake (mg of metal per g of biosorbent), V = metal solution volume (ml), C_i= initial metal concentration in the solution (mgl^{-1}), C_f= final metal concentration in the solution (mgl^{-1}) and m = amount of the added biosorbent as a wet biomass (mg) [21].

2.6.7. Resistance to Antibiotics

A sterile swab was dipped into the broth culture and any excess moisture was expressed by pressing the swab against the side of the tube. The surface of the agar plates were swabbed completely and allowed to dry for about 5 min. Antibiotic disks (levofloxacin, 5 µg; imipenem, 10 µg; chloramphenicol, 30 µg; ampicillin, 10µg; penicillin G, 10 µg; streptomycin, 10 µg; vancomycin, 30 µg; ceftriaxone, 30 µg and amikacin 30 µg)were aseptically picked by sterile forceps and placed on the agar plates. The plates were then incubated at 37°C for 48 h. Inhibition zone diameters were measured in millimeters and the results were reported [22].

2.6.8. Identification and Characterization of the Selected Bacterial Isolates

The most multiple metal resistant bacterial isolates were submitted to the phenotypic characterization through morphological, physiological and biochemical tests according to Bergey's manual for systematic bacteriology [23]. Phenotypic characteristics such as Gram's staining, motility, cultural characteristics, catalase, oxidase …etc., were studied by adopting standard procedures. Also, effect of sodium chloride, pH and temperature on growth was tested.

Bacterial isolates were submitted to genotypic characterization through 16S rRNA gene technique. The 16S rRNA gene from the strain was amplified using universal primers (27F; 5-AGA GTT TGA TCC TGG CTC AG-3 and 1492R; 5-GGT TAC CTT GTT ACG ACT T-3). The PCR products were purified and sequenced by the GATC-Biotech. Company (Germany). The sequences were compared with known sequences in the Gene Bank nucleotide database and identified as the nearest phylogenetic neighbor with the highest similarity percent [24].

3. Results

3.1. Physical and Chemical Properties of Seawater

Physical and chemical properties such as temperature, pH, salinity, dissolved oxygen and biological oxygen demand as well as, the dissolved nutrient salts concentrations at seawater samples collected from different sites of the present study area were measured during Summer 2012 and the means of the triple measured values of each parameter were recorded (Table 1).

3.2. Heavy Metals Concentrations in Water and Sediments

Mean of heavy metals concentrations in water and sediments collected from the different sites of the present study area were recorded at Table 1.

3.3. Total Viable Bacterial Counts in Seawater and Sediments

Table 1 shows the mean of the total viable bacterial count in seawater and sediments collected from the different sites of the present study area.

3.4. Screening for the Most Metal Resistant Bacterial Isolates

Isolation of single metal resistant marine bacteria was carried out on seawater nutrient agar that amended by 50 mgl^{-1} of different metals (Cd, Cu, Ni, Zn, Pb, Mn and Fe) as an initial metal ion concentration. At this concentration, 220 bacterial isolates (Cd, 24; Cu, 32; Ni and Zn, 31; Pb, 41; Mn, 37 and Fe, 24) were isolated from seawater and sediments of the study area during summer. After 24 h incubation period, the growing isolates were transferred to the higher metal concentrations. The most resistant bacterial isolates to each metal which recorded growth at the highest metal concentration were selected. As well as, the multiple metal resistance ability of the selected isolates was detected. Each single metal resistant isolate was examined to grow at high concentrations of the other heavy metals. Different isolates showed high resistance to more than one metal but only two isolates were able to grow at all tested metals concentrations. The multiple metal resistant isolates were selected. The two isolates

were coded as I_1 and I_2, respectively and presented for further studies.

Table 1. Means of physic-chemical parameters, heavy metals and bacterial count in seawater and sediments of different sites

Parameter		Sites						
		St 1	St 2	St 3	St 4	St 5	St 6	St 7
Physic-Chemical Parameters		Measuring Unit:°C (Temp)/ ‰ (Salinity)/ mg l^{-1} (DO, BOD)						
Temp	Water	31.27 ± 0.01	30.09 ± 0.09	30.96 ± 0.04	31.34 ± 0.12	30.66 ± 0.16	29.25 ± 0.09	32.56 ± 0.25
pH		8.20 ± 0.01	7.98 ± 0.01	8.21 ± 0.02	7.89 ± 0.01	8.03 ± 0.01	8.18 ± 0.02	8.11 ± 0.01
Salinity		41.61 ± 0.10	41.59 ± 0.09	41.56 ± 0.12	41.84 ± 0.02	41.96 ± 0.02	41.74 ± 0.07	42.32 ± 0.10
DO		6.62 ± 0.09	6.05 ± 0.10	6.54 ± 0.13	6.85 ± 0.21	6.76 ± 0.20	6.15 ±013	6.25 ± 0.14
BOD		2.85 ± 0.11	1.35 ± 0.15	4.15 ± 0.22	1.34 ± 0.13	1.93 ± 0.13	1.13 ± 0.23	2.13 ± 0.22
Dissolved Nutrients		Measuring Unit: µM l^{-1}						
NH_4^+	Water	2.73 ± 0.22	2.41 ± 0.28	12.56 ± 1.35	2.38 ± 0.42	3.26 ± 1.04	3.49 ± 0.27	4.32 ± 0.61
NO_2^-		0.07 ± 0.01	0.09 ± 0.05	0.99 ± 0.29	0.11 ± 0.05	0.10 ± 0.08	0.09 ± 0.03	0.12 ± 0.04
NO_3^-		0.64 ± 0.51	0.60 ± 0.45	3.17 ± 1.36	1.18 ± 0.41	0.65 ± 0.17	0.47 ± 0.35	0.43 ± 0.15
PO_4^{-3}		0.13 ± 0.08	0.08 ± 0.06	0.38 ± 0.08	0.16 ± 0.02	0.13 ± 0.05	0.20 ± 0.12	0.19 ± 0.09
SiO_4^{-4}		1.17 ± 0.54	1.10 ± 0.33	2.65 ± 0.18	1.33 ± 0.50	1.26 ± 0.14	0.67 ± 0.15	1.28 ± 0.18
Heavy Metals		Measuring Unit:µg l^{-1} (Water)/ µg g^{-1} (Sediments)						
Cd	Water	0.29 ± 0.04	0.28 ± 0.07	0.39 ± 0.03	0.32 ± 0.06	0.24 ± 0.05	0.22 ± 0.06	0.23 ± 0.04
	Sediments	2.23 ± 0.26	3.02 ± 0.77	3.86 ± 0.48	2.86 ± 0.49	2.03 ± 0.31	2.03 ± 0.10	2.59 ± 0.24
Cu	Water	1.10 ± 0.52	0.88 ± 0.30	3.85 ± 0.70	0.98 ± 0.36	1.98 ± 0.76	0.78 ± 0.28	0.68 ± 0.26
	Sediments	12.87 ± 1.14	15.37± 1.19	90.67 ± 15.04	9.78 ± 0.95	26.01 ± 3.09	12.77 ± 0.90	15.05 ± 2.63
Ni	Water	1.25 ± 0.11	1.05 ± 0.11	1.60 ± 0.45	0.78 ± 0.22	0.70 ± 0.16	0.40 ± 0.27	1.35 ± 0.26
	Sediments	27.02 ± 1.22	24.60 ± 2.70	37.63 ± 1.80	20.51 ± 1.46	22.58 ± 1.38	17.00 ± 0.45	17.69 ± 1.31
Zn	Water	1.87 ± 0.64	1.68 ± 0.57	11.36 ± 0.66	2.11 ± 1.17	2.07 ± 0.36	1.88 ± 0.60	3.12 ± 1.16
	Sediments	28.64 ± 3.58	38.43 ± 2.73	146.00 ± 35.11	27.41 ± 2.85	35.42 ± 3.68	22.93 ± 3.28	27.52 ± 1.87
Pb	Water	2.60 ± 0.67	2.22 ± 0.73	4.25 ± 1.10	2.17 ± 0.23	2.14 ± 0.59	1.88 ± 0.86	2.30 ± 1.02
	Sediments	42.91 ± 2.97	45.55 ± 3.49	72.82 ± 5.53	23.01 ± 4.96	35.69 ± 3.10	18.84 ± 2.41	23.21 ± 2.43
Mn	Water	0.20 ± 0.06	0.16 ± 0.04	0.30 ± 0.06	0.20 ± 0.04	0.18 ± 0.10	0.18 ± 0.12	0.21 ± 0.12
	Sediments	257.00 ± 20.54	196.50 ± 18.90	306.92 ± 14.24	182.67 ± 09.12	170.75 ± 15.79	134.17 ± 22.77	121.25 ± 07.48
Fe	Water	15.48 ± 1.85	12.70 ± 2.02	25.31 ± 7.94	14.47 ± 3.06	13.54 ± 4.00	14.14 ± 2.81	14.43 ± 4.34
	Sediments	13830.75 ± 282.50	11130.75 ± 825.02	12178.42 ± 855.40	5398.75 ± 409.03	10440.67 ± 455.92	6119.42 ± 1528.64	6874.42 ± 202.69
Total viable bacterial count		Measuring Unit: cfu ml^{-1} (Water)/ cfu g^{-1} (Sediments)						
Sea water		$5.2 \times 10^5 \pm 9.8 \times 10^2$	$1.3 \times 10^5 \pm 1.9 \times 10^3$	$7.1 \times 10^6 \pm 3.4 \times 10^3$	$1.9 \times 10^5 \pm 2.2 \times 10^2$	$1.4 \times 10^5 \pm 3.4 \times 10^3$	$1.7 \times 10^5 \pm 2.5 \times 10^3$	$4.1 \times 10^5 \pm 1.2 \times 10^3$
Sediments		$4 \times 10^{10} \pm 6.8 \times 10^3$	$6 \times 10^{10} \pm 4.7 \times 10^3$	$6 \times 10^{13} \pm 8.7 \times 10^3$	$7 \times 10^{10} \pm 7.8 \times 10^3$	$8 \times 10^{10} \pm 4.9 \times 10^3$	$8 \times 10^{10} \pm 9.9 \times 10^3$	$9 \times 10^{11} \pm 6.9 \times 10^3$

3.5. Characterization of the Bacterial Isolates

The two isolates; I_1 and I_2 were submitted for phenotypic and genotypic characterization. Phenotypic characteristics included morphological examination, physiological and biochemical tests. Isolate I_1 was straight rod, Gram negative, motile marine bacteria (Table 2) which couldn't grow without presence of seawater or NaCl in the growth media. It had the ability to produce different enzymes such as, gelatinase, amylase, catalase, oxidase and lipase enzymes and can utilize some sugars as a carbon source such as glucose, maltose and fructose. These phenotypic characterizations showed that isolate I_1 was expected to be *Alteromonas macleodii*. Isolate I_2 was coccoid, Gram negative, non motile marine bacteria (Table 3) which can grow at 0% and 5% NaCl and cannot grow at 10% NaCl. It wasn't able to utilize citrate. It produced oxidase, catalase and amylase enzymes. It reduced nitrate to nitrite and gave negative result with indole production test. These phenotypic characterizations showed that isolate I_1 was expected to be *Nitratireductor basaltis.*

Genomic RNA of each bacterial isolate was extracted and the 16S rRNA gene was amplified using the universal primers (27F; 5'AGA GTT TGA TCC TGG CTC AG-3' and 1492R; 5'-GGT TAC CTT GTT ACG ACT T-3'). The amplified PCR fragments were purified and then sequenced. Sequencing data of 16S rRNA were submitted and compared with known sequences in the Gene Bank nucleotide database. The results (Table 4) showed that I_1 was identified as *Alteromonas macleodii* NR 037127.1 with 95% similarity percent. While, isolate I_2identified as *Nitratireductor basaltis* NR 044414.1with 96% similarity percent.

Table 2. Phenotypic characterizations of the isolate I_1

Character	Result
Gram reaction	-ve
Cell shape	Straight Rod
Cell width	1 µm
Cell length	2 µm
Motility	Motile
Flagella	One polar
Growth in presence of NaCl	
0%	-ve
5%	+ve
10%	+ve
Growth at:	
4°C	-ve
35°C	+ve
40°C	+ve
No_3 reduction to No_2	-ve
Citrate utilization	-ve
Production of:	
Oxidase	+ve
Lipase	+ve
Gelatinase	+ve
Amylase	+ve
Catalase	+ve
Chitenase	-ve
Utilization of sugars:	
Glucose,	+ve
Maltose	+ve
Fructose	+ve
Sucrose,	-ve
Lactose	-ve

Table 3. Phenotypic characterizations of the isolate I_2

Character	Result
Gram reaction	-ve
Cell shape	Coccoid
Motility	Non motile
Growth in presence of NaCl	
0%	+ve
5%	+ve
10%	-ve
Growth at:	
4°C	+ve
35°C	+ve
40°C	+ve
No_3 reduction to No_2	+ve
Citrate utilization	-ve
Indole production	-ve
Production of:	
Oxidase	+ve
Lipase	-ve
Gelatinase	-ve
Amylase	+ve
Catalase	+ve
Urease	-ve
Utilization of:	
Glucose	+ve
Sucrose	+ve
Maltose	-ve
Fructose	-ve

Table 4. Accession number of the experimental 16S rRNA sequence and its similarity percentage to the closest known species

Strain Code	Accession no.	Most related species	Similarity (%)
I_1	NR 037127.1	*Alteromonas macleodii*	96 %
I_2	NR 044414.1	*Nitratireductor basaltis*	95 %

3.6. The Minimum Inhibitory Concentrations (MICs) of Metals

The Minimum Inhibitory Concentrations (MICs) of different metals against *Alteromonas macleodii* and *Nitratireductor basaltis* were independently detected (Table 5). The multimetal resistant isolates, *A. macleodii*and *N. basaltis* showed high degrees of resistance to different heavy metals. MICs values varied from 175 to 350 mg l^{-1} for *A. macleodii* and from 125 to 350 mg l^{-1} for *N. basaltis* against different metals. Among the tested heavy metals, Pb and Mn were less toxic, while Cd was the most toxic to both strains. *A. macleodii* was highly resistant to the most of the tested metals than *N. basaltis.* The minimum inhibitory concentrations of different heavy metals were: Cd, 175 and 125; Cu, 275 and 225; Ni, 250 and 200; Zn, 250 and 200; Pb, 350; Mn, 300 and 350; Fe, 250 mg l^{-1} for *A. macleodii* and *N. basaltis*, respectively. It was observed that, *A. macleodii* was most resistant than *N. basaltis* against different heavy metals.

Table 5. Minimum inhibitory concentrations (MICs) of different metals against *A. macleodii* and *N. basaltis*

Metal	Tested concentrations (mgl^{-1})	MICs (mgl^{-1})	
		A. macleodii	*N. basaltis*
Cd	25-200	175	125
Cu	50-400	275	225
Ni	50-300	250	200
Zn	50-300	250	200
Pb	50-400	350	350
Mn	50-400	300	350
Fe	50-400	250	250

3.7. Resistance to Antibiotics

The resistance of the multiple metals resistant isolates (*A. macleodii* and *N. basaltis*) towards the popular nine tested antibiotics was estimated to investigate the relationship between metals and antibiotics resistance ability in bacteria. The two isolates showed variable degrees of resistance towards different antibiotics, but it was observed that the highest multiple metal resistant isolate (*A. macleodii*) wasn't the highest antibiotic resistant and the reverse were correct with the second isolate (*N. basaltis*). *N. basaltis* was more resistant to the most of the tested antibiotics than *A. macleodii* (Table 6). *N. basaltis* showed resistance to vancomycin with 6 mm inhibition zone diameter followed by streptomycin (8 mm), penicillin G (8 mm), ampicillin (9 mm), ceftriaxone (9 mm) and amikacin (11mm), while it was highly sensitive to imipenem (36 mm), levofloxacin (26 mm) and chloramphenicol (17 mm). Whereas, *A. macleodii* was highly sensitive to levofloxacin with 40 mm inhibition zone diameter, followed by imipenem (35 mm) and chloramphenicol (30 mm). Moderate sensitivity was recorded for ampicillin (25 mm) and it was resistant against the other tested antibiotics.

Table 6. Resistance/sensitivity of *A. macleodii* and *N. basaltis* to different antibiotics (sensitivity is represented by the inhibition zone diameter in mm)

Antibiotic name	Disc code	Inhibition zone diameter (mm)		Resistance/Sensitivity	
		A. macleodii	*N. basaltis*	*A. macleodii*	*N. basaltis*
Ampicillin	AMP	25	9	S	R
Streptomycin	S	12	8	R	R
Imipenem	IPM	35	36	S	S
Chloramphenicol	C	30	17	S	S
Vancomycin	VA	9	6	R	R
Ceftriaxone	CRO	6	9	R	R
Amikacin	AK	11	11	R	R
Levofloxacin	Lev	40	26	S	S
Penicillin G	P	11	8	R	R

3.8. Biosorption of Heavy Metals by *Alteromonas macleodii*

The most multiple metal resistant marine bacterial isolate, *Alteromonas macleodii* was chosen for heavy metals biosorption experiments. *A. macleodii* was cultured in seawater nutrient broth for 24 - 36 h. The bacterial cells were harvested by centrifugation at 5000 rpm for 15 min then the biomass pellet was washed twice by saline water and applied to the biosorption optimization process.

3.8.1. Effect of the Physical and Chemical Factors on Metals Biosorption

The effect of salinity on biosorption process was studied on the different metals but the other parameters such pH, temperature, contact time, etc., were studied on cadmium as a representative of a toxic metals. By tested the effect of salinity on biosorption of metals by *Alteromonas macleodii*, it was observed that, distilled water was the most effective for biosorption of Cd, Cu, Ni, Mn and Fe with 83-92% removal percent, while didn't exceeded 10% in seawater. The biomass dosage of biosorbent strongly influences the extent of cadmium biosorption by *A. macleodii*. Increasing of the concentration of the biomass from 50 to 250 mg led to increase in the removal percentage of cadmium from 28% to 83%, so 250 mg was chosen for the next experiments.

The pH value of the solution is one of the most important factors affecting the biosorption process. The results of the effect of pH on Cd removal by *A. macleodii* showed that, the highest metal removal efficiency was at pH 6, therefore a pH value of 6 was chosen for the next biosorption experiments.

The metal removal by the *A. macleodii* was a rapid process, which took place within few hours. The Cd removal percentage by wet biomass of *A. macleodii* was increased from 41% to 83% with increasing the contact time from 2 to 5 h and didn't increase significantly by increasing time over 5 h where plateau pattern was observed.

The effect of temperature on biosorption of Cd by wet biomass of *A. macleodii* was investigated by testing different temperatures (25, 30 and 35°C). The results showed that the temperature had no significant effect on Cd biosorption, with optimum condition of 30°C. The results revealed the percentage of cadmium removal by 250 mg wet biomass of *A. macleodii* were decreased from 96% to 53% with the increase in Cd concentration from 25 to 200 mg l^{-1} within 5 h at pH 6.

Moreover, the results stated that, the increase in the initial metal concentration lead to an increase in metal uptake by *Alteromonas macleodii*. However, within the range of Cd concentration (C_1) from 25 to 200 mg l^{-1}, the Cd uptake (Q) by *A. macleodii* increased from 2.4 mg g^{-1} at 25 mg l^{-1} to 10.6 mg g^{-1} at 200 mg l^{-1} of Cd.

3.8.2. Biosorption of Heavy Metals in a Single Metal System

Biosorption of different heavy metals by *Alteromonas macleodii* in single and multiple metal systems was investigated.

Biosorption of different heavy metals by *Alteromonas macleodii* in a single metal system containing only one metal such Cd, Cu, Ni, Zn, Pb, Mn and Fe were studied. The results (Table 7) showed that, at the initial metal ion concentration (50 mg l^{-1}) of different metals and at the optimum condition, Mn was the highly biosorbed metal by *A. macleodii* followed by Pb, Cu, Zn, Fe, Ni and Cd with removal efficiency reached 93.4%, 92%, 91%, 91%, 86%, 85% and 83%, respectively. At the highest tested metal concentration (200 mg l^{-1}), biosoption reached Cu, 64%; Ni, 54%; Zn, 62%; Pb, 73.8%; Mn, 66%;Fe, 65%and Cd, 53%. The present data show that, the removal of different heavy metals by *A. macleodii* decreased with the increase in metal concentration.

3.8.3. Metal Uptake by the Wet Biomass of *A. macleodii*

Uptake of different heavy metals by the wet biomass of *A. macleodii* was calculated at the highly tested metal concentrations (Table 8). The maximum metal uptake by *A. macleodii* as a wet biomass were 10.6, 12.8, 10.8, 12.4, 14.8, 13.2 and 13 mg g^{-1} at 200 mg l^{-1} of Cd, Cu, Ni, Zn, Pb, Mn and Fe, respectively.

3.8.4. Biosorption of Heavy Metals in a Multisystem by *A. macleodii*

Removal of different heavy metals in a multimetal system containing a mixture of 15 and 30 mg l^{-1} of each metal by *A. macleodii* was carried out. Biosorption of the seven heavy metals was lower in multiple metal system solutions than in single metal system solutions. Approximately, 98% of Pb, 92% of Fe, 87% of Zn, 80% of Cd, 58% of Ni, 55% of Cu and 53% of Mn were removed from the multimetal system containing 15 mg l^{-1} of each metal (Figure 3). While at 30 mg l^{-1} system, 93% of Pb, 89% of Fe, 55% of Zn, 50% of Cd, 44.5% of Cu, 40% of Mn and 36% of Ni were removed (Figure 4).

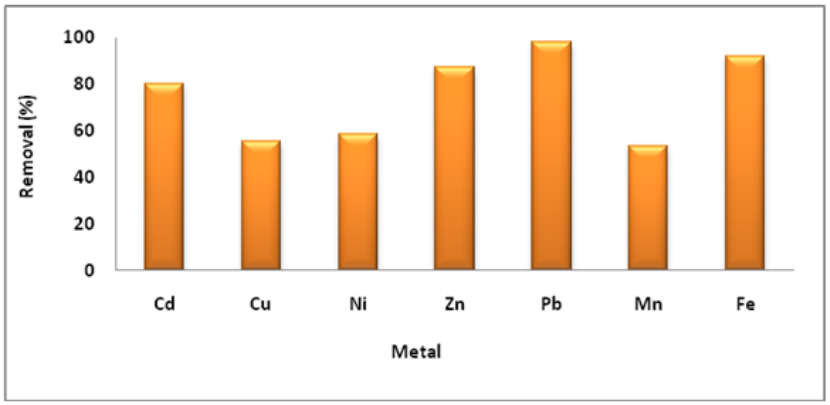

Figure 3. Biosorption of heavy metals in a multisystem containing 15 mg l^{-1} of each metal

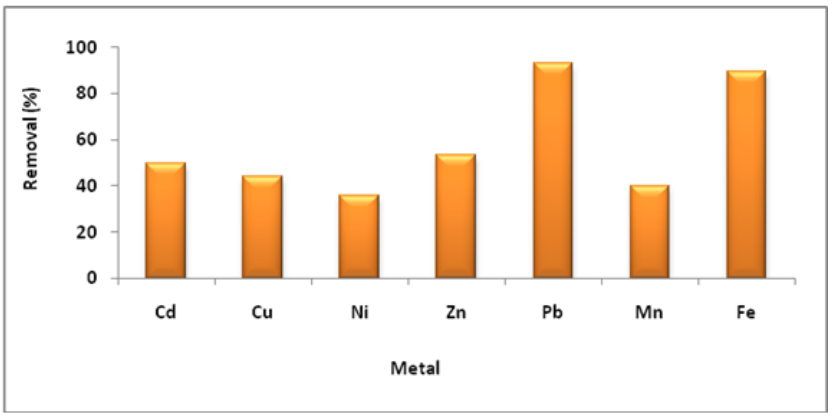

Figure 4. Biosorption of heavy metals in a multisystem containing 30 mg l^{-1} of each metal

Table 7. Biosorption of heavy metals at different concentrations by *A. macleodii*

Metal	Initial Conc. (mg l^{-1})	Final Conc. (mg l^{-1})	Removal (%)
Cadmium	50	8.5	83
	100	24.0	76
	150	48.0	68
	200	94	53
Copper	50	4.5	91.0
	100	23.0	77.0
	150	44.3	70.5
	200	72.0	64.0
Nickel	50	7.5	85
	100	28.0	72
	150	58.5	61
	200	92.0	54
Zinc	50	4.5	91.0
	100	20.0	80.0
	150	44.5	70.3
	200	76.0	62.0
Lead	50	4.0	92.0
	100	13.0	87.0
	150	27.0	82.0
	200	52.4	73.8
Manganese	50	3.3	93.4
	100	15.2	84.8
	150	33.9	77.4
	200	68.0	66.0
Iron	50	7.0	86
	100	21.0	79
	150	42.0	72
	200	70.0	65

Table 8. Metal uptake by the wet biomass of *A. macleodii* at the highest metals concentrations (200 mg g^{-1})

Metal	Metal uptake (mg g^{-1})
Cadmium	10.6
Copper	12.8
Nickel	10.8
Zinc	12.4
Lead	14.8
Manganese	13.2
Iron	13.0

4. Discussion

Heavy metals are one of the severe pollutants in natural environment due to their toxicity, persistence and bioaccumulation problems [25]. Although there are some natural sources of these metals, the majority of heavy metals found in nearshore waters are anthropogenic. Therefore monitoring and assessing the effects of anthropogenic influence is crucial for protection and sustainable maintaining of the fragile aquatic system in the Red Sea.

The introduction of heavy metals in various forms to the environment can produce considerable modifications of the microbial communities and their activities. Sediments recorded bacterial count more than water at the present study area due to high organic matter and nutrients content of the sediments.

The response of microbial communities to heavy metals depends on the concentration and availability of these metals. The ability of microbial strains to grow in the

presence of heavy metals would be helpful in the waste water treatment [26,27]. For the development of an effective bioremediation system using microbes, one of the important steps is to identify microorganisms which can survive in high levels of heavy metal.

Results of the screening process in the present investigation showed that, the most resistant bacterial isolates for different metals (*Alteromonas macleodii* and *Nitratireductor basaltis*) were isolated from the site recorded the highest concentrations of heavy metals in water and sediments compared to the other studied sites. This resistance to heavy metals is developed mainly in response to the stress associated with heavy metals in the environment [28].

Different studies had reported the ability of *A. macleodii* to resist and remove heavy metals. Biosorption of three metallic ions (Pb, Cd and Zn) in single component and bi-component mixtures in aqueous solutions by *A. macleodii* exopolysaccharides was previously reported by Loaic *et al.* [29]. As well as Takeuchi *et al.* [30] recorded that *Alteromonas macleodii* showed higher arsenic resistance (210 - 730 mg l^{-1}) than the other tested marine strains. In contrast, *Nitratireductor basaltis* was firstly isolated by Kim *et al.* [31] so, there is no available data discussed its heavy metal resistant.

Bacteria exposed to high levels of heavy metals in their environment have adapted to this stress by developing various resistance mechanism. Bridge *et al.* [32] confirmed that the microorganisms release a diverse range of specific and nonspecific metal binding compounds in response to high levels of toxic metals which can ameliorate the effect of toxic metals and mediate the uptake process. These mechanisms could be utilized for detoxification and removal of heavy metals from polluted environment [33].

High bacterial resistance to metal is an important factor to be considered for remediation of heavy metals because it is directly related to the survival and growth of bacteria in metal contaminated water. Multiple metal-resistant microorganisms are promising candidates for bioremediation because many sites are contaminated with a varied of heavy metals [34, 35].

In the present investigation, *A. macleodii* and *N. basaltis* showed high resistance to the tested common heavy metals; Cd, Cu, Ni, Zn, Pb, Mn and Fe with different MICs, these metals were appeared at the following order: Pb> Mn> Cu > Ni = Zn = Fe > Cd for *A. macleodii*; Pb = Mn> Fe > Cu > Ni = Zn > Cd for *N. basaltis*. These variations in MICs for different metals on bacterial strains may be due the differences in effect and toxicity of each metal on the bacterial cells, also solubility of metal had a strong effect on the metal resistant pattern of bacteria. Cadmium and lead appeared to be the most and the least toxic metals to the both strains. The higher resistance of *A. macleodii* may be due to the exo-polysaccharides production, which had the ability to chelate heavy metals rapidly and with high efficiency, it may increase its resistance to metals, which didn't observed in *N. basaltis*.

E. coli, isolated from soil was tested for MICs against different metals by Ansari and Malik [36]. It exhibited a maximum MICs of 32 mg l^{-1} for Hg, 200 mg l^{-1} for Cd, 400 mg l^{-1} for Zn and Cu, 800 mg l^{-1} for Ni and 1600 mg l^{-1} for Pb. Also, sewage mlti-metal resistant isolates *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Acinetobacter radioresistens* and *Pseudomonas aeruginosa* showed the following MICs, Cd (4 - 7 mM), Cr (0.7 mM), Ni (6.75 - 8.5 mM), Pb (6 mM), As (6.5 - 15 mM) and Hg (0.75 mM) [18]. While, *Pseudomonas* sprecorded high resistance to six common heavy metal, Cu, Cs, Zn, Pb, As and Hg [37]. In comparison to other reported metal resistant strains, our bacterial isolates exhibited relatively high metal resistance.

Metal resistance in microorganisms has been reported to hold an association with antibiotic resistance [38]. Previous studies had demonstrated the role of plasmids in positive in conferring resistance to both antibiotics and metals. Others have demonstrated genetic linkages (presumably by plasmids) between antibiotic resistance in *Enterobacter aerogenes* and tolerance to Cd and Zn [39]. Raja *et al.* [18] stated that, the multiple metal resistances of *Proteus vulgaris, Pseudomonas aeruginosa, Acinetobacter radioresistens* and *Pseudomonas aeruginosa*were also associated with resistance to antibiotics ampicillin, tetracycline, chloramphenicol, kanamycin, erythromycin, streptomycin and nalidixic acid.

In the present investigation, *A. macleodii* and *N. basaltis* showed resistance to most of antibiotics but the most resistant strain to heavy metals, *A. macleodii* was not the most resistant to antibiotics. The reason for this phenomenon didn't obvious, so the biochemical and molecular mechanism used by the bacterial isolates for multiple metal and antibiotic resistance needs to be investigated.

Similarly, positive correlations between tolerance to high levels of Cu, Pb and Zn and multiple antibiotic resistance were noted among bacteria from waters [40]. It is likely that both heavy metal and antibiotic resistance in bacteria isolated from metal contaminated sites are mediated by efflux pumps in their membranes [41].

Biosorption of heavy metals from aqueous solutions is a relatively new technology for the treatment of industrial wastewater [42]. The major advantages of biosorption technology are its effectiveness in reducing the concentration of heavy metal ions to very low levels and the use of inexpensive biosorbent materials. Biosorption processes are particularly suitable for the treatment of wastewater streams containing dilute heavy metal ion concentrations, or when very low concentrations of heavy metals are required [42]. Furthermore, many investigators have reported the biosorption of heavy metals into pure cultures of bacteria and algae and onto natural microbial populations as the new bioremediation technology [43].

In the present study, *Alteromonas macleodii* was introduced to the biosorption experiments as the most multiple metal resistant bacteria at the northern Red Sea. Optimization of biosorption process by *A. macleodii* was studied on cadmium as one of the most toxic metals in this investigation. Salinity, biomass concentration, pH, metal ion concentration, temperature and contact time were conducted to study the optimization process.

Although *A. macleadii* didn't grow at absence of seawater or NaCl in growth medium, salinity negatively affected its biosorption of different heavy metals except zinc and lead. Therefor, *A. macleadii* showed low biosorption for most of the studied metals (Cd, Cu, Ni, Mn and Fe) in seawater. The same results were obtained by Ruiz *et al.* [44] who used *Bacillus jeotgali*, a marine bacterium that grow at optimum salinity of 34‰. In

biosorption of Cd and Zn at salinities of 0, 10, 20, 30 and 40‰ by using distilled water and seawater (diluted or concentrated), a clear different behavior was observed for salinity of 0 compared with those of salts.

In general terms, biosorption percentages decreased in a significant way when salts were present. This behavior may be related to seawater composition which contains a mixture of cations, light metals like sodium and calcium are usually present. Although the elimination of these cations is not the purpose of biosorption studies, they can compete and interfere for binding sites with the metal ion to be eliminated [45]. At a high Na: metal ratio, sodium is more competitive and will bind all or most of the adsorption sites, whereas when the Na concentration decreases, it is less competitive leaving some adsorption sites free and available for metal [46]. Alternatively, other sorbable ions can compete with the divalent cations of interest for binding to the biomass, affecting biosorption [47]. Moreover, the anions of the metal salts that balance the positive charge of the metal ion are necessarily present in solution. These anions may also affect the metal uptake forming complexes in solution at several concentrations.

The behavior of biosorption of Zn and Pb by *A. macleodii* in saline solutions wasn't clear and need further studies. However, it is necessary to remember that heavy metals concentrations in open seawater ranged between 0 and 37 µg l^{-1} as detected at the present area of study, while the concentration used in biosorption expriments, was in the range between 50,000 and 200,000 µg l^{-1}. This fact can explain the abnormal behavior of the biosorption line at salinity of 30 - 40‰.

The biosorbent mass strongly influences the extent of metal biosorption by *A. macleodii*. Across the increment of the concentration of biosorbent, the removal percentage was increased. This observation was due to the increased adsorption surface and the availability of free adsorption sites [48]. Conversely, with increment of biosorbent concentration, the quantity of biosorbed metal ions per unit weight of *A. macleodii* decreased due to the fact that the available ions were insufficient to completely cover the available exchangeable sites on *A. macleodii* cells, resulting in low ion uptake, also the interference between binding sites due to increased biosorbent dosages cannot be overruled, as this will result in low specific uptake. This result was also suggested by Gadd *et al.* [49] and in agreement with those obtained by Aksu and Cagatay [50] and Vijayaraghavan *et al.* [51].

One of the most important factors affecting the biosorption process is the pH of the solution. The pH of the solution has a very significant effect on metal ion solubility and surface charge of the biomass [52] which in turn influences the biosorption process. In our investigation, the tested pH values were within the range of neutral to acidic medium only because alkaline pH values could not studied on Cd biosorption by *A. macleodii* as a result of biosorption experiments conducted at alkaline pH values have been reported to complicate the evaluation of biosorbent potential as a result of metal precipitation [53]. At higher pH, the solubility of metal complexes decreases to a great extent allowing metal hydroxide precipitation which may complicate the sorption process [54]. pH 6 was the optimum value for biosorption of Cd by *A. macleodii* in the present study. In general, pH 3 - 6 has been found favorable for the biosorption of metal ions by microbial biomass [55].

Several researchers have also investigated the effect of pH on biosorption of heavy metals by bacterial biomass. The optimum pH for the biosorption of Cu, Ni, Zn, Pb and Cr ions by Gram positive (*Micrococcus* sp.) and Gram negative (*Pseudomanas* sp.) bacteria was found to be 5 [56]. Furthermore, the optimum pH for Pb uptake in Gram negative capsulated and non-capsulated bacteria was determined as 4 [57]. Ianis *et al.* [58] also found the optimum pH to be 4.5 for Cu ion adsorption by *Penicillum cyclopium*. Whereas maximum biosorption capacities percentages of Cu, Pb and Cd ions were obtained at pH, 4 - 5 by biomass of *Enterobacter cloacae* which were Cu (78.98%) > Pb (67.98%) > Cd (58.93%) [59].

In the present study, an increase in the metal concentration decreased the removal percent by *A. macleadii*. This trend might be explained by the ability of active sites to fully absorb the metal ions at lower concentrations, while at higher concentrations, the available sites for biosorption were limited caused saturation of absorption sites and biosorption percent decreased but metal uptake increased [60].

The temperature of the biosorption medium could be important for energy dependent mechanisms in metal biosorption. Energy-independent mechanisms are less likely to be affected by temperature [61]. Temperature was not a primary factor to influence metal biosorption by *A. macleadii*, which indicated that the biosorption process was independent of energy. Different temperatures were obtained by Ozdemir*et al.* when they used dry biomass of the thermophilic bacteria, *Geobacillus toebii*(G1) and *Geobacillus thermoleovorans* (G2) in biosorption of different metals. The optimum temperature values obtained were 60°C for Cu, 70°C for Cd, Ni and Mn, 80°C for Zn in case of G1 and 60°C for Cu and 70°C for Cd, Ni, Zn and Mn in the case of G2 [54].

It was also observed that contact time of biosorption experiment was involved in the transfer of metal from metal solution to binding sites on the cells of *A. macleadii* through various steps until reached plateau pattern at equilibrium. The most important and rapid step is the first phase in which the bulk transport of metal ions onto biomass takes place in a few minutes or hours due to mixing and adjective flow [54]. In the present study, the experimental conditions allowed normal mixing of the metal ions and the biomass in the system which partially suppressed some kinetic factors leading the equilibrium in short time (5 h) at which binding sites were saturated by metal. Weber demonstrated that the biosorption of metal was influenced by the factors, such as time which affect mass transfer from the bulk solution to binding sites [62].

Generally, different optimum conditions for heavy metals biosorption were observed by many studies. Singh and Gadi stated that, the optimum conditions for biosorption of Ni and Cu by *Brevundimonas vesicularis* were pH, 4 -5; biomass, 100 mg and contact time, 80 min in 100 ml solution for 52% and 65.5% removal of 100 mg l^{-1} of Ni and Cu [63].

It was observed that, biosorption and uptake profile of *A. macleodii* in a single metal system solution was determined to be Pb> Mn> Fe > Ni > Zn > Cu > Cd. The variations in biosorption and uptake percentages of different metals by *A. macleodii* are probably due to

differences at the affinity of each metal for the active binding sites of the biosorbent, which are likely functional groups such as carboxyl and amine groups, should be stronger compared to other metal ions. Also, the metal solubility had a strong effect on biosorption process.

Pardo*et al*. reported that the biosorption percentage of Cd, Cu, Pb and Zn from aqueous solution by *Pseudomonas putida* was around 80%, while the adsorption percentage of *E. cloacae* was 46%, 54%, 57%, 66% and 74% for Hg, Cr, Cd, Pb and Cu, respectively [64]. Whereas, the maximum biosorption capacities of the biomass of *Enterobacter cloacae* for Pb, Cd, Cr, Hg and Cu ions were determined as 65.68% at 200 mg l^{-1}, 56.56% at 150 mg l^{-1}, 54.28% at 100 mg l^{-1}, 45.57% at 300 mg l^{-1} and 74.46% at 50 mg l^{-1} of initial metal ion concentrations, respectively [59].

Metal uptake by *A. macleodii* increased with the increase in the initial Cd concentration due to increasing the mass transfer driving force of the metal ions between the aqueous solution and *A. macleodii* phases, which lead to increase in metal ions uptake [65]. Different metal uptake values of different metals may be due to the metal solubility and speciation to binding sites and functional groups.

The non living biomass of thermophilic bacteria, *Geobacillus toebii* (G1) and *Geobacillus thermoleovorans* (G2) showed the maximum biosorption capacities of Cd, Cu, Ni, Zn and Mn in single metals system, which were 38.8, 41.5, 42.2, 29.3 and 23.2 mg g^{-1}, respectively for G2, while 29.2, 48.5, 21.1, 21.1 and 13.9 mg g^{-1} for G1 [54].

The industrial effluents generally include a mixture of metals. The presence of multi-metals in the solutions affects the removal of a particular metal by absorption due to competitive interactions with the biomass of bacterial species. The decrease in removal percent of different metals in the multi-metal system than at single metal system is due to higher competition between the same charged metals for binding sites of the biomass. This can be explained by differences in selectivity of biosorbents for metals in aqueous solutions, which have been more extensively studied by Premuzic *et al.*, metal solubility also, had a strong effect on the variable biosorption percentage of the different metals in multiple metal systems [66].

The present study showed that the total amount of metal biosorbed by *Alteromonas macleodii* in a multiple metal system was lower than those in a single metal system. In this context, many studies recorded the same results [67,68,69].

Alteromonas macleodii has been proved to be resistant to different heavy metals and had the ability to biosorb them. It biosorbed seven metals (Cd, Cu, Ni, Zn, Pb, Mn and Fe) in a multimetal system contained 15and 30 mg l^{-1} of each metal and exhibited different removal percentages: Pb> Fe > Zn > Cd > Ni > Cu >Mn at 15 mg l^{-1} and Pb> Fe > Zn > Cd > Cu >Mn > Ni at 30 mg l^{-1} of each metal.

Puranik and Paknikar reported that, metals were adsorbed by *Citrobacter* biomass in multi-metal solutions at the following order: Co < Ni < Cd < Cu < Zn<Pb [70]. While Prashar *et al.* found the order of metals biosorption as Pb> Cd > Cu > Ni [71]. Singh and Gadi observed that, when binary solutions of Ni and Cu prepared in 1:1 ratio (optimum concentration of each metal, 100 mg l^{-1}) were treated with 0.1 g of biomass of *Bacillus vesicularis*under same optimum conditions as used in single metal systems, the percentage removal is 37.0% for Ni and 51.4% for Cu which is lower in both cases than the single metal ion systems (52.4% for Ni and 65.4% for Cu) [63].

Biosorption profile of *Stenotrophomonas maltophilia* was determined to be Cu >Pb> Cd and Pb> Cu > Cd in primary and ternary solutions, respectively. Biosorption of the three heavy metals was higher in primary solutions than in ternary solutions. Approximately 22% of Cu, 24% of Cd, and 42.75% of Pb were removed from primary solutions; 16% of Cu, 8% of Cd, and 35% of Pb, were removed from ternary solutions by *S. maltophilia* [72].

5. Conclusion

Bioremediation is an eco-friendly green technology used for the cleaning of environmental pollutants to safe levels. As well as high tolerance to various heavy metals and high chelating ability of marine bacteria open new perspectives for the bioremediation technology for the removal of heavy metals from highly contaminated effluents. Biosorption as a kind of bioremediation technologies offers an economically feasible technology for efficient removal of metal(s) from aqueous solution. This study confirmed that the biosorbent prepared from *A. Macleodii* wet biomass, was low cost, easily available, eco-friendly and so could effectively remove heavy metals from industrial effluents.

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