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Bioaccumulation of Chromium by Autochthonous Bacteria Associated With The Heavy Metal- Resistant Halophyte *Arthrocnemum macrostachyum*

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*A*RTHROCNEMUM macrostachyum is a halophyte naturally growing on Manzala Lake shoreline of Egypt and able to tolerate and accumulate heavy metals. The use of metal-resistant rhizobacteria is an important technology to improve the tolerance capability of the halophytes in metal-polluted environments. A total of 33 bacterial isolates were obtained from the root system of *A. macrostachyum* and screened for resistance to 1.25 and 12.5mM of Cr(VI) in plant-based culture medium prepared from the halophyte shoot juice. The six most tolerant isolates were identified based on cell morphology and API microtube profiles. Those isolates were found closely related to *Bacillus lentus, Burkholderia cepacia, Raoultella ornithinolytica* and *Providencia retgeri* indicated on the online database. Among the strains tested for their ability to accumulate chromium, *Bacillus lentus* was the most effective with an average accumulation percentage of 17.8, *Providencia retgeri* ranked thereafter (15.1%). *Bacillus circulance* was the least with a negligible accumulation level of 0.5%. The biosorption rate of the heavy metal was contact time- and bacterial strain-dependent. Again, *Bacillus lentus* showed the highest uptake capacity of 32.8mg g^{-1}, a parameter that positively correlated with contact time (r= 0.865), this was not the case with *Burkholderia cepacia* where the correlation was negative (r= − 0.811). When introduced into an aqueous solution of 25mg L^{-1} Cr(VI), *Burkholderia cepacia* was the superior in Cr(VI) reduction (86.7%), while *Bacillus circulance* failed to reduce the heavy metal. The pH 5.5 was the most favorable for bacterial Cr accumulation which proportionally decreased as the acidity of the solution increased. This study provides more understanding of the significant contribution of the heavy metal-tolerant microbiome to improve the metal remediation efficiency of halophytic plant covers of stressed environments.

Keywords: Accumulation, *Arthrocnemum macrostachyum*, Chromium, Heavy metal-resistant bacteria, Manzala Lake, Reduction.

Introduction

Due to their low solubility and bioavailability besides the property as carcinogenic and mutagenic agents, soil contamination with heavy metals is considered a global problem for the environment and public health (Davis et al., 2011). In fact, soil pollution with heavy metals of high toxicity is obviously increased as a result of industrial activities using cadmium, chromium, copper, lead such as mining, refining, tanning, and other manufacturing processes. In addition, they are constituents in some agrochemicals including both chemical fertilizers and pesticides as well as wastewater irrigation and sewage sludge, thus causing heavy metal contamination and agricultural soils (Glick, 2010; Akcil et al., 2015).

Certain heavy metal ions and metalloids, e.g., iron, copper, manganese or zinc, act as cofactors

of many enzymes and are essential components of living matter. However, these essential components in high concentrations, i.e. non-essential, pose significant and critical threats to the environment and public health because of their toxicity, persistence and their biological accumulation in aquatic habitats (Çeribasi & Yetis, 2001; Chen et al., 2009 ; Gurel et al., 2010), bioaccumulation in edible aquatic organisms (e.g., mollusks, fishes), thus, being a health risk to various consumers, particularly the humans (Huang et al., 2006) this is beside carcinogenic effects (Waisberg et al., 2003) and the development of Alzheimer's disease (Eid & Zawia, 2016).

Among the heavy metals, chromium as a priority pollutant is very well known for its mutagenicity, carcinogenicity in addition to teratogenicity in humans, experimental animals, and plants (Huang et al., 2006; Liu et al., 2008). Actually, the extensive use of this metal in various industries, e.g. leather tanning, stainless-steel production, electroplating and wood preservatives did result in the *in situ* soil and groundwater contamination, this poses a serious threat to human health (Carolin et al., 2017). Compared to trivalent chromium compounds (Cr^{+3}) those of hexavalency (Cr^{+6}) are very toxic due to their extraordinary solubility in water, rapid permeability through the majority of biological membranes and subsequent interaction with intracellular proteins and nucleic acids (Sultan & Hasnain, 2005). Consequently, it has special and increasing concern for its pollution and treatments. Nowadays, a number of conventional chemical procedures are applied for toxic chromate pollution (Carolin et al., 2017), those encompass chemical reduction followed by precipitation, ion exchange besides adsorption on activated coal, alum, kaolinite, and ash. However, these methods are not economically feasible as they consume high levels of energy and large quantities of chemical reagents. Furthermore, the produced metal-containing chemical sludge is considered a potential source of heavy metal pollution (Mishra et al., 2017). This necessitates adopting other more safety methods for the treatment of such toxic materials. It is well established that plant-associated microbiota are having a huge potential to improve direct extraction or stabilization of toxic metal ions in contaminated environments. Indeed, the beneficial plant-microbe interweaves under metal stress conditions can be exploited to improve tolerance and remediation efficiency by phytoremediation

of toxic metals not only in polluted soil; but for cleaning up of water bodies as well (Rajkumar et al., 2013). The metal detoxification mechanisms can be divided into two broad categories. The first includes the strategies that allow plants to evade the metal stress though higher biomass production or effective uptake of essential nutrients. The second category involves the strategies that help the plants to tolerate metal stress by either immobilizing the toxic metal or enhancing the solubility, mobility, accumulation and reduction of metal during the process of detoxification (Tak et al., 2013; Gupta & Diwan, 2017; Shelake et al., 2018). Numerous microbiota were found to posses the ability to accumulate and/or reduce hexavalent chromium including *Bacillus* spp. *Exiguobacterium* spp. *Leucobacte* spp. (Sarangi & Krishnan, 2008), *Providencia* spp. (Thacker et al., 2006), *Shewanella aoneidensis* (Daulton et al., 2007) and *Achromobacter* spp. (Ma et al., 2015).

The halophytic shrub *Arthrocnemum macrostachyum* (Moric) C. Koch is commonly distributed in coastal regions along the Mediterranean basin to the Middle East and Asia. This species represents a model plant to investigate the plant-bacteria interweave specifically in terms of metal phytoremediation efficiency due to its great capability to accumulate heavy metals inside its tissues (Conesa & Schulin, 2010). Nevertheless, little is known on its associated bacteria and their role remediation specifically in a highly polluted wetlands with industrial effluents. Thus, the present study was designed to isolate and identify chromium-resistant bacteria associated with *A. macrostachyum* growing in Lake Manzala wetlands. In addition, the bioremediation capability of the isolated bacteria in terms of chromium accumulation and reduction was among the major targets of the study.

Materials and Methods

Sample collection

Representative full-grown *A. macrostachyum* plant samples with rhizosphere soils (10-20cm depth corresponding to the area of high root biomass) were taken from two heavy metal-polluted sites (31°17'.095"N 32°13'0.026"E and 31°13'.165"N 32°14'0.195"E, Fig. 1) of Manzala Lake shoreline near Bahr El-baqar drainage. Samples were gently washed with the Lake water followed by distilled water, then placed in plastic bags using plastic gloves and

immediately transported to the laboratory and stored at 4°C until processing. Plant samples were separated into roots and shoots, the former was used for bacterial isolation while growth culture media were prepared from the latter. Lake water and rhizosphere soil (adjacent to root system) samples were collected in brown glass bottles and plastic bags respectively. Chemical profiles of water and soil samples were assessed adopting the procedures of Rayment & Higginson (1992).

Isolation and identification of heavy metal-resistant bacteria

Five grams of roots from the indigenous *A. macrostachyum* of the contaminated region were sliced and blended with a little amount of saline solution for 15min in a sterilized mortar, then centrifuged for 30min at 110rpm. Resultant suspensions were transferred to flasks containing 45ml saline solution and serial dilutions were prepared. The juicy shoots of the halophyte were sliced and blended for 5min in a Waring blender (2.7g fresh weight/ ml distilled water). The produced crude juices were thoroughly filtered through cheesecloth and stored in the freezer for further use. A concentration of 3% in the lake water was prepared and the pH was adjusted at 7.0. Agar plant juice culture medium was prepared by

adding agar (1.7%, w/v) and autoclaved for 20min at 121°C (Hegazi et al., 2017). Aliquots of 200ml of suitable dilutions were surface inoculated on a plant-based culture medium, and incubation took place at 30°C for 7-14 days.

A number of colonies developed on the culture medium were picked and subjected to single colony isolation. Those were purified by repeated sub-culturing on the same culture medium and screened for sensitivity to different concentrations of the heavy metal chromium (VI). The most tolerant isolates were examined for cell morphology, Gram-reaction, and sporulation. The API microtube systems, 20 E for Enterobacteriaceae, 20 NE for non-Enterobacteriaceae and 50 CH for Bacillaceae were applied for further identification (Logan & Berkeley, 1984).

The biomass yield of the identified strains was produced by the cultivation of the individuals in nutrient broth with shaking for 72hrs at 30°C, following centrifugation at 10000rpm for 20min Cell pellets were washed twice with sterilized distilled water and resuspended in 1ml sterilized tap water (Oves et al., 2013).

Fig. 1. Map illustrating sampling sites on Manzala lake

Determination of chromium (VI) tolerance

A stock solution of potassium dichromate ($K_2Cr_2O_7$) was prepared. Representative Cr volumes were taken from the stock solutions and added to the plant-based culture medium to guarantee the concentrations of 1.25 and 12.5mM, based on precipitation and clearance of the medium after autoclaving, according to the equation (Manual, 1999): $N\,V = \grave{N}\,\grave{V}$

where, N, stock concentration; V, stock volume to be completed; $\grave{N}$, desired concentration; $\grave{V}$, desired volume.

Adopting the inoculation spot technique of Alam et al. (2011), the bacterial isolates were screened for Cr(VI) susceptibility after 7 days of incubation at 28°C. The maximum tolerable concentration (MTC) of the metal was designated as the highest concentration of metal that allowed bacterial growth (Dinu et al., 2011). The superior bacterial strains in the heavy metal resistance were assessed for Cr VI accumulation and reduction capabilities.

Chromium (VI) accumulation by bacterial strains

The method of Khodaverdiloo & Samadi (2011) was used to estimate the biosorption of chromium accumulated by the tested bacterial strains. The experiments were done in fixed volume (100ml) of Cr(VI) solution (25mg L^{-1}) in a 250ml Erlenmeyer flasks. Bacterial biomass yield of 20mg/100ml was exposed to Cr solutions for 7hrs on an orbital shaker (Wise Shake SHO-2D, Korea) at 150rpm/min (Li et al., 2018). Biomass was separated by centrifugation at 10000rpm for 15min at 4°C (Cooling centrifuge, SIGMA 1-16K, Germany). and the supernatant was analyzed for residual Cr concentration by flame atomic absorption spectrophotometer (Oves et al., 2013).The bioaccumulation was calculated as accumulation percentage (acc.%= $[(C_a\text{-}C_f)/C_a] \times 100$):

where; C_f is the final concentration of metal in the solution with bacteria, and C_a is the initial concentration of metal in the control solution (Li et al., 2018) this estimate was calculated the accumulation rate of the heavy metal was estimated at the different contact times of 10, 30, 60, 240, 420min.

The uptake capacity (AC, mg g^{-1}) expressing the amount of metal bound by the biosorbent, was calculated as follow; AC mg g^{-1} = V (C_a-C_f)/m)

where; C_a and C_f are the same as in accumulation percentage equation, m (g) is the weight of cell sorbent, and V (L) is the volume of working metal solution. Strain-free solutions were used as control (Li et al., 2018).

Chromium (VI) reduction by bacterial strains

Chromium Cr (VI) reducing activity was determined as follows: in 100ml aqueos solution of 25mg L^{-1} Cr(IV) concentration adjusted at pH 6, 20mg bacterial biomass was added, the mixture was kept at ambient temperature for 4hrs. The reduction activity of the bacterium was measured by Spectrophotometer according to the method described by Thacker et al. (2006). Hexavalent chromium specific colorimetric reagent S-diphenyl carbazide (DPC) 0.25% (w/v) was prepared in acetone (AR) to minimize deterioration (Saha & Orvig, 2010). The reaction mixture was set up in 25ml volumetric standard flask as follows; 15ml volume of each sample or standard $K_2Cr_2O_7$, (30, 25, 20, 15, 10, 5, 1mg/L), 2ml H_2SO_4 and 1ml DPC were added. The final volume was made up to 25ml by distilled water. The solution was allowed to stand for 10min. Thereafter, the absorbance of the purple-colored solution was Spectrophotometrically measured at 540nm (USLAB Double Beam UV Spectrophotometer ST-UV-1901PC, USA). Cr(VI) concentration was extrapolated from a standard curve prepared from standard solutions of potassium dichromate.

For calculating the concentration of total chromium, Cr(III) in samples was completely oxidized to Cr(VI) (Onchoke & Sasu, 2016). Here, methyl orange was used as an indicator. A volume of 15ml of each sample was pipetted out into a 100ml standard flask. One ml H_2SO_4 was added and the total volume was made up to 40ml by distilled water. The mixture was heated to boiling. Two drops of $KMnO_4$ were added to give a dark red color followed by 1ml of NaN_3 with gently boiling for 30sec. The total chromium concentration was estimated using the same colorimetric method as for Cr(VI) (Ahmed et al., 2016). The level of Cr(III) was recovered by calculating the difference between total Cr and Cr(VI).

Statistical analyses

Two replicates were set up for each parameter tested. The experimental layout was a complete randomized design and sampling was random. Data generated were statistically analyzed using

one-way analysis of variance (ANOVA) and means were compared by the least significant difference (Snedecor & Cochran, 1980). Linear regressions and correlation coefficients between contact times and either chromium accumulation or reduction were calculated.

Results

Chromium profile of the plant-soil-water system

Variable concentrations of the heavy metal were accumulated in *A. macrostachyum* plant tissues and the rhizosphere soil in close contact with the root system as well as in water of Manzala Lake. An extraordinary quantity of the metal amounted to 562.5mg kg^{-1} was recorded in the soil of the halophyte root theater, plant vegetative part ranked thereafter with considerably lower level of 63.4mg per kg plant. On the contrary, as low as 16.1mg per kg plant of the heavy metal was accumulated in the internal root tissues. The negligible chromium content of 0.36mg l^{-1} was estimated for the lake water.

Bacterial community resistant to heavy metals

Adopting the culture-dependent technique, cultivable populations (CFUs) associated with the root system of *A. macrostachyum* did successfully grow on the plant-based culture medium prepared from the plant vegetative parts. This facilitated the isolation of a great number of heavy metal tolerant members. The plant-based culture medium supported the development of

macro-colonies of up to 10^6 CFU g^{-1}, besides those of micro-sizes ($\leq$1 mm in diameter) particularly with prolonged incubation. Based on variability in cultural characteristics of developing colonies, 30 were picked up and subjected for single colony isolation. Purification was performed by repeated sub-culturing on the same medium.

Bacterial resistance to chromium

When inoculated on plant-based agar culture medium supplemented with two concentrations of chromium, out of the selected 30 isolates, 11 ones nicely tolerated 1.25mM of the heavy metal. While only 6 (20%) kept their ability to withstand the higher concentration of 12.5mM (Table 1). The growth pattern of the tested isolates was incubation time-dependent. It appeared slow along the first 7 days particularly in the presence of 12.5mM of the heavy metal, thereafter it goes somewhat faster.

Identification of chromium-tolerant rhizobacteria

The majority of the identified chromium-resistant rhizobacterial isolates were Gram-negative short rods with no spore formation (Table 2). API analyses indicated that 3 isolates are closely related to *Burkholderia cepacia* with similarity of 99.5-99.9% to those on the online database. The reminder 3 ones were belonging to *Bacillus lentus, Providencia retgeri* and *Raoultella ornithinolytica* with similarity percentages of 99.7-99.9.

TABLE 1. Comparative visible growth of bacterial isolates grown on plant-based culture medium received 2 concentrations of chromium.

Isolates	Control	Cr (IV) mM	
		1.25	12.5
7	+++	++	-
8	+++	++	-
16	+++	+++	-
17	+++	+++	+
26	+++	++	+
27	+++	++	+
28A	+++	++	+
28B	+++	+	-
30A	+++	++	+
30B	+++	+	+
31	+++	++	-
32	+++	+	-

+++, very good growth; ++, moderate growth; +, scant growth; -, no growth.

TABLE 2 Taxonomic profile of selected Cr(VI) resistant bacterial strains.

Isolate code.	Gram reaction and cell shape	Sporulation	Identification%	Strain identification
7	-ve short rod	-	99.9	*Bukhoolderia cepacia* 2
16	-ve short rod	-	99.9	*Raoultella ornithinolytica.*
17	-ve short rod	-	99.5	*Burkholderia cepacia* 3
26	-ve short rod	-	99.9	*Burkholderia cepacia* 1
28A	-ve short rod	-	99.9	*Providencia retgeri*
30B	+ve long rod	+	99.7	*Bacillus lentus*
28B	+ve coccobacilli	+	99.7	*Bacillus circulance*

Bioaccumulation of chromium (VI) by bacterial strains

The capability to accumulate the heavy metal conspicuously varied depending upon bacterial strain and contact time (Table 3). Irrespective of contact time, the highest mean accumulation of 17.8% was recorded by *Bacillus lentus* followed by *Providencia retgeri* (15.1%) and *Burkholderia cepacia* (10.8). The bacterium *Bacillus circulance* was the lowest accumulator with 0.5%. In respect to contact time extended to 420min, no remarkable differences were reported in the chromium accumulation percentages along the first 60min where the estimates hardly fluctuated between 9.5 and 10.4. Prolonged contact to 240min resulted in obvious increase of up to 44% in the metal accumulation rate, which decreased thereafter. The relationship between metal accumulation percentages and contact time could rather be expressed in the linear regressions illustrated in Fig. 2. While both criteria positively correlated (r = 0.865) for *Bacillus lentus*; but they were negatively interacted (r = − 0.811) in case of *Burkholderia cepacian*.

The uptake capacity of the heavy metal was, as well, contact time- and bacterial member-dependent (Table 4). As with the accumulation capacity, *Bacillus lentus* was the superior among all the tested microbiota with an average uptake capacity of 32.8mg g⁻¹. *Provideacia retgeri* was ranked the second with uptake capacity of 22.7mg g⁻¹. *Bacillus circulans* showed the lowest level of chromium absorption with 0.8mg g⁻¹.

The chromium reduction potential of bacterial strains

When bacterial biomass was exposed to an aqueous solution of 25mg L⁻¹ Cr(VI), the introduced bacterial strains exhibited variable abilities to reduce Cr(VI) to Cr(III) after 240min exposure (Fig. 3). *Burkholderia cepacia* exhibited the highest reduction capacity of 86.7%. On the contrary, *Bacillus circulance* failed to reduce the hexavalent metal. Other bacterial candidates successfully reduced the metal but to somewhat lower extents (37.4-57.6%).

Effect of pH on chromium accumulation and reduction

The ability of *Providencia retgeri* to accumulate and absorb the heavy metal was assessed in the aqueous solution of 25mg L⁻¹ of various pH values. The pH of 5.5 deemed the most favorable for chromium accumulation (13.5%) which decreased proportionally as the acidity of solution increased up to pH 4.5 then increased at pH 4.0 (Table 5). The reduction percentage of the heavy metal by the bacterium was the highest at pH 4.0 (66.2%) and the lowest at pH 4.5 (54.1%). Total detoxification decreased from 77.3% to 59.1% at the respective pH values of 4.0 and 4.5.

TABLE 3. Mean ± standard error of chromium accumulation perecentage by bacterial strains

Contact time (min)	Accumulation percentage (%)						
	Bacillus circulance	*Bacillus lentus*	*Burkholderia cepacia 1*	*Bukhoolderia cepacia 2*	*Burkholderia cepacia 3*	*Providencia retgeri*	**Total**
10	0.0 ± 0.0	0.0 ± 0.0	24.2 ± 7.9	14.6 ± 6.6	10.1 ± 0.4	14.3 ± 9.6	9.06 ± 2.8
30	1.2 ± 0.0	20.9 ± 0.8	15.3 ± 6.4	1.9 ± 1.0	4.6 ± 0.0	8.2 ± 1.6	8.7ᵃ ± 2.0
60	0.9 ± 0.0	15.2 ± 0.0	5.0 ± 0.0	6.7 ± 0.7	24.1 ± 4.5	4.8 ± 0.0	9.5ᵇ ± 2.0
240	0.2 ± 0.0	28.6 ± 0.6	4.5 ± 0.0	17.9 ± 0.4	4.7 ± 1.8	26.3 ± 0.0	13.7ᶜ ± 2.7
420	0.0 ± 0.0	24.1 ± 9.8	5.4 ± 0.2	6.1 ± 1.3	5.0 ± 1.3	22.0 ± 12.7	10.3 ± 3.2

Total mean with different letters are significantly different at P< 0.05 level according to LSD (value= 7).
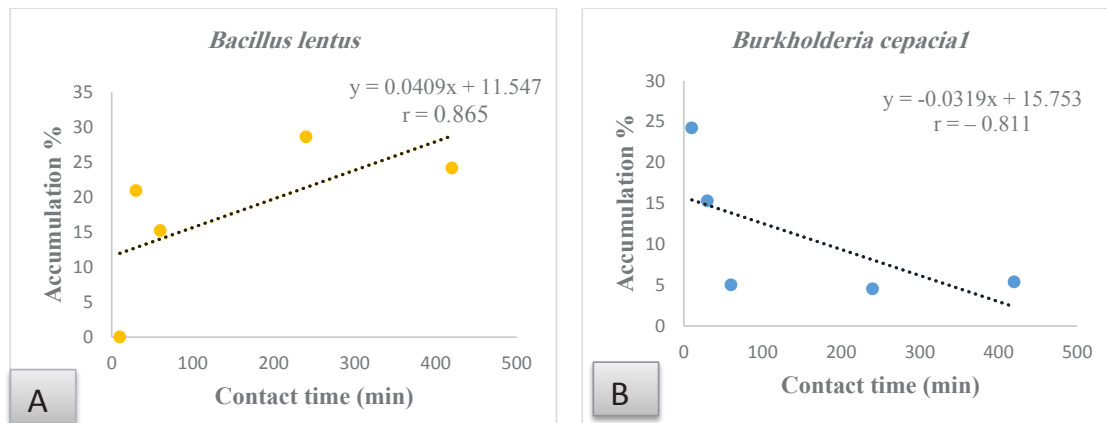
Fig. 2 . Linear regressions and coefficients of determinations among Cr(VI) accumulation percentages and contact times for *Bacillus lentus* (A) and *Burkholderia cepacia* 1 (B).

TABLE 4 . Mean ± standard error of Cr(VI) uptake capacity of bacterial cells.

Bacterial strains	Uptake capacity mg g^{-1}				
	10min	30min	60min	240min	420min
Bacillus circulans	0.0 ± 3.3	2.1 ± 0.1	1.7 ± 0.1	0.4 ± 0.01	0.0 ± 5.1
Bacillus lentus	0.0 ± 19.8	39.1 ± 1.5	27.5 ± 0.7	55.1 ± 1.2	42.3 ± 17.2
Burkholderia cepacia 1	39.6 ± 13.0	25.2 ± 10.5	8.8 ± 0.1	8.8 ± 0.1	9.9 ± 0.3
Burkholderia cepacia 2	3.5 ± 2.9	2.8 ± 1.4	10.0 ± 1.0	24.4 ± 0.5	9.0 ± 1.9
Burkholderia cepacia 3	15.4 ± 6.6	6.8 ± 0.4	32.1 ± 5.9	6.4 ± 2.4	4.0 ± 3.1
Providencia rettgeri	0.0 ± 24.2	15.4 ± 2.9	8.9 ± 0.1	50.7 ± 0.6	38.6 ± 13.3
Total	5.2 ± 6.6	15.3[a] ± 3.6	14.7[b] ± 2.8	24.4[c] ± 5.3	17.9[d] ± 6.1

Total mean with different letters are significantly different at P< 0.05 level according to LSD (value= 43).
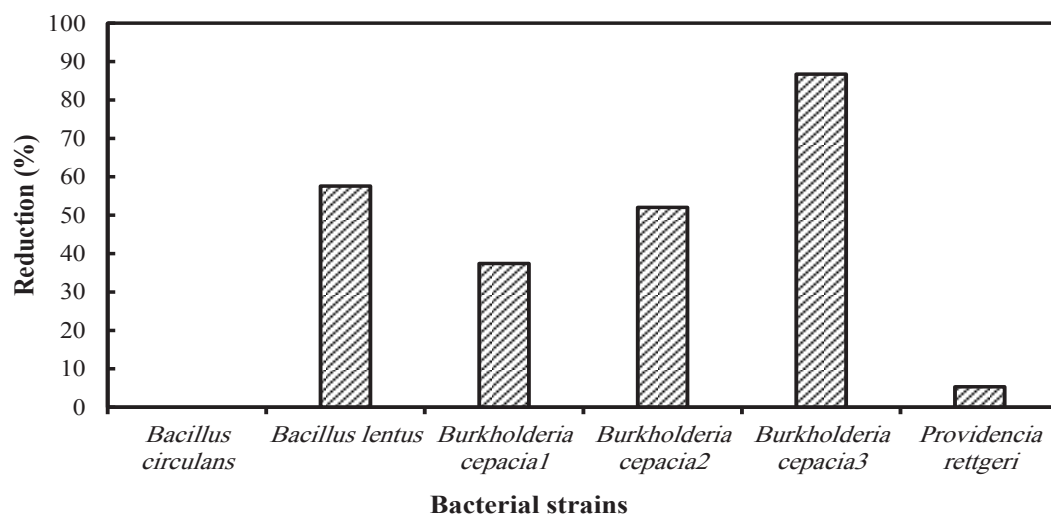

Fig. 3. Reduction percentages of chromium(VI) by bacterial strains.

TABLE 5. Effect of eques solution pH on Cr(VI) accumulation and reduction by *Providencia retgeri*.

Parameters	pH				
	4.0	4.5	5.0	5.5	6.0
Accumulation %	11.12	4.97	1.55	13.51	3.11
Reduction %	66.20	54.14	64.34	57.10	60.89
Total detoxification (%)	77.3	59.1	65.9	70.6	64.0

Discussion

Due to their potential toxicity, reactivity and soil mobility, heavy metals are of special environmental concern. The emissions of such chemical pollutants have become a serious threat to mankind. The exposure routes of heavy metals to humans encompass inhalation, dermal absorption, and ingestion (WHO, 2018). Several efforts are currently being done in an attempt to alleviate their environmental impacts. Among those, are ion exchange, chelating, precipitation, adsorption, thermal, membrane technologies and biosorption strategies. The latter, in particular, is of several advantages compared to other conventional methods recommended for heavy metal remediation because of its accessibility and efficiency (Dhanarani et al., 2016). As mentioned by Abioye et al. (2018), the important economic aspects of biosorption technology are due to that the biomass used for decontamination of heavy metal pollutants is natural, easily available, affordable and provides an application performance in comparison with conventional procedures for decontamination.

The hexavalent chromium occupies a non-tiny place on the map of priority pollutants due to its carcinogenicity and mutagenicity. On the other hand, the derivatives of trivalent Cr are insoluble at neutral pH, less toxic and mutagenic than Cr(VI). Therefore, the reduction of Cr(VI) to Cr(III) is a necessary beneficial reaction in the heavy metal-polluted environment (Mishra et al., 2017).

Phytoremediation technique involves the use of metal-resistant plants that having the ability to facilitate metal precipitation onto root surfaces and/or accumulation into them (Mendez & Maier, 2008). The halophyte *Arthrocnemum macrostachyum* is a promising plant possessing the ability of remediation in heavy metal contaminated environments due to its ability for metal uptake and accumulation in mining wastes and polluted estuaries (Conesa & Schulin, 2010). In addition, the capability to accumulate and reduce Cr VI by certain bacterial species isolated from contaminated environments under field and laboratory conditions has been assessed by many investigators (Joutey et al., 2015; Tirry et al., 2018).

Isolating bacteria from metal-polluted areas is an advantage to have bacterial members adapted to the toxicity of heavy metals. In the present study, a number of bacterial isolates were obtained from Manzala Lake water and those associated with the halophyte *A. macrostachyum* and experimented for their ability to resist the presence of Cr(VI) in plant-based culture medium. The most tolerant isolates were subjected to cell morphology and API microtube systems for identification. Three isolates were closely related to *Burkholderia cepacia* indictined on the online database, while the others were identified as *Bacillus lentus*, *Providencia retgeri,* and *Raoultella ornithinolytica*. It is important to note that the high percentages of identity of these isolates (more than 99%) with the previously described species of some isolates suggesting a significant classified diversity of cultivable bacteria associated with *A. macrostachyum* growing in the examined contaminated ecosystem. In this context, Navarro-Torre et al. (2016) isolated 48 different bacterial strains from *A. mocrostachyum* microbiome growing in a highly contaminated estuary. They found that a proportion of 40% of the rhizospheric isolates was assigned to *Bacillus spp*. It is well established that the genus *Bacillus* is one of the most predominant in the rhizospheres of plants growing in heavy metal contaminated soils, this attributes to its capability to withstand metals and NaCl (Nies & Silver, 2007). The six identified rhizobacterial strains in the present study did successfully tolerate Cr(VI) concentration of 12.5mM in plant-based culture medium, but other isolates were not able. Here, Shen & Buick (2004) reported that bacteria have the tendency to react differently under various stress conditions. They mentioned that the cellular mechanism could be broadly classified into three major categories, those are adaptability, tolerance and mutagenic

alteration. The bacterial interaction towards metal ions can be further classified into its origin and Gram's type (Thatoi et al., 2014).

Biosorption or bioaccumulation of heavy metals is a biological remediation technology that involves the removal of the metal species from a solution by inexpensive biomaterials. The majority of bio sorbents used showed appropriate biosorption capacities toward all types of metal ions, and many affordable and available materials applied for the elimination of heavy metals in various environments are mainly derived from bacteria, fungi, algae, plants and some polysaccharide materials (Mustapha & Halimoon, 2015). Results of the present study on screening of isolated bacterial species for biosorption and accumulation of Cr(VI) indicated that *Bacillus lentus* and *Providencia retgeri* exhibited the highest rates of 17.8 and 15.1% respectively, while *Bacillus circullance* showed the lowest rate of 0.5%. Chaney et al. (2007) reported that the ability of some bacterial strains to take up and accumulate the heavy metals inside their cells might be attributed to their inherent physiological characteristics related to cell wall structure. The fungus *Penicillium notatum* has a rigid and complex cell wall that contains the polysaccharides chitins and glucans beside possessing high surface-to-volume ratio that enables this microorganism to absorb the heavy metals into its cell wall. In addition, heavy metal bio-accumulators release a number of extracellular enzymes such as laccases and metal binding proteins that act as chelators having the ability to bind heavy metals and hence facilitate absorption by the cell wall. In the present study, the highest accumulation rate of chromium (VI) was observed at the 240min exposure time, with 50% of tested bacterial strains. Longer exposure for 420min decreased the accumulation rates for the majority of tested strains. This decline in metal sorption is most probably attributed to saturation of the organism-metal binding sites or could be a result of cell damage.

The reduction of Cr(VI) to Cr(III) by the six bacterial strains was assessed in the aqueous solution of 25mg L^{-1} of the heavy metal. After 240min exposure, the metal reduced by 37.4 -86.7% depending upon strain. *Burkholderia cepacia* was the superior while *Bacillus circulance* failed to reduce the metal.

Great attention has been paid to optimize the cultivation conditions for microorganisms to guarantee the proper remediation status of heavy metals in various environments. Indeed, pH conspicuously contributes in the biosorption of heavy metals, where the lower pH causes the protonation of binding sites in microbial surfaces and thereby impacts negative charge to microbial surface, which in turn contributing metal binding (Shankar et al., 2007). As low as pH 5.0 was found the optimum for the biosorption of several heavy metals such as lead using *Rhizopus nigricans* (Yan & Viraraghavan, 2003), nickel by *Penicillium chcrysogenum* (Tian-Wei et al., 2004), and copper by *Micrococcus luteus* (Leung et al., 2000). On the contrary, Nasseri et al. (2002) reported reduction in metal absorption in alkaline pH. Similar results on biosorption of chromium were reported by Goyal et al. (2003) and Shankar et al. (2007) using *Streptococcus eauisimlis* and *Aspergillus* sp. Respectively. A part of the present study has been dealt with the effect of aqueous solution pH on Cr VI accumulation and reduction by the metal tolerant strains using *Providencia retgeri* as a representative. The bacterium successfully accumulated and reduced the heavy metal at pH 4.o-5.5, the bacterial activities decreased as the acidity of the solution increased. In conformity with these findings, Sharma & Adholeya (2011) investigated the Cr(VI) reduction capability of the fungus *Paecilopmyces lilacinus* in growth medium of different pH values, results demonstrated that pH 5.5 was the optimum for the fungal reduction efficiency.

Based on the preliminary bioremediation capabilities of the studied bacterial members, both *Bacillus lentus* and *Burkholderia cepacia* are recommended for application in the phytoremediation programs of the halophyte *A. macrostachyum* in the Manzala Lake area.

Conclusion

In conclusion, the global target of the present study is to alleviate the detrimental impacts of Cr VI contamination in soil-water environments using microbe-associated phytoremediation techniques involving autochthonous plant covers. This is, actually, based on the previous attempts in this study area that indicated the successful inoculation of halophytic plants in heavy metal polluted soils with the rhizospheric bacterial consortia. This supports plant growth and

metal uptake in plant roots, thus improving the phytostabilization potential of the halophyte.

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