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Screening of Multi-Metal Tolerant Halophilic Bacteria for Heavy Metal Remediation

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A B S T R A C T

Hundred and twenty eighty halophilic bacterial isolated from the soil and water of Karnataka mangrove regions were examined for multi-metal tolerance. *Bacillus pumilus* (accession no.MF472596) was found to be tolerant against four toxic heavy metal ions (Cd^{2+}, Cu^{2+}, Fe^{3+}, and Ba^{2+}) up to 1000 ppm each. Chemical analysis was carried out by ICP-AES for Ba^{2+} and AAS for rest of the metal ions. The bioremediation efficiency against metals are as follows Fe>Cu>Ba>Cd (90%, 71%, 52% and 19% respectively) at pH 7. Altering the pH in a range of 6 to10 the bioremediation rate increased to 96%, 88%, 54% and 52% for Fe, Cu, Ba, and Cd respectively. The metal absorption efficiency increases on altering pH i.e. from 136 ppm, 52 ppm and 35 ppm to 196 ppm, 82 ppm and 60 ppm in Fe^{3+}, Ba^{2+}, Cd^{2+} respectively, whereas reduction in Cu^{2+} absorption was noted i.e. from 114 ppm to 41 ppm. This investigation justifies that specific pH exposure can play a key role in enhancing bioremediation activity of bacterial isolate towards metal ion.

Introduction

Industrial activities release many toxic metals to the environment, many of these pollutants are not easily degradable rather persist in environment complicating their remediation.

These create toxic effects in human (Umrania, 2003), specially when they get accumulated in water bodies available for domestic purpose above the permissible limit (Ba-0.3mg/l, Cu-2.0mg/l, Cd-0.003mg/l as per WHO and 0.2mg/l for Fe as per EU) (Lenntech, 2017). This contaminated water interferes with the health and growth of crops, lowering their quality and marketability (Augusto-Costa and Pereira-Duta, 2001).

Remediation and leaching by microbes are gaining attention in the last two decades (Umrania, 2003), as they provide an alternative and eco-friendly method than of the physiochemical techniques in which a huge amount of toxic sludge is left at the end. Microbes carry out bio-remediating by three ways: bioaccumulation, biotransformation, and biodegradation (Bestetti *et al.*, 1996). They interact with the metal ion, changing the chemical form by simple oxidation or reduction process (Noghabi *et al.*, 2007; Choi *et al.,* 1996; Ellis *et al.,* 2003). Sometimes the resistant processes are mediated at plasmid level (Zolgharnein *et al.*, 2007).

Recent studies have documented the significance of microbe in remediation (Sobhy *et al.,* 2014; Yan and Viraraghavan, 2003; Umrania, 2006; Kozdra and Van Elsas, 2001; Valls and DeLorenzo, 2002; Ajaz *et al.*, 2010). Metal exposure leads to tolerant microbial sp. belonging to genera of *Bacillus, Corynebacterium, Arthrobacter, Pseudomonas, Ralstonia, Alcaligenes, and Burkholderia.* (Sobhy *et al.*, 2014; Yan and Viraraghavan, 2003; Umrania, 2006; Kozdra and Van Elsas, 2001; Valls and DeLorenzo, 2002; Ajaz *et al.*, 2010). In our study, we have randomly selected four heavy metal ions of which cadmium belongs to the most toxic group, barium to the minor toxic group whereas iron and copper are essential elements but a higher concentration even of the essential metal in the human body can lead to fatal effects.

Iron is essential for erythropoiesis as it is the key component of hemoglobin, myoglobin, heme enzyme, metalloflavoprotein and mitochondrial enzyme.

Overloading of iron in vital organs can lead to cirrhosis, cardiac collapse, cyanosis, metabolic acidosis and pneumoconiosis (Doherty *et al.*, 2006). Even premature death cases and neurogenerative diseases are seen (Atli Arnarson, 2017).

Copper has been used for many centuries. It is a key component for several metalloenzymes (Kamza and Gitlin, 2002). Its deficiency is uncommon inhuman. High concentration of copper intake can cause gastrointestinal distress resulting in, diarrhea, nausea, stomach cramps. Injection of a large number of copper salts may produce hepatic necrosis and death (Pizzaro *et al.,* 1999).

Barium an alkaline earth metal is relatively abundant in nature. High barium doses result in intractable vomiting, severe diarrhea, gastrointestinal hemorrhage and sometimes cardiac arrest leading to death (ASTDR, 2005b). Profound hypoleukemia and muscle weakness leading to flaccid paralysis are an indication of barium poisoning (Johnson and VanTassell, 1991).

Cadmium ranks in a close relationship next to lead and mercury as one of the most toxic elements (Jarup *et al.,* 1998). The main source of cadmium is through food for the community, low serum ferritin level in human are noticed for twice the level of cadmium in them (Berglund *et al.,* 1994) once absorbed efficiently retained in human body damaging the kidney, causing chronic pulmonary disease, cardiovascular effects and causing bone demineralization (Bernard, 2008). Cadmium compounds are contemplated to be human carcinogenic (NTP, 2004; Takeuchi, 1977).

The present study was carried out with halophilic microbes of Karnataka mangrove region with an objective to search for promising multi-metal resistant halophilic microbes in order to use for remediation from any toxic site from.

Halophytes of such hypersaline regions are selected for bioremediation due to their metabolic differences than that of terrestrial ones and follow (Oren, 2002; Ventosa *et al.*, 1998; Roberts, 2005; Mevarech *et al.,* 2000; Tehei *et al.,* 2002) they follow compatible solute strategies which can be put into effective use for remediation.

The isolate was identified as *Bacillus pumilus* (accession no. MF472596) has shown potential for remediation against Cd^{2+}, Cu^{2+}, Fe^{3+}, Ba^{2+} and was taken up for study in various parameters to get the maximum result.

Materials and Methods

Atomic Absorption Spectrophotometer, SHIMADZU, AA600, was used for bioremediation analysis. UV Spectrophotometer, Agilent Technology, Carry 60, was used for spectrophotometric studies. Sonicator Probe, Life core, ENUP-500, used for sonicating the bacterial cells. All media are of Hi-media company and all chemical used are of analytical grade. Molecular analysis was carried out in Trans-Disciplinary University of Health Science and Technology and *Eurofins* genomics India, Bangalore.

Isolation of bacteria

The bacterial strains were isolated from the water and sediment samples, collected during Pre Monsoon (June-July) and Post Monsoon (October-November) season of Mangroves regions from three districts (at twenty different sites) in the Coastal region of Karnataka [i.e. Honavar (14.26°-74.44°), Kumta (14.49°-74.39°) and Karwar (14.84°-74.11°)].

Sediments samples were taken at a depth of 5cm and 40cm from the root region of various trees sp. like *Sonneratia alba*, *Kandelia candel*, *Rhizophora spp*, *Avicennia spp*. and water samples were collected at a depth of 30-100 cm. The samples were incubated in Hi-media halophilic broth (M591-500G) for 12 days for enrichment and isolation of extreme halophiles.

Following ten-fold serial dilution technique in a Hi-media halophilic agar plates, halophilic bacterial isolation was carried out (Rath and Subramanyam, 1996) by incubating aerobically at 37^{o}C for 48 hours (Das *et al.*, 2012). Pure cultures were obtained by repeated streaking over the nutrient agar plates and preserved in glycerol at -20°C and on nutrient agar slants at 4°C for further use.

Stock solutions preparation

Stock solutions of Cadmium, Iron, Barium, and Copper (1000 mg/L) were prepared from corresponding metal salts (i.e. $CdCl_2$, $FeSo_4.7H_2O$, $BaCl_2.2H_2O$, $CuSO_4.5H_20$). The glassware used for this purpose were leached in 2N HNO_3 and rinsed several times with distilled water before use to avoid any metal contamination. The Fe^{2+} is oxidized to Fe^{3+} in presence of nitric acid. 2ltrs of a stock solution of each metal ions was prepared in distilled water and acidified with HNO_3 (10-20 ml of 2% HNO_3) to prevent precipitation and was sterilized at 121°C for 15 min.

Metal tolerance study of isolates

Various concentrations of heavy metals i.e. 100-1000 (mg/L) were prepared in a final volume of 10 ml in Hi-media nutrient broth, to which 1 ml of 24 hr old isolated bacterial cultures were inoculated at 37°C for 24 h. The tubes were observed for turbidity which was further analyzed by pipetting out 5ml of the sample and analyzing under a UV-spectrum. A loopful of the cultures was streaked onto the nutrient agar plate containing respective metal concentration to check for the viability. The most potent isolate showing maximum tolerance to the metals was screened by this qualitative method (Pardo *et al.*, 2003).

16S rRNA sequence analysis

Bacteria used for our study were preliminarily identified using ABIS online tool based on the cultural, morphological and biochemical characterization. Further identification was carried out using 16S rRNA gene sequencing. Bacterial genomic DNA was extracted (Peng *et al.*, 2005). The DNA was used as a template for PCR using universal primers. These purified products are a template in cycle sequencing (Pitcher *et al.,* 1989). The amplified 16S rRNA gene was purified with

QIAGEN Inc. kit and electrophoreses on 1% agarose gel Sequencing was carried out in *Eurofins* (Suganthi *et al.*, 2013; Zhou *et al.*, 1996; Achenbach and Woese, 1995). BLAST program was used to access the DNA similarities and multiple sequence alignment and molecular phylogeny were performed using *Bio Edit* software.

Optimization of growth parameters

Growth characterization

Overnight grown bacterial culture in Luria Bertani medium with 5% salt conc. was used as inoculums for the analysis of growth pattern.

It was inoculated in different Erlenmeyer flasks; each containing 100 ml of nutrient broth supplemented with 1000 ppm of different metal solutions incubated at 37°C. 5ml of bacterial suspension from each of the flasks was pipetted out after every 2 h and analyzed at 620 nm to monitor the growth pattern (Fig. 4).

Effect of pH on the isolate

Bacillus pumilus was set incubated with varying pH environments (i.e. 2, 4, 6, 8 and 10). 5ml of bacterial suspension was pipetted out after every 2 h and analyzed at 620 nm to monitor the growth pattern and tolerance (Fig. 5).

Effect of pH on metal absorption

To check the pH effect on bioremediation, the biomass of *Bacillus pumilus* was set incubated at different metal concentrations with varying pH environments (i.e. 2, 4, 6, 7, 8 and 10). 5ml of bacterial suspension from each of the flasks was pipetted out after the incubation period and analyzed at 620 nm (Silva *et al.*, 2009) (Fig. 6).

Optimization of metal uptake by the isolate

Based on the spectrophotometer analysis, the following parameters were chosen for the isolate to be tested under AAS and ICP-AES for metal reduction.

Remediation of metals by the organisms at pH 7

One milliliter of the freshly prepared aliquot of the isolate was incubated in 100 ml of nutrient broth media containing the highest tolerating concentration of respective metal ion $CdCl_2$, $FeSO_4.7H_2O$, $BaCl_2.2H_2O$, and $CuSO_4.5H_2O$. The media was adjusted to pH 7 and the cultures were incubated at 37°C for 48 h. The incubated cultures were centrifuged at 6500 xg for 20 min, supernatants were used for the determination of the residual metal ion contents by using AAS or ICP-AES (Abou Zeid *et al.*, 2009; Kermani *et al.*, 2010). Controls without inoculation of the bacteria were prepared to detect the initial metal conc.

Effect of contact time

Media containing metal solutions adjusted to pH 7 and inoculated with selected isolate was incubated at 37°C for 72 h. The initial and residual conc. of metal within the media was measured as mentioned earlier.

Uptake of metal by the organisms at pH 7 (following cell disruption method)

The metal uptake at pH 7 at an optimized temperature and incubation period by the *Bacillus sp*. The cultures were centrifuged at 6500 xg for 20 min. The pellets were washed with de-ionized water three times and the supernatant was discarded. The pellets were sonicated at 70 kHz for 15 min at 2 min interval and centrifuged at 10000 xg for 20min. Bacterial free suspensions were ensured by passing the supernatant through a

22μm syringe filter and determined under AAS or ICP-AES for metal uptake (Volesky *et al.*, 1995).

Effect of highest and lowest pH values

As per the spectrophotometer analysis, the highest and lowest pH range which the isolate could tolerate for each metal were selected respectively and observed for bioremediation. After 48h incubation the incubated cultures were centrifuged at 6500 xg for 20 min. The supernatants were used for the determination of the residual metal ion contents by using AAS or ICP-AES (Silva *et al.*, 2009; Abou Zeid *et al.*, 2009). Controls without inoculation of the bacteria were prepared to detect the initial metal conc.

Uptake of metal by the organisms at highest and lowest pH (following cell disruption method)

A comparative study was carried out on the uptake of metal at the highest and lowest pH at an optimized temperature and incubation period. The cultures were centrifuged at 6500 xg for 20 min. The pellets were washed with de-ionized water three times and the supernatant was discarded. The pellets were sonicated with 70 kHz for 10mins with 2 min interval in between and centrifuged at 10000 xg for 20min. The supernatants were passed through a 22μm syringe filter and analyzed under AAS or ICP-AES for metal uptake (Abou Zeid *et al.*, 2009; Kermani *et al.*, 2010; Volesky *et al.*, 1995).

Results and Discussion

Hundred twenty eight halophilic isolates were tested for multi-metal tolerance revealing that cadmium is non-tolerable for the majority of the mangrove isolates. Only *Bacillus pumilus* was found tolerant to all metals was selected for further study. The selection is based on the isolate growth on the nutrient agar plate containing the same conc. of metal, not on spectrophotometric analysis.

Molecular characterization

For phylogenetic analysis, the 16S rRNA gene sequence of a single band of *mw* was obtained [Fig. 1 (a, b)]. This gene sequence, when compared with those retrieved from the GeneBank database, revealed the closest prokaryotic relative of the heavy metal resistant bacteria, KBORMPorg to be *Bacillus pumilus* in NCBI BLASTN. Sequences alignment edition were done using Bioedit (Version 7.2.6). Using the Bootstrap method tree topologies were evaluated in MEGA 6 software providing confidence estimation through phylogenetic tree topologies about the isolate, the sequence was deposited in GenBank under accession number MF472596 (Figure 2).

Spectrophotometer analysis of the *B.pumilus* on various metal tolerances

At 620 nm the isolated was analyzed and found that *B. pumilus* can tolerate up to 1000 ppm of all metals (Figure 3).

Growth characterization

The growth pattern of *B. pumilus* in the presence and absence of metals has been shown in Figure 4.

Effect of pH on the isolate

The growth pattern and tolerance towards various pH by *B. pumilus* been shown in Figure 5.

Effect of pH on metal tolerance

pH range from 6-10 for the microbe inoculated for 48h is found to be effective in interacting with the metals (Fig. 6).

Optimization of metal uptake by the isolate

Remediation of metals by the organisms at pH 7

Up to 1000 ppm, the isolate had shown tolerance towards Cadmium, Barium, Iron and Copper at pH 7 in 48 h. On analyzing with AAS its was found, 90% of metal reduction in the case of Iron, followed by Copper 71%, 50% reduction was found in Barium (ICP-AES analyzed) whereas only a 19% reduction was found in the case of Cadmium.

Effect of contact times

At pH 7, the isolate was incubated for a period of 72 h with the highest tolerating conc. of the Cadmium, Barium, Copper, and Iron to which the reduction was found to be 22%, 52%, 80% and 90% respectively when analyzed under AAS and ICP-AES (only for Barium). There is no effect found in the case of iron and a minimal effect in case of Barium.

Uptake of metal by the organisms (following cell disruption method)

Following the same metal conc. (1000 ppm for Cd, Ba, Fe, and Cu) the isolate was grown at pH 7; the cells were disrupted following sonication technique, to detect the uptake of the above metals by the isolate. All the filtered supernatant was analyzed in AAS and ICP-AES. The uptakes of different metal by the isolate are arranged in ascending orders: Cadmium, Barium, Copper, and Iron i.e. 35ppm; 52 ppm; 57ppm, 136ppm respectively.

Effect of highest and lowest pH values

Following the spectrophotometer analysis of the isolate for the tolerance of pH at highest and lowest level is considered, it was tested for metal remediation at the same ppm conc. as above (i.e. 1000 ppm for Cd, Ba, Fe and Cu). pH 6 is the lowest range for the isolate to tolerate Copper, Barium, Iron in which the reduction of metal ranges from 88%, 54%, and 91% respectively. pH 10 is the highest for all metals i.e. Copper, Iron, Cadmium and Barium in which the metal reduction ranges from 88%, 96%, 52% and 52% respectively. pH 7 remain the lowest tolerating range for the isolate in case of Cadmium with a metal reduction of 19% was observed. From the above, we can say that pH plays a key role in metal remediation.

Uptake of metal by the organisms at highest and lowest pH (following cell disruption method)

The isolate grown in the optimized pH was subjected for metal uptake following the above technique. The uptake of different metals by the isolate in varying pH subjected for comparison. pH 6 is the lowest range for the isolate to tolerate metals like Copper, Barium, and Iron in which the metal uptake ranges from 41 ppm, 82 ppm, and 140 ppm, respectively. pH 10 is the highest for all the metals i.e. Copper, Iron, Cadmium and Barium in which the metal uptake ranges from 22 ppm, 196 ppm, 60 ppm and 52 ppm respectively. pH 7 remains the lowest tolerating range for the isolate in case of Cadmium in which the metal uptake is about 35.1 ppm respectively.

Bacillus pumilus tolerate all the four heavy metals up to 1000 ppm. The resistivity of the microbe towards heavy metals was checked by incubating in different metal solution concentration (Yan and Viraraghavan, 2003; Hall, 1999). The selection procedure of the isolate was based on the growth of bacterial colonies on a nutrient agar plate containing respective metal ions. The isolate has shown least tolerance towards Cd, whereas good affinity is observed in the case of Fe, Cu, and Ba. The variation in the resistant mechanism of different microbes is the cause of the varying intolerance towards different conc. of heavy metals.

The BLAST hits of KBORMPorg obtained from 16s rRNA gene sequence indicate its close relation with *Bacillus pumilus species* (accession nos. MF472596).

Spectrophotometric data reveals the isolate showed a profound growth pattern in the absence of metals except Barium. The growth of the isolate can be seen up to 36-40 hr after which it is found to be in standard phase till 48^{th} hr before touching the decline phase. The media without metal and Barium supplement, the isolate achieved log phase at a much lower time in comparison to the growth in the presence of other metals. Presence of Barium, the growth of the isolate is found to be higher than other. The isolate shows highest absorbance value towards all metals in alkaline condition whereas in case of cadmium acidic pH is ineffective, which are taken for effective remediation parameter. The evaluation of pH in our work is based on Tehei and Valls conclusion, states the number of cell surface sites available to bind cations, as well as metal speciation, and are affected due to pH variation (Yan and Viraraghavan, 2003). Ajaz and co-workers reported that pH can greatly influence heavy metal removal by microbes (Jalali *et al.*, 2002; Pardo *et al.*, 2003; Hornung *et al.*, 2009; Cappuccino and Natalie, 2002; Pitcher *et al.*, 1989; Acinas *et al.*, 2004; Tamura *et al.*, 2011; Felsenstein, 1985) by influencing the metal speciation and solution chemistry as well as surface properties of bacterial cells.

The selected isolate subjected to five different parameters for analyzing the remediation of selected heavy metals under AAS as follows;

Remediation of metals by the organisms at neutral pH.

Effect of contact times.

Uptake of metal by the organisms (analyzed by cell disruption method).

Effect of highest and lowest pH values.

Uptake of metal by the organisms at highest and lowest pH (following cell disruption method).

Following Haq *et al.*, AAS and ICP-AES analyzing procedure the selected isolate *Bacillus pumilus* was prepared by first subjecting it to its highest tolerating conc. of the selected heavy metals at pH 7 for a period of 48 h. The supernatant was removed at the end of 48 h of the incubation period by centrifugation method and diluted to 1ppm and acidified with HNO_3 (Strandberg *et al.*, 1981).

The chemical analysis data revealed the removal percentage of each of the heavy metals in descending order Fe>Cu>Ba>Cd, 90%, 71%, 52%, 19%, which made clear about the bioremediation of the metals by the isolate *Bacillus pumilus*.

The culture pellets were thus collected and rinsed thrice with PBS and lysed by applying sonication with amplitude of 100 for a period of 20 min. with 45 sec interval after every 3-4 min. and acidified with HNO_3 and set for AAS analysis.

The above results corroborate with the work of Haq and co-workers who reported about 86% removal of cadmium (100 mg/l) from medium within 24 h by E. Cloacae (Haq *et al.*, 1999).

Another report suggests a Cd removal by E. Cloacae bacteria isolated from tobacco could reduce only 29% of Cadmium from the medium (Sahoo *et al.*, 2016).

Fig.1 Molecular Characterization

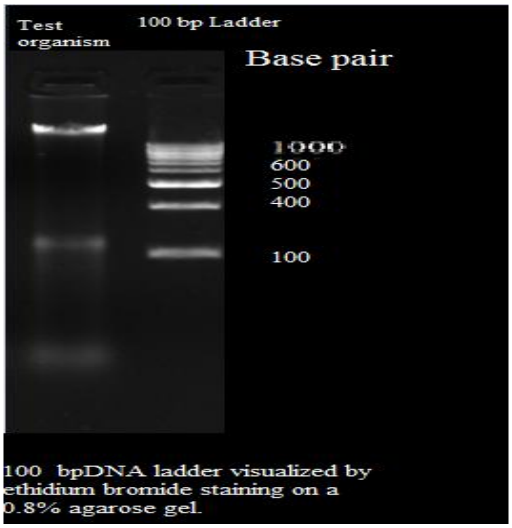


FIG.1(a). Agarose gel electrophoresis of DNA Sample

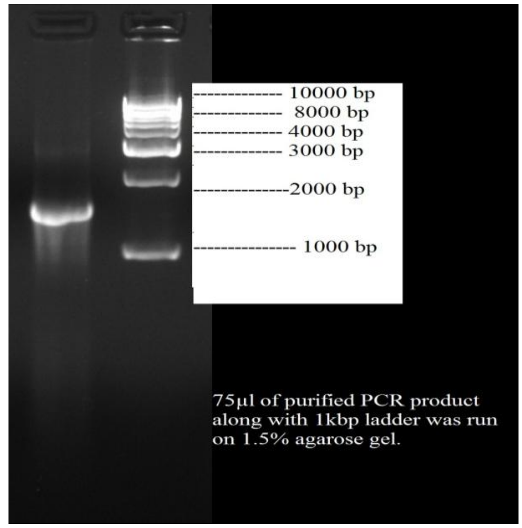


FIG 1(b). Agarose gel electrophoresis of PCR-amplified DNA product.

Fig.2 Phylogenetic tree based on 16s-rRNA gene partial sequences obtained from the NCBI nucleotide sequence database

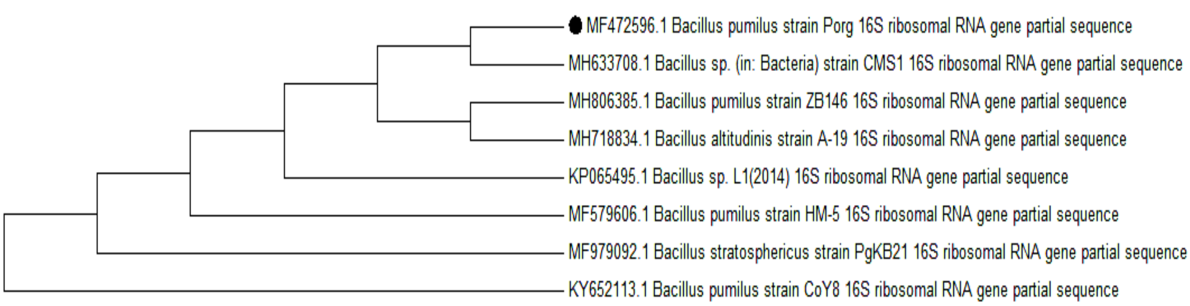


Fig.3 Spectrophotometer analysis of the *B.pumilus* on various metal tolerances

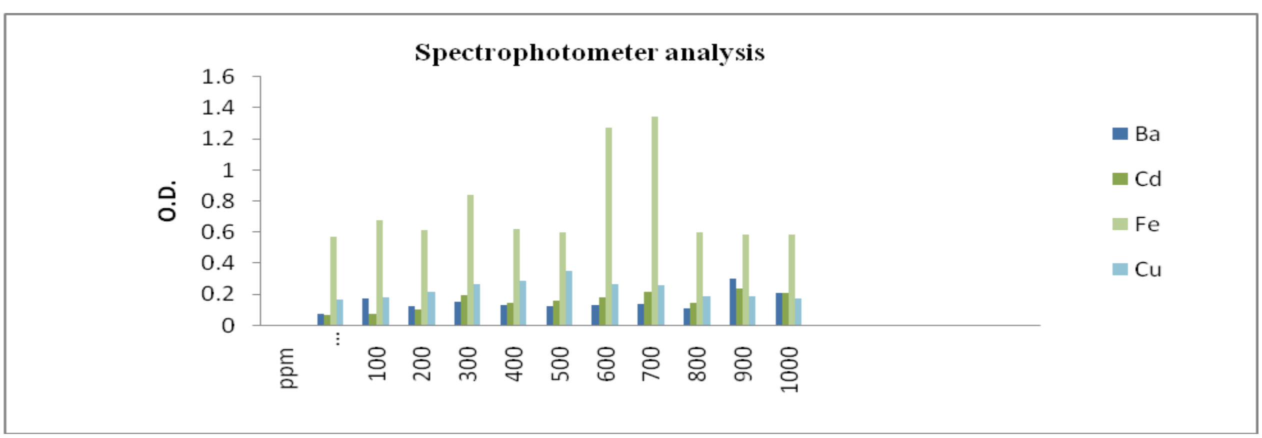
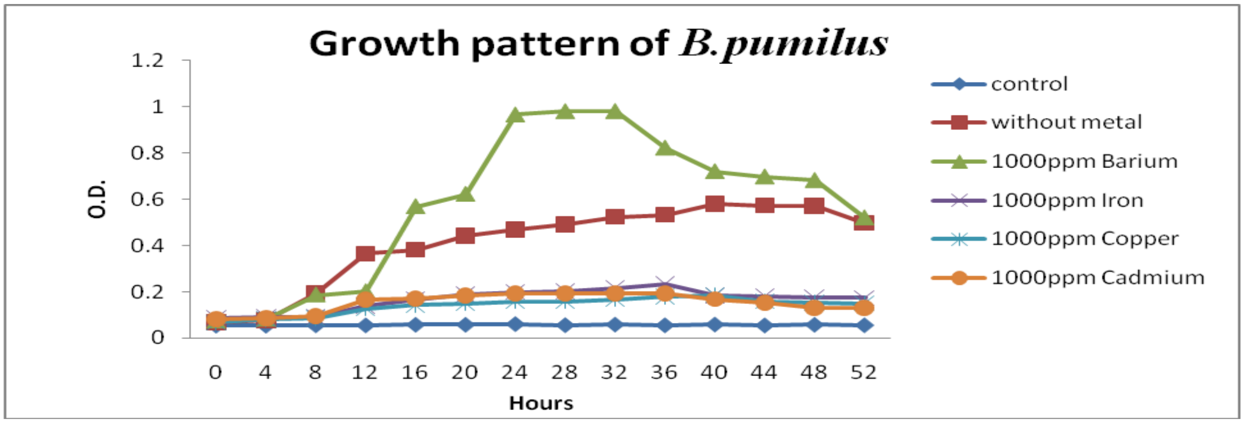


Fig.4 Growth pattern of *B.pumilus*



Effect of pH on the isolate

Fig.5 Growth pattern and pH tolerance of *B.pumilus*

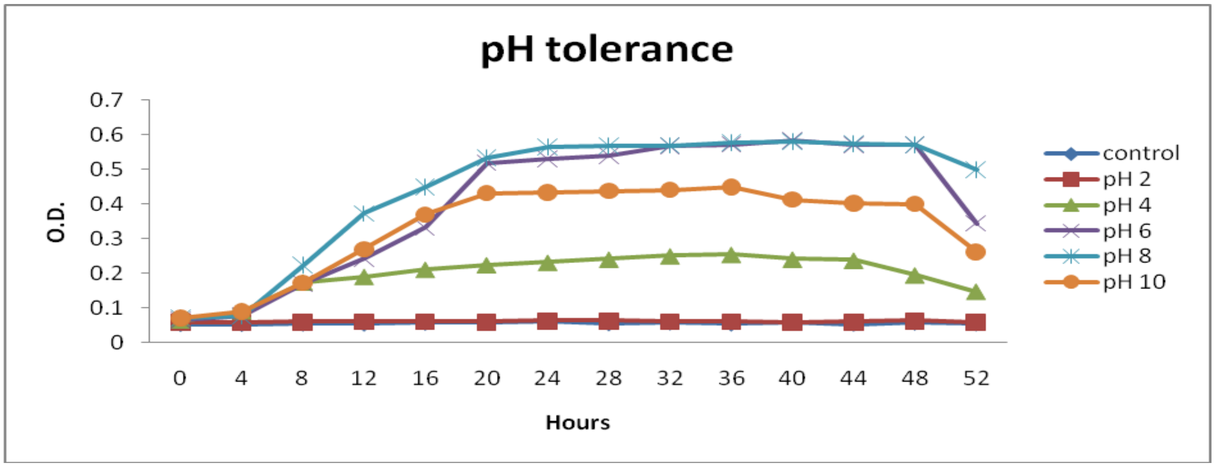
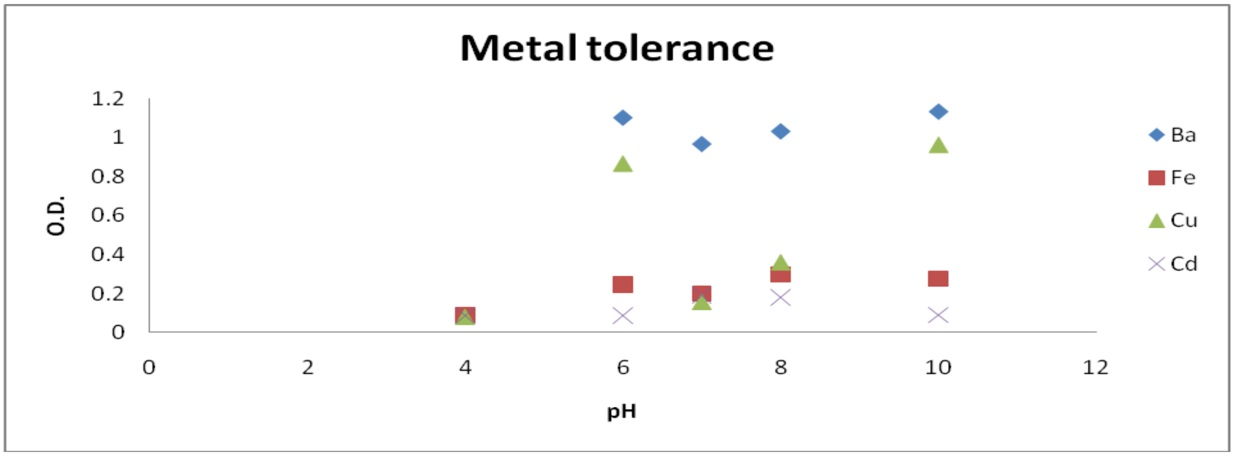


Fig.6 Metal tolerance at different pH and time



Effect of pH on metal tolerance

Bezverbnaya and Odokuma studied resistant to the heavy metals toxicity by *Bacillus sp.* and *Aeromonas sp.* concluding that the persistence of these isolates in the presence of the respective heavy metals may be as a result of the possession of heavy metal resistant plasmids (Bezverbnaya *et al.,* 2005; Odokuma and Oliwe, 2003). Castillo-Zacarías and co-workers who isolated phenol-resistant bacteria in Monterrey, México from industrial polluted effluents found a Cd^{2+} removal rate of 23 to 78% by *E. cloacae*, 23 to 64% by *P. aeruginosa* and 24 to 64% by *K. pneumoniae* (Castillo-Zacarías *et al.,* 2011).

Kermani and co-workers had reported about cadmium resistant *Pseudomonas aeruginosa* which tolerate up to a concentration of 80 mg/L (Kermani *et al.,* 2010). Similar results are obtained on *Vibrio harvei* as studied by (Abd-Elnaby *et al.,* 2011). H. Al Daghistani reported four microbial species *Bacillus sphaericus, Bacillus pumilus, Panibacillus alvae and G. sterothermophilus* which have shown a copper remediation of 87.5%, 81%, 65.4% and 79.6% respectively (Al-Daghistani, 2012). Shetty and co-workers showed a remediation of 40-70% against copper ion by using *Pseudomonas sp*. (Shetty and Rajkumar, 2009; Vullo *et al.,* 2008; Kumaran *et al.,* 2011). Srikumaran *et al.,* reported a reduction of 62.8% of iron by using a *Pseudomonas sp*. isolated from Uppnar estuarine region (Kumaran *et al.,* 2011).

Metal ion binding to the cell surface may be due to covalent bonding, electrostatic interaction, Van-der Waals forces, extracellular precipitation, redox interaction or combination among the processes (Blanco *et al.,* 2000). The negatively charged groups on the bacterial cell wall adsorb metal cations, which retained by mineral nucleation (Wase and Forster, 1997). The contact time between the metal solute and the bacterial cells is an important factor affecting the metal uptake.

In this study, a minimal change in heavy metal remediation in noticed on increasing the contact time from 48 h to 72 h. In the case of Ba, 50% to 52% and Iron from 90.4% to 90.8%, a moderate increase in effect is seen in the case of Cd i.e. from 19% to 22%. Effective metal remediation was found in case of Cu i.e. 71.97% to 88.7%. Our result agrees with the results obtained by El-Shanshoury *et al.,* (2012), who had carried the work with *B.anthracis* (El-Shanshoury *et al.,* 2012).

Surface activity and kinetic energy of the solute became more efficient in sorption activity with the rise in temperature, which promote the active uptake or attachment of the metals to the cell surface, respectively (Sag and Kutsai, 2000). Remediation of metals by *B. pumilus* was found to be decreased with increasing temperature above 40°C, which disagree with the results obtained by Mameri and co-worker (Mameri *et al.,* 1999; Prescott *et al.,* 2002; Uslu and Tanyol, 2006) in our case.

AAS analysis for the sonicated cell for Fe was 136.27 ppm, Cu 114.7 ppm, Ba 52.18 ppm and Cd 35.1 ppm, which clearly confirms the metal absorption capability.

Babich and Jalali found the pH value as one of the main factors in the bioremediation efficiency and binding to microorganisms (Babich and Stotzky, 1985; Lopez *et al.,* 2000). We have set a highest and lowest pH tolerating level by the isolate towards each metal. pH 6 is the lowest range for the isolate to tolerate metals like Copper, Barium, Iron in which the reduction of metal ranges from 88.7%, 54%,91.2% respectively. pH 7 remain the lowest tolerating range for the isolate towards Cadmium in which the reduction was found at 19%.

pH 10 is the highest for all metals i.e. Copper, Iron, Cadmium and Barium in which the metal reduction ranges from 88%,96.2%, 52% and 52% respectively. The absorption of four metal ions by *B.pumilus* at their lowest pH tolerance ability is as follows Fe>Ba>Cu>Cd, with uptake values of 140.23 ppm, 82.31 ppm, 41.79 ppm and 35.1 ppm respectively. Similarly, at the absorption of heavy metals at their highest pH tolerance ability, is as follows Fe>Cd>Ba>Cu, with different uptake values of 196.21 ppm, 60.3 ppm,52.18 ppm and 22.13 ppm respectively. pH variation plays a critical role in the remediation of metals from the respective solutions. An increase in remediation percentage was noticed in all the cases.

The uptake of the metals like Cd and Fe by the isolate has increased when subjected to an alkaline pH, the Barium uptake is higher in acidic pH whereas Cu removal by the isolate has increased with pH alteration but the metal uptake was greatly affected i.e. 114.7 ppm (pH 7) to 22.13 ppm (pH 10).

From the results, it could be concluded that the bacterial flora isolated possessed potential in respect of bio-remediation activity The isolate tolerate for several metals, which could be exploited at mining sites or industrial wastewater contaminated regions where the metals are present either individually or in combination under various physiochemical parameter.

A rise in temperature was ineffective in metal remediation but increasing the contact time has given positive effect in bioremediation of heavy metals. The study suggested that altering the pH plays a major role not only in sorption potential of the bacterium but also bio-absorption capacity has increased towards Fe and Cd.

The multi-metal tolerating *Bacillus sp.* thus appeared to be a suitable candidate in an eco-friendly method for heavy metal ion removal from the environment. So, further exploration of the mangrove regions seems effective in finding multi-metal resistant or tolerating microbial species.

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References

Abd-Elnaby, H., Abou-Elela, G.M., El-Sersy, N.A., 2011. Optimization, economization and characterization of cellulase produced by marine *Streptomyces ruber*. *Afr J Biotechnol.* 10(3): 412-3423.

Abou Zeid A.A., Hassanein A.W., Hedayat, S.M., Fahid, G.A.A., 2009. Biosorption of some heavy metal ions using bacterial species isolated from agriculture waste water drains in *Egypt.J. Appl. Sci. Res*. 5(4): 373-383.

Achenbach L, and Woese C., 1995. 16S and 23S rRNA-like primers. In: Sowers KR, Schreier HJ, editors. Archaea, A Laboratory Manual, Methanogens. New York: Cold Spring Harbor Laboratory Press, Cold Spring Harbor. pp. 521–52.

Acinas, S.G., Marcelino, L.A., Klepac-Ceraj, V., Polz, M.F., 2004. Divergence and redundancy of 16S rRNA sequences in genomes with multiple rrn operons. *J Bacteriol.* 186(9): 2629-35, PMID: 15090503.

Ajaz, H.M., Arasus, R.T., Narayananb, V.K.R., Zahir, H.M.I., 2010. Bioremediation of heavy metal contaminated soil by the *Exigobacterium* and Accumulation of Cd, Ni, Zn and Cu from Soil environment. *Inter.J.Biol.Technol*. 1(2): 94-101.

Al-Daghistani, H., 2012. Bio-Remediation of Cu, Ni and Cr from Rotogravure Wastewater Using Immobilized, Dead, and Live Biomass of Indigenous Thermophilic *Bacillus Sp. The Internet Journal of Microbiology*. 10(1): 1-10.

ASTDR, 2005b. Toxicological profile for Barium (update). Agency for toxic substances and disease registry, Atlanta, Georgia. pp. 1-197.

Atli Arnarson, 2017. The dark side of Iron- why too much is harmful, healthline.

Augusto-Costa, A.C., and Pereira-Duta, F., 2001. Bioaccumulation of copper, zinc, cadmium and lead by *Bacillus sp., Bacillus cereus, Bacillus sphaericus and Bacillus subtilis Braz J Microbiol*. 32: 32-50.

Babich, H., and Stotzky, G., 1985. Heavy metal toxicity to microbe mediated ecological process: A review and application to regulatory policies. *Environ. Res.* 36, 111-137.

Berglund, M., Akesson, A., Nermell, B., 1994. Intestinal absorption of dietary cadmium in women depends on body iron stores and fibre intake. *Environ Health Perspect*. 102, 1058-66.

Bernard, A., 2008. Cadmium and its adverse effects on human health. *Indian J Med Res*. 128(4): 557-64.

Bestetti, B., Reniero, D., Galli, E., 1996. Mercury resistance in aromatic compound degrading *Pseudomonas* strains. *FEMS Microbiol Ecol*. 20: 185-194.

Bezverbnaya, I.P., Buzoleva, L.S., Khristoforvova, S., Russ., 2005. Study on Genomic and Cadmium resistance potential isolate. *J Mar Biol.* 31: p 73-77.

Blanco A, Sanpedro MA, Sanz B, Llama MJ, Serra JL., 2000. Environmental biotechnology and cleaner bioprocesses, Olguin EJ, Sanchez G, Hernandez E, CRC Press, Taylor & Francis group. pp. 135-151.

Cappuccino, J.G., and Natalie, S.A., 2002. Laboratory manual on Microbiology. *Benjamin cummings publisher*. ISBN 0805376488.

Castillo-Zacarías, C.J., Suárez-Herrera, M.A., Garza-González, M.T., Sánchez-González, M.N., López-Chuken U.J., 2011. Cadmium increases catechol 2,3-dioxygenase activity in *Variovorax* sp. 12S, a metal-tolerant and phenol-degrading strain. *Afr J Microbiol Res*. 5, 2627-31.

Choi, J., Lee, J.Y., Yang, J., 1996. Biosorption of heavy metala and uranium by starfish and *Pseudomonas putida*. *J. Hazard. Mater*. 161(1): 157-162.

Das, S., De, M., Ray, R., Chowdhury, C., Jana, T.K., De, T.K. 2012. Microbial Ecosystem in Sunderban Mangrove Forest Sediment, North-East Coast of Bay of Bengal, India. *Geomicrobiology Journal*. 29(7): 656-66.

Doherty, M.J., Healy, M., Richardson S.G., 2006. Total body iron overload in welder’s siderosis. *Occup Environ Med.* 61, 82-85.

Ellis, R.J., Morgan, P., Weightman, A.J., Fry,.J.C., 2003. Cultivation dependent approaches for determining bacterial diversity in heavy metal-contaminated soil. *Appl. Environ Microbiol.* 69, 3223-230.

El-Shanshoury, A.R., Elsik, S.E., Ateya, P.S., Ebeid, E.M., 2012. Synthesis of lead nanoparticles by *Enterobacter sp*. and avirulant *Bacillus anthracis* PS2010. *Ann. Microbiolo*. 62(4): 1803-10.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*. 39(4): p 783-791, doi: 10.1111/j.1558-5646.1985.tb00420.x. PubMed PMID: 28561359.

Hall, T.A., 1999. A user friendly biological sequencing aligment editor and analysis program for windows 95/NT. *Nucleic Acid Symp.Ser*. 41, 95-98.

Haq, R., Zaidi, S.K., Shakoori, A.R., 1999. Cadmium resistant *Enterobacter cloacae* and *Klebsiella sp.* isolated from industrial effluents and their possible role in cadmium detoxification. *World J Microbiol Biotechnol.* 15, 283-290.

Hornung, R.W., Lanphear, B.P., Dietrich, K.N., 2009. Age of greatest susceptibility to childhood lead exposure: a new statistical approach. *Environ. Health Prospect*. 117(8): 1309-12.

Jalali, R., Ghaufourian, H., Sepehr, S., 2002. Removal and recovery of lead using non-living biomass of marine algae. *J. Hazard, Material*. 92(3): 253-62.

Jarup, L., Berglund, M., Elinder, C.G., 1998. Health Risk of Cadmium Exposure-A review of the literature and a risk estimate. *Scand J Work Environ Health*. 24(1): 1-51.

Johnson, C.H., and VanTassell, V.J., 1991. Acute barium poisioning with respiratory failure and rhabdomyolysis. Ann Emerg Med. 20, 1138-42.

Kamza, I., and Gitlin, J.G., 2002. Copper chaperones for cytochromes c oxidase and human disease. J Bioenerg Biomembr. 34, 381-88.

Kermani, A.J.N., Ghasemi, M.F., Khosravan, A., Farahmand, A., Shakibaie, M.R., 2010. Cadmium bioremediation by metal-resistant mutated bacteria isolated from active sludge of industrial effluent. *Iran J Environ Health Sci Eng.* 7, 279-286.

Kozdra, J.J., and Van Elsas, J.D., 2001. Structural diversity of microbial communities in arable soils of a heavy industrialized area determined by PCR-DGGE fingerprinting and FAME profiling. *Appl. Soil Ecol*. 17: 31-42.

Kumaran, A., Sundaramanickam, Bragadeeswaran, S., 2011. Absorption studies on heavy metals by isolated bacterial strain (*Pseudomonas sp.*) from Uppanar estuarine water, Southeast coast of India. *Journal of applied sciences in environmental sanitation.* 6 (4): 471-476

Lenntech, B.V. 2017. WHO/EU drinking water standards comparative table. https://www.lenntech.com/who-eu-water-standards.htm.

Lopez, A., Lazaro, N., Priego, J.M., Marques, A.M., 2000. Effect of pH on the biosorption of nickel and other heavy metals by *Pseudomonas fluorescens* 4F39J. *Ind. Microbiol. Biotechnol*. 24, 146-151.

Mameri, N., Boudries, N., Addour, L., Belhocine, D., Lounici, H., Grib, H., Pauss, A., 1999. Batch zinc biosorption by a bacteria non-living *Streptomyces rimosus* biomass. *Water Res.* 33, 1347-54.

Mevarech, M., Frolow, F., Gloss, L.M., 2000. Halophilic Enzymes: Proteins with a Grain of Salt. *Biophys. Chem*. 86, 155–164.

Noghabi, K.A., Zahiri, H.S., Yoon, S.C., 2007. The production of a cold induced extracellular biopolymer by *Pseudomonas fluoresence* BM07 under various growth conditions and its role in heavy metals absorption Process. *Process Biochem*. 42(5): 847-55.

NTP, 2004. Cadmium and cadmium compounds. National Toxicology Program Report on carcinogens. 11th edition: III: pp. 42-44.

Odokuma L.O., and Oliwe, S.I., 2003. Effect of concentration and contact time on heavy metal uptake by three bacterial isolate. *Glob J Environ Sci*. 2, 72-76.

Oren, A., 2002. Halophilic Microorganisms and Their Environments, *Kluwer Academic Publishers*.

Pardo, R., Herguedas, M., Barrado, E., Vega, M., 2003. Biosorption of cadmium, copper, lead and zinc by inactive biomass *Pseudomonas putida*. *Anal. Bioanal. Chem*. 37(1): 26-32.

Pardo, R., Herguedas, M., Barrado, E., Vega, M., 2003. Biosorption of cadmium, copper, lead and zinc by inactive biomass *Pseudomonas putida*. *Anal. Bioanal. Chem*. 37(1): 26-32.

Peng, Y., Yang, X., and Zhang, Y., 2005. Microbial fibrinolytic enzymes: an overview of source, production, properties, and thrombolytic activity in vivo. *Appl. Microbiol. Biotechnol.* 69: 126–132. doi:10.1007/s00253-005-0159-7. PMID: 16211381.

Pitcher, D.G., Saunders, N.A., Owen, R.J., 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol*. 8(4): 151–156.

Pizzaro, F., Olivares, M., Gidi, V., Araya, M., 1999. The gastrointestinal track and acute effect of copper in drinking water and beverages. *Rev Environ Health*. 14, 231-38.

Prescott, L.M., Harley, J.P., Klein, D.A., 2002. Microbiology, 5th edition. New York, US, McGraw-Hill Higher Education. 95-112.

Rath, C.C., and Subramanyam, V.R., 1996. Thermo tolerant enzyme activities of Bacillus species isolated from the hot springs of Orissa. *Microbioscience.* 86(348): 157-161.

Roberts, M.F. 2005. Organic Compatible Solutes of Halotolerant and Halophilic Microorganisms, *BMC Saline Systems*. 1, 1–5.

Sag, Y., and Kutsai, T., 2000. Determination of biosorption heats of heavy metal ions on *Zoogloes ramigera* and *Rhizopus arrhizus*. *Biochem. Eng. J*. 6: 145-151.

Sahoo, S.R., Maharana, A.K., Rath, S.N., Mohanty. D., Ray P., 2016. Study on Genomics And Cadmium Resistance Potentiality of *Enterobacter* Isolated From Narcotic Containing Agent, *RJPBCS*, 7(1): 603- 10.

Shetty, R., and Rajkumar, S.H., 2009. Biosorption of Cu (II) by Metal Resistant *Pseudomonas sp.* Int. *J. Environ Res*. 3(1): 121-128.

Silva, R.M.P., Rodríguez, A.A., Montes De Oca, J.M.G., Moreno, D.C., 2009. Biosorption of chromium, copper, manganese and zinc by *Pseudomonas aeruginosa* AT18 isolated from a site contaminated with petroleum. *Bioresource Technology*. 100(4): 1533-38.

Sobhy. E., Elsilk, Abd., EL-Raheem, R., El-Shanshourny, Perihan S., Ateya., 2014. Accumulation of some heavy metals by metal resistant avirulent *Bacillus anthracis* PS2010 isolated from Egypt. *AJMR*. 8(12): 1266-76.

Strandberg, G.W., Shumate, S.E., Parrott, J.R., 1981. Microbial cells as biosorbents for heavy metals: accumulation of uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*. *Applied and Environmental Microbiology*. 41 (1): 237-245.

Suganthi, C., A. Mageswari, S. Karthikeyan, M. Anbalagan, A. Sivakumar, K.M. Gothandam., 2013. Screening and optimization of protease production from a halotolerant *Bacillus licheniformis* isolated from saltern sediment. Journal of Genetic Engineering and Biotechnology. 11(1), 47-52.

Takeuchi, T., 1977. Neuropathology of minamata disease in Kumamoto, especially at chromic stage, in Roisin L., ShiakiH., Greevic, N. (eds). *Neurotoxicology*. New York, Raven Press. pp. 35-46.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods, *Mol Biol Evol*. (Oct), *Epub*, (May 4), 28(10): 2731-9, doi: 10.1093/molbev/msr121.

Tehei, M., Franzetti, B., Maurel, M.C., Vergne, J., Hountondji, C., Zaccai, G., 2002. The Search for Traces of Life: the Protective Effect of Salt on Biological Macromolecules. *Extremophiles*. 6, 427–430.

Umrania, V, V. 2006. Bioremediation of toxic heavy metals using acido-thermophilic autotrophes. *Bioresource Technology*. 97: 1237- 42.

Umrania, V.V., 2003. Bioremediation on toxic heavy metals using acidothermophilic autotrophies. *Indian J Biotechnol*. 2: p 451-64.

Uslu, G., and Tanyol, M., 2006. Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (ll) ions onto

Pseudomonas putida, effect of temperature. *J Hazard Material*. 135, 87-93.

Valls, M., and DeLorenzo, V., 2002. Exploting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS Microbiol*. 26, 327.

Ventosa, A., Nieto, J.J, Oren, 1998. A. biology of moderately halophilic aerobic bacteria. *Microbiol. Mol. Biol. Rev*. 62, 504–544.

Volesky, B., May-Phillips, H.A., 1995. Biosorption of heavy metals by *Saccharomyces cerevisiae*. *J. Appl. Microbiol, Biotechnol.* 42, 797-806.

Vullo, D.L., Ceretti, H.M., Daniel, M.A., Silvana, A.M., Ramı´rez, Zalts, A., 2008. Cadmium, zinc and copper biosorption mediated by *Pseudomonas veronii* 2E. *Bioresource Technology*. 99, 5574–558.

Wase J, and Forster CF., 1997. Bio-sorbents for metal ions. London, Taylor & Francis, p 238.

Yan, G., and Viraraghavan. T. 2003. Heavy metal removal from aqueous solution by fungus *Mucor rouxii*. *Water Res*. 37, 4486–96.

Zhou J., Bruns M.A., Tiedje J.M., 1996. DNA recovery from soils of diverse composition. *Appl Environ Microbiol.* 62 (2): 316-322

Zolgharnein, H., Azmi, M.L.M., Saad, M.Z., Mutalib, A.R., Mohamed, C.A.R., 2007. Detection of plasmids in heavy metal resistance bacteria isolated from the Persian Gulf and enclosed industrial areas, Iran. *J. Biotechnol*. 5: 232-39.

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