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Study on Role of *Pseudomonas aeruginosa* on Heavy Metal Bioremediation

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ABSTRACT

Targetted removal of environmental toxins by use of biological processes is termed as bioremediation. It could either be intrinsic or engineered depending on the requirement of the conditions. Steep industrialization has led to a large amount of effluents being let off into the environment. A major part of these effluents contain heavy metals. This research was aimed at quantifying the bioremidiative capabilities of Pseudomonas aeruginosa. The experiment was carried out as a two phase study; the first phase consisted of quantifying the bioremediation for Cu, Cr, Fe and Zn at three different concentrations such as 5%, 10% and 15%. Maximized results were obtained with 15% metal concentration of Copper and Zinc which showed reduction of free ion concentration approx. 79.1% at 48hrs, 52.4% at 72 hr of incubation respectively. Chromium biodegradation was observed 41.6% at 72 hr of incubation with 5% metal concentration and as the metal concentration was increasing the reduction of Cr ion was decreasing. Reduction of free ion concentration of iron was observed 61.0% in 10% solution only after 24 hrs of incubation. Phase two experiment was carried out on actual on site conditions with effluents from leather, paper and steel industries to validate the research. The organism showed maximum bioremediation of 44.6% for Cr in the effluents from the leather industry and 18.0% for Cr in effluent of pulp and paper industry where as the Cr concentration was very low in effluent of steel industry still bioremediation was taking place. In effluent of steel industry the maximum bioremediation of iron was 37.3%, followed by 11.1% in pulp and paper industry, where as iron bioremediation was very less in leather industry almost 3.5%. The Cu concentration in effluent of all the three industries was almost negligible, although Pseudomonas aeruginosa was remediating metal even at very low concentration. The results obtained from present investigation indicate that Pseudomonas aeruginosa could be an effective measure for remediation of heavy metals.

Keywords: *Pseudomonas aeruginosa, Bioremediation, Heavy metals, Resistance, Environment, Effluent*

INTRODUCTION

Problems related to heavy metal pollution are on the rise, especially due to the number of industries that are coming up and the amount of discharge that they are releasing into the water bodies. Not only does this pollute the environment, but it also makes waste water treatment costly. One of the major components of industrial discharges that go untreated into the environment, are the heavy metals. Presence of most heavy metals over a certain threshold has proven to be extremely deleterious for the soil and the biota it supports.

Several strategies are being devised for effective removal of such hazardous components, but there is still room for more developed strategies.

One such approach being extensively exploited is that of Bioremediation, wherein suitable microorganisms with tolerance towards toxic levels of heavy metals are used to take-up metals through the acquisition of specific resistance systems such as efflux and uptake mechanisms, extracellular precipitation pumps[1,2,3] and many more targeted species include *Bacillus licheniformi*, *Brevibacillus laterosporus* and almost 45 strains *of Pseudomonas*[4] Some build up tolerance naturally; others are genetically modified to give high remediation results. Since there is very little input and no harmful by product, this technique has an upper hand over the physio-chemical techniques.

Two classes of bioremediation technologies have been developed. One is the intrinsic, which makes use of microorganisms occurring naturally to degrade contaminants and do not need engineered interventions at the site. Intrinsic remediation depends on the activity of indigenous microorganisms. The second approach involves engineered intervention, usually to improve the rate of bioremediation by introducing manipulated and modified processes such as adding microorganisms and supplying nutrients. The basic idea behind engineered remediation is to alter environmental conditions for enhancing microorganisms activity. Therefore, conducting degradation of the contaminants in engineered processes occurs at lower risks and is cost effective.

Bacterial bioremediation has been an approach which has been extensively applied these days as an alternative to other conventional methods being used for heavy metal remediation. The complexity has always been in quantifying the level of removal achieved through these approaches, as that is an driving factor for cost incurred. The conventional method for quantification involves atomic spectrophotometer to determine the biodegradation by any agent. This procedure is time taxing and expensive and it also requires advanced equipment. Our approach with colorimetric analysis thought being less specific than other approaches is relatively cheaper and requires minimal instrumentation

In this study, we have focused on the role of *Pseudomonas aeruginosa* in heavy metal removal from the surrounding medium. *Pseudomonas aeruginosa* is member of the Gamma Proteobacteria class of Bacteria. It is a Gram negative, aerobic rod belonging to the bacterial family *Pseudomonadaceae*.

Pseudomonas is characterized as a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm[5] Almost all strains are motile by means of a single polar flagellum. The bacterium thrives in soil and water, and on surfaces that are in contact with soil or water. Its metabolism is respiratory and never fermentative, but it will grow in the absence of O_2, if NO_3 is available as a respiratory electron acceptor. It has minimal nutritional needs. *P. aeruginosa* possesses the metabolic versatility for which they are employed to take up a variety of substances, ranging from phenol contaminants to oil spill remediation. It is also known to show high resistance towards salinity, antibiotic, chromate and salinity resistance when tested in tannery[6].

Hussein *et al*[7] isolated *P. aeruginosa* from industrial discharge in his attempt to isolate metal resistant strains that were tolerant against Chromium, Copper, Nickel and Cadmium contamination. Iron limiting Casamino acid media was used for selective isolation since it can induce the fluorescent siderophores of the *pseudomonas* species. Eighteen colonies were selected and purified. The isolates were screened for their metal resistance on Casamino acid media containing different metal concentration of each Cr, Cu, Cd and Ni which ranged between 1 to10 mM/lit. The bacterial isolates were capable to grow and resist high level of metal toxicity.

Wastes from mining contain high fractions of heavy metal ions that leach the land of its fertility[8,9]. A study conducted by Nagashetti *et al*[10] identified high levels of zinc, cesium, lead, arsenate and mercury resistance in eight copper resistant *Pseudomonas* strains. The bacterial strain was tested for metal tolerance with wide range of hexavalent chromium, copper and zinc concentrations (250 ppm, 500 ppm and 1000 ppm). The results indicated that after 7 days incubation, the bacteria survived upto 1000 ppm hexavalent chromium, copper and zinc concentration. The experiment showed percent removal of hexavalent chromium, copper and zinc after treatment with all microorganisms. Chromium absorbed at 250 ppm was 0.31 mg, similarly at 500 ppm it was 0.42 mg while for 1000 ppm it was a good 1.07 mg. The titration based analysis for the zinc and copper absorbed by the *Pseudomonas aeruginosa* are as follows: 0.25 mg & 0.28 mg at 250 ppm, 0.41mg & 0.51 mg at 500 mg respectively.

MATERIALS AND METHODS

Isolation and characterisation of *Pseudomonas aeruginosa*

Soil sample was taken from Mango orchid, Amity University, Lucknow, weighed and diluted in 100ml of distilled water. A homogenous filtrate was obtained that was serially diluted and poured over sterile King's B Media plate[11] Greenish yellow fluorescent colonies of *P. aeruginosa* were observed that were characterised and subcultured.

Metal uptake by bacteria at varying Metal concentrations

0.01M metal solution of Zinc, Ferrous, Cupric and Chromium salt solution was prepared.

Each of these metals solutions were added to Luria Broth media[12] in 5%, 10% and 15% concentrations to observe organism growth and uptake.

After an interval of 24 hrs, the broth was centrifuged, the biomass was weighed and the supernatant was treated with ammonia to obtain a specific colour. The absorbance of treated supernatant was recorded at 480 nm wavelength in the UV spectrophotometer.

Determination of Metal Uptake in Industrial Effluent by *P. aeruginosa*

Effluents from the following three industries were taken to validate the study: paper mill, steel mill and leather industry.

The effluents were filtered and initial concentration of metals were analysed under an atomic spectrophotometer. These were inoculated with *Pseudomonas aeruginosa* and its growth was observed. After 78 hrs, the broth was centrifuged, the biomass was settled down, weighed and the supernatant was subjected to atomic absorption spectrophotometry (at IITR, Lucknow) to determine the uptake of metal ions.

RESULTS

The results of the present investigation showed consistent data in favour of aims and objectives. These results confirmed that varying levels of ion concentration affect the level of uptake of free heavy metal ions by bacterial species *Pseudomonas aeruginosa.*

Metals uptake at varying concentration by *Pseudomonas aeruginosa*

The uptake profile of *Pseudomonas aeruginosa* with respect to time of incubation in presence of heavy metal was observed. At 24 hr *Pseudomonas aeruginosa* showed negligible growth in presence of Chromium, Copper, Iron and Zinc representing lag phase, from 24 hr till 72 hr it showed exponential growth phase, whereas after 72 hr decrease in bacterial population was observed.

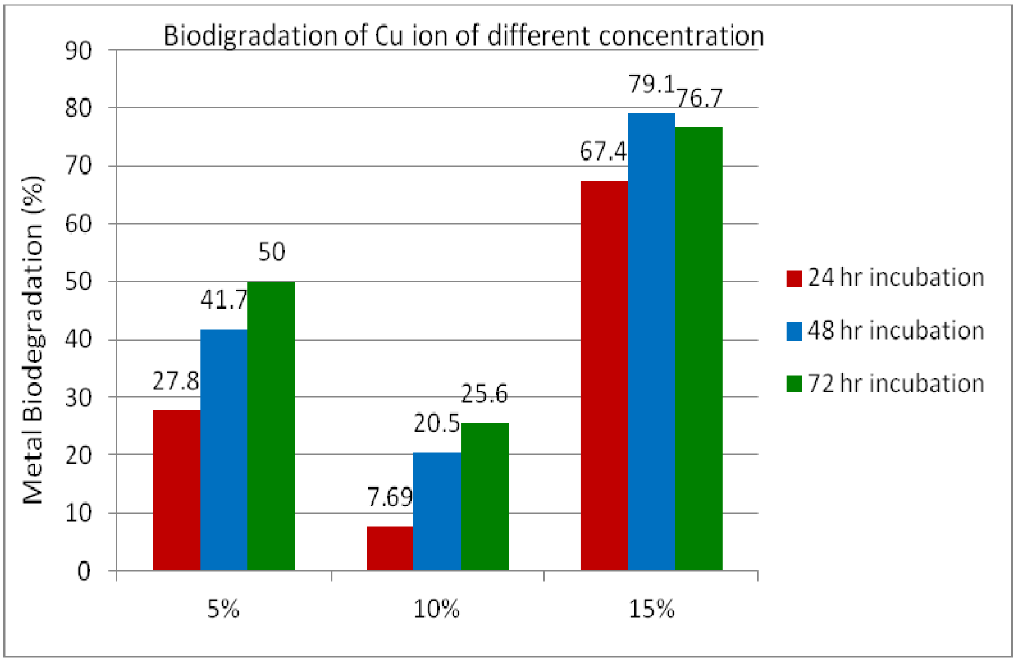
Effect of Copper concentration:

In case of 5%, and 10% Copper exposures, a gradual decrease in O.D confirms the reduction in free Cu in the broth. Maximum uptake of Cu was observed at its 15% concentration which was calculated to be 79.1% after 48 hr of incubation and after 48 hr incubation the growth of microbe was almost constant and micro organism started releasing the absorbed metal back in broth after 48 hr.

Table 1: Comparative absorption of Copper at different concentrations

Cu Concentration	Incubation time (hr)	Readings	
		O.D. (at 480nm)	Weight of Biomass (gm)
5%	Control	0.036	
	24hr	0.026	6.815
	48hr	0.021	6.837
	72hr	0.018	6.841
10%	Control	0.039	
	24hr	0.036	6.835
	48hr	0.031	6.843
	72hr	0.029	6.845
15%	Control	0.043	
	24hr	0.014	6.793
	48hr	0.009	6.836
	72hr	0.01	6.838

Fig. 1: Metal Biodegradation (%) at its different % concentration of Cu ion by *P. aeruginosa*



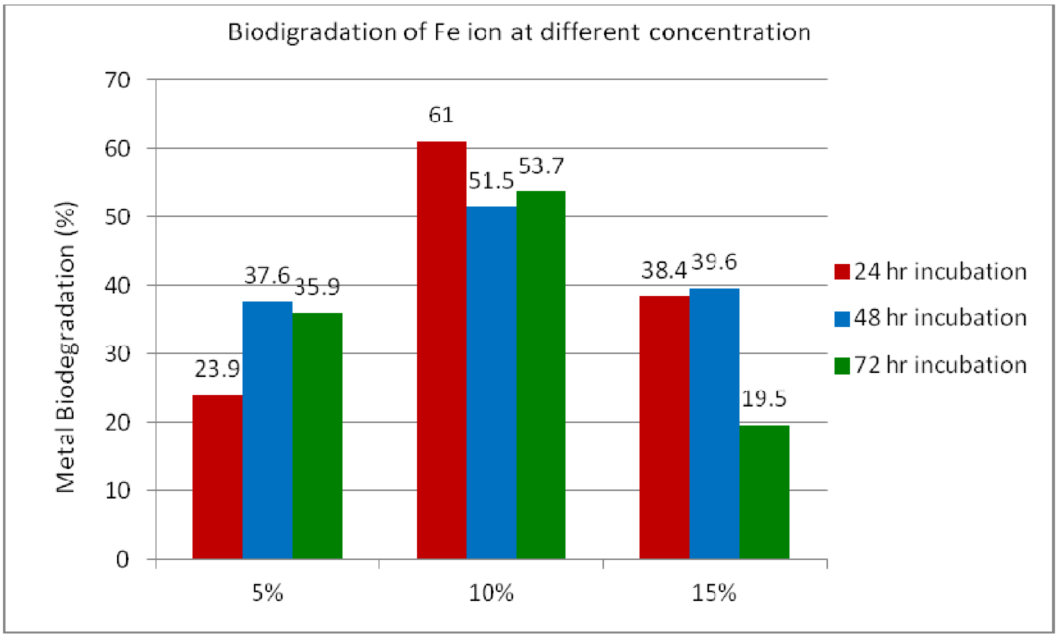
Effect of Iron concentration:

The *Pseudomonas aeruginosa* showed maximum iron uptake at 10% concentration, which was calculated to be 61.0% only after 24 hr of incubation. The decrease in OD at 5% and 15% Fe concentration also showed some uptake, although it was comparatively less than the uptake at 10%.

Table 2 Comparative absorption of Iron at different concentrations

Fe Concentration	Incubation time (hr)	Readings	
		O.D. (at 480nm)	Weight of Biomass(gm)
5%	Control	0.117	
	24hr	0.089	6.645
	48hr	0.073	6.841
	72hr	0.075	6.845
10%	Control	0.136	
	24hr	0.053	6.922
	48hr	0.066	6.922
	72hr	0.063	6.923
15%	Control	0.164	
	24hr	0.101	6.315
	48hr	0.099	6.743
	72hr	0.132	6.715

Fig. 2: Metal Biodegradation (%) at its different % concentration of Fe ion by *P. aeruginosa*



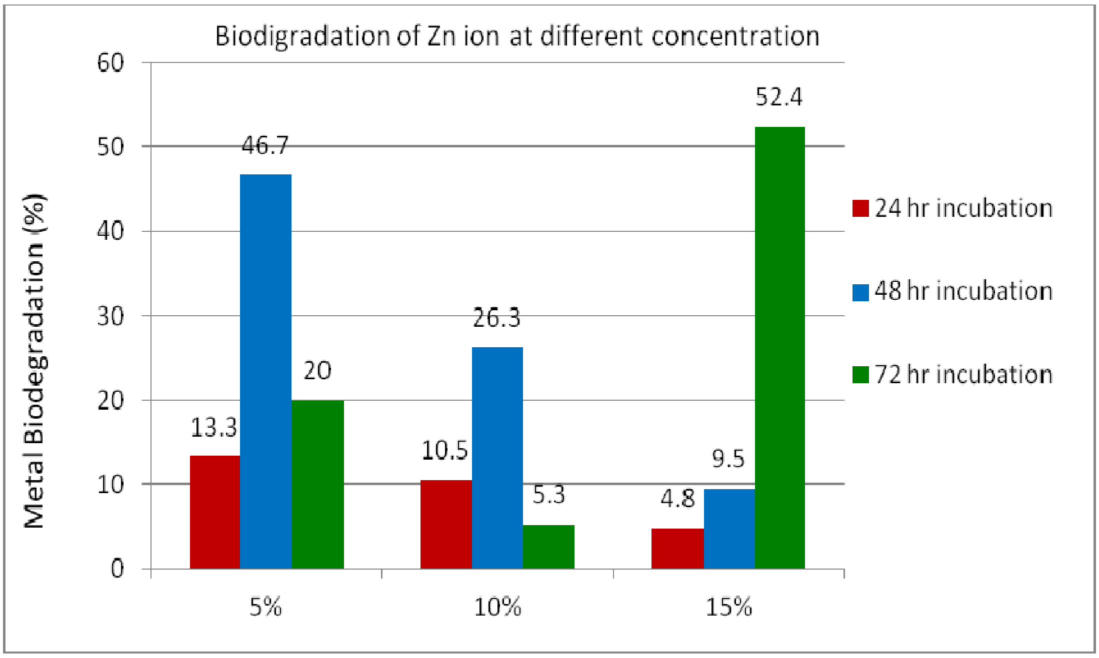
Effect of Zinc concentration:

The micro-organism showed the zinc uptake almost at all the concentrations, the highest uptake of Zn metal was observed at 15%, which was calculated to be 52.4% after 72 hr incubation.

Table 3: Comparative absorption of Zinc at different concentrations

Zn Concentration	Incubation time (hr)	Readings	
		O.D. (at 480nm)	Weight of Biomass(gm)
5%	Control	0.015	
	24hr	0.013	6.135
	48hr	0.008	6.837
	72hr	0.012	6.831
10%	Control	0.019	
	24hr	0.017	6.237
	48hr	0.014	6.743
	72hr	0.018	6.735
15%	Control	0.021	
	24hr	0.02	6.739
	48hr	0.019	6.816
	72hr	0.01	6.858

Fig. 3: Metal Biodegradation (%) at its different % concentration of Zn ion by *P. aeruginosa*



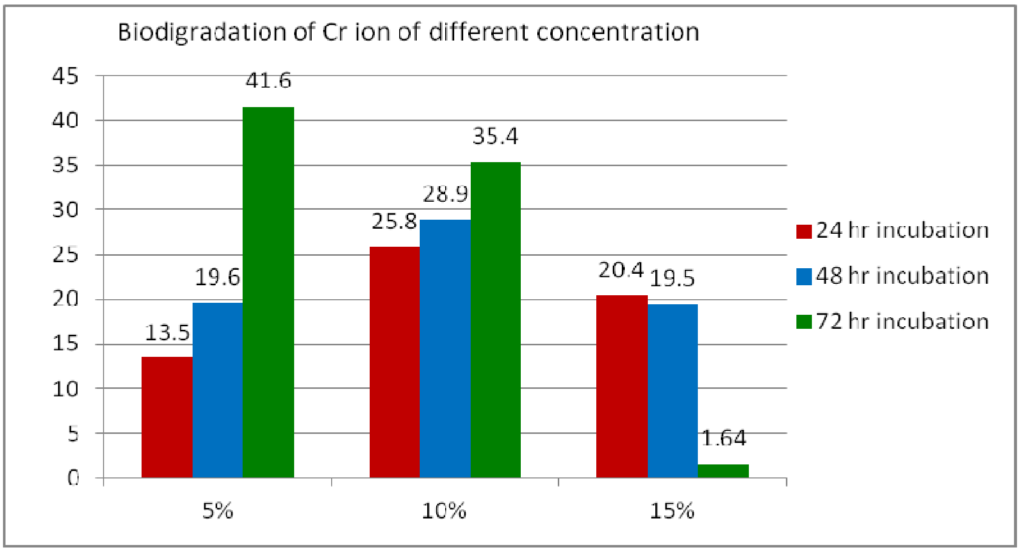
Effect of Chromium Concentration:

The best uptake of chromium was observed in 5% solution which was estimated approx 41.6%, after 72 hr incubation, although the chromium uptake was decreasing as the chromium ion concentration was increased. *P. aeruginosa* was showing biodegradation of Cr metal best at low concentration.

Table 4: Comparative absorption of Chromium at different concentrations

Cr Concentration	Incubation time (hr)	Readings	
		O.D. (at 480nm)	Weight of Biomass (gm)
5%	Control	0.245	
	24hr	0.212	6.832
	48hr	0.197	6.852
	72hr	0.143	6.855
10%	Control	0.271	
	24hr	0.201	6.835
	48hr	0.223	6.911
	72hr	0.175	6.923
15%	Control	0.367	
	24hr	0.292	6.834
	48hr	0.332	6.836
	72hr	0.361	6.837

Fig. 4: Metal Biodegradation (%) at its different concentration of Cr ion by *P. aeruginosa*



Metals uptake of industrial effluents by *Pseudomonas aeruginosa*

In this present study metal absorption of Cu, Cr and Fe ions by bacteria strain was observed on the effluents of three different industries. The atomic spectrometry results confirm the reduction in these ions in the effluent.

The results indicate that *Pseudomonas aeruginosa* was most effective in Chromium (44.6%) bioremediation in the effluent of leather industry, while maximum iron bioremediation was 37.3% in the effluent of steel industry. In the effluent of pulp and paper industry the bioremediation of chromium was 18.0% and iron was 11.1%; whereas bioremediation of chromium was very less even its original concentration was very low in effluent of steel industry. The iron bioremediation was 3.5% in the effluent of leather industry. The Cu bioremediation in the effluent steel industry was observed to be 5.4% which was very less. The Cu concentration was almost negligible in rest of the two industries (leather and paper pulp), although *Pseudomonas aeruginosa* was degrading metal even at very low concentration. The metal uptake results are indicated in the Table 5.

Table 5: Metals degradation of different industrial effluents by *Pseudomonas aeruginosa*

Metals →	Cr			Cu			Fe		
Sample source (Industry) ↓	Crude (mg/l)	Treated (mg/l)	% degradation	Crude (mg/l)	Treated (mg/l)	% degradation	Crude (mg/l)	Treated (mg/l)	% degradation
Leather	0.215	0.119	44.6	-0.002	-0.002	-	0.114	0.110	3.5
Pulp &Paper	0.163	0.134	18.0	0.002	-0.006	-	0.002	0.002	11.1
Steel	0.001	0.001	0	0.050	0.045	9.2	0.243	0.152	37.2

DISCUSSION

On increasing Copper toxicity, 79.1% of metal uptake was observed at 15% metal concentration, which is the highest uptake result across all the four metals tested on a varying concentration scale from 5% to 15%.

Since Iron and Zinc ions are required as the essential micro nutrients for the survival of cell, they are efficiently absorbed by the cell.

Iron ions were showing absorption almost at all concentration of metal from 5% to 15% and maximum metal absorption was 61% in 10% Fe solution. The absorption of Zn ion was maximum 52.4% in 15% Zn solution after 72 hr incubation, whereas Zn ion was almost well absorbed in 5% and 10% solution also even within 48hr incubation. Copper was best absorbed by *P. aeruginosa* at high concentration where as Chromium was absorbed only till a certain toxicity limit, after which the uptake decreases.

These results are in concurrence with the results of similar studies carried out by Nagashetti *et al*[10], Kolembkiewicz *et al*[8] and Kabata-Pendias *et al*[9] that proved Cr to be the heavy metal showing maximum affinity towards bioremediation by *P. aeruginosa*.

Hussein *et al*[7] observed maximum metal uptake for Cu and then Cr in laboratory media. Similar results were observed in the present study also, where as in industrial effluents of tannery the chromium uptake was found to be maximum 44.6% where as copper uptake was found 9.2% in the effluent of steel industry.

The copper concentration in the effluent of tannery, paper and steel industry was present in very low concentration but was showing some uptake of Cu metal (9.2%) in the effluent of steel industry although the concentration was only 0.05 mg/l in crude effluent. The metal uptake of Fe was observed in all the industrial effluent and the maximum was observed 37.2% in the effluent of steel industry.

CONCLUSION

During the (first phase) first set of experiments that consisted of quantifying the bioremediation for Cu, Cr, Fe and Zn, maximised results were obtained with 15% solution of Copper which showed a 79.2% reduction in free ion concentration and Chromium showed 41.6% reduction with 5% ion concentration. (Phase two) The second set of experiments replicated actual on site conditions with effluents from leather, paper and steel industries to validate the research. The organism showed maximum bioremediation of 44.6% Cr for effluents from the leather industry as the Cr metal concentration in effluent was low 2.15% w/v. Although the copper concentration was very low (0.5%w/v) in steel industry still was showing some reduction (9.2%).

From the present investigation it could be concluded that the uptake profile of the heavy metal tolerant (Cu, Zn, Fe and Cr) *P. aeruginosa* had an extended effect on the availability of free ions in the test solution over a period ranging from 0-72hrs. The isolate is a potent heavy metal tolerant strain with capability to grow in high concentrations of Zn, Cu, Fe and Cr.

Therefore strain has a great capability to show tolerance towards heavy metals. The result obtained had indicated for potential heavy metal remediation and reconfirmed the capacity of the isolate to be a suitable candidate to be used for heavy metal pollutants bioremediation. The study must be explored further to know the genetic and molecular behaviour of *Pseudomonas aeruginosa* towards heavy metal uptake, utilization and transformation. The isolate can be further utilized and tested in-vitro and in-vivo as a potent candidate for metal removed from contaminated sites like industrial effluents.

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