DOI:10.26524/krj.2022.6

Kongunadu Research Journal 9 (1): 38-46, 2022
Publisher: Kongunadu Arts and Science College, Coimbatore.
ISSN 2349-2694,
https://krjournal.com/index.php/krj/article/view/535

RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION AND OPTIMIZATION OF *STREPTOMYCES ALBOGRISEOLOUS* NGP2 AND ITS PROSPECTS ON HEAVY METALS DEGRADATION

Vishnupriya, B.*, Anbarasi, G. and Manjudevi, M.
PG and Research Department of Biotechnology, Kongunadu Arts and Science College (Autonomous), Coimbatore – 641029, Tamil Nadu, India

ABSTRACT

The biosorbing ability of actinomycetes from the marine sediments of South Indian Coastal region, Chennai, Tamilnadu, India was examined and identified as *Streptomyces albogriseolous* NGP 2 (JX843531) by 16S rRNA gene sequence analysis. The isolate showed the potential metal removing capacity by incubating it with various concentrations of heavy metals such as Copper (Cu), Zinc (Zn), Chromium (Cr) and Cadmium (Cd). Simultaneously, various optimization properties such as pH, initial metal concentration and contact time were analyzed. The pH 7.0 was recorded as an optimum for the removal of Cr and Zn, for Cd and Cu it was recorded as 6.0. The biosorption of the heavy metals Cu and Zn by the strain was found to be highest and recorded as 92% and 90.6% respectively, while the lowest sorption was found for Cd (85 %) at 30 mg/l concentration. The effectiveness of the removal of heavy metals was best studied by Langmuir and Freundlich adsorption isotherm models. The regression Coefficients also significantly expressed in the removal process. The results revealed that the isolate has the potential capability for the removal of heavy metals.

Keywords: Actinomycetes, Biosorption, Langmuir and Freundlich, Optimization properties

1. INTRODUCTION

The waste water from the mines and metal refineries contains hazardous heavy metal ions which cause serious illness to the human as well as environment's health and wealth[7]. Based on the statistical report, India generates 62 million tonnes of waste per year and emits greenhouse gases at maximum limit[23]. Four major industries such as fertilizers, tannery, pesticide and chemical industries are responsible for causing more pollution. The discharged effluents after treatment also contains highest amount of heavy metals[22]. The heavy metals are arsenic, cadmium, chromium, copper, lead, nickel and zinc which interfere with our metabolic process and accumulate in body[14]. A number of conventional waste water treatment techniques were employed for the removal of pollution such as chemical coagulation, adsorption and activated sludge[6]. With this backdrop, the effective alternate method of removal mechanism achieved by microorganism as biosorbent[15]. Biosorption was done with various microorganisms such as bacteria, fungi, bio film, algae, genetically engineered microbe and immobilized microbial cell. This process highly depends on the optical structure of the cell wall which found as primary mechanism[25]. Among them actinomycetes specifically *Streptomyces* are of much more interest because of their ability to survive in heavy metal contaminated sites through the production of metal ion chelators such as Siderophores[18] also they have special feature of stability.

For the better understanding of absorption mechanism, Langmuir and Freundlich equations widely used. The Langmuir model suspects that without any interaction between adsorbed ions on a homogenous surface leads to the adsorption of metal ions. The adsorption proceeds until a complete monolayer formed[20]. The Freundlich model fully based on adsorption on a heterogeneous

*Correspondence: Vishnupriya, B., PG and Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore - 641029, Tamil Nadu, India. E.mail: bvishnupriya_bt@kongunaducollege.ac.in

surface[9]. It has been broadly perceived and affirmed that its intra particle mass exchange rate which speaks to the bottleneck and in controlling the rate of whole sorption process. Finding of molecule size and its structures are hence extremely critical. Models have been produced to decide both the quantity of adsorption destinations required to tie each metal particle and the rate of adsorption, utilizing a batch reactor mass balance and the Langmuir hypothesis of adsorption to surfaces or continuous dynamic systems[18].

The aim of the present study was to investigate the biosorptive potential of *S. albogriseolous* NGP 2 and effects on various factors such as metal concentrations, contact time and pH through Langmuir equation.

2. MATERIALS AND METHODS

2.1. Collection and isolation of actinomycetes

The marine sediment was collected from the coastal region of marine, Chennai, Tamilnadu, India located at 13.05^0 N 80.28^0 E and 2-3 depth by using grab sampler. The collected sediment was serially diluted and spread over starch casein Agar (SCA) plates and incubated at 28 + 2^0 C for 7 days[3]. After incubation six actinomycetes were isolated and tested for heavy metal tolerance.

2.2. Selection of heavy metal tolerant strains

To determine metal tolerance actinomycete strains were aseptically streaked on SCA plates supplemented with 100-2000 µg/ml of copper sulphate ($CuSO_4.5H_2O$), zinc sulphate ($ZnSO_4$), potassium dichromate (K_2 Cr_2 O_7), cadmium sulphate ($CdSO_4$) and checked for growth after incubation at 28±2^{0}C for 72h with shaker (160r/min). After incubation the growth was detected by measuring the optical density at 600nm. The maximum tolerance level (MTL) was described as the highest concentration of individual metal supporting growth of actinomycetes.

2.3. Biochemical characterization of actinomycetes

Among six actinomycetes, strain NGP 2 showing the highest MTL values were selected and characterized morphologically and biochemically. Properties of the strain NGP 2 that included gram's reaction, methyl red test, voges – proskauer test, caseinase test, cellulase test, deaminase test, sugar fermentation, nitrate reduction test, gelatinase test were determined by the standard method given in Bergey's manual of determinative bacteriology[12].

2.4. 16 s rRNA identification

The partial sequencing of 16s rRNA gene chain of the strain NGP 2 was carried out by automated DNA sequence model ABI 3100 according to the protocol provided by the manufactures (ABI PRISM 3100 Genetic analyzer user's manual) using universal primers, oligo 1F (5'-GAG TTTGATCCTGGCTCAG - 3'). Later, nucleotide sequence data was deposited in the Gen-Bank. The online program BLASTn was used to find out the related sequences with known taxonomic information in the databank at NCBI website (http//www.ncbi.nlm.nih.gov/BLAST) to accurately identify the strain NGP 2[24].

2.5. Biomass production

Biomass of *S. albogriseolous* NGP 2 was produced by growing actinomycete culture in starch casein broth (pH 7.0) at 28± 2^0 C for 72 h. Cells were harvested by centrifugation at 6000 rpm for 20 min. Dry biomass prepared by vacuum oven drying at 90^0 C and then used for biosorption studies.

2.6. Metal solutions

A stock solution of $CuSO_4.5H_2O$, $ZnSO_4$, $K_2Cr_2O_7$, and $CdSO_4$ was prepared in fixed volume (100 mg/ml) of single metal ion solution in 100 ml conical flask by dissolving appropriate quantities of pure metal powders in 1% nitric acid.

2.7. Metal sorption

A batch equilibrium method was used to determine the sorption of copper, zinc, chromium and cadmium by *S. albogriseolous* NGP 2[13]. The strain was exposed to metal solution for 72h on an orbital shaker 160 rpm and centrifugation at 6000rpm for 15 min and the supernatant was analysed for residual metal concentration by flame atomic absorption spectrophotometer. Measurement of metal uptake, the amount of metal bound by the biosorbent was calculated by $Q = V(Ci - Cf)/M$. Where, Q = Metal ion uptake capacity (mg/g), V = Solution volume (ml), Ci = Initial concentration of the metal in solution (mg/g), Cf = Final concentration of the metal in solution (mg/g), M = Dry weight of biosorbent (mg). Sorption models were chosen for comparison with experimental data.

The Langmuir model, $Q = Qax\,bCf / 1 + bCf$, in which Qmax = the maximum metal uptake under the given conditions, b = a constant related to the affinity between the biosorbent and sorbate.

The Freundlich model, $Q = kCf\,(1/n)$. K and n are Freundlich constants, which correlated to the maximum absorption capacity and adsorption intensity respectively.

2.8. Initial metal concentration

To examine the effect of initial metal concentration, the experiments were performed at different initial metal concentrations such as 30, 60, 90, 120 and 150 mg/l by using 100mg dried biomass of *S. albogriseolous* NGP2 incubated for 60 min on orbital shaking incubator at 160r/min [10].

2.9. Contact time on biosorption

To determine the equilibrium time required for biosorption was performed using 100 mg cell biomass from the initial metal concentration of each metal ion in 100 ml of metal solution. The metal solutions were taken at the desired intervals (from 0 to 60 min) and subsequently centrifuged at 6000 rpm for 10 min. The heavy metal concentration in the supernatant was analysed by flame atomic absorption spectroscopy[18]

2.10. pH on biosorption

In order to evaluate the impact of pH on biosorption, NGP2 strain was subjected to different pH (varying between 2 and 10) then allowed to contact with metal solutions of the corresponding pH 2,4,6,8 and 10. For contact time analysis, 10 ml of cell suspension (10 mg of dry cell biomass) was mixed with 100 ml aliquots of metal solutions in a 250 ml Erlenmerfer flask with appropriate controls. Flasks were incubated on an orbital shaker incubator (120 rpm) at 32±2°C. Samples of metal solutions were removed from each flask at different time intervals (0-72h) and were analysed for residual metal content.

3. RESULTS AND DISCUSSION

3.1. Screening and selection of heavy metal tolerant bacteria

In this study, total of six strains able to grow in the presence of toxic metals (Cu, Zn, Cr and Cd) on SCA medium. These strains grown in nutrient broth amended with varying concentrations of different heavy metals showed a variable tolerance level to the tested metals. Strain NGP2 was selected due to high degree of metal tolerance, ability to produce significantly higher amounts of biomass (Figure 1). Among various metals with different concentrations, strain NGP2 could survive at 1300, 1200, 800 and 600 mg/l of Cu, Zn, Cr and Cd respectively. In addition, varied growth behaviour of strain NGP2 was observed when it was grown in nutrient broth treated with fixed concentration at 100 mg/ml of each metal (Figure 2).

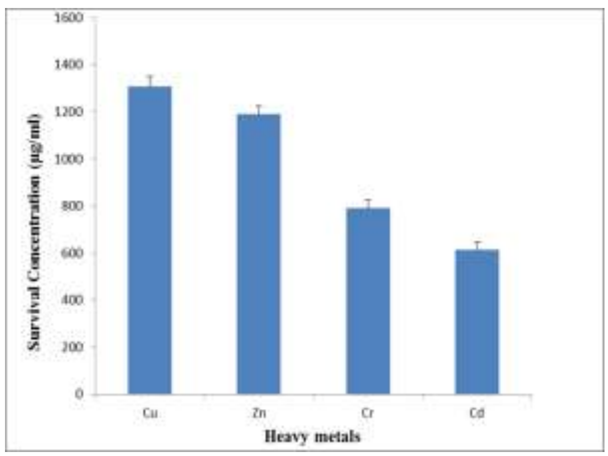

Fig. 1. Heavy metal tolerant pattern of strain NGP 2. *Streptomyces* was inoculated with different concentrations of heavy metals and analyzed its survival parameters

All the heavy metals were absorbed by the tested strain effectively. Cu and Zn were removed at 45h, whereas Cr and Cd were removed at 35 and 25h respectively. Similar to our study, 40 actinomycetes isolated from high metal content soils in Iran, showed highly resistance to the heavy metals. Among them, 13 isolates were selected as high resistant actinomycetes that showed resistance to 140 mM $ZnCl_2$, 7 mM $CuSO_4$, 9.2 mM $CdCl_2$, and 60 mM $NiCl_2$[10]. The bioremediation efficiency against metals are as follows Fe>Cu>Ba>Cd (90%, 71%, 52% and 19% respectively) by the bacteria *Bacillus pumilus* isolated from water of Karnataka mangrove region[8].

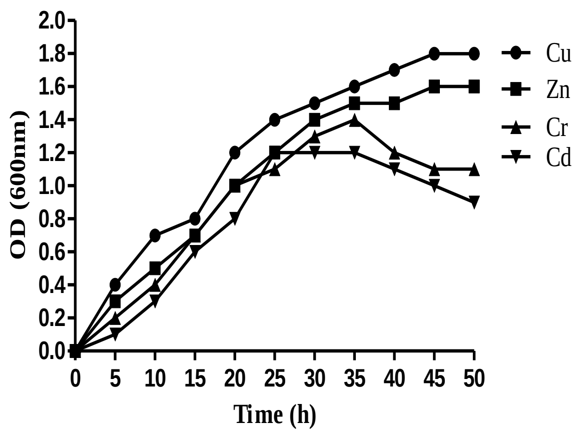

Fig. 2. Growth pattern of the strain NGP 2 at 100 mg/ml of metal.

3.2. Molecular identification of the strain NGP2

The selected strain was characterized and identified by using standard morphological, and biochemical tests (Table 1). On the basis of the characteristics observed for strain NGP2 and compared with those listed in Bergey's manual of determinative Bacteriology, presumptively identified as *Streptomyces Sp*[31]. For the further validation, the strain was subjected to 16srRNA gene sequence analysis[17]. The sequence was submitted to Gen-Bank (JX843531). A phylogenetic tree constructed by MEGA 4 software based on 16srRNA partial sequence. The metagenomics of actinomycetes based 16SrRNA and Genbank database and the results were showed that 17 bands were closely related with five genera of actinomycetes[16].

Table 1. Morphological and Biochemical characteristics of the strain NGP 2

Tests Performed	Characteristic Observed
Accession number	JX843531
Morphology	
Colony Color	White
Colony Shape	Cocci
Pigment	Nil
Biochemical Test	
Gram Staining	+ ve
Methyl Red Test	+ve
Voges-proskauer Test	+ve
Caesinase Test	+ve
Cellulase Test	+ve
Deaminase Test	-ve
Sugar Fermentation	+ve
Nitrate Reduction Test	-ve
Gelutinase Test	+ve

3.3. Biosorption profile

The Langmuir and Freundlich isotherms of heavy metals biosorption by the biomass of strain *S. albogriseolous* NGP 2 are presented in table 2.

The Langmuir and Freundlich absorption constants were evaluated from the isotherms with correlation coefficients (>0.98). Both models described a better absorption process as indicated by correlation coefficient (r^2)[35]. In the Langmuir isotherm *b* is a Langmuir constant related to energy of sorption. If value of *b* is higher than the affinity of biosorbent is enhanced for metal ions. In this study, the Langmuir constant *b* value was highest for Cu (0.143) and lowest for Cd (0.035). According to the *b* value observed here, the biomass could absorb the metal ions in the order: Cu > Cr > Zn > Cd.

Adsorption partition constant of metals were further determined by Freundlich isotherm where *K* and *n* are constants. *K* is the degree of adsorption. Conceptually, when *K* value is low it indicates minimal adsorption of heavy metals whereas the higher *K* value suggests greater sorption ability[36]. In the study, *K* value was highest for Cu (3.559) and it was lowest for Cd (2.322). The value of $1/n$ was lowest for Cu (0.693) but was highest for Cd (0.794) suggesting maximum biosorption of Cu and least of Cd. All values favoured Freundlich isotherm and correspondingly the order of adsorption were similar to Langmuir isotherm. Activated Teff Straw (ATS) was studied for the biosorption of heavy metals such as Cr, Cd, Pb, Ni and Cu. The adsorption isotherm was checked with Langmuir and it could be fitted well. The R_T value was less than one, indicating that the adsorption of the metal ion onto ATS is favourable[5]. Similarly, the removal of nickel ions from aqueous solutions using carboxymethyl cellulose-graft-poly (acrylic acid) CMC-g-PAA hydrogel as an adsorbent was studied[33]. Isotherm for the adsorption of nickel on CMC-g-PAA hydrogel was developed and the equilibrium data fitted to the Langmuir and Freundlich isotherm models[2]. Batch adsorption experiment was carried out on the targeted metal ions Cu (II), Co (II) and the results were analyzed by the Langmuir and Freundlich equation at different concentrations (100–1000 mg/l) and the characteristic parameters for each adsorption isotherm were determined[29]. In another study, polypyrrole-based activated carbon was prepared by adsorption of lead (II) from aqueous phase solution and analyzed initial ion concentration, pH, contact time, and adsorbent dose. The Freundlich isotherm equation (R2 = 0.9950) calculated maximum capacity, qm, determined from the Langmuir model was 50 mg/g[30].

Table 2. Langmuir and Freundlich isotherm parameter

Metal	Langmuir Parameters			Freundlich Parameter		
	Qmax	b	r^2	k	1/n	r^2
Cu	41.49	0.143	0.98	3.559	0.693	0.99
Zn	32.28	0.044	0.96	2.477	0.759	0.98
Cr	75.51	0.061	0.97	2.992	0.702	0.98
Cd	52.02	0.035	0.95	2.322	0.794	0.96

3.4. Effect of initial metal concentration, contact time and pH on biosorption

The effect of initial metal concentration on metal biosorption of *S. albogriseolus* NGP2 was evaluated under reaction condition, set at pH 6 and 30±2°C for equilibrium time half an hour as shown in figure 3. Here, it was observed that the rate of biosorption decreased with increased metal ion concentration[32]. The maximum biosorption of metal was recovered at a low initial metal ion concentration for example it was 92% for Cu at 30 mg/l, while it was 82.7% at 150 mg/l. A trend similar to Cu was also observed for other metals. At lower concentrations, all metal ions present in the solution, interact with the binding sites than at higher ion concentration. Similar results have been reported by others. *Gemella sp*., *Micrococcus sp*. and *Hafnia sp* isolated from Chittagong city, Bangladesh. Among them *Gemella sp*. and *Micrococcus sp*. showed resistance to Lead (Pb), chromium (Cr) and cadmium (Cd), where *Hafnia sp.* showed sensitivity to cadmium (Cd). Degrading potentiality was assessed using Atomic Absorption Spectrophotometer where *Gemella sp*. and *Micrococcus sp*. showed 55.16 ± 0.06% and 36.55 ± 0.01% reduction of Pb respectively. On the other hand, moderate degradation of Cd was shown by *Gemella sp*. (50.99 ± 0.01%) and Micrococcus sp. (38.64 ± 0.06%). The above results corroborate with other study that, 86 % removal of Cd from medium within 24h by *Enterobacter Cloacae*[11]. Another report suggests that, 29 % of Cd was removed by *E. Cloacae* bacteria isolated from tobacco[21]. Rhizophere microbes play a vital role in phytoremediation by siderphore production, acidification, releasing plant growth harmone and through redox changes[26]. Heavymetal uptake capacity was reported as 99% of Pb and Cr containing 10mg Pb and Cr in the medium, 77% of Cd was absorbed in the medium containing 10mg of Cd by *A. flavus* and *R. pusillus* [28]

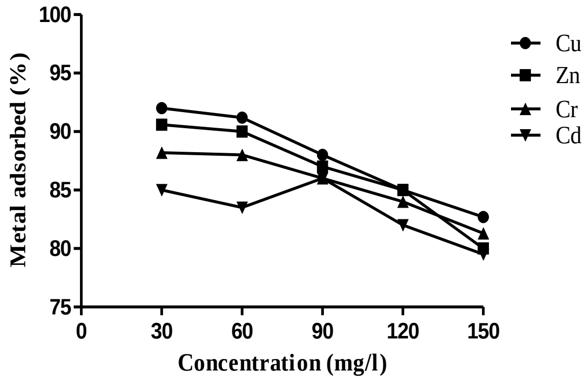

Fig. 3. Effect of initial metal ion concentration on biosorption of the strain NGP 2.

The contact time is one of the important factors of biosorption process. The biosorption of Cu, Zn, Cr and Cd by actinomycete biomass is shown in figure 4. In the experiment, the initial sorption rate was highest, after 30 min, the order of biosorption rate was Cu>Zn>Cr>Cd. Likewise, the heavy metals effect on *Rhodococcus opacus* biomass growth is accordingly, Cd > Ni > Pb > Cu > Zn > Fe. The total time for biodegradation increased from 144 to 216 h in the presence of Fe, Zn, Cu, or Pb, and it was up to 240 h in the presence of Cd or Ni[1]. It is reported in another work, the effect of contact time on the biosorption uptake capacity of Zn(II), Co(II) and Cd(II) ions onto *A. niger* at the initial concentration of 10 mg/l, biomass dosage of 2 g/l and at pH 5 [27]

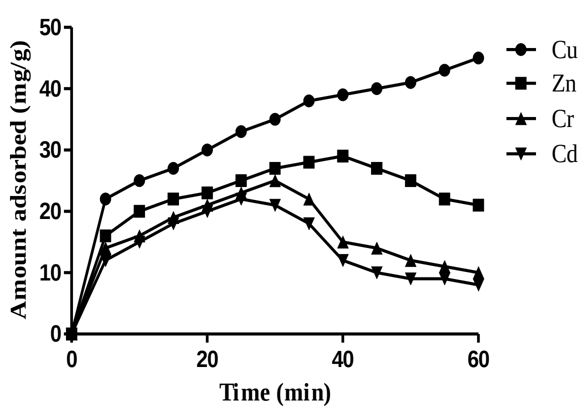

Fig. 4. Biosorption of heavy metals by the strain NGP 2 over the reaction time

pH plays vital role in the biosorption process. The experiment was carried out with varying pH to evaluate their effect on biosorption capacity of microbial biomass using a fixed concentration of Cu, Zn, Cr and Cd (Figure 5). The biosorption capacities for each metal ion increased with an increase in pH. The optimum pH for Cr and Zn removal was 7, while for Cd and Cu it was 6. According to the work of[4], the optimum pH for adsorption of Cr (VI) ranged from 1 to 3, using carboxymethyl cellulose-based hydrogel as adsorbent. Most of the living organisms have been shown to absorb heavy metals such as Cd and Cu at a low pH, due to their physiological properties[19].

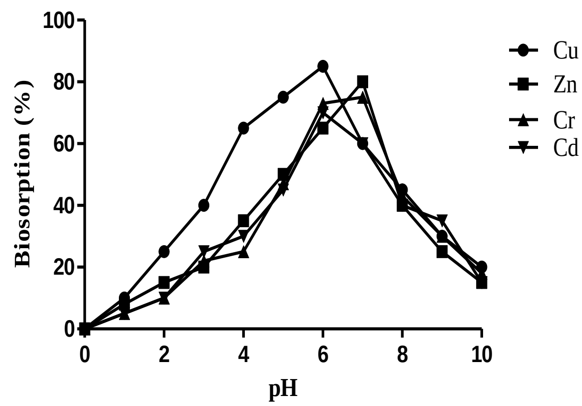

Fig. 5. Effect of pH on biosorption of the strain NGP 2 at different pH

4. Conclusion

The present research aimed at removal of heavy metals such as copper, zinc, chromium and cadmium at different concentration by *S.albogriseolus* NGP2. The optimum pH for chromium and zinc removal was pH 7, while for cadmium and copper it was 6 and the optimal contact time was 60 min for each metal by strain. Biosorption data fitted well with the Langmuir and Freundlich adsorption isotherm equations and indicated sufficient biosorption by the test strain at varying metal ion concentration. This study validates that the biomass of *S. albogriseolus* NGP2 could be used as an inexpensive and highly efficient reliable biosorbing bio-agent for effectively removing heavy metals.

Acknowledgement

The authors would like to thank Department of Science and Technology (DST-FIST) (Grant No. DST-FIST/120/2012) India for providing fund for establishing laboratory facilities to Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.

REFERENCES

1. Aburas, M. M. A. (2016). Bioremediation of toxic heavy metals by waste water actinomycetes. *Int. J. Curr. Res*, 8: 24870-24875.
2. Ajenifuja, E., Ajao, J. A., & Ajayi, E. O. B. (2017). Adsorption isotherm studies of Cu (II) and Co (II) in high concentration aqueous solutions on photocatalytically modified diatomaceous ceramic adsorbents. *Applied Water Science*, *7*(7): 3793-3801.
3. Alghamdi, A. A., Al-Odayni, A. B., Saeed, W. S., Al-Kahtani, A., Alharthi, F. A., & Aouak, T. (2019). Efficient adsorption of lead (II) from aqueous phase solutions using polypyrrole-based activated carbon. *Materials*, *12*(12): 2020.
4. Anah, L., & Astrini, N. (2018, June). Isotherm adsorption studies of Ni (II) ion removal from aqueous solutions by modified carboxymethyl cellulose hydrogel. In *IOP Conference Series: Earth and Environmental Science* (Vol. 160, No. 1, p. 012017). IOP Publishing.
5. Basha, S., Jhala, J., Thorat, R., Goel, S., Trivedi, R., Shah, K., ... & Jha, B. (2010). Assessment of heavy metal content in suspended particulate matter of coastal industrial town, Mithapur, Gujarat, India. *Atmospheric Research*, *97*(1-2): 257-265.
6. Caeiro, S., Costa, M. H., Ramos, T. B., Fernandes, F., Silveira, N., Coimbra, A., ... & Painho, M. (2005). Assessing heavy metal contamination in Sado Estuary sediment: an index analysis approach. *Ecological indicators*, *5*(2): 151-169.
7. Desta, M. B. (2013). Batch sorption experiments: Langmuir and Freundlich isotherm studies for the adsorption of textile metal ions onto teff straw (Eragrostis tef) agricultural waste. *Journal of thermodynamics*, *2013*.
8. Dhote, J., Ingoleb, S., & Chavhan, (2012) A., Review on Wastewater Treatment Technologies. Interantional journal of Engineering Research & Technology 1: 1-10.
9. Diep, P., Mahadevan, R., & Yakunin, A. F. (2018). Heavy metal removal by bioaccumulation using genetically engineered microorganisms. *Frontiers in bioengineering and biotechnology*, *6:* 157.
10. Divakar, G., Sameer, R. S., & Bapuji, M. (2018). Screening of multi-metal tolerant halophilic bacteria for heavy metal remediation. *Int. J. Curr. Microbiol. App. Sci*, *7*(10): 2062-2076.
11. Gavrilescu, M. (2004). Removal of heavy metals from the environment by biosorption. *Engineering in Life Sciences*, *4*(3): 219-232.
12. Hajahmadi, Z., Younesi, H., Bahramifar, N., Khakpour, H., & Pirzadeh, K. (2015). Multicomponent isotherm for biosorption of Zn (II), CO (II) and Cd (II) from ternary mixture onto pretreated dried Aspergillus niger biomass. *Water resources and industry*, *11:* 71-80.
13. Hamedi, J., Dehhaghi, M., & Mohammdipanah, F. (2015). Isolation of Extremely Heavy Metal Resistant Strains of Rare Actinomycetes from High Metal Content Soils in Iran. *International Journal of Environmental Research*, *9*(2).
14. 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 Journal of Microbiology Biotechnology* 15: 283-290
15. Holt, J. G., Krieg, N. R., Sneath, P. H. A., & Staley, J. T. (1994). Bergey's Manual of Determinative Bacteriology 9th Edition, Williams and Wilkins. *Baltimore, Maryland, USA*.
16. Hussein, H., Ibrahim, S. F., Kandeel, K., & Moawad, H. (2004). Biosorption of heavy metals from waste water using Pseudomonas

sp. *Electronic journal of Biotechnology*, *7*(1): 30-37.

17. Jaishankar, M., Tseten, T., Anbalagan, N., & Mathew, B. B. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisipilinary Toxicology*, 7: 60-72.
18. Javanbakht, V., Alavi, S. A., & Zilouei, H. (2014). Mechanisms of heavy metal removal using microorganisms as biosorbent. *Water Science and Technology*, *69*(9): 1775-1787.
19. Krishnamurti, G. S., McArthur, D. F. E., Wang, M. K., Kozak, L. M., & Huang, P. M. (2005). Biogeochemistry of soil cadmium and the impact on terrestrial food chain contamination. In *Biogeochemistry of trace elements in the rhizosphere* (pp. 197-257). Elsevier.
20. Marathe, K. R., Patil, R. H., Vishwakarma, K. S., Chaudhari, A. B., & Maheshwari, V. L. (2019). Protease inhibitors and their applications: An overview. *Studies in Natural Products Chemistry*, *62:* 211-242.
21. Marathe, K., Chaudhari, A., Kamalaja, K., & Maheshwari, V. (2016). Magnesium dependent proteinaceous protease inhibitor with pesticidal potential from alkali-halotolerant Streptomyces spp.: optimization of production using statistical tools. *Biocatalysis and Agricultural Biotechnology*, *5:* 58-68. 31.
22. Niţã, I., Iorgulescu, M., Spiroiu, M. F., Ghiurea, M., Petcu, C., & Cinteza, O. (2007). The adsorption of heavy metal ions on porous calcium alginate microparticles.
23. Ok, Y. S., Yang, J. E., Zhang, Y. S., Kim, S. J., & Chung, D. Y. (2007). Heavy metal adsorption by a formulated zeolite-Portland cement mixture. *Journal of Hazardous Materials*, *147*(1-2): 91-96.
24. Oves, M., Khan, M. S., & Zaidi, A. (2013). Biosorption of heavy metals by Bacillus thuringiensis strain OSM29 originating from industrial effluent contaminated north Indian soil. *Saudi journal of biological sciences*, *20*(2): 121-129.
25. Oyewole, O. A., Zobeashia, S. S. L. T., Oladoja, E. O., Raji, R. O., Odiniya, E. E., & Musa, A. M. (2019). Biosorption of heavy metal polluted soil using bacteria and fungi isolated from soil. *SN Applied Sciences*, *1*(8): 1-8.
26. Pham, V. H. T., Kim, J., Chang, S., & Chung, W. (2022). Bacterial biosorbents, an efficient heavy metals green clean-up strategy: Prospects, Challenges, and Opportunities. *Microorganisms*, *10*(3): 610.
27. Qayyum, S., Khan, I., Meng, K., Zang, X., Zhao, Y., Gu, Q., & Peng, C. (2016). Bioaccumulation of heavy metals from aqueous solution using indigenous fungal isolates.
28. Rusmana, I., & Lestari, Y. (2015). Metagenomic of actinomycetes based on 16S rRNA and nifH genes in soil and roots of four Indonesian rice cultivars using PCR-DGGE. *HAYATI Journal of Biosciences*, *22*(3): 113-121.
29. 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. *RESEARCH JOURNAL OF PHARMACEUTICAL, BIOLOGICAL AND CHEMICAL SCIENCES*, *7*(1): 603-610.
30. Seneviratne, M., Seneviratne, G., Madawala, H. M. S. P., & Vithanage, M. (2017). Role of rhizospheric microbes in heavy metal uptake by plants. In *Agro-environmental sustainability* (pp. 147-163). Springer, Cham.
31. Sharma, P., Dutta, D., Udayan, A., Nadda, A. K., Lam, S. S., & Kumar, S. (2022). Role of microbes in bioaccumulation of heavy metals in municipal solid waste: Impacts on plant and human being. *Environmental Pollution*, 119248.
32. Sommer, C. (2012). *Investigations of the production, purification, activation and characterization of novel, recombinant, highly active Transglutaminases* (Doctoral dissertation, Halle (Saale), Martin-Luther-Universität Halle-Wittenberg, Diss., 2012).
33. Sreedevi, P. R., Suresh, K., & Jiang, G. (2022). Bacterial bioremediation of heavy metals in wastewater: A review of processes and applications. *Journal of Water Process Engineering*, *48*, 102884.

34. Swaminathan, M. (2018). How can India's waste problem see a systemic change. *Economic and Political Weekly*, *53:* 16.
35. Tamura, K., Dudley, J., Nei, M., & Kumar, S., MEGA 4: (2007) Molecular Evolutionary Genetics Analysis software version 4.0. *Molecular Biology Evalution,* 24: 1596-1599.
36. Timková, I., Sedláková-Kaduková, J., & Pristaš, P. (2018). Biosorption and bioaccumulation abilities of actinomycetes/streptomycetes isolated from metal contaminated sites. *Separations*, *5*(4): 54.

About The License