THINK INDIA JOURNAL

ISSN:0971-1260
Vol-22-Issue-37-December -2019

Degradation of Heavy Metals by Deltamethrin Treated Soil Microorganisms

Anupam Kumar*, Avinash Jyoti, Digvijay Singh, Himanshu Singh

[1]*Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India*
**Corresponding author: anupam.kumar167@gmail.com*

Abstract

The proverb that "everything in excess is opposed to nature" hysterics immaculately when exposed to the applications of pesticides and their effects on heavy metals and possess antagonistic properties outside the suggested Environmental Protection Agency parameters. Heavy metals, which causes several physiological happenings in higher organisms but excess of heavy metals is bad and lead to severe health challenges. Pollution caused by heavy metals presents a key universal apprehension in the atmosphere as a consequence of their very delayed or no biodegradation, persistence in the environment and accumulation in the biological systems. Heavy metal derived toxicity needs to be prevailed which is mainly due to many humanoid actions, the prolonged & extensive usage of synthetic pyrethroids pesticides and allied heavy metals used in various preparations and formulations. Bioremediation is a promising technology as a result of its possible cost effectiveness and environmental compatibility. In our present study, one bacterial isolate and two isolates of fungus wewed by zinc (Zn) & least to mercury (Hg). Higher biosorption capacity of fungal biomass of Aspergillus flavus was observed at 800 ppm conc. of copper and zinc. Similarly, higher biosorption capacity of fungal biomass of Penicillium citrinum was observed at 50 ppm conc. of zinc. Thus, we can conclude that Bacillus subtilis, Aspergillus flavus and Penicillium citrinum are being ubiquitous, showed the potential to be used as biosorbents for the removal of higher concentrations of different heavy metals in the environment.

Keywords*: Heavy metals, Pesticides, Bioremediation, Deltamethrin*

INTRODUCTION

Heavy metal contamination is a key universal apprehension in the environment as a consequence of these hazardous contaminants, which tend to accumulate in the a live creatures includes microbes, vegetations, animals and human beings and are accountable for numerous physiological and metabolic ailments[1, 2]. Heavy metals are non- biodegradable pollutants & can be demarcated as the elements with metallic assets (such as conductivity, ductility, stability as cations, ligand specificity etc.), with atomic masses ranging from 54.63 to 200.59 & with a density greater than 5g cm^{-3}. Heavy metals are extremely hazardous & accumulate in the

ISSN:0971-1260
Vol-22-Issue-37-December -2019

biological systems & concentrate in the food chain at each trophic level [3]. The natural causes of heavy metals (HM) such as releases of volcanoes, transference of inland particulates an denduring of metal augmented rocks as an outcome of extended disclosure to air, significantly inserts advancedamount of HM to soils. Instead, anthropologicalactions such as exploitation of metals associated pesticides, exploitation of mines & smelters, metal-enriched waste sludge in the agriculture [4,5], metallurgical industries, fossil fuel combustion, military training & weapons etc., contribute greatly to contaminate the soil with heavy metals. Copper, zinc &nickel are essential trace metals in the human body, but its excessive intake from outside adversely affect the human health [6].Agronomicperformsfor instanceunnecessary usage of phosphatic manuresand fertilizers intended foroptimum production of harvests, widespread application of toxic pesticides are main cause of soil pollution [7]andresults into depredation of humans and other life forms under the precept "if little is good, a lot more will be better" [8]. Deltamethrin-pyrethroid insecticides formulation includes copper (Cu), zinc (Zn) & mercury (Hg) heavy metals. Pyrethroids are synthetic analogs of naturally occurring pyrethrum, isolated from *Chrysanthemum* flower [9, 10]. Pyrethroid insecticides have been introduced in the market in 1980, contributed greatly to pest control & agricultural output and account for appx. 25% of the global insecticide market. At present, 16 pyrethroid insecticides are certified for use in the USA in varied formulations of agricultural or consumer products [11]. To diminish the ecological and public health dangers allied with pyrethroid use, it is required to develop quick and effective approaches to eliminate or minimize the amount of heavy metals derived from insecticides in the environment [12]. Various techniques are available for the remediation of contaminated environment includes precipitation, electrolysis, membrane filtration, ion exchange, reverse osmosis, sorptive flotation, & adsorption with the activated carbon [13]. Among various approaches which are functional for remediation of polluted environment, the biotic approach that is constructed on metabolic activity of the pyrethroids-degrading microorganisms appears to be the utmost operative method [12]. Thus, the ideal way out of the reduction of pollution for these delayed or non-biodegradable metals is "bioremediation" which is a competent and promising approach in command to deal with the pollutants existing in the environment. Outcome of various studies have proven that pyrethroids and heavy metals derived from them can be successfully removed from media & soil by potential microorganisms, belongs to different taxonomic groups [12]. Bioremediation techniques are more advantageous as compared

ISSN:0971-1260
Vol-22-Issue-37-December -2019

to conventional methods as it can be implemented on site, therefore, minimizing personnel risks. It minimizes the sludge disposal problems due to generation of less effluent volume when compared to traditional approaches [14].Microbial organisms can sustain in all environments as a result of their distinctive capability to adopt the toxicants existence in the environment and utilize them as energy source or they exploit heavy metals. Soil micro floras are best recognized to perform a crucial part in the deployment and control of metallic cations, thus changing their accessibility to the different types of plants [15].

Material and Methods

Soil samples were taken from a vegetable cultivation field having history of insecticide application at Ranipur village, situated in Phagwara (Pb). Approximately a total of 500 g of fresh soil was taken from three different spots in the farm. A sterilized hand trowel was used to dug approximately 6-inch-deep, mixed up the soil in the hole and collected together from different spots in the field in a sterile polyethylene bag. Moist soil sample was then, air dried at room temperature and kept in 4°C prior to begin the experiment. Fig 1 is showing the soil sample collection and sieving of soil sample followed by air dry [16].

Fig 1: Collection of soil sample

Physiochemical characterization of the sample

Soil sample was characterized for its physiochemical properties from soil testing laboratory in Punjab Agricultural University (PAU), Ludhiana, Punjab.

Preparation of Growth medium

ISSN:0971-1260
Vol-22-Issue-37-December -2019

The most frequently used growth media were used for cultivation & enumeration of bacteria and fungi. Appropriate amount of media powder was suspended in respective amount of distilled water. To dissolve the medium completely, it was heated till boiling. The prepared media was sterilized prior to use, at 121ºC at 15 psi for 15 minutes. Media used for bacterial and fungal isolation were Nutrient Agar (NA), Nutrient Broth (NB), Soya bean Casein Digest Agar, Potato Dextrose Agar (PDA), Potato Dextrose Broth (PDB), Sabouraud Dextrose Chloramphenicol Agar, Rose Bengal Agar (with Chloramphenicol) [17].

Isolation of soil microflora by serial dilution & plating method

Enumeration of bacteria and fungi were done by consecutive dilution and plating technique. Almost 0.1 ml of each diluted microbial culture was then spreaded over the surface of solidified media or liquid media for growth of Bacteria and Fungi, using sterile glass spreader. Plates were then incubated until colonies appeared, at 35-37ºC &25-27 ºC for bacteria and fungi respectively [18].

Physical characterization of bacterial & fungal isolates

The bacterial colonies were examined on the basis of colony shape, colony height, colony margin, surface refraction, opacity and color and, fungal colonies were examined on the basis of their texture, color, shape, surface appearance& form of conidia, phialides and conidiophores [17, 18].

Biochemical Characterization of Bacterial isolates

In order to identify bacteria, various biochemical reagents were used. Availability of large number of biochemical procedures for bacterial identification is there. Gram staining, Lactophenol Cotton Blue Staining, Catalase Test, Indole Test, Citrate test, Urease Test, Methyl Red (MR) Test, Voges-Proskauer (VP) test, Triple Sugar Iron Agar (TSIA) Test, MacConkey

ISSN:0971-1260
Vol-22-Issue-37-December -2019

Agar Test, Starch Hydrolysis, Motility Test, Bacterial Identification- 16S r RNA Sequencing, and Fungal Identification via 18S rRNA Sequencing [19, 20].

Preparation of Heavy Metal Solution

The 1000 ppm stock solutions of Cu, Zn and Hg were made in double distilled water using Copper (II) Sulphate pentahydrate (CuSO4.5H20) (Titan Biotech), Mercuric Chloride (HgCl2) (Qualikems), Zinc Sulphate heptahydrate (ZnSO4.7H20) (Central Drug House). The 50, 100, 400 & 800 ppm solutions of these heavy metals were prepared from 1000 ppm stock solution. And, added to sterilized nutrient broth & potato dextrose broth in order to make its concentration 50, 100, 400 & 800 ppm solution [17, 18].

Heavy Metal uptake by Bacterial isolate from liquid media

Bacterial isolate was further evaluated for its toleration to heavy metals at higher concentrations of respective heavy metals (Cu, Zn & Hg) in sterilized nutrient broth containing 50, 100, 400 & 800 ppm were dispensed in 100 ml lots to conical flasks of 250 ml capacity. The conical flasks were then, incubated at 37℃ for time period of 24 hrs. The bacterial growth was measured turbidometrically at an interval of 48 hrs. Using UV-vis spectrophotometer (Double beam SL 210) at 645nm, 540nm & 485nm, readings were taken & analyzed for bacterial adaptation to different heavy metals. All the experiments were conducted in triplicate & avg. values were taken for analysis.

Heavy Metal uptake by Fungal isolates from liquid media

Fungal isolates, were further evaluated for its toleration to heavy metals at concentrations of 50, 100, 400 & 800 ppm of respective heavy metals (Cu, Zn & Hg). Fungal inoculums of 24 hours old fungal cultures were taken & inoculated into conical flasks containing 50, 100, 400 & 800 ppm concentrations of each Cu, Zn & Hg heavy metals and flasks were then put on orbital shaker incubator at 28℃ for 96 hrs. at 150 rpm. Harvesting of fungal growth was being done after 96 hrs. of incubation by filtration process using Whatman filter no. 42, followed by rinsing of harvested fungal biomass with (dd/w) double distilled water 3-4 times & dried at 80°C for 18 hrs. in hot air oven. After drying the fungal biomass, it was weighed & estimation of

ISSN:0971-1260
Vol-22-Issue-37-December -2019

concentration of heavy metals was being done by digestion process, using nitric acid & per chloric acid in 3:1. Further, filtration of processed fungal biomass was performed using Whatman filter (no. 42) and filtrate volume was completed upto 50 ml in the volumetric flask. Estimation of heavy metal concentration in digested fungal biomass was done using UV-vis spectrophotometer (Double beam SL 210) at 645 nm, 540nm. All set of the experimentations were performed in triplicate and average values considered for data analysis [19, 20]. The uptake of heavy metals by fungal biomass was evaluated using the following equation:

$$Q_e = (Ci - Cf)V / 1000M$$

Where, Q_e is conc. of HM accumulated by fungal biomass in (mg/g), *Ci* is initial conc. of HM (mg/L), *Cf* is the final conc. of HM (mg/L), *V* (L) is the volume of the aqueous medium & M is the dry weight (g) of the fungal biomass.

Results and Discussion

Physiochemical properties of soil sample

The physiochemical properties of soil sample used for isolation of microbial species were analysed from soil testing laboratory of Punjab Agricultural University (PAU) Ludhiana, and are presented in table no. 1

Table 1: Soil physical and chemical testing report

SOIL TEST REPORT			
Test	**Result**		**Remarks**
Presence of small elements in the soil	Type of soil	Mara	White color in the field, Using good/canal water
	Alkalineparts	7.8 (ok)	
	Salty substances	0.92 (white color)	
	Zooid carbon category (percent)	0.990 (more)	
	Phosphorus (kg/acre)	26.3 (too much)	

ISSN:0971-1260
Vol-22-Issue-37-December -2019

	Potash catergory (kg/acre)	360 (much)	
	Zinc	3.26	Is not the lack of small elements
	Iron	36.10	
	Manganese	6.28	
	Copper	2.36	

Morphological and Biochemical Characterization of Deltamethrin resistant Bacteria

On the basis of morphological & staining characteristics of bacterial isolate, isolated on nutrient agar & soya bean casein digest agar, bacterial isolate was microscopically observed as gram-negative rod-shaped bacteria exhibiting opaque/dull white color, dull surface refraction, medium sized flat & irregular colonies with lobate margins. The bacterial isolate was assumed to belong to the family of *bacillus spp. that* was further conformed by various biochemical tests followed by molecular characterization by 16S rRNA gene sequencing & finally, conformed to be *Bacillus* subtilis**.** Morphological and gram staining results are shown in fig 1.

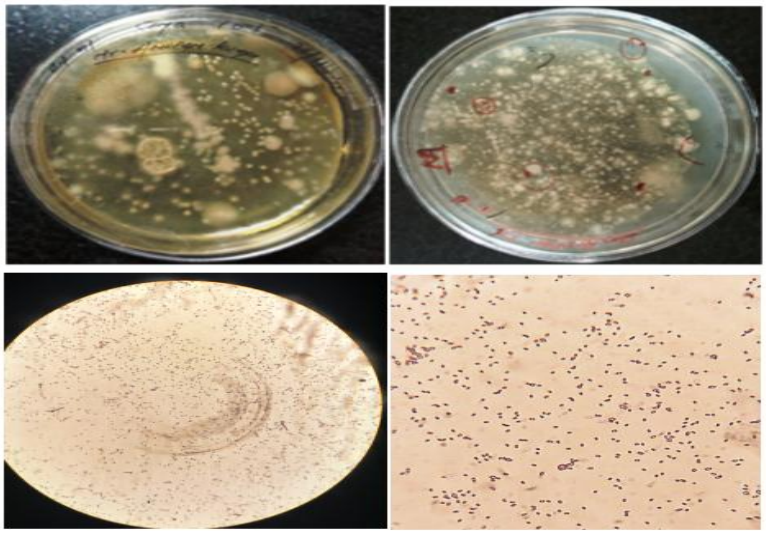

Fig 1: Morphological and microscopic observations of *Bacillus subtilis*

Molecular Characterization of Bacterial isolate by 16S rRNA Gene Sequencing

ISSN:0971-1260
Vol-22-Issue-37-December -2019

On the basis of molecular characterization of bacterial isolate by 16S rRNA Gene Sequencing, isolate was conformed as ***Bacillus subtilis.***Obtained phylogenetic tree is shown in fig 2.

Sequence of *Bacillus subtilis*

CCAAATCTTCTTCCTTCATTCCATGCTGTCGAGCGGACAGATGGGAGCTTGCTCCCTG
ATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACTGGGAT
AACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAAC
ATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTG
GTGAGGTAATGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGG
CCACACTGGGACTGAGCCGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTT
CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGG
ATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTT
GACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATAC
GTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCT
TAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGA
ACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAG
ATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGG
AGCGAAGCGTGCGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACG
ATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAG
CACTCCGCCTGGGGAGTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGCCC
GCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCATCGCGAGAACTTACCAGGTC
TTGACATCCTCTGACAATCCTAGAGATAGGACGTCCCCTTCGGGGGCAGAGTGACAG
GTGGTGCATGCTGTCGTCAGCCGTGTCGTGAGATGTTGGGTTAGTCCCGCAACGAGC
GCATCCCTTGATCTTAGTTGCCAGCAATTCAGTGGTACTCTAGGTGACTTGCCGGTG
ACATACGAGAGTGGGATGACGTCAATCATCATGCCCTTATGACTGGCTACACACCTC
TACATTGACGGACATGGCATCGTAACCGCGGAGGTTAAGCCAATCCCACAAACTCT
GG

Phylogeny Tree of *Bacillus subtilis*

ISSN:0971-1260
Vol-22-Issue-37-December -2019

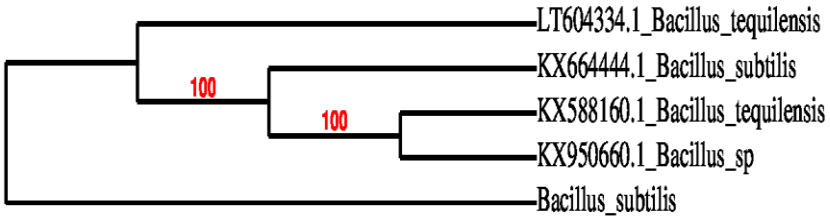

Fig 2 : Phylogeny Tree of *Bacillus subtilis*

Morphological and Biochemical Characterization of Deltamethrin resistant Fungi

Based onthe microscopic analysis & morphological characteristics of fungal isolates, isolated on sabouraud dextrose chloramphenicol agar and rose bengal agar medium, fungal isolates were assumed to be their belonging to the family of *Aspergillus spp. and Penicillium spp.* Colonies characteristics and microscopic analysis of *Aspergillus* were observed as woolly, yellow-brown to olive colored, granular in shape, floccose texture exhibiting long conidiophores, rough just beneath the globose vesicle and circumferentially raised phialides. It was further confirmed by molecular characterization by 18S rRNA gene sequencing & finally, conformed to be *Aspergillus flavus.*Morphological and stained results of *A. flavus* is shown in fig 3. Biochemical results are shown in table 2.

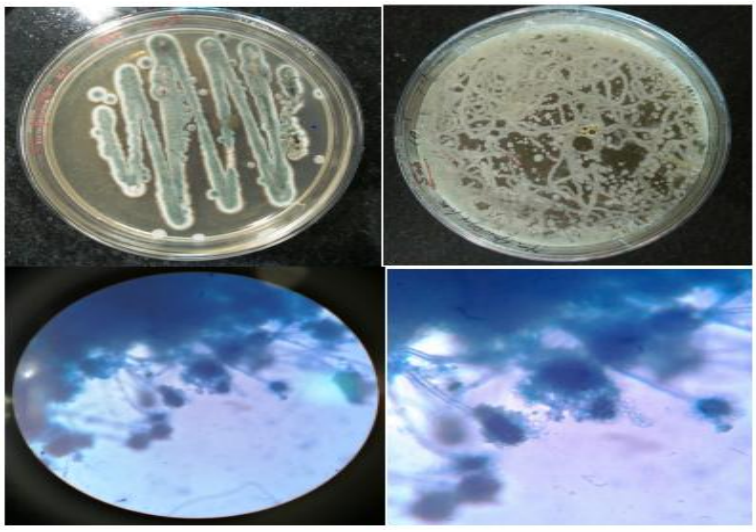

Fig 3:Morphological and microscopic observations of *Aspergillus flavus*

ISSN:0971-1260
Vol-22-Issue-37-December -2019

Similarly, colonies characteristics & microscopic analysis of *Penicillium spp.* were observed as green/ grayish-green with a white lining, velvety to powdery surface varied colors of exudates on the surface, reverse side shows a pale cream to yellow color or more intense reddish-brown. It was further conformed by molecular characterization by 18S rRNA gene sequencing & finally, conformed to be *Penicillium citrinum.*In fig 4 the morphological and stained results are shown.

Fig4: Morphological and microscopic observations of *Penicillium citrinum*

Table2: Biochemical tests and results of bacterial sample

S. No	Biochemical Test	Results	Remarks
1.	Motility Test	+ Ve – *Bacillus subtilis*	Motile; medium got turbid
		-Ve – *Staphylococcus aureus*	Non-motile; no turbidity
2.	Indole Test	+Ve - *E. coli*	Cherry red ring formed
		-Ve - *Bacillus subtilis*	Reagent layer remained yellow

ISSN:0971-1260
Vol-22-Issue-37-December -2019

3.	Citrate Test	+Ve - *Bacillus subtilis*	Colour changed to Prussian blue
		-Ve - *E. coli*	Color persisted green
4.	Urease Test	+Ve - *Staphylococcus aureus*	Color changed to pink
		-Ve - *Bacillus subtilis*	No change in color
5.	Methyl Red (MR) Test	+Ve - *E. coli*	Color changed from yellow to red
		-Ve - *Bacillus subtilis*	No change in color
6.	Voges-Proskauer (VP) Test	+Ve - *Bacillus subtilis*	Colour changed to red
		-Ve - *E. coli*	No change in color
7.	Starch Hydrolysis Test	+Ve - *Bacillus subtilis*	Transparent clear zones around colony
		-Ve - *Staphylococcus aureus*	No clear zones
8.	Catalase Test	+Ve - *Bacillus subtilis*	Immediate bubbling
		-Ve - *Streptococcus pyrogens*	No bubbling

Molecular Characterization of Fungal isolates by 18S rRNA Gene Sequencing

On the basis of molecular characterization of fungal isolates by 18S rRNA Gene Sequencing, isolates were conformed as *Aspergillus flavus* and *Penicillium citrinum.* In fig 5 and fig 6 phylogenetic tree have been shown while in fig 7 standard curve obtained respect to heavy metals are shown.

Sequence of *Aspergillus flavus*

ISSN:0971-1260
Vol-22-Issue-37-December -2019

AGTTTACTGATTGCGATACAATCAACTCAGACTTCACTAGATCAGACAGAGTTCGTG
GTGTCTCCGGCGGGCGCGGGCCCGGGGCTGAGAGCCCCCGGCGGCCATGAATGGCG
GGCCCGCCGAAGCAACTAAGGTACAGTAAACACGGGTGGGAGGTTGGGCTCGCTAG
GAACCCTACACTCGGTAATGATCCTTCCGCAGGTTCACCTACGGAAGGATCATTACC
GAGTGTAGGGTTCCTAGCGAGCCCACCCCCCCCGGTTTTTTGGACCCTAGTTGGTTTC
GGGGGCCCCCATTTATGGGCCGGGGGGCTTTAGCCCGGGCCGGGCCCCCGGGGAAA
AC

Phylogenetic tree of *Aspergillus flavus*

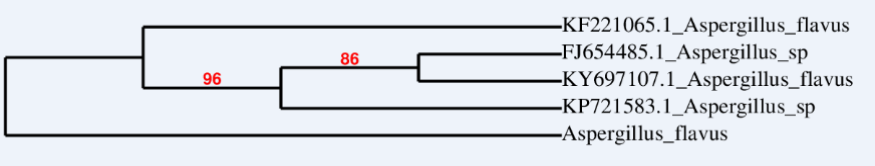

Fig 5: Phylogenetic tree of *Aspergillus flavus*

Sequence of *Penicillium citrinum.*

TTAGAATCGTTGATGAGTTTTACTAATTTCGTTATAGGTCTCAGACTGCAACTTCAGA
CAGCGTTCAGGGGGGCCGTCGGCGGGCGCGGGGCCCGCCGAGGCAACATAGGTTCG
GGCAACACGGGTGGGAGGTTGGGCCCCGAGGGGCCCGCACTCGGTAATGATCCTTC
CGCAGGTTCACCTACGGAAG

Phylogenetic tree of *Penicillium citrinum.*

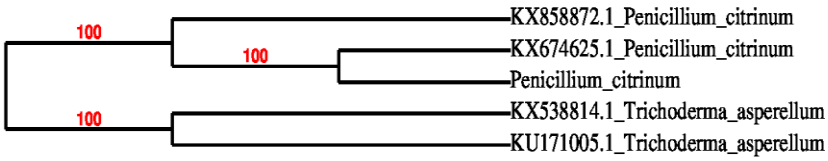

Fig 6: Phylogenetic tree of *Penicillium citrinum.*

Calibration curves of Copper, Zinc & Mercury

ISSN:0971-1260
Vol-22-Issue-37-December -2019

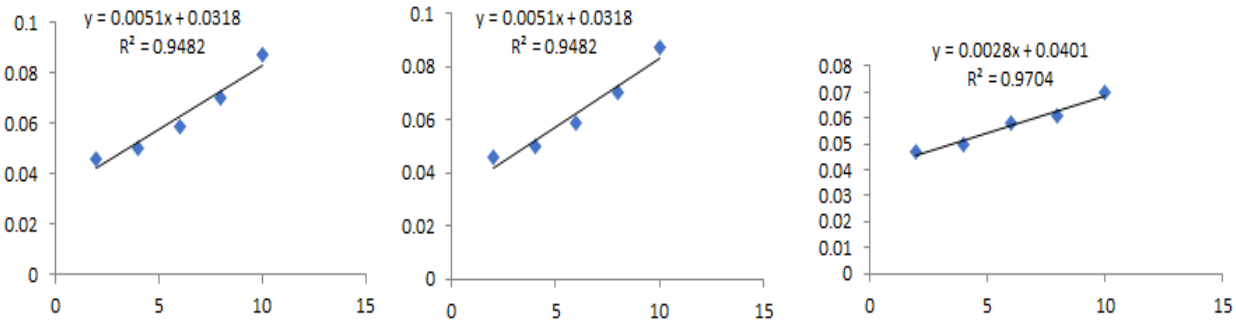

Fig 7: Standard curve of Cu, Zn and Hg

Bacterial adaptation to heavy metals in the liquid media

Bacterial isolatehas shown maximum adaptability to copper (Cu) at 50 ppm concentration followed by zinc (Zn) & mercury (Hg). At 50 ppm concentrations of Cu, Zn & Hg, *Bacillus subtilis* acquire the absorbance values of 0.9901, 0.9500 and 0.3647 respectively; at 100 ppm concentrations, 0.877, 0.9204 and 0.1689; at 400 ppm concentrations, 0.7456, 0.4050 and 0.1210; & at 800 ppm conc. 0.4385, 0.1879 and 0.0775 respectively has been gained by *Bacillus subtilis.* In fig 8 effects of heavy metals on the growth of *B. subtilis* is depicted. The graphical representation of absorbance vs different concentrations of heavy metals (Cu, Zn & Hg) is depicted as follows:

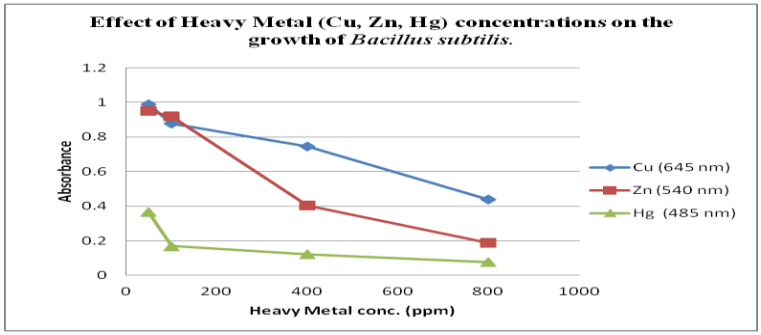

Fig 8: Graphical representation of the effect of different concentrations of heavy metals on the growth of *Bacillus subtilis.*

Cell dry weight of fungal biomass of *Aspergillus flavus and Penicillium citrinum* in the liquid media

ISSN:0971-1260
Vol-22-Issue-37-December -2019

Dried biomass weight (g) of *Aspergillus flavus*, at 50ppm conc. of Cu, Zn and Hg was noted as 0.22, 0.22 and 0.23g respectively; at 100 ppm conc. 0.28, 0.32 nd 0.25 respectively; at 400 ppm conc. 0.19, 0.29 and 0.20g respectively; & 0.20, 0.28 and 0.35g at 800 ppm conc. respectively. In fig 9 cell dry weight of fungal isolates were shown.

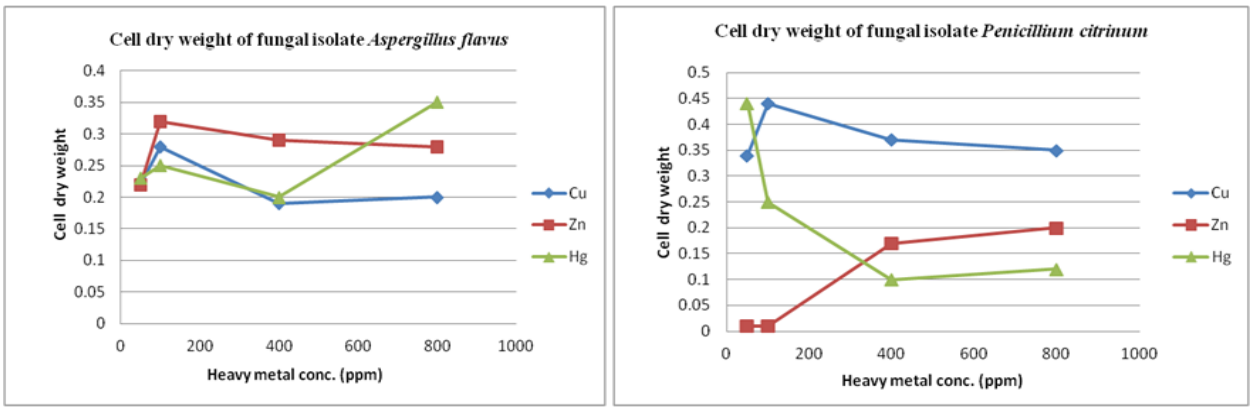

Fig 9: Cell dry weight (g) of fungal biomass of *Aspergillus flavus*and *Penicillium citrinum*

Similarly, dried biomass weight (g) of *Penicillium citrinum*, at 50ppm conc. of Cu, Zn and Hg was noted as 0.34, 0.01 and 0.44g respectively; at 100 ppm conc. 0.44, 0.01 nd 0.25g respectively; at 400 ppm conc. 0.37, 0.17 and 0.10 respectively; & 0.35, 0.20 and 0.12g at 800 ppm conc. respectively.

Effect of Biosorption capacity (q_e) of *Aspergillus flavus* and *Penicillium citrinum* on different heavy metal (Cu, Zn, Hg) concentrations

Fungal enzymes tend to degrade the HMs by incorporating them in their metabolic pathways & by utilizing them as their carbon and energy source. Two fungal isolates were isolated from deltamethrin contaminated soil, designated as *Aspergillus flavus* &*Penicillium citrinum*, were further tested for their tolerance to higher concentrations of Cu, Zn and Hg heavy metals. Higher biosorption capacity (q_e) of *Aspergillus flavus* was observed for copper (Cu) at 400 and 800 ppm followed by Zn at 800 ppm concentration. At 50ppm conc. of Cu, Zn and Hg, biosorption capacity (q_e) of the *Aspergillus flavus*,was observed as 23.03, 21.80 and 21.50 mg/g respectively; at 100 ppm conc. 34.05, 29.70 and 39.70 mg/g respectively; at 400 ppm conc. 209.40, 137.10 and 199.80 mg/g respectively; & 398.90, 284.16 and 228.40 mg/g at 800 ppm concentration respectively. In fig 10 biosorption capacity of fungal isolates are mentioned.

ISSN:0971-1260
Vol-22-Issue-37-December -2019

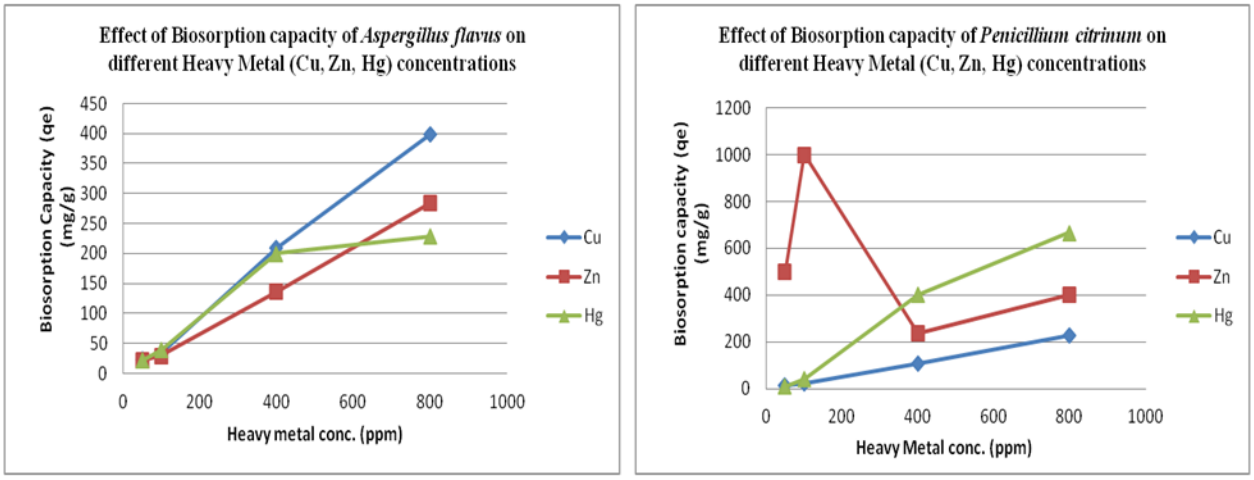

Fig 10: Graphical representation of the biosorption capacity (q_e) of *Aspergillus flavus*and *Penicillium citrinum*at different concentrations of heavy metal.

Higher biosorption capacity (q_e) of *Penicillium citrinum* was observed for Cu at 50 ppm followed by Hg at 800 ppm concentration. At 50ppm conc. of Cu, Zn and Hg, biosorption capacity (q_e) of the *Aspergillus flavus*was observed as 14.70, 501.70 and 11.05 mg/g respectively; at 100 ppm conc. 22.70, 1001 and 39.70 mg/g respectively; at 400 ppm conc. 108.13, 235.40 and 399.80 mg/g respectively; & 228.60, 400.08 and 666.50 mg/g at 800 ppm concentration respectively

Conclusion

Agricultural practices, sewage waste & industrial effluents pose to be the key sources for pollution in environment by heavy metals. Increased industrial practices have severed the problem by effecting microbes at all levels. Mining wastes, plastic manufacturing, electroplating, fertilizer generating plants & metallurgical methods are all playing active role in causing heavy metal damage to the surrounding. Considering all above problems created by the contamination of the environment, this study was done to isolate such microbes which have the potential to lessen the toxic effects of heavy metals and shown resistance towards deltamethrin pesticide. Biochemical methods prove helpful in giving exact and credential identification of the bacterial isolates. Further, 16s r RNA and 18s r RNA gene sequencing provide identification of genus and species for isolates that do not fit in any recognized biochemical profiles of strains creating very low likelihood towards acceptable identification according to the known commercial systems.

ISSN:0971-1260
Vol-22-Issue-37-December -2019

The presence of three microbes namely *Bacillus subtilis, Aspergillus flavus* and *Penicillium citrinum*were confirmed as potent agent to degrade the heavy metals and they can resist towards deltamethrin pesticide inthis study.

Acknowledgements

Authors are thankful to Lovely Professional University for providing fund and lab facilities to conduct the experiments. Authors also thankful to Punjab Agriculture University, Ludhiana for soil analysis.

References

1. Rajendran, P., Muthukrishnan, J. and Gunasekaran, P., "Microbes in heavy metal remediation", Indian Journal of Experimental Biology, vol. 41, pp.935-944, 2003.
2. Banerjee, S., Gothalwal, R., Sahu, P.K. and Sao, S., "Microbial observation in bioaccumulation of heavy metals from the ash dyke of thermal power plants of Chhattisgarh, India", Advances in Bioscience and Biotechnology, vol. 6(02), p.131, 2015.
3. Siddiquee, S., Rovina, K., Azad, S.A., Naher, L., Suryani, S. and Chaikaew, P., "Heavy metal contaminants removal from wastewater using the potential filamentous fungi biomass: a review", J Microb Biochem Technol, vol. 7(6), pp.384-395, 2015.
4. Qayyum, S., Khan, I., Meng, K., Zang, X., Zhao, Y., Gu, Q. and Peng, C., "Bioaccumulation of heavy metals from aqueous solution using indigenous fungal isolates", Indian Journal of Geo-Marine Sciences, vol. 45(4), pp.499-507, 2016.
5. Smith, S.R., "A critical review of the bioavailability and impacts of heavy metals in municipal solid waste composts compared to sewage sludge", Environment international, vol. 35(1), pp.142-156, 2009.
6. Chen, S., Deng, Y., Chang, C., Lee, J., Cheng, Y., Cui, Z., Zhou, J., He, F., Hu, M. and Zhang, L.H., "Pathway and kinetics of cyhalothrin biodegradation by Bacillus thuringiensis strain ZS-19", Scientific reports, vol. 5, pp.8784-8784, 2015.

ISSN:0971-1260
Vol-22-Issue-37-December -2019

7. Oves, M., Saghir Khan, M., Huda Qari, A., Nadeen Felemban, M. and Almeelbi, T., "Heavy metals: biological importance and detoxification strategies", Journal of Bioremediation and Biodegradation, vol. 7(2), pp.1-15, 2016.

8. Aktar, W., Sengupta, D. and Chowdhury, A., "Impact of pesticides use in agriculture: their benefits and hazards", Interdisciplinary toxicology, vol. 2(1), pp.1-12, 2009.

9. Ali, Z., Gilani, R., Hussain, H. and Hussain, I., "Quantitative determination of Deltamethrin in milk, blood and urine of domestic animals", IOSR J. Appl. Chem.(IOSR-JAC) e-ISSN, pp.2278-5736, 2013.

10. Rehman, H., Aziz, A.T., Saggu, S.H., Abbas, Z.K., Mohan, A. and Ansari, A.A., "Systematic review on pyrethroid toxicity with special reference to deltamethrin", Journal of Entomology and Zoology Studies, vol. 2(6), pp.60-70, 2014.

11. Shafer, T.J., Meyer, D.A. and Crofton, K.M., "Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs", Environmental health perspectives, 113(2), pp.123-136, 2004.

12. Cycoń, M. and Piotrowska-Seget, Z., "Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review", Frontiers in microbiology, vol. 7, p.1463, 2016.

13. Ghosh, A., Ghosh Dastidar, M. and Sreekrishnan, T.R., "Recent advances in bioremediation of heavy metals and metal complex dyes", Journal of Environmental Engineering, vol. 142(9), p.C4015003, 2015.

14. Yadav, K.K., Gupta, N., Kumar, V. and Singh, J.K., "Bioremediation of heavy metals from contaminated sites using potential species A review", Indian J. Environ. Prot, vol. 37(1), p.65, 2017.

15. El Hameed, A.H.A., Eweda, W.E., Abou-Taleb, K.A. and Mira, H.I., "Biosorption of uranium and heavy metals using some local fungi isolated from phosphatic fertilizers", Annals of Agricultural Sciences, vol 60(2), pp.345-351, 2015.

ISSN:0971-1260
Vol-22-Issue-37-December -2019

16. Akbar, S., Sultan, S. and Kertesz, M., "Bacterial community analysis of cypermethrin enrichment cultures and bioremediation of cypermethrin contaminated soils", Journal of basic microbiology, vol. 55(7), pp.819-829, 2015.

17. Sharma, A., Gangola, S., Khati, P., Kumar, G. and Srivastava, A., "Novel pathway of cypermethrin biodegradation in a Bacillus sp. strain SG2 isolated from cypermethrin-contaminated agriculture field", 3 Biotech, vol. 6(1), p.45, 2016.

18. Chen, S., Lai, K., Li, Y., Hu, M., Zhang, Y. and Zeng, Y., "Biodegradation of deltamethrin and its hydrolysis product 3-phenoxybenzaldehyde by a newly isolated Streptomyces aureus strain HP-S-01", Applied microbiology and biotechnology, vol. 90(4), pp.1471-1483, 2011.

19. Janda, J.M. and Abbott, S.L., "16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls", Journal of clinical microbiology, vol. 45(9), pp.2761-2764, 2007.

20. Kumar, S., "Isolation, characterization and growth response study of chlorpyrifos degrading bacteria from cultivated soil", Int. J. of Adv. Engg,vol. 2(3), pp.199-203, 2011.