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RESEARCH ARTICLE

BIOSORPTION OF HEAVY METALS USING *ASPERGILLUS* SPECIES ISOLATED FROM CONTAMINATED SOIL.

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Abstract

With the rapid development of industries heavy metal pollution has become one of the major global concerns due to their toxicity and threat to human life and environment. This work evaluated the heavy metal biosorption potential of *Aspergillus* sp. (*Aspergillus flavus* and *Aspergillus fumigatus*) isolated from contaminated soil of Bhagwanpur industrial area, Haridwar. The heavy metal concentrations were determined after digestion of soil samples. The results indicate the heavy metal resistant fungi that were isolated and screened for the biosorption potential. The minimum inhibitory concentration (MIC) of Pb, Cr, Ni and Zn was determined by agar diffusion method in 25, 50, 100, 200 and 400 ppm concentrations. In this study, adsorption of Zn, Ni, Cr and Pb were investigated and two sp. of *Aspergillus* were identified *viz. Aspergillus flavus* and *Aspergillus fumigatus*. The results showed that *Aspergillus flavus* and *Aspergillus fumigatus* could biosorb all the metals in the order Zn>Ni>Cr>Pb and Pb>Cr>Zn>Ni respectively.

Introduction:-

Heavy metals, a major category of globally distributed pollutants, are natural elements that have been extracted from the earth and harnessed for human industry and product for millionaire. Hutton and Symon [1] termed heavy metal as the group of metals and metalloids having an atomic density greater than 4 g/cm^3 or 5 times or greater than water. They are toxic, persistent, non-biodegradable and tend to accumulate in organisms or concentrate in food chains (bioaccumulation). Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag), copper (Cu), chromium (Cr), nickel (Ni), iron (Fe) and the Platinum group elements. It affects both flora and fauna species severely. Heavy metals affect many biological processes such as respiration, photosynthesis, reproduction and metabolism, which cause a partial or total damage to living organism [2], [3].

Contaminants vary in their tendency to end up in the water held in the soil or in the underlying ground water (by leaching through the soil), volatize (evaporate) into the air and binding tightly to the soil [4]**.** Also, the waste electrical and electronic equipment contains substantial quantities of valuable materials which can be source of potential environmental contaminants [5]. Heavy metal pollution of water is one of the major environmental pollution. Many industrial wastewaters contain various heavy metals like lead, zinc, nickel, copper and chromium. Heavy metal pollution of soil and wastewater is a significant environmental problem and relates the negative impact on human life [6].

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Soil and water are the precious natural resources on which sustainability of human life depends in many ways. Water bodies receiving the industrial effluent have high biological oxygen demand (BOD), chemical oxygen demand (COD) and chlorides level that is above the concentration prescribed by the standard institute. Soil also get contaminated with the accumulation of heavy metals and metalloids through the emission from industries, mine tailing, disposal of metal wastes etc. Soil is serving as sink for the pollutants discharge into the environment [7].

Heavy metal contaminated soil and wastewater are the sources of metal resistant micro-organisms [8]. All these resistant microbes can affect the mobility and reactivity of metals which detoxify them and hence preventing further metal contamination [9]. There are number of resistant micro-organisms (Bacteria, fungi, algae and yeast) which have the capacity to degrade the pollutant. Degradation of heavy metal using bioaccumulation by microbes is substantially grown technology during recent years.

Fungal biomass is also used as biosorbent and has the potential for removal of heavy and radionuclide from the polluted sites. The cell wall of fungal species and their components have functional groups *i.e.* carboxyl, hydroxyl, sulphahydryl etc, and play major role in the sequestrations of metals. Hetrotrophic fungi such as *Mucor sp., Aspergillus sp., Penicillium sp. and Fusarium sp.*, can remove both type of metal species *i.e.* soluble and insoluble form of metals [10].

The search for new and innovative technology for the remediation of heavy metal pollution has attracted the attention to the biosorption potential of certain micro-organism. The use of microorganisms as biosorbent of heavy metals from contaminated soil and industrial wastewater is a potential alternative over the chemical methods. Biosorption is the removal of material (like compounds, molecule, atoms, ions, etc) by inactive, dead biomass (material of biological origin) due to "high attractive forces" which is present between the two [11]. Biosorption is passive, metabolism-independent process in contrast to bioaccumulation *i.e.* metabolism-dependent process [12]. According to the metabolism of different microbial cells, biosorption may be completed in many ways depending on different metabolic activity of cells. Biosorption may be extracellular accumulation, cell surface sorption and intracellular accumulation. In the present study, fungal specieswere isolated from contaminated soil sample of industrial area and further evaluated for biosorption capacity of lead, chromium, nickel and zinc in laboratory.

Material and methods:-

Sampling Site:-

Soil samples were randomly collected from the industrial area of Bhagwanpur which is located in Haridwar district and also famous for its Industrial setup or Industrial area. It is situated at 30.06941^{o} N 77.83997o E. For all analysis composite sample was formed by mixing the collected samples from different depth and sample was stored in polythene bags 4°C for further analysis.

Heavy metal analysis of soil samples:-

Sample was digested by taking 1 g of soil in 250 ml glass beaker with 8 mL of aqua regia on a sand bath for 2 h. After evaporation to near dryness the samples were dissolved with 10 mL nitric acid and diluted to 50 mL using distilled water. Total metal concentration (Cr, Ni, Co, Cu, Fe, Cd, Pb, and Zn) of digested soil samples was analyzed by using AAS.

Isolation and screening of heavy metal resistant fungal strains:-

The fungal strains were isolated from soil samples by serial dilution method and maintained in agar slants. Isolates were screened on Martin's agar enriched with 25 ppm of different heavy metals and incubated at 25°C for 72 hrs [13]. After incubation growth was observed and two fungal isolates were used for biosorption potential. The different fungal isolates identified by the colour, shape, size and their conidia, mycelium pattern by the Lactophenol cotton blue stain.

Minimum inhibitory concentration:-

The minimum inhibitory concentration (MIC) of Pb, Cr, Ni and Zn at which no growth occurred was determined by agar diffusion method [14]. Martin's agar containing fungal strain was poured in petri-plates and allowed to solidify. After solidification five partitions were formed with bore well depth of 2 mm. Metal solutions (10 µl) of Pb, Cr, Ni and Zn having concentration 25, 50, 100, 200 and 400 ppm were poured in wells separately. These petri-plates were incubated at 25°C for 72 hrs, and after incubation plates were observed.

Biosorbent Preparation:-
All the strains were inoculated separately into 100 mL martin's broth in 500 mL conical flasks and incubated on a shaker at 150 rpm for 4 – 7 days at 28°C ± 1°C.

The cells were grown to late exponential phase, harvested by centrifugation (REMI, India) at 10,000 rpm for 30 min at 4°C and washed three times with deionized water. Cell suspensions for assay of biosorption potential of live microbe were prepared by resuspending the cell pellet in deionized water. Biomass concentrations in cell suspensions were determined by drying an aliquot in a pre-weighed aluminum foil container to a constant weight at 80°C [15].

Biosorption experiment:-
Dried and powdered living biomass (1gm ± 0.1) of two isolates was inoculated separately into 1 L of metal solution containing 25 to 400 ppm of Ni, Cr, Zn and Pb. Metal-free and biosorbent-free solutions were prepared as controls. The flasks containg metal ion solution was kept on rotatory shaker for 72 h at 25°C and 150 rpm. After 72 hrs, cells were harvested from the medium and content of supernatant for heavy metals were analyzed with proper digestion and dilution by AAS. The optimum pH and temperature was maintained for the growth of microorganisms in batch culture [16].
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Biosorption capacity i.e. amount of metal ion (mg) bioabsorbed/g of dried biomass was calculated using the following equation:

$$Q = \frac{C_i - C_f}{M} \times V$$

Whereas Q = mg of metal ions uptake per gram biomass (mg/g), Ci is initial concentration of metallic ions (mg/L); C_f = final concentration of metal in solution; M is dried mass of biosorbent in the reaction mixture (g) and V is volume of reaction mixture (ml).

Result and Discussion:-

Heavy metal concentration:-
The Heavy metals are found naturally in soil and are essential part of all living organisms [17]. They are environmental contaminants and would affects the all ecosystems. Soil microorganisms like bacteria, fungi, algae and yeast are known best to remove all heavy metal contaminants from soil as well as in water. The bioadsorption mechanism includes the process of ionexchange, co-ordination, complexation, chelation, adsorption, micro-precipitation and diffusion with the help of cell membrane and wall.

Among all different heavy metals, eight heavy metals were analyzed during the present study (Table 1). The concentrations of eight heavy metals were Chromium (10.3 ± 1.08 ppm), Lead (4.55 ± 1.12ppm), Nickel (7.35 ± 0.32 ppm), Zinc (29.26 ± 1.14 ppm), Cobalt (1.5 ± 0.07ppm), Copper (3.5 ± 0.23 ppm), Cadmium (0.05 ± 0.016 ppm) and Iron (0.52 ±0.03 ppm) respectively. The concentration of Zinc, Lead, Chromium and Nickel were analyzed above the permissible limit recommended by WHO standards. This has adverse effect on ecosystem while Cobalt, Copper, Cadmium and Iron were found almost within the limit in the study area. More or less similar values of heavy metal concentrations were recorded by Sharma and Raju, [18].

Isolation and screening of fungal isolates:-
From soil sample fungal strains were isolated and screened based on their resistant capacity against four heavy metals (Pb, Zn, Cr and Ni). The identified resistant fungi were*Aspergillusflavus*and *Aspergillusfumigatus.*These fungal strains showed resistant growth against 25 ppm concentration of each metal (Pb, Zn, Cr, & Ni) individually.

All strains were cultured for resistivity against the five different concentrations of heavy metals viz. 25, 50, 100, 200 and 400 ppm. Table 2 showed the minimum inhibition concentration (MIC) of fungal isolates at five concentrations. The resistance level were in three resistant levels i.e., least (+), moderate (++) and high (+++) and measured by comparing diameter of zone of metal contained media against no metal contained (control) media. On the basis of MIC, isolates were evaluated for metal biosorption potential. Varying concentration of metal tolerance in fungi heavy metal contaminated sites has been reported by other workers [19].

Biosorption potential:-

In the present study dried biomass of *Aspergillus flavus* and *Aspergillus fumigatus* was used for the biosorption of four metals viz. Pb, Zn, Cr and Ni at five initial metal concentrations of 25, 50, 100, 200 and 400 ppm. The biosorption capacity of *Aspergillus flavus* was high for Zn at 400 ppm (183.2 ± 1.52 mg/g) and minimum for Pb at 25 ppm (13.6 ± 1.66 mg/g). In case of *Aspergillus fumigatus* maximum biosorption was observed for Pb at 400 ppm (225.1 ± 0.8 mg/g) and minimum for Ni at 25 ppm (12.8 ± 0.16 mg/g). Thus, *Aspergillus flavus* and *Aspergillus fumigatus* could biosorbed metal in the order Zn>Ni>Cr>Pb and Pb>Cr>Zn>Ni. Biosorption study revealed that biosorption capacity increases with the increase in initial concentration of metal. The variation in the biosorption capacity for various metals varied with the difference in the biomass, and biosorption conditions like pH, temperature and other environmental conditions [20]. In present study biosorption capacity of *Aspergillus fumigatus* was found maximum as compared to *Aspergillus flavus* (Table 3 and 4).

This divergence is due to the intrinsic ability and affinity of organism for different metals. This unique feature of microorganisms is due to its chemical composition of cell wall [21].

Conclusion:-

The present study concludes that heavy metals resistant fungi *Aspergillus fumigatus and Aspergillus flavus* are capable to remediates Cr, Zn, Pb and Ni metals from the contaminated soil. Tolerance capacity of fungal isolates was different in higher concentrations of heavy metals. Both the *Aspergillus sp*. Showed different sorption pattern for lower to higher concentration. At 400 ppm concentration *Aspergillus flavus* and *Aspergillu sfumigatus* were least resistant for Zn and Pb metals. Biosorption treatment of contaminated sites is a new way of safe, reliable and cost-effective method with no secondary product or sludge production.

Table 1:-Heavy metal concentration in soil sample

Heavy metal	**Concentration**
Cr ppm	10.3 ± 1.08
Pb ppm	4.55 ± 1.12
Ni ppm	7.35 ± 0.32
Zn ppm	29.26 ± 1.14
Co ppm	1.5 ± 0.07
Cu ppm	3.5 ± 0.23
Cd ppm	0.05 ± 0.016
Fe ppm	0.52 ± 0.03

Table 2:-Minimum inhibition concentration of *Aspergillusflavus* and *Apsergillus fumigates*.
('+' least resistant; '++' moderate resistant; '+++' highly resistant; '-'No growth)

Metals	**Initial concentration (ppm)**	***Aspergillus flavus***	***Aspergillus fumigates***
Pb	25	+	++
	50	+	+
	100	+	+
	200	-	++
	400	-	+
Zn	25	+++	++
	50	++	+++
	100	+	+++
	200	+	++
	400	+	-
Cr			
	25	++	++
	50	+	++
	100	+	+
	200	-	+
	400	-	+

Ni	25	+++	++
	50	+++	++
	100	++	+
	200	++	-
	400	-	-

Table 3:-Biosorption capacity (mg/g) of *Aspergillus flavus* for lead, Zinc, chromium and nickel
(All values are (Mean ± S. E))

Metal concentration (ppm)	**Pb**	**Zn**	**Cr**	**Ni**
25	13.6 ± 1.66	13.9 ± 0.03	15.3 ± 0.15	15.8 ±0.08
50	32.3 ± 0.33	23.8 ± 0.12	33.4 ± 0.58	28.6 ± 0.26
100	62.4 ± 1.78	58.2 ± 3.5	60.2 ± 0.12	41.2 ± 0.89
200	-	106.3 ± 0.33	-	79.6 ± 0.88
400	-	183.2 ± 1.52	-	-

Table 4:-Biosorption capacity (mg/g) of *Aspergillus fumigatus* for lead, Zinc, chromium and nickel
(All values are (Mean ± S. E))

Metal concentration (ppm)	**Pb**	**Zn**	**Cr**	**Ni**
25	22.5 ± 0.21	12.9 ± 0.66	15.6 ±2.8	12.8 ±0.16
50	41.6 ± 2.9	23.8 ± 0.12	26.1 ± 0.55	23.3 ± 0.14
100	81.6 ± 0.88	47.7 ±0.17	45.2 ± 0.26	37.1 ± 1.73
200	156.6 ± 2.4	75.06 ± 0.54	89.9 ± 0.32	-
400	225.1 ± 0.8	-	175.2 ± 2.18	-

References:-

1. Hutton, M. and Symon, C. (1986). The quantities of Cadmium, Lead, Mercury and Arsenic entering the U.K. Environment from human activities. *Sci. Total Environment*. 57:129-150.
2. Mulligan, C.N., Yong, R.N. and Gibbs, B.F. (2001). Remediation technologies for metal-contaminated soil and ground soil and groundwater: an evaluation. *Engin.Geol*. 60:193-207.
3. Anderson, D. (2003). Introduction to heavy metal monitoring, European environment agency (EPA), environment assessment report No. 10, Europe's environment:- the 3rd assessment, published on web by EEA
4. Shayley, H., McBride, M., and Harrison, E. (2009). Sources and impacts of contaminated in soils. *Cornell waste Management Institute*. 1-6.
5. Wajer, P.A., Hischier, R. and Eugster, M. (2011). Environmental impacts of the Swiss collection and recovery systems for waste electrical and electric equipment (WEEE): A follow-up. *Science of the total environment*. 409:1746 – 1756.
6. Cheng. (2003). Effects of heavy metals on plants and resistance mechanisms. *Environmental Science and Pollution Research.* 10 (4): 256 - 264.
7. Shokoohi, R., Saghi, M.H., Ghafari, H.R. and Hadi, M. (2009). Biosorption of iron from aqueous solution by dried biomass of activated sludge. *Iranian Journal of Environmental Health Science & Engineering.* 6(2): 107-114
8. Gadd GM. (1993). Interactions of fungi with toxic metals. *New phytol*. 124:25–60
9. Tsezos M. and Volesky, B. (1981).Biosorption of uranium and thorium. *Biotechnol. Bioeng.* 23:583-604
10. Heinfling A., Bergbauer, M. and Szewzyk U. (1997). Biodegradation of azo and phthalocyanine dyes by Trametesversicolor and Bjerkanderaadusta. *Applied Microbial Biotechnology.* 48:261-266
11. Volesky, B. and Holan, Z. (1995). Biosorption of heavy metals. *Biotechnol. Progr*. 11: 235-250.
12. Hussien, H., Faraz, S. and Moawad, H. (2003). Isolation and Characterisation of Pseudomonas resistant to heavy metals contaminants. *Arab Journal of Biotechnology*. 7:13-22.
13. Aneja, K. R. (2010). In: Experiments in microbiology, plant pathology and biotechnology (4th edition). New age international (pvt) ltd., New Delhi.
14. Hansson, P.J., Edwards, N.T. and Andrews, J.A. (2003). Effect of different tree species on soil properties.Journal of Applied Ecology.23: 657-666.

15. Puranik, P. R. and Paknikar, K. M. (1999): Biosorption of lead, cadmium and zinc by Citrobacter strain MCM B-181: characterization studies. Biotechnol. Prog., 15: 228-237
16. Cybulski Z, Dzuirla E, Kaczorek E, Olszanowski A, (2003). The influence of emulsifiers on hydrocarbon biodegradation by pseudomonadacea and Bacillacea strains. Spill Science and technology bulletin 8:503-507.
17. Dalal, R. C. and Moloney, D. (2000). Sustainabbility indicators of soil health and biodiversity. In: Management for sustainable ecosystems. Brisbame: Centre for conservation biology, University of Queensland, 101-108.
18. Sharma, M. S. R. and Raju, N. S. (2013). Correlation of heavy metal contamination with soil properties of industrial areas of Mysore, Karnataka, India by CLUSTER analysis. *International Research Journal of Environment Science.* 2(10): 22-27.
19. Ashida, J. (1965). Adaptation of fungi to metal toxicants. *Annu Rev Phytopathol.* 3:153
20. Gupta, R., Ahuja, P., Khan, S., Saxena, R. K. and Mohapatra, M. 2000. Microbial bio- sorbents: meetings challenges of heavy metal pollution in aqueous solution. Curr. Sci. 78 : 967-973
21. Gadd, G M (1993). Interaction of fungi with toxic metals. New Phytol 124: 25-60.