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MYCOREMEDIATION OF HEAVY METALS PRESENT IN CONTAMINATED AGRICULTURAL SOIL

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ABSTRACT

In this study, Mycoremediation technique was used to analyse the potential of mushroom in absorbing heavy metals from contaminated soil. A small village Sitagarha located in Hazaribag, Jharkhand was selected as sampling site. Soil sample was collected from two different sites – from field where cow manure was used as fertilizer and the other soil sample where chemical fertilizer was mixed. A control sample was also collected from nearby barren land where no cultivation was carried out since decades. After initial screening, three heavy metals Cu, Cd and Zn were selected to carry out further studies. The soil sample was processed in laboratory and analysed in AAS to detect the concentration of heavy metals. Results showed the presence of heavy metals as – Cd > Cu > Zn. Further these soil samples were mixed along with straw and spawn to prepare a mushroom cultivation bag as mushroom has the property to absorb heavy metals. The result of absorbance of heavy metals by mushroom was somewhere an indication that mycoremediation proofs to be an important tool to fight against heavy metals.

***Keywords**:* Chemical fertilizer, Heavy metals, Mushroom, Mycoremediation.

Introduction

Mycoremediation is composed of two words – myco means “fungi” and remediation means “restoring” which means removal of any contaminants using fungi. It is a form of Bioremediation working on fungi-based-technology to decontaminate our environment where we reside. Mycoremediation is used in the removal of contaminants mainly from soil and water. Mycoremediation is a promising technology which removes toxic metals through its fruiting body. Fungi have been proved to be the most effective, cheap and environmental friendly remedy for removal of toxic metals from soil and water (Asiriuwa *et al.*, 2013). Mycoremediation is counted amongst the cheapest solution of remediation. Mushroom are saprophytic and they lack chlorophyll hence they cannot carry the process of Photosynthesis. Mushroom is a macrofungi and it acts as an effective biosorbent of toxic metals. Mushroom consists of fruiting body which is composed of cap, a spore forming part called sporophore and stipe (stalk). Mycelium which is the vegetative part of mushroom plays main role in the uptake of heavy metals from the substrate. Previously reported investigation reveal that mushroom contain higher amount of heavy metals as compared to agricultural crops (Demirbas, 2001). Soil is the earth’s skin and we humans are going on polluting it. It is a universal truth that heavy metals are present everywhere in soil whether in minute or higher concentration. Day by day the increasing population has led to rapid industrialization which has ultimately led to increase in environmental pollution. Our mother nature is diminishing day by day. Hence, it is our responsibility to protect our nature. Anthropogenic activities have been one of the major reason due to which soil is subjected to various pollutants (Facchinelli *et al*., 2001 and Jonathan *et al.*, 2004). Through present investigation an attempt has been made to examine the level of contamination of heavy metal in agricultural field of Sitagarha where farmers use chemical fertilizer in huge amount for better yield and quality whenever they cultivate vegetables and crops. The residues of these chemical fertilizer in the soil contain toxic heavy metals which decrease soil fertility, enters into food chain and ultimately into human body. Humans on consumption of such toxic metals leds to accumulation of these toxic metals and hence human health is uplifted to a state of high risk (Denti *et al*., 1998; Sandaa *et al*., 1999; and Arantzazu *et al*., 2000). This article reports research conducted to assess the accumulation of selected heavy metal (Cu, Cd & Zn) present in the contaminated sampling area. The present study focused on bioaccumulation of heavy metals by mushroom which revealed how effective mushroom are to absorb heavy metals from soil. It was also seen that heavy metals vary greatly in different composition of agricultural soil.

Materials & Methods

Study Area:

The site selected for this study was Sitagarha situated in Block – Churchu, District –Hazaribag, State – Jharkhand, Pincode - 825303. Soil sample was collected from Sitagarha. The location lies between Latitude 23.99385°N and Longitude – 85.3927°E. It is a small village containing almost weaker section of people.The people residing in Sitagarha are mainly tribals. The tribal people are socio-economically backward as compared to the non-tribals. They have very low literacy rate as a result, sources of earning are limited and hence agriculture, labouring and forestry is the main source of livelihood. They possess their own culture, way of life, source of livelihood, religious beliefs which are quite different from the others sections of the Indian communities. The tribal women are very hardworking than the tribal men and hence contribute significantly towards their family income. The status of tribal people in terms of education, employment and health is low as compared to people belonging to the general population. The economic as well as the lifestyle of tribal people is nature centric. They use to cultivate vegetables in large amount and locally sell them to earn money. Hence, they draw most of the resources from the Agriculture and Forest.

Sample collection:

Simple random sampling method was followed for sample collection. In this study two soil samples were collected from agricultural fields of Sitagarha along with control sample. Control was collected from the area near by agricultural field where no cultivation has been done since decades. The first sample was collected from the agricultural field where farmer has been using only cow manure (Sample 1) as fertilizer mainly for cultivation of different vegetables. The second sample was collected from the field where farmers used only chemical fertilizers (Sample 2) during cultivation. The sample was collected with sterilized plastic spatula. After removing 3-4cm top layer of soil sample was collected and then was transferred to a pp bag ensuring that the bag was air tight.

Sample preparation and Analysis:

All three samples collected were air dried to remove the moisture content. Over-sized particles were removed. After drying the samples were crushed in a clean dry sterilized mortar and pestle into fine soil. The soil was sieved through 2 mm sieve. 3g of sieved soil was weighed and then digested with a mixture of 10ml conc. HCL and 3.5 ml conc. HNO_3. The mixture was left overnight without any heating process under the switch-on fume cupboard and heated for 2 hours at 104° C on the next day. Distilled water was added to the digested sample and then filtered using a Whatman filter paper and the final volume was set to 100 ml using distilled water. The solution was then transferred into sampling bottles for analysis. Further the concentration of Cu, Cd, and Zn was analysed using in Atomic Absorption Spectrophotometer (Ogundele *et al*., 2015).

Preparation of Mushroom bag mixed with soil sample: The straw was dipped in water overnight (For 1kg straw, 10L water was required). Formaldehyde was added in water and mixed well (1ml formaldehyde/1L water).The next day the wet straw was taken out and allowed to dry leaving a little moisture to it. The same day the mushroom bag was prepared. pp bags were tied with rubber at its base to give it a round shape. First layer of straw was made in the bag around 3cm in height. Then the soil samples were layered above the straw and then spawn was embedded in the soil samples so that when fruiting body starts to grow it absorbs the heavy metals present in soil sample. Then, again a layer of straw was added on top. Finally, the bag was tied tightly with a rubber band and then some holes were made in the layer of the spawn to let the mushroom grow outward. The mushroom bags were kept in a dark room (23°C) as required for better cultivation oyster mushroom *(Pleurotus ostreatus)*. Water was sprinkledat regular time interval of 2 days to maintain the moisture up to 80%.Within 10 days mycelium was seen growing and the bag turned to be whitish. After 2-3 day, fruiting bodies starts growing. Fruiting bodies was plucked on 3rd day and 7th day to check the difference in concentration of absorbed heavy metals.

Determination of the total Heavy metal in the Mushroom:

5 g of mushroom from each bag of sample was taken and dried in the oven at 105°C for 1 hour, 1 g of the dried sample was weighed into a 50 mL beaker and placed in a muffle furnace set at 500°C. The mushroom was left to ash for 3 hrs. Then 10 mL of the ashed mushroom sample was taken and dissolved in 20% HNO_3 and heated gently on a hot plate for about 5mins. The sample was then allowed to cool and then filtered into 5mL flask. The heavy metals in the sample were then determined using Atomic Absorption Spectrophotometer.

Results and Discussion

This study was an attempt to check the level of concentration of heavy metal in the agricultural field of Sitagarha. Results revealed that there is quite a high concentration of heavy metals in soil sample collected from the field where chemical fertilizer was used since years as compared to soil sample collected from the field where cow manure is used as fertilizer. The presence of Cadmium in high amount is also a matter of concern as Cadmium is extremely toxic to human, and adversely affects kidneys, and bones (Greenpeace, 2008). Cadmium is used in the manufacture of pesticides, herbicides used in agriculture (Alloway *et al*., 1998).

Sampling site :

Fig.1 shows the map of sampling site i.eSitagarha.Churchu is one of the Block of Hazaribag where Sitagarha is located.

Fig.1 : Map of Hazaribag

Soil sample :

Fig. 2 and 3 shows soil sample from cow manure field (Sample 1) and chemical fertilizer (Sample 2) field respectively. By seeing the colour and texture of the soil sample in Fig.2 and 3 one can easily identify the soil sample containing cow manure.

Analysis of concentration of heavy metal present in soil samples:

The amount of total heavy metal present in the contaminated soil sample can be seen in Table1. The results collaborates the finding of Jyothish *et al*. which showed that high concentration of heavy metal are present in chemical fertilizer. Table1 shows the concentration of Cu, Cd and Zn to be 54.34 ppm, 2.81 ppm and 78.62ppm respectively in soil sample containing chemical fertilizer. In soil sample containing cow manure reveals the concentration of Cu 31.02 ppm, Cd 1.25 ppm and Zn 44.21 ppm. The amount of all three heavy metals was high in sample containing chemical fertilizer. This shows that regular use of chemical fertilizer may lead to accumulation of heavy metal in agricultural soil in high concentration in the coming years which may not be safe for human health it enters into the food chain.

Mushroom bag preparation cultivation of mushroom:

Oyster Mushroom (*Pleurotus ostreatus*) bag was prepared as shown in Fig.4,5 and 6 along with soil sample containing cow manure and chemical fertilizer respectively. Oyster spawn was embedded in the soil sample so that when fruiting body grows it absorbs heavy metal from the soil sample as reported by Asiriuwa *et al.* mushroom are capable of absorbing heavy metals. The layering of straw, soil sample and spawn can be seen clearly in the Figure 4, 5 and 6.

Fig.2 : Sample 1

Fig.3 : Sample 2

Fig.4 : Straw+ Control+Spawn

Fig.5 : Straw+ Sample1+Spawn

Fig.6 : Straw+ Sample 2+Spawn

Analysis of Heavy metal absorbed by mushroom:

The result in Table 2 shows the concentration of heavy metal absorbed by the fruiting body of mushroom on 3rd and 7th day of fruiting. The result clearly shows how effectively heavy metals are absorbed by mushroom. The concentration of Cu, Cd and Zn is shown in the bar diagram in Fig.8, 9 and 10 respectively. After detection, it was observed that mushroom absorbed highest concentration of Zn (17.44ppm) from the soil sample containing chemical fertilizer. The concentration of accumulation of Cu, Cd and Zn was gradually seen to increase day by day in the fruiting body of mushroom

Copper (Cu): On 3rd and 7thday of fruiting, Fig.8 reveals the concentration of Cu present in control, sample 1 and sample 2. On 3rd day of fruiting, the concentration of Cu in control was 0.008ppm, sample 1 was 0.324ppm and sample 2 was 0.831ppm. On 7th day, the concentration was increased to a level of 0.027ppm, 7.62ppm 12.13ppm in control, sample 1 and sample 2 respectively. Soil contaminated with heavy metal directly or indirectly has adverse effects. It directly affects by decreasing the crop yield and productivity and indirectly by entering the food chain. Copper is indeed regarded as essential metal for the body but in high doses it can cause anaemia.

Cadmium (Cd): Fig.9 reveals the concentration of Cd on 3rd and 7thday of fruiting. On 3rd day of fruiting, the concentration of Cd in control was 0.003ppm, sample 1 was 0.081ppm and sample 2 was 0.108ppm. On 7th day, the concentration was increased to 0.011ppm, 0.481ppm 0.972ppm in control, sample 1 and sample 2 respectively. In human body, Cadmium is known to affect several enzymes as seen in renal damage which results in proteinuria is the result of Cadmium adversely affecting enzymes responsible for reabsorption of proteins in the kidney tubules.

Zinc (Zn): Fig.10 shows the highest concentration of Zn among all the selected heavy metals. The absorption rate was high in case of zinc. On 3rd day of fruiting the concentration of Zn in control was 0.013ppm, sample 1 was 0.664ppm and sample 2 was 0.1.42ppm. On 7th day, the concentration was increased to 0.094ppm, 010.32ppm 17.44ppm in control, sample 1 and sample 2 respectively. The main reason behind increase in zinc concentration is due to increase in anthropogenic activities such as mining, coal, and waste combustion and steel processing. From the present study it is evident that oyster mushroom are capable of absorbing heavy metals from contaminated soil. On the other hand the technology proofs to be a great boon to the society since it is very cost effective.

Table 1 : Concentration of heavy metal of the contaminated soil samples (in ppm)

Heavy metal	Control	(Sample 1)	(Sample 2)
Cu	0.132	31.02	54.34
Cd	0.040	1.25	2.81
Zn	0.215	44.21	78.62

Table 1: Concentration of heavy metals uptake by mushroom (in ppm)

Heavy metal	Control		Sample 1		Sample 2	
	3rd Day	7th Day	3rd Day	7th Day	3rd Day	7th Day
Cu	0.008	0.027	0.324	7.62	0.831	12.13
Cd	0.003	0.011	0.081	0.481	0.108	0.972
Zn	0.013	0.094	0.664	10.32	1.42	17.44

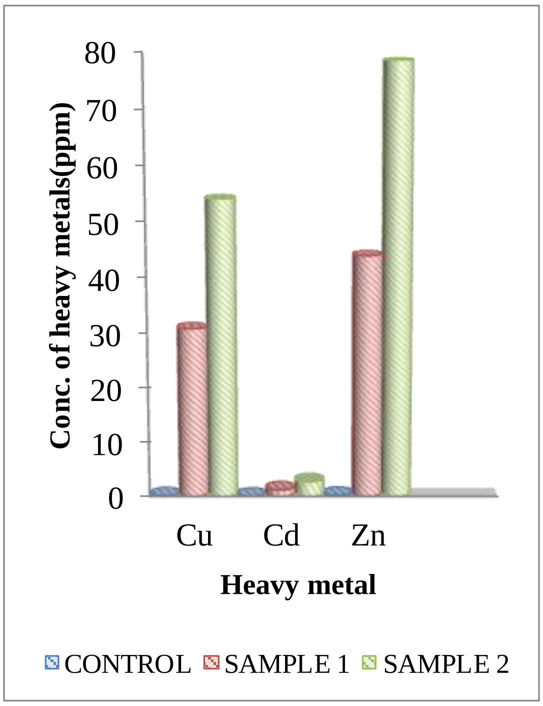

Fig.7:Concentration of amount of heavy metals present in soil samples

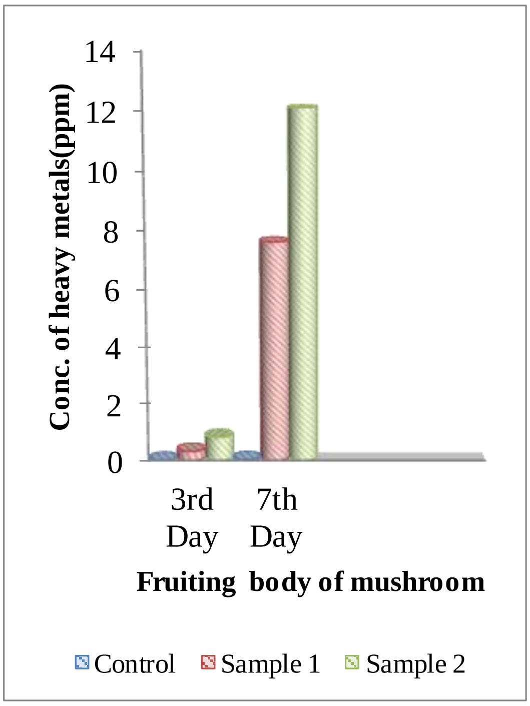

Fig.8: Concentration of Cu absorbed by mushroom (ppm)

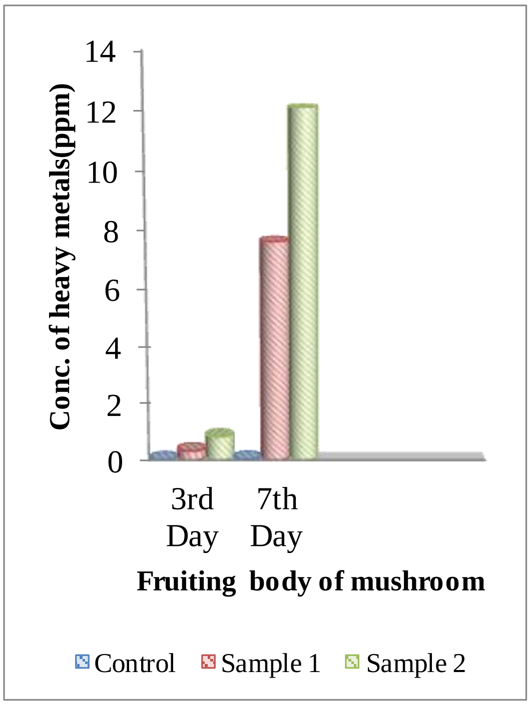

Fig.9: Concentration of Cd absorbed by mushroom (ppm)

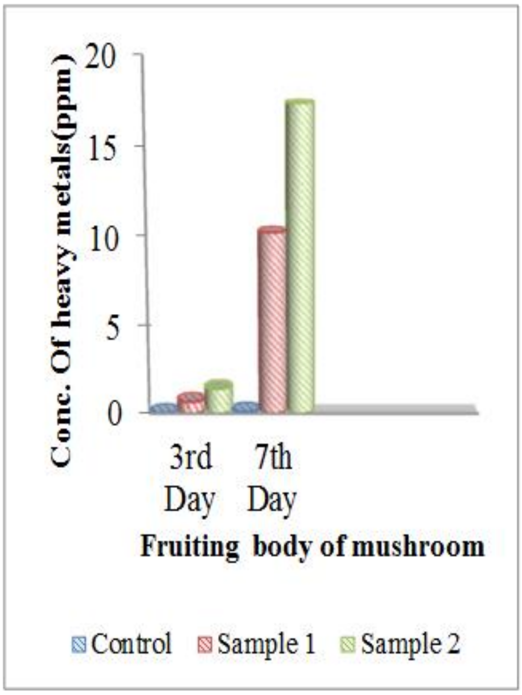

Fig.10: Concentration of Zn absorbed by mushroom (ppm)

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