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Efficacy of Pleurotus Species for Mycoremediation of Heavy Metals – A Case Study of Vinoba Bhave University, Hazaribag

Manisha Shalini Besra[1]* Prashant Kumar Mishra[2]

[1,2] Department of Biotechnology, Vinoba Bhave University, Hazaribag, Jharkhand, India

Abstract – In this study, Mycoremediation, which is regarded as a powerful tool for remediation of heavy metal is used, to remediate the soil from heavy metal contamination. Soils contain heavy metals in minute range which ultimately leads to accumulation in high concentration after a certain time period. Hence, to fight against this problem a cost effective as well as eco-friendly technique is required to be implemented. Mycoremediation somewhere is a perfect answer to the solution as it does not require much energy input and gives quick results. To study how efficient Mycoremediation is, 3 soil samples were collected from three different stations of Vinoba Bhave University campus, Hazaribag. Three heavy metals Cu, Cd and Zn were selected after initial screening and further study was carried out. All 3 soil samples were processed in lab and then analysed in AAS to detect the presence of Cu, Cd and Zn. The soil sample collected from 3 different stations contained overall range of Cu in 42.06mg/kg – 59.61mg/kg, Cd was in range of 0.91mg/kg – 3.61mg/kg while Zn was 43.11mg/kg – 64.32mg/kg. Soil samples were further mixed in an oyster mushroom bag as the genus has been reported to accumulate high levels of heavy metals in their mycelium. Later, mushrooms were harvested on 3rd, 7th and 10th day and then tested to detect the level of absorption of heavy metals. Zn showed the highest rate of absorption amongst the three heavy metals. As reported in earlier investigation oyster mushrooms can serve as a great bioremediator tool for heavy metal absorption. Hence, the present study clearly focuses on the bioaccumulation of heavy metal by oyster mushroom (Pleurotus ostreatus). The absorbed concentration reveals how effective mushroom can be as a remediator tool in case of heavy metal pollution.

Keywords: Mycoremediation, Heavy Metals, Soil, Oyster Mushroom, Bioaccumulation.

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INTRODUCTION

Soil may be defined as a product of interaction of several factors such as the influence of organisms, climate, minerals present etc. Soil acts as an emerging medium in different function it performs. Increased rate of anthropogenic activities have deteriorated the soil entity (Facchinelli *et al.*, 2001, Jonathan *et al.*, 2004). The presence of toxic metal in soil is becoming a matter of concern as these toxic metal reduces the fertility of the soil, enters food chain which eventually leads to severe health problems (Denti *et al.*, 1998, Sandaa *et al.*, 1999). Although various chemical and mechanical methods for removal of heavy metals are available but all of them are costly, energy consuming and complicated. Mycoremediation on the other hand is an eco-friendly and cost effective techniques where fungal hypae especially mushroom are utilised for biosorption of heavy metals from substratum. These trace metal can be removed by a promising technology "Mycoremediation" which uses fungi as a remediation tool. Mycoremediation is listed amongst the best tool to remove heavy metals from soil and water. It comes under the category of very cost effective technique and gives quick results. Pleurotus species is commonly known as Oyster mushroom and it belongs to Basidiomycetes of fungi. Although approximately 70 species of this genus have been identified. 7 of them are commonly found. Typical of mushroom, they are Saprophytic and are mainly found in rainy season especially in forest ecosystem. They are highly adaptive to different climatic condition (Agrahar Murugkar & Subbuakshmi 2005) and they prefer different agricultural waste for their growth. Pleurotus species is an important edible mushroom but in recent past their capacity of Biosorption has attracted a large number of researchers (Tay et al., 2011; Das et al., 2007, Dulay et al., 2015; Arbanah et al., 2012; Prasad et al., 2013; Favero et al., 1990) etc. have indicated that different portions of oyster mushroom differently accumulate heavy metals. Keeping

these factors in mind, an attempt was made to examine the level of contamination of heavy metals in the campus of Vinoba Bhave Universiy, Hazaribag. Soil samples were collected to examine the presence of heavy metal. As we know everything in excess in harmful; increased rate of heavy metal in soil is harmful for the plants.

MATERIALS & METHODS

Sample collection:

Vinoba Bhave University campus was selected as the sampling area. The University was established in the year 1992. Soil samples were collected from 3 different stations i.e. Samraat Ashok Bhawan garden, Vehicle stand and Botanical garden of the University campus. For sample collection, simple random sampling method was applied. Sterilized plastic spatula was used to collect the soil samples. After removing 3-4cm top layer of soil, sample was collected in a pp bag ensuring that the bag was air tight. Five replica of experiment was maintained and average was taken.

Determination of Physio-chemical properties:

Soil samples collected from 3 stations were characterized by testing its physio-chemical properties; texture, pH, particle size, organic C, organic matter, P, N, K and cation exchange capacity were determined by standard methods.

Sample preparation and Analysis:

The soil samples were air dried to remove the moisture content. Oversized particles were removed and then crushed in a clean sterilized dry mortar and pestle to generate fine soil. The obtained fine soil was sieved through 2mm sieve. Thereafter, 3g soil sample was weighed and digested with a mixture of 10ml conc.HCl and 3.5ml conc.HNO_3. The mixture was left overnight without any kind of heating process under switch on fume cupboard. On the next day, it was heated for 2hrs at 104°C. Distilled water was added to the digested sample and was filtered with whatman filter paper and then using distilled water, final volume was set to 100ml. The solution was further transferred into sampling bottles for analysis of concentration of Cu, Cd and Zn using AAS (Ogundele *et al*., 2015).

Preparation of Mushroom bag mixed with the soil sample:

Standard protocol was followed to prepare oyster mushroom bags. 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, wet straw was decanted and allowed to dry leaving around 60 – 70% moisture in it. The same day mushroom bag was prepared. pp bags were taken and first layer of straw was made in the bag around 3cm in height. Then, a layer of soil sample was added and then spawn was embedded in the soil samples. Then, again a layer of straw was added on top to cover the spawn completely. The bag was tightly packed and few holes were made in the layer of the spawn to let the fruiting body grow outward. The mushroom bags were kept in a dark room (23°C) as required for better cultivation oyster mushroom *(Pleurotus ostreatus)*. Water was sprinkled at a time interval of 2 days, to maintain the moisture up to 80%. Spawn run was observed within 15 days and after few days mycelium was seen growing and the bag turned to seems whitish. After 2-3 day, fruiting bodies start growing. Fruiting bodies was harvested on 3rd day, 7th day and on 10th day to check the difference in concentration of absorbed heavy metals.

Determination of the total Heavy metal in the Mushroom:

5g of oyster mushroom from each bag of sample was harvested on 3rd day, 7th day and on 10th day and dried in the oven at 105°C for 1 hour, 1g of the dried sample was weighed into a 50 mL beaker and then placed in a muffle furnace, set at 500°C. The mushroom was left to ash for 3hrs and then 10 mL of the ash mushroom sample was 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 sample were then analysed in Atomic Absorption Spectrophotometer (Asiriuwa *et al*., 2013).

RESULTS AND DISCUSSION

Physio-chemical properties of soil samples:

Physiochemical properties of soil samples collected from different sites of Vinoba Bhave University campus is presented in **Table 1**. Texture of soil in Samrat Ashok Bhawan garden, Vehicle stand and Botanical garden is sandy loam. As the distance of 3 stations is not much, hence, variation in texture is not observed. Percentage of sand in garden and botanical garden is 33% and 37% respectively. Percentage of sand in vehicle stand is much higher and was recorded 64%, clay was 6% and 7.3% in garden soil and botanical garden soil. Percentage of clay was comparatively high in vehicle stand and was recorded 16%. Silt was 32% and 37% in Samrat Ashok Bhawan garden soil and botanical garden soil respectively. Whereas, silt content in soil collected from vehicle stand was 47%, once again higher than the earlier 2 values. pH of Samrat Ashok Bhawan garden soil and Botanical garden soil was 6.6 and 6.3 which indicated acidic nature. pH of soil collected from vehicle stand was 7.4 i.e slightly alkaline. Carbon content was high in 2 garden sites i.e Botanical garden and Samrat

Ashok Bhawan garden. Carbon content near vehicle stand was much low i.e 8.2%. Higher Carbon content in 2 garden sites is obviously because of decaying leaves nd high microbial activities which is not upto that mark in vehicle stand. This explanation is further substantiated by the value of organic matter recorded in Samrat Ashok Bhawan garden i.e 32% and Botanical garden i.e 38%. Nitrogen content was also higher in soil from Samrat Ashok Bhawan garden and botanical garden as compared to the soil present in vehicle stand. The overall difference in soil property is also reflected in electric conductivity which is much higher in soils of garden than soil collected from vehicle stand.

Detection of heavy metal concentration in study sample:

Range of heavy metals and their mean for three heavy metals i.e. Cu, Cd and Zn presenting **Samrat Ashok Bhawan garden** is presented in **Table 4.2.** Cu ranged from 42.06 – 48.26mg/kg. The mean value was 44.9 ± 3.17 mg/kg. Range of Cd in this sample was 3.18 – 3.61mg/kg. Mean value for Cd 3.34 ± 0.23 mg/kg. Zn ranged from 49.33 – 54.81mg/kg. Mean value for Zn was 52.16 ± 2.74 mg/kg.

Range and mean of heavy metals in soil of **Vehicle stand** is recorded in **Table 4.3.** Here, Cu ranged from 54.34 – 58.13mg/kg. Mean value for Cu was 56.19 ± 1.8mg/kg. Concentration of Cd was much low and it varied from 0.91 – 1.24mg/kg. The mean of the value was 1.09 ± 0.16mg/kg. Concentration of Zn varied from 43.11 – 46.14mg/kg. The mean value was 44.52 ± 1.52 mg/kg.

Range and mean of three heavy metals is depicted in **Table no. 4.** The sample in this case collected from **Botany garden**. Cu here ranged between 52.30 – 59.61mg/kg. Mean value was 55.04 ± 3.77mg/kg. Range of Cd was on lower side and it varied from 1.21 – 1.93mg/kg. Mean value was 1.5 ± 0.37mg/kg. Zn in this sample varied from 56.91 64.32 mg/kg. The mean value was 59.85 mg/kg.

Table 4.5 describes range of heavy metals and mean concentration present in **mixture** of soil (equal proportion) collected from 3 selected stations i.e Samrat Ashok Bhawan garden, Vehicle stand and Botanical garden. The data presented is mean of 5 replicas. Range of Cu varied from 38.16 – 48.72 mg/kg. The mean value was 43.14 ± 5.2mg/kg. Cd ranged from 1.62 – 3.34mg/kg and the mean was 2.02 ± 1.06 mg/kg. Similarly, Zn was recorded 41.48 - 51.62 mg/kg. The mean of 5 replicas was 47.15 ± 5.17 mg/kg. The variation was analysed statistically and was found significant.

Detection of the total Heavy metal in the Mushroom on 3rd, 7th and 10th day:

Absorption of heavy metals by Oyster mushroom was recorded after 3 days, 7 days and 10 days. The mean for absorption from sample of **Samrat Ashok Bhawan garden** is presented in **Table no. 4.6.** Mean absorption of Cu was 4.79 ± 2.6mg/kg after 3days whereas the mean of absorption recorded after 7days was 17.22 ± 4.6 mg/kg. The experiment was extended to 10th day to see the possible change in absorption of heavy metals. Slight variation was observed in case of Cu where 0.06mg/kg of these heavy metals was absorbed further. Mean absorption of Cd after 3 days was recorded 0.05 ± 0.0006 mg/kg whereas after 7 days the total absorption was recorded 0.82 ± 0.14mg/kg. Absorption of Cd on 10th day was more as compared to the value of 7th day and the increase value was 0.06mg/kg.Uptake of Zn in this sample was 4.71 ± 0.02mg/kg after 3 days whereas the total absorption increases 17.52 ± 0.9mg/kg. Zn on the other hand, exhibited a lower value of concentration on 10th day i.e.

Similar observation was made with the soil sample recorded from **Vehicle stand** which is presented in **Table 4.7.** Total absorption for all three heavy metals was noted down after 3days and 7days. Cu was absorbed 4.06mg/kg which further increased to 14mg/kg after 7days. On 10th day Cu absorption increased by 0.95 mg/kg as compared to the value recorded on the 7th day. Cd uptake was 0.02 mg/kg and 0.24 mg/kg after 3days and 7days respectively. Increase in Cd content in experimental condition after 10th day was recorded 0.04 mg/kg. 5.32mg/kg and 16.63mg/kg was the total uptake of Zn recorded after 3 days and 7days respectively. Zn further reduced 15.53 mg/kg as compared to 16.63 mg/kg recorded after 7days.

Table 4.8 represents mean concentration of 3 heavy metals absorbed by mushroom from soil of **Botanical garden** after 3days, 7days and 10 days. Absorption of Cu recorded was 6.02mg/kg after 3 days and 18.41mg/kg after 7 days. In this set of experiment Zn absorption increased by 0.21mg/kg after 10days. Cd absorption was 0.04 mg/kg and 0.62 mg/kg after 3days and 7days respectively. Increase in Cd absorption took place by 0.9mg/kg .7.24 mg/kg and 21.41 mg/kg of Zn were absorbed by Oyster mushroom from the soil of Botany garden after 3days and 7days respectively. Content of Zn was reduced by 2.1mg/kg after 10days.

Table 4.9 is depicted for **mixture** of soil from all the three stations selected during this study. Almost similar trend was observed in the mixture of sample taken into consideration. Cu absorption was 5.39 mg/kg after 3days and 15.33 mg/kg after 7days. Absorption of Cu increased from 15.33

mg/kg to 16.28 mg/kg after 10days. Likewise 0.06 mg/kg and 0.51 mg/kg was absorption of Cd after 3days and 7days respectively. Cd enhanced by 0.11 mg/kg when absorption period was extended to 3days further. Our test material Oyster mushroom observed 8.34 and 18.36 mg/kg of Zn after 3days and 7days respectively. Zn content after 10days once again decreased by 2.25 mg/kg.

Table 4.1.: Physio-chemical properties of soil samples

S. No.	Property	Garden Soil	Vehicle Stand	Botanical Garden
1.	Texture	Sandy loam	Sandy clay	Sandy Loam
2.	Sand (%)	33 ± 2.02	64 ± 5.43	37 ± 2.7
3.	Clay (%)	6 ± 0.3	16 ± 3.02	7.3 ± 1.4
4.	Silt (%)	32 ± 1.8	47 ± 4.11	37 ± 1.4
5.	pH	6.6 ± 0.3	7.4 ± 0.2	6.3 ± 0.21
6.	C (%)	14 ± 2.8	8.2 ± 1.3	24 ± 2.02
7.	Organic matter (%)	32 ± 2.3	12.3 ± 1.7	38 ± 4.01
8.	N (%)	8 ± 1.2	2.01 ± 0.8	14.2 ± 2.8
9.	P (mg/kg)	2.32 ± 0.3	1.36 ± 0.5	3.01 ± 1.2
10.	K (Cmol/kg)	1.02 ± 0.04	0.37 ± 0.01	1.41 ± 0.7
11.	CEC (Cmol/kg)	21.3 ± 3.1	7 ± 1.33	19.02 ± 2.31

Table 4.2: Range and Mean concentration of heavy metals from Samrat Ashok Bhawan garden

Heavy metal	Range (mg/kg)	Mean ± S.D (mg/kg)
Cu	42.06 - 48.26	44.29 ± 3.17
Cd	3.18 - 3.61	3.34 ± 0.23
Zn	49.33 – 54.81	52.16 ± 2.74

Table 4.3: Range and Mean concentration of heavy metals from University vehicle stand

Heavy metal	Range (mg/kg)	Mean ± S.D (mg/kg)
Cu	54.34 – 58.13	56.19 ± 1.89
Cd	0.91 – 1.24	1.09 ± 0.16
Zn	43.11 – 46.14	44.52 ± 1.52

Table 4.4: Range and Mean concentration of heavy metals from University Botanical garden

Heavy metal	Range (mg/kg)	Mean ± S.D (mg/kg)
Cu	52.30 – 59.61	55.04 ± 3.77
Cd	1.21 – 1.93	1.5 ± 0.37
Zn	56.91 – 64.32	59.85 ± 3.9

Table 4.5: Range and Mean concentration of heavy metals when mixed in equal proportion of sample 1 + 2 + 3

Heavy metal	Range (mg/kg)	Mean ± S.D (mg/kg)
Cu	38.16 – 48.72	43.14 ± 5.2
Cd	1.62 – 3.24	2.02 ± 1.06
Zn	41.48 – 51.62	47.15 ± 5.17

Table 4.6: Mean concentration of 3 heavy metals absorbed by oyster mushroom after 3 days, 7 days & 10 days in Samrat Ashok Bhawan garden soil sample

Heavy metal	Mean ± S.D (mg/kg)		
	3rd Day	7th Day	10th Day
Cu	4.79 ± 0.026	17.22 ± 4.6	17.28 ± 2.1
Cd	0.05 ± 0.006	0.82 ± 0.14	0.88 ± 0.16
Zn	4.71 ± 0.027	17.52 ± 0.98	16.39 ± 0.74

Table 4.7: Mean concentration of 3 heavy metals absorbed by oyster mushroom after 3 days, 7 days & 10 days in University vehicle stand

Heavy metal	Mean ± S.D (mg/kg)		
	3rd Day	7th Day	10th Day
Cu	4.06 ± 0.73	14 ± 2.8	14.95 ± 2.8
Cd	0.02 ± 0.063	0.24 ± 0.02	0.28 ± 0.01
Zn	5.32 ± 0.031	16.63 ± 0.43	15.53 ± 0.40

Table 4.8: Mean concentration of 3 heavy metals absorbed by oyster mushroom after 3 days, 7 days & 10 days in Botanical garden

Heavy metal	Mean ± S.D (mg/kg)		
	3rd Day	7th Day	10th Day
Cu	6.02 ± 0.12	18.41 ± 0.48	18.62 ± 0.31
Cd	0.04 ± 0.002	0.62 ± 0.08	0.71 ± 0.02
Zn	7.24 ± 0.35	20.41 ± 0.32	18.31 ± 2.1

Table 4.9: Mean concentration of 3 heavy metals absorbed by oyster mushroom after 3 days, 7 days & 10 days in mixture (sample 1 + sample 2 + sample 3)

Heavy metal	Mean ± S.D (mg/kg)		
	3rd Day	7th Day	10th Day
Cu	5.39 ± 0.9	15.33 ± 1.2	16.28 ± 0.8
Cd	0.06 ± 0.009	0.51 ± 0.01	0.62 ± 0.03
Zn	8.34 ± 0.2	18.36 ± 0.03	16.11 ± 0.01

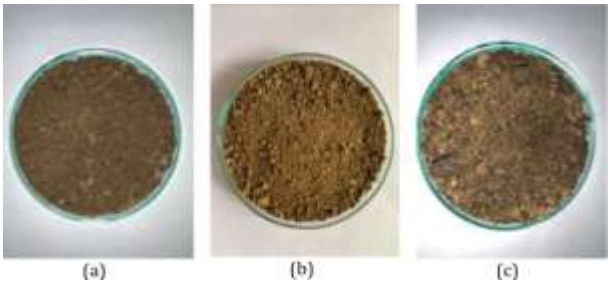

Fig.1: (a) Soil sample from Samrat Ashok Bhawan garden (b) Soil sample from Vehicle stand (c) Soil sample from Botanical garden

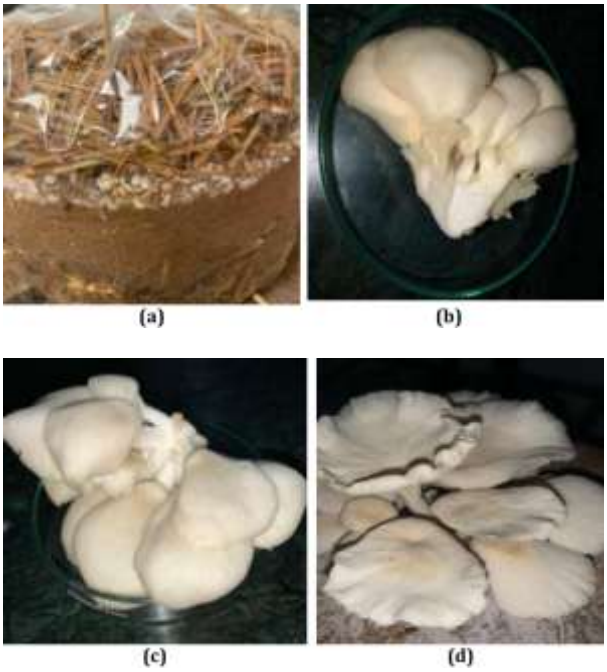

Figure 2 (a) Oyster mushroom cultivation bag (b) Fruiting body (3rd day) (c) Fruiting body (7rd day) (d) Fruiting body (10th day)

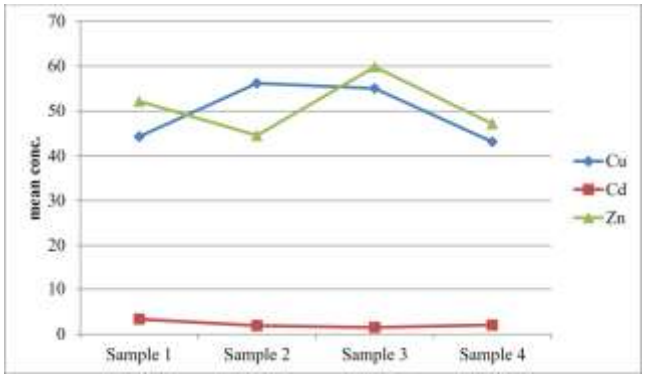

Fig.3: Mean concentration of heavy metal in all 4 soil samples

Note: Sample 1 – Soil sample collected from Samrat Ashok Bhawan garden

Sample 2 – Soil sample collected from University Vehicle stand

Sample 3 – Soil sample collected from University Botanical garden

Sample 4 – Mixture of all 3 soil samples

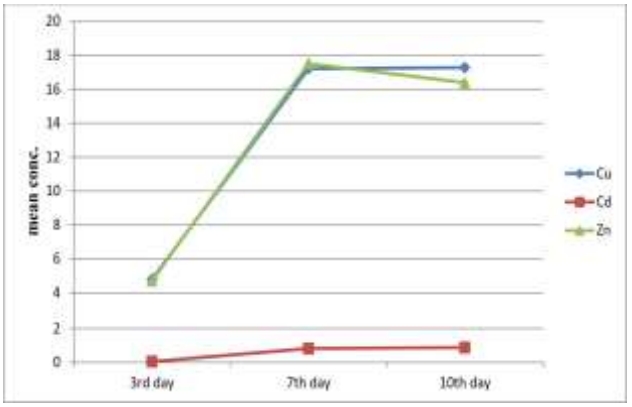

Fig. 4: Absorption of heavy metals by Oyster mushroom from sample of Samrat Ashok Bhawan garden on 3rd, 7th day and 10th day.

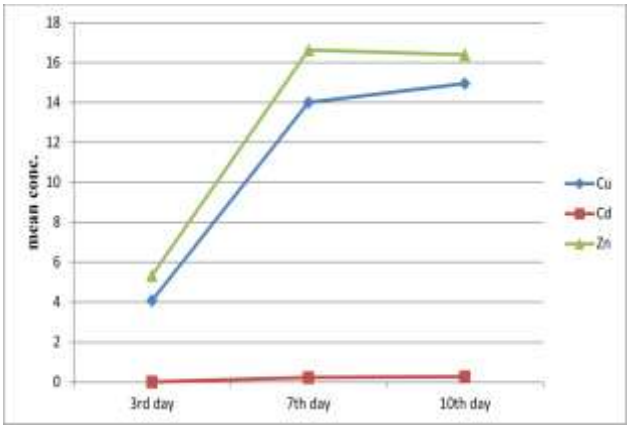

Fig. 4: Absorption of heavy metals by Oyster mushroom from sample of University Vehicle stand on 3rd, 7th day and 10th day.

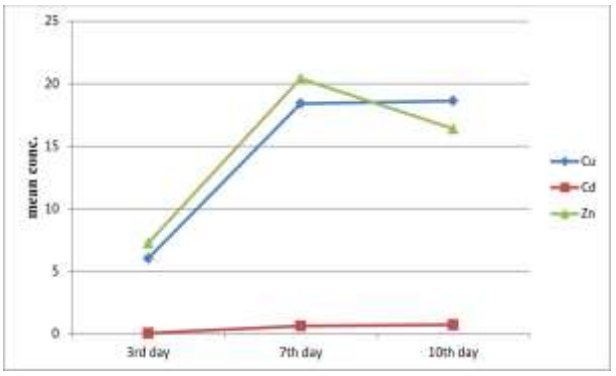

Fig. 5: Absorption of heavy metals by Oyster mushroom from sample of University Botanical garden on 3rd, 7th day and 10th day.

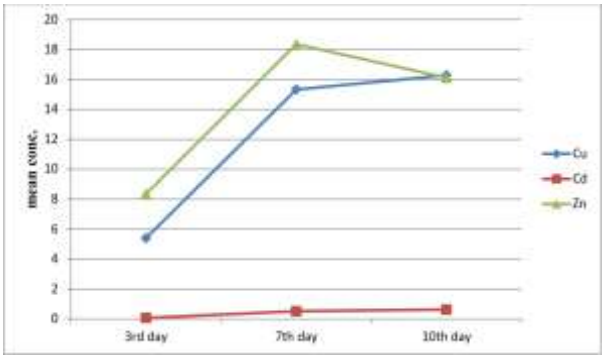

Fig. 6: Absorption of heavy metals by Oyster mushroom from sample of Mixture of all 3 soil samples on 3rd, 7th day and 10th day.

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Corresponding Author

Manisha Shalini Besra*

Department of Biotechnology, Vinoba Bhave University, Hazaribag, Jharkhand, India

mani4nov@gmail.com