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Potential of Spent Bags of Milky Mushroom *Calocybe Indica* in Bioaccumulation of Heavy Metals from Tannery Effluent

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Abstract: ***In Present study, Biosorption of heavy metals by spent bags of milky mushroom mycelium along with paddy straw were used for effluent treatment. To enhance the treatment effect, co-metabolites like Carbon Source (Glucose, Sucrose), Nitrogen Source (Ammonium Chloride, Urea), and Phosphorous Source (Orthophosphoric acid) were added. After treatment with fungal mycelium, the effluent were analysed by UV -Visible Spectrophotometer to determine the growth and Atomic Absorption Spectrophotometer for biosorption of heavy metals by the fungal mycelium and treated effluent. The toxicity of treated effluent was studied by germinating green gram seeds, the root and shoot lengths were measured and seedling index vigour were calculated. Bioaccumulations of heavy metals were comparatively high in presence of glucose and phosphorous as co-metabolites sources as observed from Atomic Absorption Spectrophotometer analysis.***

Keywords: Bioaccumulation, Atomic Absorption Spectrophotometer, Co-metabolites Glucose and Phosphorous, Bioassay

1. Introduction

Environmental pollution is one of the major problems of the world and it is increased day by day due to urbanization and industrialization. Large amount of toxic and recalcitrant pollutants produced by the industrial process are disposed into water reservoirs, as a contaminated effluent. These leads to environmental damages and directly influencing the aquatic ecosystem and in turn human being. Heavy metals contamination is significantly affecting human being too the ecosystems (Margesin & Schinner, 2001)[1]. The ultimate solution for pollution abatement is bioremediation which is the most efficient strategy to manage and recover the contaminated environment (Ahemad&Khan, 2011)[2].

Bioremediation is a way of cleaning up heavy metals using biomass (or micro organism) through the process of biodegradation, biosorption, bioaccumulation and bioconversion operating in different ways (Kulshreshtha et al., 2014; Mose et al., 2016). Biosorption is a passive process and heavy metals get adsorbed on the surface of the biosorbent (Velasquez and Dussan, 2009) exhibiting the tolerance of biosorbent towards heavy metals.

Mycoremediation is a process of using fungi to return an environment contaminated by pollutants to a less contaminated state (Asiruwa et. al., 2013) Biosorbent from mushrooms can be prepared from mycelium or fruit body (live or dead) and spent mushroom substrate (SMS). The factors like the presence of microbial population, the availability of contaminants to these organisms, metal ion concentration and environmental factors like temperature, pH and the presence of nutrients affect the biosorption process in totality (Prakash, 2017). The potential of fungal biomass as biosorbent has been accepted for the removal of heavy metals and radionuclides from polluted waters because of their excellent metal binding properties and tolerance towards metals (Yazdani et al.2010; Salman et al., 2014). *Pleurotus* species have also been assessed for the removal of different heavy metals from chemical laboratory wasted in the form of live mycelia (Arbanah et al., 2012, 2013)

Numbers of basidiomycetes are reported to have heavymetals ions removal from wastewater since their mycelium excretes enzymes that breakdown complex substance into simpler molecules and absorb heavy metals (Gadd, G M., 2000). The present study is to see their efficiency of biosorption of heavy metals by spent bags of *Calocybe indica* from tannery effluent by adding different co-metabolites as source (Kamalasankari S A M & Karpagam S, 2017) and heavy metals absorptions from tannery effluent by milky mushroom were determined.

2. Materials and Methods

2.1 Collection of Samples

The sample was collected from the tannery industries in 10 liters clean can and stored at room temperature. After Harvesting spent bags of Milky mushrooms were collected from Indis milky mushrooms, Karumpukuppam, Ambetkar Nagar, New Gummidipoondi, Thiruvallur – 601 201. Fungal mycelium along with paddy straw was used for effluent treatment.

2.2 Mycoremediation of Tannery Effluent by Spent Bag of *CALOCYBE INDICA*

Untreated effluent (100ml) was amended with 1mM Co-metabolites namely Carbon (Glucose and Sucrose), Nitrogen (Ammonium chloride and Urea), and Phosphorous (Orthophosphoric acid) were added in the concentration of 1mM. To this 10g of Fungal mycelium along with paddy straw was inoculated. Set up was maintained for seven days. After seven days, the treated effluent was filtered with

muslin cloth. The wet weight and dry weight of the fungal mycelium were determined and tabulated. The treated effluent was analyzed at 660 nm in UV-Visible Hitachi Spectrophotometer.

2.3 Heavy Metal Analysis of Treated Effluent

2.3.1 Hot plate method (acid digestion of water sample)

Samples of 5ml was mixed with 5ml of Conc. Nitric acid and heated slowly until the content reduced to half in a Hot Plate. To the residue, add 2ml of H_2O_2 were added and heated until it reduced to half of the volume. The sample was transferred to standard flask and made up to 50ml, by adding distilled water and analyzed for Cr, Cd, Zn, Cu, Fe and Pd. Water was taken as blank and the procedure repeated. The Atomic Absorption Spectrophotometry was heated with cathode lamp, the air acetylene flame was ignited and instrument was calibrated with different working standards (Vanloon & Lichwa, 1973).

2.3.2 Soxhlet method (acid digestion of dried fungal mycelium):

Dried fungal mycelium powder of 2 gm were taken in round bottom flask, to this 5ml of HNO_3, 2ml of HCl and 1 ml of H_2O_2 were added. It was kept in soxhlet for acid digestion and heated for 30 minutes until the sample become colorless. Then, the colorless content was filtered and transferred to standard flask and made up to 50ml by adding distilled water and analyzed for Cr, Cd, Cu, Zn, Fe, and Pb in Atomic absorption spectrophotometer and readings were tabulated and uptake of heavy metals by fungal mycelium were calculated using the equation (Raman et al., 2014) :

$$q_e(Mg/g) = \frac{1000CV}{W}$$

Whereas q_e is concentration of heavy metal accumulated by fungal mycelium in (mg/g), C is concentration of heavy metal (ppm); V (ml) is the volume of the tannery effluent, and W is the dry weight of the Fungal mycelium.

2.4 Bioassay

Green gram seeds were germinated using treated effluent, root length and shoot length were measured and seedling vigour index were calculated.

Seedling Vigour Index = (Shoot length + Root length) x Germination Percentage.

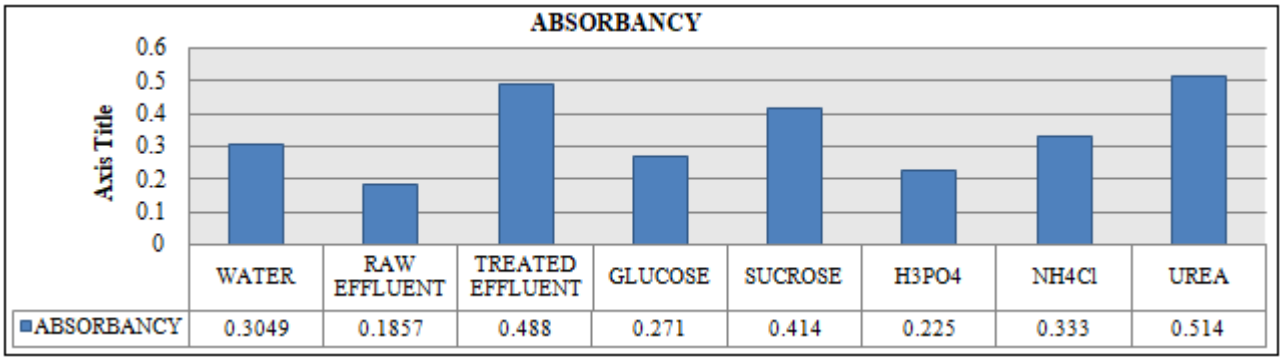
3. Result and Discussion

3.1 Growth Analysis

The growth rate of *Calocybe indica* was studied in response to tannery effluent. Simultaneously the bioremediation efficiency of fungi was studied. The effect of additional sources of co-metabolites like carbon source and fertilizers on the rate of bioremediation proves to be effective. Both Glucose and Phosphorous source enhance the bioremediation efficiency as observed from the growth rate of the fungus (Kamalasankari S A M & Karpagam S, 2017) and the spectral readings (Graph 1).

3.2 UV – Visible Spectrophotometery Reading

One of the effluents was read at 660nm and water as blank. The filtrate of fungal mycelium was also subjected to spectrophotometer analysis at 660nm. The reading were noted and represented on graph. (Graph.1)



3.3 Heavy Metal Analysis of Tannery Effluent by Atomic Absorption Spectrophotometer

Heavy metals were analyzed in both treated effluent and the fungal mycelium by acid digesting both the samples. Estimation of heavy metal analyzed by AAS. Uptake of heavy metals was high in the presence of Glucose and Phosphorous Source. The readings were tabulated (Table1 &2).

Table 1: Heavy metals analysis of Treated effluent and Fungal mycelium

S. No.	Sample	Chromium Cr (ppm)	Cadmium Cd (ppm)	Zinc Zn (ppm)	Iron Fe (ppm)	Magnesium Mg (ppm)	Copper Cu (ppm)
Absorbancy (nm)		359.0nm	237.9 nm	213.6 nm	239.3 nm	285.1 nm	324.5 nm
1.	Effluent	10.65	1.97	1.69	10.16	0.70	0.36
2.	Effluent + FM	9.9	8.27	4.23	8.12	2.26	5.02
3.	Mycelium	ND	2.3	3.76	87.6	1.23	ND
4.	Eff+Gl+Fug	8.32	7.8	4.94	3.96	1.75	ND
5.	Mycelium	34.95	34.95	80.81	204.01	26.66	ND
6.	Eff+Su+Fug	13.18	19.32	14.61	4.51	6.64	ND

7.	Mycelium	3.47	4.25	3.42	96.39	2.51	ND
8.	Eff+P+Fug	12.2	13.24	10.18	2.88	5.58	ND
9.	Mycelium	12.2	23.4	18.81	110.53	20.3	ND
10.	Eff+NH_4Cl+Fug	12.88	15.96	10.42	2.24	4.72	ND
11.	Mycelium	2.79	4.15	2.99	103.6	3.47	ND
12.	Eff+Urea+Fug	12.2	19.58	24.87	3.06	5.79	ND
13.	Mycelium	7.17	1.59	2.17	104.56	3.38	ND

Table 2: Uptake of heavy metals by Fungal Mycelium:

S.No.	*Samples*	*Cromium*	*Cadmium*	*Zinc*	*Iron*	*Magnesium*	*Copper*
1.	Eff+ Fug	ND	401.39	656.19	15,287.95	214.65	ND
2.	Eff+Fug+Glu	5844.48	5844.48	13379.59	34115.38	4458.19	ND
3.	Eff+Fug+Sug	649.81	795.88	640.45	18050.56	470.03	ND
4.	Eff+Fug+P	1812.77	3476.96	2794.94	18,483.27	3016.34	ND
5.	Eff+Fug+NH_4Cl	466.55	693.97	500	15393.75	580.26	ND
6.	Eff+Fug+Urea	1257.89	278.94	380.70	18,343.85	592.98	ND

ND = not detectable*

3.4 Bioassay

The toxicity of treated effluent was estimated by germination of green gram seeds, where as it shoot length and root length were measured and also Seedling Vigour Index were calculated (Table 3).

Table 4: Bioassay and seedling vigour index of treated effluent on the germination of green gram seeds

S.NO.	Samples	No. of Seeds	No. of Seeds Germinated	Germination Percentage	Root Length (cm)	Shoot Length (cm)	Seedling Vigour Index (VI)
1.	Control (Water)	30	27	90	10.9	2.7	253.9
2.	Raw Effluent	30	6	20	.3	-	66
3.	Treted Raw Effluent	30	15	50	1.6	-	80
4.	Glucose	30	24	80	1.9	-	152
5.	Sucrose	30	18	60	1.1	-	6
6.	H_3PO_4	30	24	80	2.5	-	200
7.	NH_4Cl	30	24	80	2.6	-	208
8.	UREA	30	9	30	.8	-	24

In present study, Mycoremediation of tannery effluent by spent bags of *Calocybe indica* is enhanced in presence of Glucose and Phosphorous as Co-metabolites source, as observed from Atomic Absorption Spectrophotometer reading of treated effluent.

Calocybe indica has the biosorption efficiency of heavy metals at highest applied concentration (Surumbar Kuzhali, 2012). The Merit of this technology is it uses relatively low cost, low – technology techiniques (Abioye, 2011 and Sharma 2011). It is proved that mushrooms have different abilities of biosorption, bioremediation, biodegradation and toxicity reduction (Kamalasankari S A M & Karpagam S, 2017) .

4. Conclusion

Mycoremediation is one of way to remove heavy metals from tannery effluents. Biosorption potential of macro fungi for heavy metals is being assessed. The cultivation of edible mushroom on agricultural and industrial wastes may thus be a value added process capable of converting these discharges, which are otherwise considered to be wastes, into foods and feeds. Besides producing nutritious mushrooms, the Spent bags after harvesting the mushrooms can be used for the biodegradation process. Thus, the present study indicates the harvested spent bags of Macro Fungi can be used as a Mycoremediator.

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