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Studies and absorption of physiochemical and heavy metals from tannery effluents using macro and micro fungi

K.Divya[1], R.Vidya[2]*

[1] School of Biosciences and Technology, Department of Biomedical sciences,
[2]*VIT School of Agricultural Innovations and Advanced Learning (VAIAL),
Vellore Institute of Technology, Vellore – 632014, Tamil Nadu, India.
Email.Id: rvidya@vit.ac.in

Abstract

Tanneries engender innumerable toxins reliant on the progression recycled. The foremost pollutants and impurity related to this division obligated conversed beneath. Bioremediation by means of fungi is widely travelled. Mycelial biomass of cultivated mushroom *Pleurotus florida* and fungi *Aspergillus niger* and *Alternaria alternata* were poised from TNAU Coimbatore and imperiled for bio adsorption studies. The bio adsorption process were carried out in four different concentrations and examined regularly for the change of color. At the end of seventh day the effluent samples were analyzed for Physico chemical parameters and heavy metal analysis. The results showed a significant degradation of pollutants as adsorbed by the fungi. Physicochemical parameters like (Color, odour, turbidity, TDS, BOD, COD, Calcium, magnesium, nitrate) of tannery effluent were estimated. The results of the study revealed that physico chemical parameters of the effluent before treatment were found to be above the permissible limits for disposal as prescribed by Central pollution control board (CPCB). After adsorption study using fungus the physico chemical parameters have been reduced and found below the acceptable limits of Central pollution control board (1995 CPCB), The Bureau of Indian Standards (BIS 1983) and World health organization (WHO 1996). With the intention of the effluents can reused for agricultural and aquaculture purposes.

Keywords – Bioadsorption, *Pleurotus florida*, *Aspergillus niger*, *Alternaria alternata*

INTRODUCTION

Leather industries are highly water demanding industries and generate huge amount of effluent containing high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), inorganic impurity (sodium, calcium, nitrate, ammonia, sulfide, and chloride), dissolved and balanced solids, and other pollutants such as vegetable or synthetic tannins, suffocated oils, chromium, pentachlorophenol and surfactants (Sharma and Malaviya 2013).The ejection of these highly oxygen challenging and colored wastewater not only cause depletion of dissolved oxygen but also reduce transparency of receiving water bodies thereby threatening the aquatic ecosystems. Similarly, chromium toxicity is also one of the major causes of environmental hazards caused by tannery effluents as it causes severe diarrhea, ulcers, eye and skin irritation, kidney dysfunction and lung carcinoma (Malaviya and Singh 2011). Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms. These biological treatment methods are attractive due to their cost effectiveness; diverse metabolic pathways and versatility of microorganisms (Ajao 2011;Malaviya and Singh 2014 and 2015)**.** Fungi are often recognized for their superior ability to produce a large variety of extracellular proteins, organic acids and other metabolites (Lilly and Basnett 1958) . Fungal systems appear to be the most appropriate in the treatment of colored and metallic effluents (Ezeronye and Okerentugba 1999)

Leather industry is one of the important industries that cause water pollution since the effluent generated from various industries has high amount of organic substances. Industrial effluent when discharged into water bodies alter the physical, chemical and biological characteristics of water and depletes the dissolved oxygen, increases alkalinity, suspended solids and sulphides which are injurious to fish and other aquatic life. Heavy metals in the effluent are one of the most hazardous environmental pollutants. Disposal of such wastes with high pollution load into water courses or onto land with or without prior treatment creates a great problem to the environment. Biological treatment is the most widely used method for removal as well as partial or complete stabilization of biologically degradable substances present in waste waters (Faiza Kauser 2014). They have potential use as biosorbent for removal of heavy metals particularly chromium from industrial waste waters (Grueninger et al 1984; Akthar NM and Mohan PM. 1995). Effluent contaminated by metals and chlorinated organic compounds are much difficult to remediate. The tannery industries pollute due to the inherent manufacturing processes as well the type of expertise employed in the manufacture hide and membrane into leather. During the tanning process at least 300 kg of chemicals were added per ton of hides. Physicochemical methods were in use for removal of heavy metals from the effluents such as

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precipitation with hydroxide and carbonates (Sujatha 1995). These methods are responsible for production of effluents and the management processes are expensive.

Fungi degradation:

The attributes that distinguish filamentous fungi from other life forms determine why they are good biodegradables. The Mycelial growth habit gives a competitive advantage over single cells such as bacteria and yeasts, especially with respect to the colonization of insoluble substrates. Fungi can rapidly branch through substrates, literally digesting their way along by secreting a battery of extracellular degradative enzymes (Aftab and Noorjahan 2006). Hyphal penetration provides a mechanical adjunct to the chemical breakdown affected by the secreted enzymes. The high surface to cell ratio characteristic of filaments maximizes both mechanical and enzymatic contact with the environment.

It is very important to develop and apply inclusive methods of waste water management to facilitate the sustainable use of water resources; conserve environmental truthfulness and progressive livelihood on a long term basis. Different Physico- chemical and biological methods had been developed for the removal of heavy metals from waste water some of which are precipitation of ion exchange, electrolysis, reverse osmosis, solvent extraction etc. The bio absorption technique using micro and macro fungi from tannery effluent is attempted in this study.

HEAVY METALS

Heavy metal contamination has increased sharply over the last century due to increasing industrialization imposes stress on organisms and their presence pose environmental disposal problems due to their non-degradable and persistent nature. The introduction of cleaner technologies is not only socially responsible but has also been shown to lead to increased productivity, competitiveness and profitability. Heavy metals such as cadmium, lead and mercury are known for their high toxicity and impact as they have been connected to major health hazards. These could be followed by arsenic and chromium as they were used industrially in large quantities for a long time. Arsenic has been reported as the largest poisoning in the history of mankind and millions of people in Bangladesh and India drink and irrigate cultures with contaminated water. (Volesky 2007).

2. MATERIALS AND METHODS

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The effluents were collected from the tannery industry which is located in Sipcot Industrial Estate, Ranipet, RANITEC (CETP) and it was stored in a bottle. Prior to the collection, the sample water bottle was rinsed with nitric acid and sterile water (Atri et al 2000; Lakhanpal 1996). The samples were taken to the laboratory as early as possible and it has to be protected from direct sunlight during transportation. The samples were stored in refrigerator and analyzed (Lakhanpal 1996; Bennet et al 2000, Gupta et al 2000 and Awofolu et al 2006). After collecting the sample, physical, chemical parameters were carried out from the sample.

2.1 Biodegradation of tannery effluent

From the collected samples 20ml, 40ml, 60ml and 80ml of the effluent was measured and taken in separate containers and to that respective fungal cultures were inoculated. Two different cultures such as *Aspergillus niger* , *Alternaria species* and the mushroom *Pleurotus florida* were added. Mushrooms were washed and for each concentration 20g of mushroom was added in 250 ml of broth for biosorption study. After inoculation the flasks were kept in an orbital shaker for 7 days at 37ºc. After 7days incubation, the physical and chemical parameters were estimated.

2.2 Tannery effluents using Alternaria species

2.2.1 Physical examination

The appearance of tannery effluents were found to be brownish color and it had agreeable odour and turbidity was found to be 6.00 NTU and the total dissolved solids was 1200mg/l. The obtained physical examinations are above the acceptable limits. The Acceptable limit prescribed for turbidity by (WHO 1996; CPCB 1995 and BIS 1983) is 5NTU and the level prescribed for total dissolved solids was 500 mg/l. After degradation using *Alternaria* the color of the effluent became colorless and there is no odour and the turbidity level decreased to 3 NTU. The percentage level of degradation is 50%. The total dissolved solids have been reduced to 356 mg/l.

2.2.2 Chemical examination

Chemical examinations were observed for pH, alkalinity as $Caco_3$, total hardness, Calcium, Magnesium, Sodium, Potassium ,Iron, Manganese , Nitrate , Fluoride, Chloride, Phosphate, BOD and COD. The properties which had decreased were taken into account. After degradation using *Alternaria* species at 4 different concentrations the pH had been decreased from 7.22 to 7.06. Calcium from 128 to 12 mg/l, Magnesium 72 to 15 mg/l. Nitrate from 102 to

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16 mg/l, BOD from 58.7 to 2.7mg/l and COD from 152.7 to 8.2mg/l. The above levels are below the acceptable limits prescribed by (CPCB 1995 and WHO 1996) as mentioned in a table 2.

2.2.3 Degradation observed when *Alternaria* is used

The heavy metal pollutant degradation was reduced to 72% for calcium, in magnesium 76% degradation, Nitrate 76% degradation and for BOD there was 73% and COD 93% respectively as shown in figure 4,5,6,7 and 8.

2.3 Tannery effluent using *Aspergillus niger*

2.3.1 Physical examination

The appearances of tannery effluents were found to be brownish color and it had agreeable odour, turbidity was found to be 6 NTU and the total dissolved solids are 1200mg/l. The obtained physical examinations are above the acceptable limits prescribed by (WHO 1996; BIS 1983 and CPCB 1995). The limits prescribed for turbidity is 5 NTU and for TDS is 500 mg/l. After degradation with *Aspergillus niger* the color became colorless and there was no odour, turbidity level reduced to 2 NTU and total dissolved solids reduced to 29 mg/l and the percentage of degradation was 85%.at the highest concentration of 80mg/l. The degradation was below the acceptable limit (WHO 1996; BIS 1983 and CPCB 1995).

2.3.2 Chemical examination

Chemical examinations were observed for pH, alkalinity as $Caco_3$, Total hardness, Calcium, Magnesium, Sodium, Potassium ,Iron, Manganese , Nitrate , Fluoride, Chloride, Phosphate, BOD and COD. After degradation using *Aspergillus niger* at 4 different concentrations the pH had decreased from 7.39 to 7.06, it was 28% of degradation, Calcium from 128to 69 mg/l, Magnesium from 72 to 42 mg/l, and Nitrate from 102 to 30 mg/l, BOD decreased from 58.7 to 21.3 mg/l, and COD from 152.7 to 57.5 mg/l. The level was observed to be below permissible limits as mentioned in table 2 Prescribed by (CPCB 1995 and WHO 1991).

2.3.3 Degradation observed when *A.niger* is used

The heavy metal pollutant degradation was reduced to 54% for calcium, in magnesium 44% degradation, Nitrate 78% degradation, For BOD there was 59 % and COD 82% respectively as shown in figure 4,5,6,7 and 8.

2.4 Tannery effluents using *Pleurotus florida*

2.4.1 Physical examination

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The appearances of tannery effluents were found to be brownish color and it had agreeable odour, turbidity was found to be 6 NTU and the total dissolved solids was 1200mg/l.The obtained physical examinations before degradation are above the acceptable limits (WHO 1996; BIS 1983 and CPCB 1995). The limits for turbidity are 5 NTU and for TDS is 500 mg/l. After degradation with *Pleurotus florida* the color became dark brownish, there is no odour, turbidity level became 4.3 NTU and total dissolved solids reduced to 226 mg/l at the highest concentration. The degradation was below the acceptable limit (WHO 1996; BIS 1983 and CPCB 1995).

2.4.2 Chemical examination

Chemical examinations were observed for pH, alkalinity as $Caco_3$, Total hardness, Calcium, Magnesium, Sodium, Potassium ,Iron, Manganese , Nitrate , Fluoride, Chloride, Phosphate, BOD and COD. After degradation using mushroom *Pleurotus florida* at 4 different concentration the pH had decreased from 10.3 to 7.80, Calcium from 128 to 26 mg/l, Magnesium from 72.0 to 31mg/l and nitrate from 93 to 46 mg/l, BOD from 58.7 to 31 mg/l and COD from 153 to 92 mg/l respectively. The level was observed to be below permissible limits as mentioned in table 2 Prescribed by (CPCB 1995 and WHO 1991).

2.4.3 Degradation observed when *Pleurotus florida* is used

The heavy metal pollutant degradation was reduced to 45% for Calcium, in Magnesium 44% degradation, Nitrate 58% degradation, for BOD there was 52 % and COD 59% respectively as shown in figure 4,5,6,7and 8.

3. Heavy metal analysis

The concentration of chromium, lead and zinc present in the effluent sample were analyzed by using Atomic absorption spectroscopy (within 24 hours of sample). The samples were mixed well and filtered through a filter paper. The filtrate was transferred to a conical flask with a capacity of 500ml and 5% of concentrated nitric acid was added to create a acidic condition. The flasks were heated on a hot plate so that the volume of samples will be reduced to 25ml.Then the samples were allowed to cool and transferred to an acid rinsed volumetric flask. The samples along with blank were analyzed by AAS.

RESULTS AND DISCUSSION

The present study tells about the pollution of tannery effluent by its Physico chemical properties. From this study it is evident that the macro fungi are available having the capacity of effective biosorption of physical, chemical properties which can be taken as ecofriendly method.

Bioremediation uses microorganisms to catalyze the degradation of waste without disturbing the environment. In recent years the use of microorganisms to recover metals from waste stream is a known strategy which is employed for landfill applications. Fungus will secrete extracellular enzymes. The decomposition of lignocelluloses is the most degradative enzyme in carbon cycle. The ability of fungi to transform a wide variety hazardous chemical has aroused inside using bioremediation. Many researchers had found the effectiveness of isolated fungi belonging to the genus Aspergillus to degrade pollutants from tannery waste water. Fungi play an appropriate role in treatment of colored and metallic effluents (Ezeronye 1999).

Degradation of physical properties:

Color and Odour: The color and odour of tannery effluents were changed from brown to colorless and odour to odorless because the fungus decomposed the toxic pollutant present in the effluent. **(**Jamal Mohamed 2002 and Aftab &Noorjhan 2006).

Total dissolved solids: The total dissolved solids of effluent had been decreased from toxic level when treated with fungus because the consumption of inorganic and organic matter by microbes for their food. (Hulmes et al 1993).

Turbidity: The turbidity level had been tainted from toxic level because the filamentous fungi entrap the suspended solid particles in waste water (Alam et al 2001, Fakhrul razi & molla 2007)

Degradation of chemical properties:

Degradation in calcium mg/l:

Three different fungi were used in 4 different concentrations such as 20mg/l, 40 mg/l, 60mg/l and 80mg/l. After treatment for seven days the degradation levels for calcium were noted for all three fungi. In *Alternaria* the degradation had found to be high in concentrations of 60mg/l and 80mg/l. The percentages of degradation were found as 78% in concentration of 60mg/l and 95% in 80mg/l concentration.

In *A.niger* the degradation were found in 60mg/l and 80mg/l. The percentage levels of degradation were as 65% in 60mg/l and 86% in 80mg/l.

Pleurotus florida showed increasing degradation as the concentration increases. The degradation was 50% in 20mg/l and 55% in 40mg/l, 65% of degradation in 60mg/l and 66% in 80mg/l respectively as mentioned in fig :4. The figures were compared with untreated effluent.

The degradation of calcium is due to the reality the calcium nutrients that needs by fungi for growth purpose (walker G.M 2004).

Degradation in magnesium mg/l:

Three different fungi were used in 4 different concentrations such as 20mg/l, 40 mg/l, 60mg/l and 80mg/l. After treatment for seven days the degradation levels for magnesium were noted for all three fungi. In *Alternaria* the degradation had found to be high in concentrations of 60mg/l and 80mg/l. The percentages of degradation were found as 80% in concentration of 60mg/l and 95% in 80mg/l concentration.

In *A.niger* the degradation were found in 60mg/l and 80mg/l. The percentage levels of degradation were as 70% in 60mg/l and 96% in 80mg/l.

Pleurotus florida showed increasing degradation as the concentration increases. The degradation was 55% in 20mg/l, 58% in 40mg/l, 59% of degradation in 60mg/l and 60% in 80mg/l respectively as mentioned in fig: 5. The figures were compared with untreated effluent.

The degradation of magnesium is due to the nutrients that needs by fungi for growth purpose (walker G.M 2004).

Degradation in nitrate mg/l:

Three different fungi were used in 4 different concentrations such as 20mg/l, 40 mg/l, 60mg/l and 80mg/l. After treatment for seven days the degradation levels for nitrate were noted for all three fungi. In *Alternaria* the degradation had found to be high in concentrations of 60mg/l and 80mg/l. The percentages of degradation were found as 81% in concentration of 60mg/l and 95% in 80mg/l concentration.

In *A.niger* the degradation were found in 60mg/l and 80mg/l. The percentage levels of degradation were as 70% in 60mg/l and 98% in 80mg/l.

Pleurotus florida showed increasing degradation as the concentration increases. The maximum degradation was found in highest concentration at 80mg/l and the percentage level of degradation was 65% respectively as mentioned in fig: 6. The figures were compared with untreated effluent.

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The nutrients are bio stimulation and important element of enzymes and proteins for living microorganisms (Atlas R.M 1984)] during the study the degradation of nitrate was due to the nutrients that stimulate growth for fungi (Mollea.C et al 2005 and Potin O et al 2004)

Degradation in BOD mg/l:

Three different fungi were used in 4 different concentrations such as 20mg/l, 40 mg/l, 60mg/l and 80mg/l. After treatment for seven days the degradation levels for BOD were noted for all three fungi. In *Alternaria* the degradation had found to be high in concentrations of 60mg/l and 80mg/l. The percentages of degradation were found as 82% in concentration of 60mg/l and 85% in 80mg/l concentration.

In *A.niger* the degradation were found in 60mg/l and 80mg/l. The percentage levels of degradation were as 65% in 60mg/l and 86% in 80mg/l.

Pleurotus florida showed increasing degradation as the concentration increases. The maximum degradation was found in highest concentration at 80mg/l and the percentage level of degradation was 75% respectively as mentioned in fig: 7. the figures were compared with untreated effluent.

The BOD level had been decreased by fungus it shows efficient degrading capability of fungus by degrading the contaminants as they use it for their growth and reproduction.(Hossain &Das 2011)

Degradation in COD mg/l:

Three different fungi were used in 4 different concentrations such as 20mg/l, 40 mg/l, 60mg/l and 80mg/l. After treatment for seven days the degradation levels for COD were noted for all three fungi. In *Alternaria* the degradation had found to be high in concentrations of 60mg/l and 80mg/l. The percentages of degradation were found as 82% in concentration of 60mg/l and 95% in 80mg/l concentration.

In *A.niger* the degradation were found highest concentration in 80mg/l. The percentage levels of degradation were noted as 96% in 80mg/l.

Pleurotus florida showed increasing degradation as the concentration increases. The maximum degradation was found in highest concentration at 80mg/l and the percentage level of degradation was 65% respectively as mentioned in figure 8. The figures were compared with untreated effluent.

COD parameter had been decreased because of the fungus has the capability of degrading the contaminants as it used for their growth and reproduction (Poonkothai and parvatham 2005)

Heavy metal degradation:

Chromium and Zinc Degradation:

The chromium is important structural element of the surface of fungal cell found. It is a structural element for fungus cell wall like chitin and chitosan. These cell wall when its active will uptake the chromium and zinc (Padma et al 2007).

Lead Degradation:

The metal lead is degraded because the fungus synthesize some enzyme which is essential for uptake of lead. (Faryal et al 2007).

CONCLUSION

Microorganisms such as algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents . (Volesky.B 1986). Strong biosorbent behavior of certain micro-organisms towards metallic ions is a function of the chemical make-up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells. Some types of biosorbents would be broad range, binding and accumlating the majority of heavy metals with no specific activity, while others are specific for certain metals.

The enzymatic treatment falls between the physicochemical and biological treatment process. It has technological advantages and requires economical considerations to apply it on a large scale. It has some potential advantages over the conventional treatment (Jamal Mohamed 2002 ; Ninnekar 1992). Recent research has focused on the development of enzymatic processes for the treatment of wastewaters, solid wastes, hazardous wastes and soils in recognition of these potential advantages (Karam 1995). A large number of enzymes (e.g. oxidoreductases, cellulolytic enzymes, etc.) from a variety of different sources have been reported to play an important role in an array of waste treatment applications (Sangave 2006a; Pandit and Klibanov 1980).Thus from the above results it can be inferred that the maximum reduction of toxic substances was recorded in biotreated sample using fungus *Alternaria sp* (90%), *A. niger* (85%) and *Pleurotus florida* (50%) compared to untreated effluent. Thus it may be concluded from the above study that *Alternaria* and *Aspergillus niger* showed a maximum degradation on the given effluent sample, even *Pleurotus florida* showed a degradation on par at concentrations of 20, 40, 60 and 80(mg/l). As there was increase in fungal biomass concentration there was a subsequent increase in percentage degradation in the samples. By using these fungi a bulk amount of untreated effluent can be degraded and this treated water can be reused for agricultural purpose.

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Figure captions:

Fig.1: (A) Tannery effluent inoculated with *Alternaria* - after degradation compared with control.

(B): closer view of degraded effluents

Fig.2 (A) Effluents inoculated with *A. niger*

(B) Effluents after degradation

Fig. 3 (A) *Pleurotus florida* before degradation

(B) *Pleurotus florida* after degradation

Fig.4. Percentage degradation in effluent A - Calcium with different fungi

Fig.5. Percentage degradation in effluent B - Magnesium with different fungi and C- Nitrate with different fungi

Fig.6. Percentage degradation in effluent D- BOD with different fungi and E- COD with different fungi

Fig.1: (A) Tannery effluent inoculated with *Alternaria* - after degradation compared with control.

(B): closer view of degraded effluents

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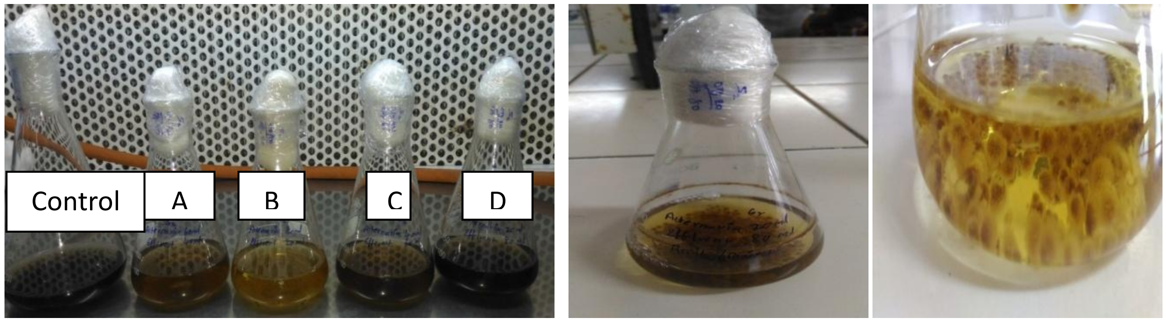
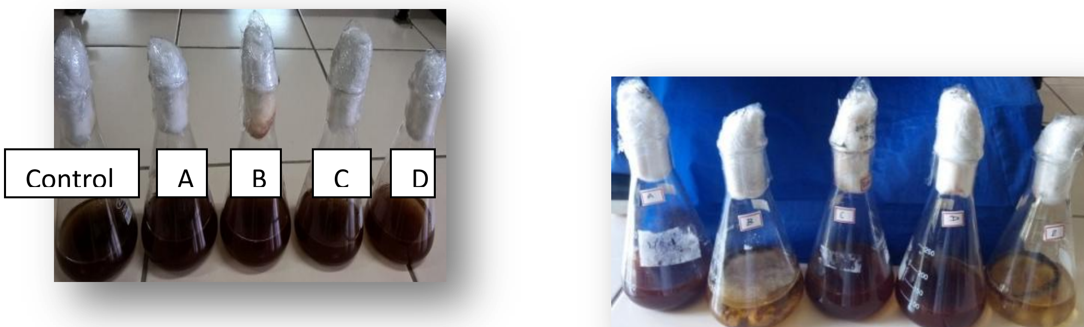


A- 20 mg/l, B – 40 mg/l, C- 60 mg/l and D- 80 mg/l

Fig.2 (A) Effluents inoculated with *A. niger* (B) Effluents after degradation



A- 20 mg/l, B – 40 mg/l, C- 60 mg/l and D- 80 mg/l

Fig. 3 (A) *Pleurotus florida* before degradation (B) *Pleurotus florida* after degradation

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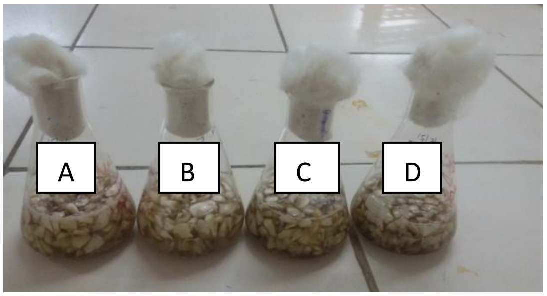

A- 20 mg/l, B – 40 mg/l, C- 60 mg/l and D- 80 mg/l

Fig.4. Percentage degradation in effluent A- Calcium with different fungi.

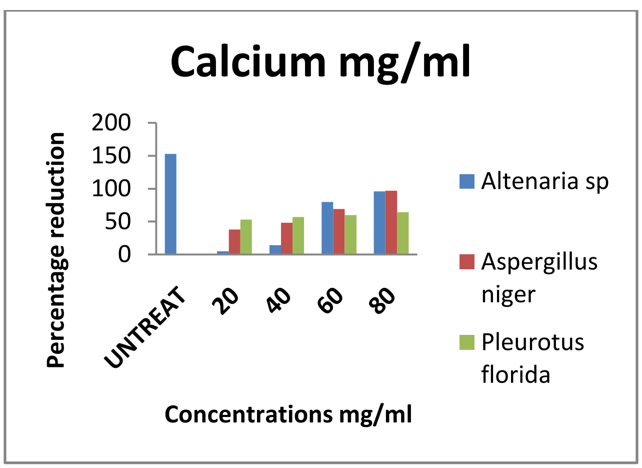

Fig.5. Percentage degradation in the effluent B- Magnesium with different fungi C- Nitrate with different fungi

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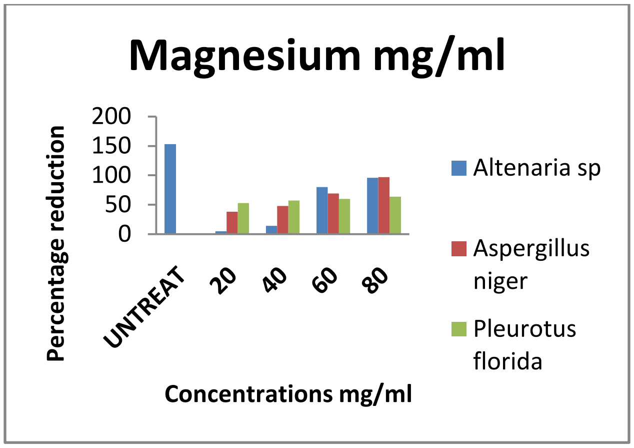


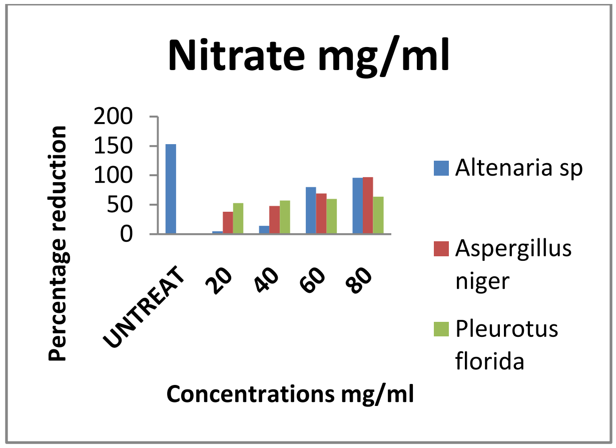
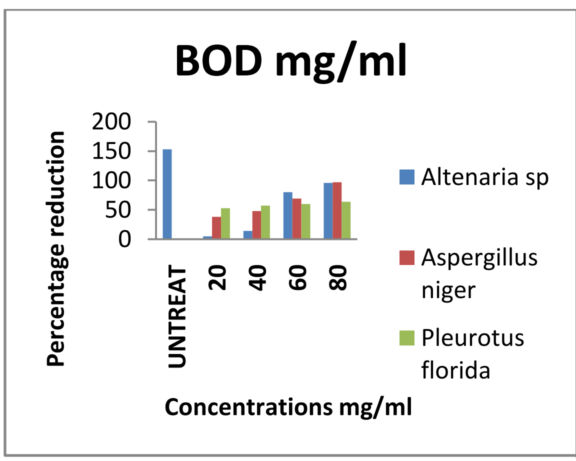


Fig.6. Percentage degradation in the effluent D- BOD with different fungi E- COD with different fungi



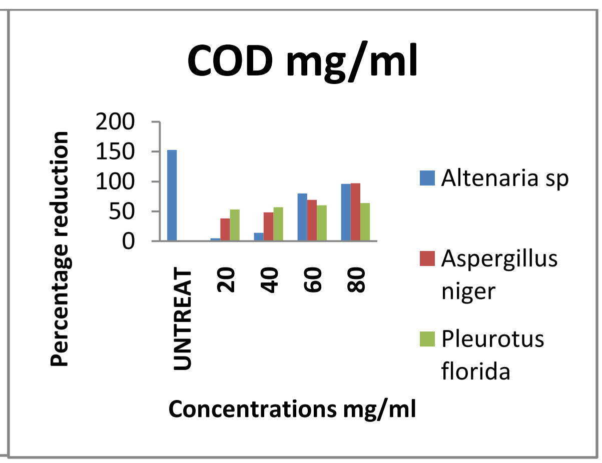


Table Captions:

Table 1. Degradation of effluent with different fungi-Physical properties

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Table 2. Degradation of effluent with different fungi – chemical properties

Table 1: Degradation of effluent with different fungi-Physical properties

S.NO	Chemical properties (mg/ml)	*Alternaria sp*		*Asp.niger*		*Pleurotus florida*		Acceptable Limit of CPCB and WHO (mg/ml)
		Initial value (mg/ml)	After Degradation	Initial Value (mg/ml)	After Degradation	Initial Value (mg/ml)	After Degradation	
1	Turbidity	6	3 NTU	6	2 NTU	6	4.3 NTU	5 NTU
2	Color	Brownish	Colorless	Brownish	Colorless	Brownish	Colorless	15(Pt.Co.Scale)
3	Odour	Agreeable	No odour	Agreeable	No odour	Agreeable	No odour	Agreeable
4	TDS	1200 mg/l	356 mg/l	1200 mg/l	29 mg/l	1200 mg/l	22 mg/l	500 mg/l

Pt.Co.Scale – Platinum cobalt scale

TDS – Total Dissolved solids.

Table 2: Degradation of effluent with different fungi – chemical properties

S.NO	Chemical properties (mg/ml)	*Alternaria sp*		*Asp.niger*		*Pleurotus florida*		Acceptable Limit of CPCB and WHO (mg/ml)
		Initial value (mg/ml)	After Degradation	Initial Value (mg/ml)	After Degradation	Initial Value (mg/ml)	After Degradation	
1	Calcium	128	12.5±0.70	128	67±2.12	128	70.5±7.7	250
2	Magnesium	72	13.5±2.12	72	40±2.12	72	28±4.2	30-100
3	Nitrate	102	15±1.41	102	29±1.4	102	45±1.4	100
4	BOD	59	2±0.21	59	20±1.4	59	18.5±3.0	30
5	COD	153	15±3.6	153	56±1.4	153	91±1.41	250

The values are expressed in triplicates in mean and standard deviations (±).

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Table 3: Heavy metal degradation by fungus

Heavy metals	**Initial level**	***Alternaria* degradation**	***Aspergillus niger* degradation**	***Pleurotus florida* degradation**
Chromium	2.32 mg/l	9.9 µg/ml	6.8µg/ml	6.5 µg/ml
Zinc	1.70 mg/l	10.4 µg/ml	9.3µg/ml	7.6 µg/ml
Lead	1.83 mg/l	6.4 µg/ml	6.2 µg/ml	5.8 µg/ml