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Effect of Hexavalent Chromium and Aluminium in Fresh Water Fish *Ictalurus Punctatus* and Bioremediation by Using Dead Fungal Biomass

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Abstract: *Fish serum may reflect status of many biochemical processes in the metabolism. Heavy metals may alter serum biochemical parameters in fishes. In this study, the effect of Cr(VI) and Al(III) in fresh water fish I.Punctatus and neutralize these metals by using dead fungal biomass. Activity of serum enzymes AST, ALT, ALP, CPK, LDH and α – amylase in aluminium and chromium exposed fish. The above study suggests that serum biochemical parameters could be used as important and sensitive biomarkers in ecotoxicological studies concerning the effects of metal contamination and fish health. To remove these heavy metals from environment by using dead fungal biomass. In this investigation, the following batch experiments study carried out by the adsorption range of metal ions [Cr(VI) &Al(III)] in optimum pH 5.2 & 5.5, various concentration of metal ions completely adsorbed with in 8hours contact time.*

Key Words*: Ictalurus punctatus, Hexavalent chromium, Aluminium, Serum enzymes, Bioremediation, Fungal biomass.*

I. Introduction

Chromium as one of the major pollutants of the environment is available in nature as an odourless, steel gray hard metallic element. It is the seventh most abundant element on the earth and twenty first most abundant element the rocks [McGrath and Smith, 1990]. Elemental chromium is not usually found pure in nature and principally occurs as the mineral chromite FeOCr2O3 or chrome iron stone in which form is extremely stable. Chromium continues to be in widespread use in industry, paints, metal plating as corrosion inhibitor and its particulates enter the aquatic medium through effluents discharged from tanneries, textiles and dyeing industries [Patolla et al., 2005]. Chromium exists in nature as stable hexavalent and trivalent forms. The hexavalent chromium is more toxic than trivalent chromium. Cr(VI) compounds found to be mutagenic and carcinogenic in a variety of test systems [Vutukuru S 2005]. Aluminium is the most abundant metal and the third most abundant element, after oxygen and silicon in the earth's crust [ATSDR 2006]. Aluminium is released to the environment by both natural processes and anthropogenic sources. Aluminium may enter natural waters via coal strip, mining activities, water treatment facilities using aluminium sulphate (alum) as a coagulant for suspended solid particles, industrial wastes and acid rain fall [Neville 1985]. However, when aluminium becomes available to organisms through acidification of surface waters, it is toxic to fish [Driscoll et al., 1980]. The main effects of aluminium exposure in fish are respiratory and ion regulatory disturbances [Genesmer 1999]. In toxicological studies of chronic and acute exposure, changes in concentrations and enzyme activities often directly reflect cell and organ damage in specific organs [Casillas et al., 1983].This is of serious environmental concern as Cr(VI) and Al(III) persists indefinitely in the environment complicating its removal. The persistent nature makes its accumulate in the food chain which with time reach harmful levels in living beings resulting in serious health hazards such as irritation in lungs and stomach, cancer in digestive tract (Cr), low growth rates in plants and death of animals. Therefore, removal of Cr(VI) and Al(III) from waste water prior to its discharge into natural water systems.

The aim of this present study is to investigate the chronic effect of hexavalent chromium and aluminium in fish Ictalurus punctatus and the conventional physico-chemical techniques used for the removal of Cr(VI) and Al(III) by using fungal dead biomass Agaricus bisporus, Agaricus silvicola and Agaricus campestris.

II. Materials And Methods

Collection of water samples

For the assessment of surface water quality, water samples were collected in polyethylene bottles from water bodies distributed in Ambur, Ranipet,Tuticorin and Thirumullaivoyal area labelled as test sample (T) and control sample (C) respectively and was assessed in Varshins en-test laboratory, Chennai.

Analysis of water samples

Both the samples were preserved and the physico-chemical characteristics of the water used for holding and experiments such as pH, temprature, BOD,COD, chlotide, sulphate, phosphate, iron, ,anganese, fluoride, hexavalent chromium, total chromium and aluminium were analyzed by spectrophotometer, colorimeter and gravimetric methods [Thangarajan 1999].

Fish collection and maintance

Ictalurus punctatus were used for the toxicity tests. The fish samples were collected from Ambur, Ranipet ,Tuticorin (test) and Thirumullaivoyal (control). They were collected in oxygenated polyethylene bags and transported to the laboratory and immediately transferred into glass aquria of 100L capacity containing well-aerated unchlorinated ground water. The fish were allowed to acclimate for 7 days before the experiments. They were fed with rice bran during the acclimation period. The fish were subsequently transferred into 50L glass aquaria for easy handling during the experiments. Only fish which were healthy and showed active movements were used for the experiments [Yang et al., 2003]. No differentiation was made between the sexes.

Blood sample collection

Ten fishes from each group (test & control) were removed and blotted. The blood samples were taken from each fish by puncture of the caudal vessel. Blood samples were centrifuged for10 minutes at 3000rpm [Heath1987, Vosyliene 1997] to obtain serum for the analysis of biochemical parameters such as ALT, AST, ALP, CPK LDH and Amylase by chemo auto analyser [Canli 1996, Markovich 1999].

Biomass sample collection

Agaricus bisporus (button mushroom), Agaricus sivicola (wood mushroom), Agaricus campestris (field mushroom) was collected from Ambur, Ranipet, Surapet., Also these mushrooms are easily available in local market.

Preparation of the dead fungal biomass

The non-living biomass of A.bisporus, A.silvicola, A.campestris was used as a biosorpent for the sorption of Cr(VI) and AL(III) ion from an aqueous solution. The mushroom spawn was available in Paddapai biotechnology lab as well as these mushrooms were easily available in local market and also cultivate from contaminated sites (Ambur, Ranipet,Tuticorin). The mushrooms were washed with tap water and deionized water to remove dust and other impurities. The mushrooms are sundried and then dried in an oven at 80°C for 12hours. After that the biomass was crushed and sieved to used as a biosorbent. The dried biomass was stored in a desiccator and used for the following experiments.

Experimental work

The Cr(VI) and Al(III) stock solutions were prepared by dissolving their corresponding analytical grade salts of $K_2Cr_2O_7$, $Al(NO_3)_3$ in deionised water. The ion concentrations in stock solutions were about 100mg/l. For obtaining of adsorption isotherms, a series of flasks (250ml., as batch experiments) . In experiments to screen efficient biomass, 2g/l dead fungal biomass of three species were contacted with 10mg/l at 28°C of chromium concentration in solution and 10mg/l of aluminium concentration in solution. In experiments, to find the effects of the pH, a pH of 2, 4, 4.5, 4.8, 5.2, 5.5 were used. In experiments to find the effect of biomass concentration on the chromium and aluminium removal, biomass concentrations of 10, 30, and 50mg/l were employed. The flasks were agitated on a shaker at 200rpm at room temperature. Expect for a pH, shift experiment the solution pH was maintained at the desired value using the addition of 0.5M H_2SO_4 or 1M NaOH. The change in the working volume due to the addition of NaOH or 0.5M H_2SO_4 was negligible. The experiments were performed at room temperature. In the temperature range of 10-50°C there are no, or very minor changes to the surface and chemistry of the groups involved in sequestering the metal ions from solution [Sanyahumbi et al., 1998]. The solution was intermittently sampled and centrifuged at 3000rpm for 5minutes, then the chromium and aluminium concentration of the supernatant were analyzed. The concentration of unabsorbed hexavalent chromium and aluminium in the biosorption media was determined at Cr(VI) 540nm in a spectrophotometer using 1,5-di phenyl carbazide reagent. In aluminium 535nm in a spectrophotometer using erichrome cyanine reagent.

Statistical analysis

All the serum biochemical parameters given as mean and standard error.

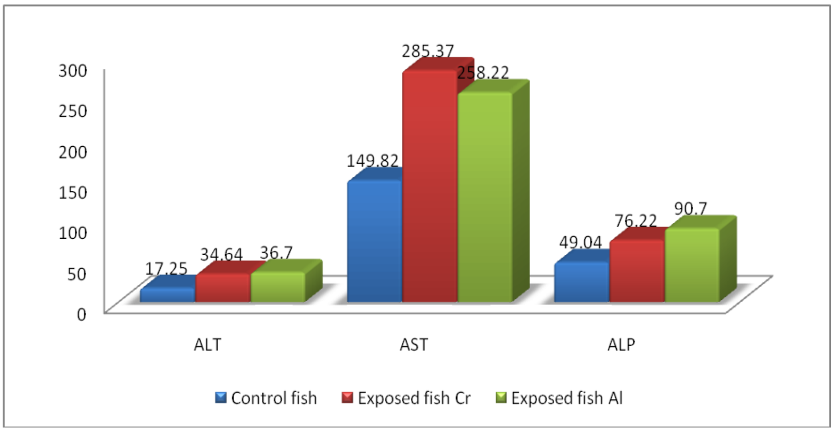
III. Results And Discussion

Serum enzymes

"Figure 1" - Comparison of ALP,AST and ALT in control and test sample.

Aspartate transaminase and alanine transaminase activity increase in Cr and Al-exposed fish. The control values of ALT and AST activities in serum were measured as 17.25±5.21 and 149.82±32.04 U/L, respectively.

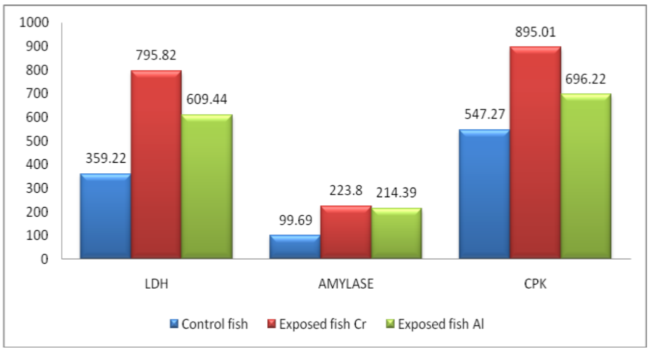
Transaminases like ALT and AST play a significant role in protein and amino acid metabolism and they may release into the plasma following tissue damage and dysfunction. Zikic et al 2001, showed that plasma AST and ALT activities increased in Cd – exposed fish Carassius auratus gibelio, the authors indicated that liberation of these transaminases into the circulation might occur due to damage of the liver, kidney, heart and other tissues in the state of stress influenced by metals. It was suggested that serum enzymes ALP,AST and ALT could be used as sensitive biomarkers in ecotoxicology due to provide an early warning of potentially hazardous alterations in contaminated aquatic oraganisms [Levesque et al.,2002, Vaglio et al., 1999, De La Torre FR et al., 2000].
"Figure 1" - Comparison of ALP,AST and ALT in control and test sample.



Alkaline phosphatase activity was changed significantly by Cr (76.22±11.75) and Al(90.07±18.42) exposed fish compared to control fish(49.04±9.01) from figure1. Alkaline phosphatase is a polyfunctional enzyme acts as a transphosphorylase at alkaline pH and plays an important role in mineralisation of the skeleton of aquatic animals and in membrane transport activities [Bernt et al., 2001]. ALP enzyme is a sensitive biomarker to metals since it is a membrane bound enzyme related to the transport of various metabolites [Lakshmi et al., 1991]. Ochmanski and Barabasz 2000, reported that the increase in the activity of ALP in blood might be due to the necrosis of liver, kidney and lung.

"Figure-2" - comparison of LDH, Amylase and CPK in control and exposed fish

Figure 2 shows that increased activity in amylase, CPK, LDH compared to (Cr and Al) control fish. The increased amylase activity shows that damage of the amylase secretary cells (Pancreatitis). The elevated LDH levels shows that impairment of oxidative phosphorylation in mitochondria and development of cellular hypoxia, providing energy in the absence of oxygen and re-oxidation of NADPH by lactate dehydrogenase [Murray et al., 2003]. The result reveals increased LDH activity in test sample which is comparable to the study done by shiffman et al 1959, where the elevation of LDH in muscle and liver of fresh water fish after the exposure of hexavalent chromium in Salano gairdneri [Shiffmann et al]. LDH is an anaerobic enzyme involved in the conversion of pyruvate to lactate in Embden Meyehoff pathway. The increased level may be due to an alternative pathway in conversion of lactate to pyruvate for the production of glucose, which is a major source of energy during stress induced by heavy metals [Vagilo and Vandriscina 1999].



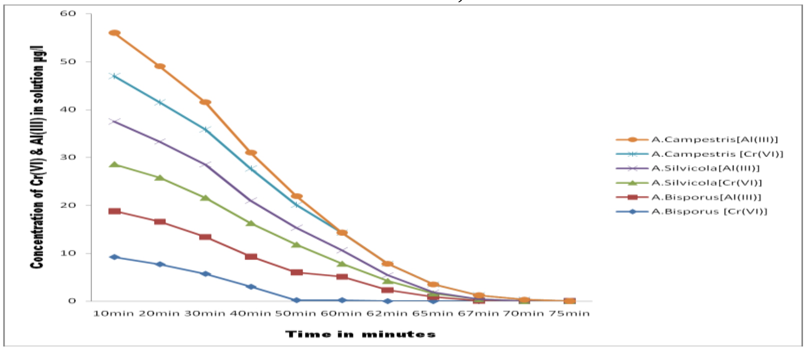
A significant increased CPK activity in Cr and Al exposed fish compare to control fish; it shows that the cell response to the increasing energy needs to cope with Cr and Al toxicity. The transfer of phosphate group from creatine phosphate to Atp in order to regenerate ATP is done by CPK [Murray et al 2003]. CPK is found in high concentration in skeletol muscle, myocardium and brain which appears to be sensitive measure of myocardial infraction and muscle diseases, but remains normal in liver disease [De La Torre et al 2000]

IV. Bio sorption Studies

Screening for efficient fungal dead biomass in removal of chromium and aluminium

To screen the efficient amount of dead fungal biomass for Cr(VI) removal and Al(III) removal, the time dependant concentration of chromium and aluminium was measured in batch system containing three species of mushrooms (A.bisporus, A.silvicola,A.campestris). Figure 1, shows that initial removal of chromium and aluminum dependant species are A.bisporus effectively removed chromium with in 62minutes and A.campestris complely removed aluminium with in 60minutes. A.silvicola 70minutes in chromium and 67minutes in aluminium. A.campestris effectively removed chromium with in 75minutes and agaricus bisporus completely removed aluminium in 70minutes. The above experiments are carried out by aqueous solution. Volesky et al 2003 showed that wild mushroom and meadow mushroom removed Cr(VI) and Al(III)from aqueous solution 98.9% & 98.2% respectively. Tomko et al showed that Amentia muscaria effectively removed aluminium from aqueous solution(96.5%). In this presnt study, Agaricus bisporus effectively removed from chromium in aqueous solution in 62minutes and Agaricus campestris completely removed from aluminium in aqueous solution in 60minutes.

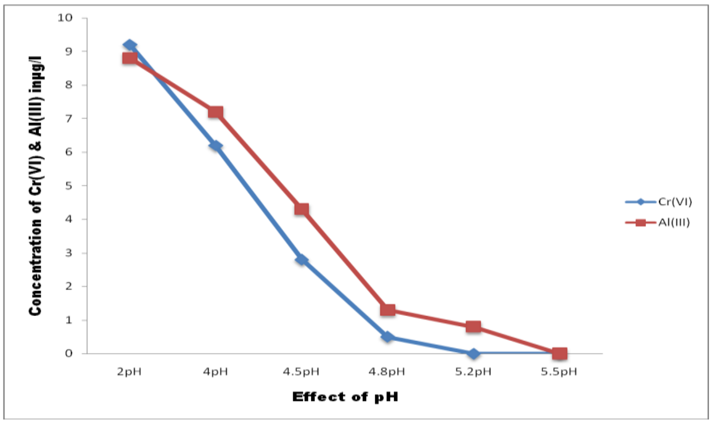
FIGURE 1,



Effect of pH

Biomass of Agaricus bisporus exposed to heavy metal ions, exhibited maximum sorption for Cr(VI) in the pH range of 4.0 – 5.5. The effect of pH on the adsorption of aluminium with dead fungal biomass of agaricus campestris was identified, when the pH value raised from 2 – 5.5. Figure2 shows that, removal of chromium from the aqueous solution at pH5.2, removal of aluminium from aqueous solution at pH 5.5. Beveridge 1986, showed that, the reduction in metal ions displayed by fungus at pH5.8 can be explained on the basis that at higher pH values the metal ions may accumulate inside the cells, and or the intra fibular capillarity's of the cell walls by a sorption or micro precipitation mechanism in the study of chromium adsorption using living biomass. The rate of aluminium sorption onto the fungi biomass sorbent will be varied with available pH values of solution when ion exchange development and application is one of the sorption processes [Gadd 1987 & Park D 2005].

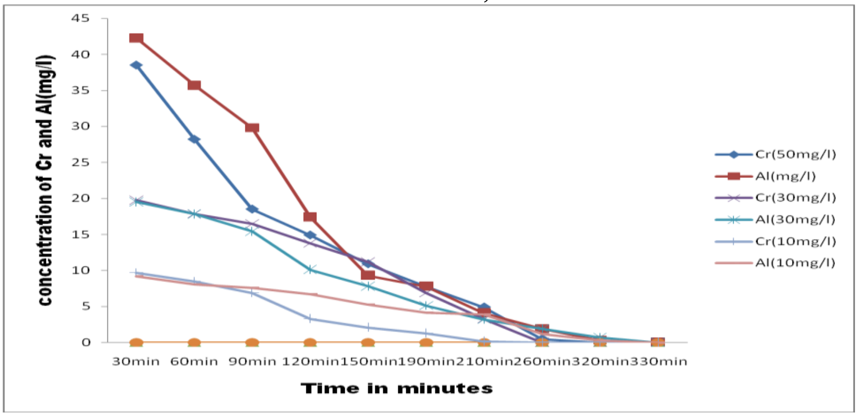
FIGURE 2



Effect of concentrations

In order to examine the effect of concentration of chromium and aluminium10, 30, 50mg/l at pH 5.2 and 5.5 at 2g/l of dead fungal biomass A.bisporus. Figure 3 shows that, the Cr(VI) concentration decreased sharply and finally the chromium disappeared in the solution. During the removal of Cr(VI), the solution optimum pH 5.2. Park 2005 reported that the amount of protons disappearing in the solution was in proportion to the amount of removal chromium. In this present study, the initial concentration of 10mg/l was completely removed in (Cr) 33minutes and (Al) 38minutes, while the complete removal of 30mg/l concentration of chromium and aluminium in 192and 215minutes respectively. 50mg/l concentration of chromium 308 and 328minutes in contact time. Saglam 2001 showed that four metal ions (Ni, Cu, Cr,Zn) was removed at various time intervals at pH nad temperature 25ºC for 6hours. In this present investigation, (A.bisporus and A.Campestris) the adsorption of chromium and aluminium ions showed that the saving power and consumption times.

FIGURE 3,



V. Conclusion

As a result, serum biochemistry could be used as a sensitive tool to assess the aquatic impact in contaminated ecosystems and also would be beneficial in determining the baseline health of and physiology of aquatic organisms. For this purpose, our results may provide useful data for further investigations, nevertheless it should be noted that, during advanced researches, appropriate biomarkers must be selected due to the variable responses to pollutants. In conclusion, the use of dead fungal biomass in the removal of heavymetals like chromium and aluminium from contaminated water works out to be cheaper, easy availability, easy biomass preparation. Because of these advantages the fungal biomass is found to be the most preferable biomass that can be used for removal of heavy metals present in the industrial effluents, and other contaminated water bodies.

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