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Phycoremediation potential of spirulina platensis on cadmium induced toxicity in oreochromis mossambicus

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Abstract
Heavy metals are one of the important environmental pollutants. The contamination of aquatic environments by heavy metals has become a global problem. Heavy metals have adverse effect on growth and reproduction and cause osmoregulatory stress. Spirulina capable to bound heavy metals of ionic in comparison to macro algae, spirulina has biosorption capacity much higher than micro algae. The work undertaken in the present study mainly centres around the toxic effect of cadmium chloride on the protein, glucose, lipid peroxidation product malondialdehyde and phycoremediation potential of spirulina on Oreochromis mossambicus. The result showed that the fish at spirulina treatment had high amount of protein (13.2±0.224), blood glucose level (0.930±0.408) and low level of malondialdehyde (13.2±0.224) in liver tissue. Cadmium treated fish had low level of protein, blood glucose. And the amount of cadmium is more concentrated in kidney compared with muscles of fish. The result demonstrated that the spirulina can be detoxify the cadmium toxicity in fish.

Keywords: Phycoremediation potential, spirulina platensis, cadmium induced toxicity, oreochromis mossambicus

Introduction
It is now realized that environmental problems have increased exponentially in recent decades mainly because of rapid growth of human population and increased demand for several house hold materials. While on other hand it has created number of health hazards. The toxic chemicals discharged into air, water and soil get into food chain from the environment. By entering into the biological system they disturb the biological processes leading to health abnormalities in some cases to fatal consequences.

Next to air, water is essential constituent of life support system and its quality plays an important role in maintenance of heath. Rapid industrialization fast growth in population and non-judicious use of natural resources has resulted into many fold increase in water pollution problem, besides sewage, agricultural, mining, discharges and other house hold residues. Industrial effluents contain a variety of toxic pollutions, including suspended solid organic compound, pesticides and toxic metal compound. Variety of contaminants include toxic heavy metals are present in rivers and reservoirs they are not biodegraded and therefore their accumulation in fish oyster, clams, muscle.

Heavy metals are most important environmental pollutant. The contamination of aquatic environments by heavy metal becomes a global problem. Heavy metals are the member of loosely defined subset of elements that exhibit metallic properties. This group mainly includes transitional metals some metalloids, actinides and lanthanides. There are around 35 elements, in this group 23 which are heavy metals. Metal pollution is a worldwide problem tremendous increase in use of heavy metal over the past few decades as inevitably result in an increased flux of metallic substance in the aquatic environment industrial waste constituents the major source of metal pollution in aquatic ecosystem. A large part of these elements exert their toxic effect by generating Reactive Oxygen Species (ROS) causing oxidative stress. Most of the heavy metals are toxic or carcinogenic in nature and pose a threat to human health and the environment. The mineral salt content in water has great influence on toxicity of salt of heavy metals, especially carbonate hardness, since many salt of heavy metals from with it insoluble complex as result of toxicity is decreased. Ions play a great role to reduce the toxicity of these compounds. Cadmium is an extremely toxic metal commonly found in industrial work places. Cadmium is used in electroplating and galvanizing due to its non-corrosive and cumulative

nature. It is used as colour pigments for paints, plastics and as a cathode material for nickel cadmium batteries. Higher level of cadmium has also been detected in sewage sludge, various vegetables and animal feeds and their tissue. Cadmium is bio accumulative that means it is persistent and increase with the time since it is non-biodegradable. Cadmium wastes washed into aquatic bodies accumulate in aquatic biomass, they are concentrated up to the food chain. Cadmium is toxic even if absorption by ingestion is low, chronic exposure to high level of cadmium in food has caused bone diseases including osteoporosis and Osteomalacia. Long term ingestion by a Japanese population, of water and food associated with a crippling condition 'Itai Itai' disease. Other consequences of cadmium exposure are anaemia, yellow discolouration of the teeth, rhinitis, anosmia, damage to olfactory nerve. Cadmium is high doses include structural and functional alterations in various vital organs include liver, kidney, gill and intestine of fishes. Cadmium produces wide variety of acute and chronic effects in aquatic animals. Its prime site is kidney.

Stress is generalized response attributed to the fact that fish commonly have a complex of adaptive reaction to cope with stressors. Stress can be considered to be generalized response it can be modulated by specific stressor condition such modifying factor may be either a non-stressor or inherent to stressor trait. Stress induce a variety of complex change in fish physiology include defence mechanism which may compromise the ability to resist disease. Metals have adverse effect on growth and reproduction and causes osmoregulatory stress.

Proteins are the much abundant organic constituents of the living matter. They largely determine the functional properties of living matter and which determine whole someness and quality. They are the main structural and functional molecules of cell and also the chief regulators of life activities. Protein content in fish depends on species type, age, nutritional status, and reproductive stage. The high biological value of fish protein contain considerable quantities of almost all essential aminoacid required for man include lysine, arginine, leucine, isoleucine, valine, threonine, methionine, tryptophan etc.

Glucose is a simple sugar found in plants and animals tissue essential to the animal diet. It is transported by blood and lymph to all the cell of body where is broken down to produce ATP main source of energy for cellular processes. Glucose is the major source of energy for body cell. Glucose is a monosaccharide and preferred source of energy for RBC, brain and nervous system. It plays a role in creation or synthesis of other substance like glycol lipid or glycoprotein.

Lipid peroxidation is considered as the main molecular mechanism involved in oxidative damage to cell structure and in the toxicity process that leads to cell death. Lipid peroxidation is a complex process occur in both plant and animals. It involves information of propagation of lipid radicals uptake of oxygen arrangement of double bond is unsaturated lipids and eventual destruction of membrane lipids with production of varying breakdown products. In lipid peroxidation unsaturated lipids are oxidised to form additional radical species as well toxic by product harmful to host ecosystem. Malondialdehyde MDA a major degradation product of lipid peroxidise has attracted much attention as a marker for assessing the extent of lipid peroxidation.

Spirulina platensis:- is an aquatic microorganism often referred as alga through it more closely resembles bacteria. It is used as food supplement for malnutrition. It is a blue green multicell alga which can't be seen by naked eyes, It consist of 60-70% protein in dry weight, protein element consist of 18 types of aminoacid and vitamin A, B, E. Spirulina can be consumed by humans and other animals. It is a simple and easy digestible food without any risk to administrated to children. It is very useful nutritious food for prevention and symptomatic treatment such as antiviral activity, blood nourishment, enhance immunity against bacteria or foreign substance for body and recovery during convalescent period, it help to improve the digestive system, reduce cancer and increase anti-oxidant capacity. It contain high chlorophyll which helps to remove toxin from blood and boost immune system. Spirulina plays an important role in detoxifies the body from heavy metals, pollutants and harmful radiation. Reduce stress, anxiety, nervousness memory problem. Spirulina also regulate blood glucose level.

Materials and Method

The present study was analyse the effect of cadmium on total protein content, glucose. MAD which is the product of lipid peroxidation in Oreochromis mossambicus. This species proved to be meaningful one for toxicological studies.

Oreochromis mossambicus is commonly known as Java Tilapia or Japanese fish. Tilapia is a fresh water fish native to Africa and Middle east. It is a exotic fish. The fry feed phytoplankton and zooplankton but adult through chiefly herbivorous. Tilapia in healthy condition were collected from Thrissur district, Fish with a length between 10 cm to 15 cm and weight 15 gm. The fishes were kept in a large rectangular tank and were allowed to acclimatize to the laboratory condition for a period of about 2 week. During this period fishes were fed with artificial food.

The toxicant selected for the study was cadmium chloride. 1000 ppm stock solution was prepared by dissolving known quantity of in 50 ml volumetric flask. The concentration of cadmium was 100ppm. Calculated volumes of the stock solution were added to the water to get required concentration of the respective toxicant. The duration of the work was six month.

Spirulina indoor culture

Micro algal cultures were developed in 1L conical flask and 3L Haufkin's flask in the lab. Algal culture is very complex process; and it should be done very carefully in a hygienic condition for pure cultures. Preparedly sterilized The culture flasks filled 3/4th with one day old boiled sea water at 34 ppt salinity were, enriched with nutrients and inoculation was done with 2% to 10% of pure cultures in each flask without any contaminations. The flask was shaken well, cotton plugged and kept in controlled temperature conditions of 24^{o} C and allowed the algae to grow. Flasks were kept on wooden racks with fluorescent light. Every day cultures were shaken well to avoid settling of algae.

1. Control group -Three control tub each containing 8 litre water with three fishes without cadmium.
2. Experimental group- Experimental tub each containing 8 litre cadmium treated water with three fishes.
3. Spirulina treated group-Three tubs each containing spirulina treated 8 litre of water with three fishes.

Quantitative Analysis of Cadmium

5 g of each fish sample that were separated from kidney and meat. They were digested in 10 ml concentrated nitric acid in open glass container for 24 hours at room temperature. The following day the pre-digested sample was heated at 80

degree C for 5 hours. Samples were then cooled to room temperature and volume was adjusted to 50 ml with distilled water. Diluted sample were stored poly ethylene bottle and were analysed using flame Atomic Absorption Spectroscopy (AAS) (Varian) at KFRI.

Protein estimation done by Lowrey method (Lowry *et al.*, 1951). Estimation of Glucose by Somogy – Nelson method. Estimation of Malondialdehyde, Lipid peroxidation Product by John A Buege and Steven. P.

Result

Table 1 shows the level of protein in the muscle of fish. Protein is significantly decreased, when compared with normal and cadmium treated. But in the case of spirulina treated showed a non-significant relation when compared to normal. But significant relationship showing between spirulina and cadmium treated fish.

Table 1: Protien In Muscle (mg/ml)

Tissue	Group	Mean ± SD
Muscle	Normal	12.4 ±0.272
	Cadmium treated	10± 0.222
	Spirulina treated	13.2 ± 0.224

Table 2 shows that blood glucose level was significantly higher than that of spirilina treated fish. Blood glucose level was at lowest level at cadmium treated fish.

Table 2: Blood Glucose (mg/ml)

Tissue	Group	Mean ± SD
Blood	Normal	0.716 ± 0.479
	Cadmium treated	2.29± 0.375
	Spirulina treated	0.930± 0.408

Table 3 shows MDA is significantly increased through the experiment when compared with normal and cadmium treated.

Table 3: Malondialdehyde In Liver Tissue (mol/g protein)

Tissue	Group	Mean ± SD
Liver	Normal	12.4 ± 0.272
	Cadmium treated	14± 0.222
	Spirulina treated	13.2± 0.224

From the table 4, it can be observed that the amount of cadmium is more concentrated in kidney compared with muscles of the fish. In spirulina treated group show a tendency to overcome the cadmium toxicity, it shows a non significant relation showed between normal and spirulina treated group.

Table 4: Quantitative Analysis of Cadmium In Fish

Tissue	Group	AAS reading
Muscle	Normal	0.019
	Cadmium treated	0.051
	Spirulina treated	0.015

Tissue	Group	AAS reading
Kidney	Normal	0.020
	Cadmium treated	0.079
	Spirulina treated	0.018

In this present investigation revealed that highest amount of cadmium is accumulates in kidney of the cadmium exposed fish, compared with control group of fishes.

Discussion

In aquatic ecosystem heavy metals are considered as the most important pollutants, since they are present through out the ecosystem and are detectable in critical amount. Cadmium is heavy metal and possesses high toxicity at very low level of exposure and has acute and chronic effect on aquatic environment.

Smet and Blust (2001) [37] reported that cadmium accumulate on the tissue of cyprinus carpio in following order: kidney>liver>gills. Cadmium posses nephron toxication in man and animals. Kidney is the principle target organ of cadmium toxicity and chronic cadmium exposure in almost all animal species is characterized by varying degree of renal damage.

Proteins are the functional molecules of the living system. They are concerned with all physiological and vital functions. From the nutritional point of view protein is most constituents of fish which determines its whole soreness and quality. Fish proteins contain all essential aminoacids in good proportion and it enhances the nutritive value of fish. Protein content depends upon species type, age, nutritional status and reproductive stage etc.

Jacobes (1977) [14] suggested that the protein metabolism is considered one of the most sensitive physiological systems that responds to environmental stress and issued as diagnostic tool to determine the physiological phase of cell. Heavy metal contamination exerts an extra stress on metabolically active tissue and organs. These metals can decrease total protein depending on species of fish concentration and duration of exposure.

The total protein content in the fish Oreochromis mossambicus decreased due to cadmium poisoning. Due to environmental stress, cell stops or at least slow down most of its original function such as transport process, DNA, RNA and protein synthesis. A particular set of protein called stress protein as preferentially expressed under these restrictive conditions (Bindu and Kuzhiveli (2006) [5].

Suggested that the fish can utilize the stored proteins to overcome the toxic stress. On the toxic stress the level of key enzyme involved in protein metabolism gets changed. Proteins are mainly involved the architecture of the cell, which is the chief source of nitrogenous metabolism. Thus, the depletion of protein fraction in tissues may have been due to their degradation and possible utilization for metabolic purpose. Increase in free aminoacid level was the result of breakdown of protein in energy and impaired incorporation of aminoacid in protein synthesis.

Hilmya (1984) [12] reported that when a heavy metal entered the fish it may synthesis and secrete binding protein in liver may be due to rapid utilization of body protein for energy requirement to overcome the impact of cadmium. The decreased level of protein may be attributed to the spontaneous utilization of aminoacid in various catabolic reaction inside the system of organism to impact the heavy metal stress (Jha 1991) [16].

In the present study also the protein in fish Tilapia exposed to cadmium reduction in protein content reached to maximum. The reduction in protein content indicated proteolysis in tissue which forms aminoacids and used in TCA cycle for energy production during stress condition. The other reason is to met energy demand during pollution stress mobilization of protein might have taken place.

Moselhy (2001) [25] suggested that blood glucose level has been used as an indicator stress. The increased glucose level has been explained through the metabolic process called gluconeogenesis, through while the body produces glucose from non-carbohydrates source like protein and fat. Banaee (2013) [2] suggested that hepatic cells have many vital functions such as secretion of bile, detoxification and synthesis of several components of blood plasma, storage of glycogen and release of glucose. Most researchers have reported that the increased blood glucose is usually observed in fish under undesirable conditions and it helps the animal by providing energy substrates to vital organs to cope with the increased energy demand. In the present work, also agreed that cadmium may cause to increasing total glucose level in cadmium exposed fish, to compare with control group.

Suggested that lipid peroxidation is one of the manifestation of oxidative damage and has been found to play an important role in toxicity of many xenobiotics. Malondialdehyde known lipid peroxidation indicator has been found to increasing in liver and kidney after exposed to cadmium. Auto oxidation or non-enzymatic oxidation of poly saturated fats lead to the formation of MDA. Obalahjamakala and Usha A Rani (2012) [26] suggested that the lipid peroxidation was enhanced during cadmium exposure which may due to the interaction of cadmium with membrane phospholipids and thus causing membrane disorganisation and further fragility. The enhance of lipid peroxidation due to inhibition on activity level of antioxidants which more concerned with defence against free radical induction of cadmium in toxification.

Allengil and Martynore V.G *et al*., (1995) [1] reported that chronic treatment with cadmium induced oxidative damage and increased lipid peroxidation as well as alternation of antioxidant defence system. An increase the level of lipid peroxidation product (MDA) lead to the formation of ROS. As consequence of enhanced lipid peroxidation lead cellular injury in animal tissue. In the present study increased level of MDA found in liver of fishes exposed to cadmium as compared to control, which is an indicator of lipid peroxidation process.

Pinero (2001) [30] reported that spirulina platensis is a cyanobacteria used many countries as nutritional supplement for human and animal consumption. It is a powerful food rich in protein, carbohydrate, fatty acid, vitamin and minerals. Spirulina helps to protect against certain nutritional deficiencies and plays an important role in heavy metal absorption.

Mazo (2004) [23] reported that spirulina platensis considered a valuable additional food source of some macro and micro nutrient including high quality protein iron, fatty acid, carotenoids and vitamins. The antioxidant mechanism of β-carotene has been suggested to be a single oxygen quenching, free radical scavenging and chain breaking during lipid peroxidation. The metalo protective role of spirulina may be attributed due to the presence of β-carotene. Spirulina contain vitamin C, E and β-carotene this phytochemical constituent may reduce the toxicity and enhance radical scavenging property ie, reduced oxidative stress.

Find that the technique of binding metals irons to the biomass of spirulina which are nutria finally significant in animals. The process of binding micro elements to the biomass is based on the ability of biological materials to bind metal ions by either metabolically mediated or purely physiochemical pathway of uptake. Because of negative surface charge and membrane composition, spirulina have nutria absorbents of metal ions. Cell wall contain polysaccharide protein and lipid contain many functional group that can form coordination complex with metal cation and functional group able to interact with metal ions in aqueous solution.

Suggested that spirulina has all biochemical in its constitution that can build healthy immune system which scavenges free radicals. Spirulina extract possess antiviral activities that may potential clinical interest. It plays in prevention of cancer, cellular aging infectious disease and reduce immune system efficiency as well as playing as important part of in functioning of medulla.

K.A Premkumar and S.K Panchiappan *et al*. (2001) [17] reveals that role of spirulina reducing the oxidative stress may be due to the presence of several active compounds. The active compounds in spirulina provoke the activity of free radical scavenging enzyme system and provide protection against cadmium induced tissue damage. B Carotene act as powerful quencher of scavenger of free radicals and vitamin E spirulina prevent cadmium induced lipid peroxidation.

Bermejo (1997) [4] demonstrated that spirulina protein extract possessed an excellent antioxidant activity. Resulted showed that the protein extract of spirulina scavenged hydroxyl and peroxyl radicals and also had inhibitory activityagainst lipid peroxidation scavenging of these free radicals by spirulina can be effective protection for living organism against oxidative stress.

The anti oxidant and protective effect of spirulina against cadmium induced toxicity in respect to lipid peroxidation. The protective effect of spirulina against cadmium induced oxidative stress could be either indirect through the enhancement of the activity of GSH peroxidase and superoxidase dismutase (free radical scavengers) or direct by inhibiting peroxidation of lipid and scavenging of free radicals. These characteristics are due to the high concentration of antioxidant components of spirulina platensis (Simsek 2009) [34].

Jha (1991) [16] showed that the stressed environment of fish may lead to the denaturing of many protein it will cause to the decrease of protein in tissue. The decreased amount of protein is due to rapid utilization of protein for the energy required to over come the heavy metal (1984). But spirulina treated showed a significant relation between cadmium treated fish at the sametime spirulina treated showed a tendency towards to normal. This is non-significant relation.

Moselhy (2001) [25] suggested that blood glucose level has been used as indicator of stress. The increased glucose level has been explained through the metabolic process called gluconeogenesis. In the present work total glucose level in cadmium exposed fish in increased to compare with spirulina treated group.

Found that autooxidation or non-enzymatic oxidation of polyunsaturated fat lead to the formation of MDA.

Prem Kumar and Pachappan *et al*., reveals that spirulina the activities of free radical scavenging enxyme and provides protection against cadmium induced tissue damagespirulina can play an important role in phytoremediation, phytoextraction and biosorption (Ozer 1994) [28].

Phytoremediation is defined a process of decontaminating soil and aquatic systems by using plants of algae to absorb heavy metals. The use of aquatic plants especially micro and macro algae has received much attention due to the ability to absorption of metals and taking up toxic elements from the environment or rendering them less harmful.

Biosorption is the ability of the organism to bound toxic

heavy metals on the cell wall. Phytoextraction is the subprocess of phytoremediation in which plants remove dangerous elements of or compound from water or soil most usually heavy metals.

The present investigation clearly revealed from tables, there was a decreased in the amount of protein and increased in blood glucose and lipid peroxidation product and simultaneously spirulina treated group showed tendency to overcome the toxicity of cadmium. So the evidences are showed that spirulina can reduced the risk of heavy metal toxicity.

Conclusion

In the present study reveals that the cadmium accumulate on more in kidney when compared with muscles of fish. In spirulina treated fish showing a non-significant relationship between normal and cadmium treated. Protein, glucose and MDA content in fish both experiment and control were established. In present observation clearly indicated that cadmium induced oxidative stress and inhibit protein metabolism in fish. The amount of blood glucose and MDA in the fish was found to increase in cadmium treated fish when compared with normal.

Protein metabolism is considered as one of the most sensitive physiological system to environmental stress. The decreased amount of protein may be due to rapid utilization of body protein for energy requirements, so as to withstand the impact of cadmium. The increased amount of blood glucose in cadmium treated fish compared to control. Glucose is use a indicator of stress due to presence of cadmium effect increase in the level of catecholamine, activating glycogenolysis and gluconeogenesis with net result of increasing plasma glucose to fish.

Lipid peroxidation has been considered as first step of cellular membrane damage by heavy metals. Malondialdehyde the indicator has been found to increase in liver and kidney after cadmium exposure. In our study administration of cadmium chloride at fish revealed significant increase of lipid peroxidation in liver and therefore it is suggested that cadmium as toxicant may induce oxidative stress by producing hydroxyl radicals.

Spirulina platensis is an aquatic blue green multicell alga, which showed a tendency to normalize or a non-significant relation in the case of protein glucose and lipid peroxidation (MDA) when compared with cadmium exposed and spirulina treated. The hazardous of heavy metal pollution have received much attention because they cannot be eliminated from ecosystem, but they persist in the sediment. The present study clearly elucidate the properties of spirulina(Antioxidant capacity that is scavenging ability of free radical, phycoremediation, biosorption, antiviral activity and Phyto extraction) through which reduce the chance of risk of heavy metal toxicity by economically.

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