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Research Article

Spirulina platensis and *Chlorella vulgaris* Assisted Bioremediation Heavy Metal Contaminated Aquatic Ecosystem

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ABSTRACT

Living organisms require trace amounts of some heavy metals including copper, lead, magnesium, vanadium, zinc etc. Human activities have influenced bio-chemical and geological cycles. Metal ions become toxic in nature when they are beyond tolerance limit. In aquatic ecosystem, fishes and microbes have close, intimate and unseparated contact from the embryonic to adult stage. Bioremediation is therefore an eco-friendly and efficient method of reclaiming environments contaminated with heavy metals by making use of the inherent biological mechanisms of microorganisms and plants to eradicate hazardous contaminants. Microbes play a key role in controlling the speciation and cycling of metals in water. Bio-availability, toxicity and reactivity of metals is greatly influenced to have a better understanding of the major factors that link microbial activity to the bio-geo-chemistry of metals. Micro-organism and other natural products plants and animals and there by- products capable of cycling metals for bio-remediation of contaminated site without any side effect on environment. This investigation discusses the toxic effects of heavy metal pollution and the mechanisms used by microbes for environmental remediation. It also emphasized the importance of modern techniques and approaches in improving the ability of microbial enzymes to effectively degrade heavy metals at a faster rate, highlighting recent advances in microbial bioremediation for the removal of heavy metals from the environment.

Keywords: *Chlorella vulgaris; Spirulina platensis; Labeo rohita; Clarias batrachus; Channa punctatus*

INTRODUCTION

Among the pollutants heavy metals are regarded as one of the most serious pollutants are due to their environmental persistence and tendency to concentrate in aquatic organisms. Heavy metals are chemical elements with a specific gravity that is at least five times greater than specific gravity of water and the pollution of ecosystem by heavy metal is an important problem. Heavy metals constitute some of the most hazardous substances that can bio-accumulates. The accumulation of heavy metals in the viscera, precipitation leads into chronic illnesses and cause significant damage to various organisms including induced stress, lipid per oxidation, protein denaturation, The physiological, cellular and molecular mechanisms too used to regulate and detoxify environmental heavy metal toxicity on a variety of organisms but a clear understanding about the mechanism is awaited and expects further studies to establish a clear understanding on the above matter and through food and water, heavy metals/pollutants invariably find a place in the organisms including humans. Heavy metal induces oxidative damage in different organs by increasing per-oxidation of membrane chemistry and altering the antioxidant system of the cells/tissues. Interaction of metal ions with the cell organelles causes injury to cellular components.

MATERIAL AND METHODS

Alive, healthy, mature, disease-free and active *Labeo rohita* (Ham.), *Clarias batrachus* (Linn.) and *Channa punctatus* (Bloch.) 120-130gm of 18-20 cm (standard length) were obtained from few selectedlocal ponds to avoid ecological variation and acclimatized in the laboratory condition for a period of seven days and were subjected

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for various exposures and investigations.

Determination of safety sublethal and lethal concentration

Safety, sub-lethal concentrations of copper was determined on *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* by the probit analysis method. Higher concentration of copper was used and slowly reduced the amount of concentration to know the LC 50/100 value for 96-hour exposure.

Acute studies

The *Labeo rohita, Clarias batrachus* and *Channa punctatus* (120- 130 gm) of 18-20 cm (standard length) were taken separately and kept in twenty groups and each group consist of forty eight fish species. No food was given to the above fish species during this period (08, 16 and 24 hrs). The first set of *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* were exposed to sub-lethal and lethal concentration of copper and zinc the detail were described somewhere else. Preparation of tissue extract

The terminations of the experiment preparation of tissue extract and enzyme assays were described elsewhere.

Statistical analysis

The experiments with acute and chronic studies were repeated at least seven times separately to subject the data for analysis of variance.

RESULTS

The results enlightened that the combined influence of both the microbes [*Chlorella vulgaris* and *Spirulina platensis*] heavily decreased the toxic influence of copper and zinc on carbohydrate enzymes [phosphoglucomutase, hexokinase, phosphoglucoisomerase and Phosphofructokinase] in brain regions [cerebrum, diencephalons, cerebellum and medulla oblongata] in *Labeo rohita* (sub-lethal concentration of Zn-0.72 mg/ltr., Cu-0.10 mg/ltr), *Clarias batrachus* (sub-lethal concentration of Zn-2.75mg/ltr., Cu-0.50 mg/ltr), and *Channa punctatus* (sub-lethal concentration of Zn-2.90mg/ltr.,Cu-0.80mg/ltr) under chronic studies.

The sub-lethal copper concentration [in presence of two microbes] inhibited the phosphoglucomutase to a significant extent at thirty days exposure than in cerebrum, medulla oblongata and cerebellum in comparison to 15 and 45 days exposure in *Labeo rohita*. In *Clarias batrachus* the fall in phosphoglucomutase was maximum in diencephalons at 30 day exposure followed by cerebrum, medulla oblongata at 15 days exposure and cerebellum at 30 days exposure than at 45 days exposure.

In *Channa punctatus* the fall in phosphoglucomutase was highest in diencephalons at 30 days of exposure than in cerebrum, medulla oblongata and cerebellum at 15 days of exposure than at 45 days of exposure under chronic studies.

The combined influence of *Chlorella vulgaris* and *Spirulina platensis* was experimented on sub-lethal concentrations of copper toxicity in which hexokinase registered optimum fall in diencephalons at 30 days of exposure followed by cerebrum and medulla oblongata at 15 days of exposure and cerebellum at 30 days of exposure than at 45 days of exposure in *Labeo rohita*. In *Clarias batrachus* the hexokinase fall was recorded in diencephalons to a great extent at 15 days of exposure than in cerebrum, medulla oblongata and cerebellum in comparison to 30 and 45 days exposure. The hexokinase maximum fall was at 30 days exposure in diencephalons followed by cerebrum, medulla oblongata and cerebellum at 15 days of exposure than 45 days exposure under chronic studies in *Channa punctatus*.

The phosphoglucoisomerase fall was optimum in diencephalon accompanied by cerebrum, medulla oblongata and cerebellum at 15 days exposure in *Labeo rohita* exposed to sub-lethal concentrations of copper in microbe's presence. In *Clarias batrachus* the phosphoglucoisomerase fall was highest in diencephalons at 30 days exposure to sub-lethal concentrations of copper in comparison to cerebrum, medulla oblongata and cerebellum at 15 days of exposure. The fall in phosphoglucoisomerase was noticed in diencephalons at 15 days of exposure accompanied by cerebrum, medulla oblongata and cerebellum under chronic studies in *Channa punctatus* than at 30 and 45 days of exposure.

The fall in phosphofructokinase was maximum in diencephalons at 30 days of exposure to sub-lethal concentrations of copper in presence of two microbes [*Chlorella vulgaris* and *Spirulina platensis*] in comparison to cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) in *Labeo rohita*. In *Clarias batrachus* the fall in phosphofructokinase was noticed in diencephalons than in cerebrum, medulla oblongata and cerebellum at 15 days of exposure. The fall in phosphofructokinase was optimum at 30 days in diencephalon accompanied by cerebrum, medulla oblongata and cerebellum at 15 days exposure to sub-lethal levels of copper in the microbes presence in *Channa punctatus*.

At 30 days exposure to sub-lethal concentrations of zinc the presence of two microbes affected phosphoglucomutase at 30 days in diencephalons than in cerebrum, medulla oblongata at 15 days and cerebellum in the *Labeo rohita* (30 days exposure). In *Clarias batrachus* the variations recorded in the phosphoglucomutase was prominent in diencephalons at 30 days followed by cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) than at 45 days exposure. The phosphoglucomutase fall was significant at 30 days in diencephalons in comparison to cerebrum, medulla oblongata (15 days exposure) and cerebellum under long term studies in *Channa punctatus*.

The maximum fall in hexokinase in the presence of two microbes exposed to sub-lethal concentrations of zinc was in diencephalons at 30 days than cerebrum, medulla oblongata and cerebellum (15 days exposure) in *Labeo rohita* in comparison at 45 days of exposure. In *Clarias batrachus* the fall in hexokinase was noticed in diencephalons at 30 days prominently in comparison to cerebrum, medulla oblongata and cerebellum than at 15 and 45 days exposure. In *Channa punctatus* to the fall in hexokinase was significant in diencephalons at 30 days exposure accompanied by cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) under long term studies.

The fall in phosphoglucoisomerase was maximum at 30 days in diencephalons in comparison to cerebrum, medulla oblongata (15 days) and cerebellum (30 days) exposed to sub-lethal concentrations of zinc in the presence of two microbes in *Labeo rohita* than at 45 days of exposure. In *Clarias batrachus* the phosphoglucoisomerase fall was highest in the diencephalons at 30 days of exposure to zinc in the presence of microbes than 15 and 45 days of exposure than in cerebrum, medulla oblongata and cerebellum (15 days exposure). The sub-lethal concentrations of zinc manifested optimum enzyme variation in diencephalons at 30 days than at 15 and 45 days exposure accompanied by cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) under long term studies in *Channa punctatus*.

The sub-lethal concentrations of zinc in presence of two microbes described earlier manipulated phosphofructokinase to a marked extent in diencephalons at 30 days exposure than in cerebrum, medulla oblongata (15 days exposure) and cerebellum (30 days exposure) in *Labeo rohita*. In *Clarias batrachus* also the diencephalon phosphofructokinase registered highest fall at 30 days exposure than in cerebrum, medulla oblongata (15 days) and cerebellum (30 days) exposure to sub-lethal level of zinc in presence of microbes.

The trend in phosphofructokinase fall exposure to sub-lethal concentrations of zinc in the presence of two microbes in *Channa punctatus* is more of less similar to *Labeo rohita* and *Clarias batrachus* under chronic studies in Figures 1-3.

DISCUSSION

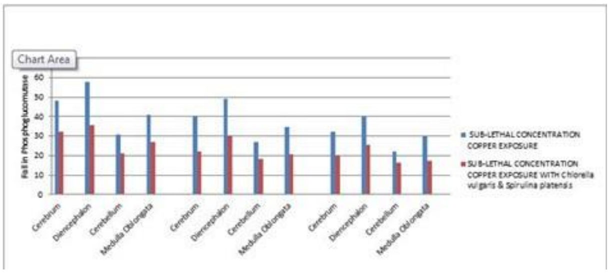

Figure 1: Combined influence of *Chlorella vulgaris* and *Spirulina platensis* on copper metal (sub-lethal) caused toxicity in three fresh water teleosts Phosphoglucomutase-chronic studies.

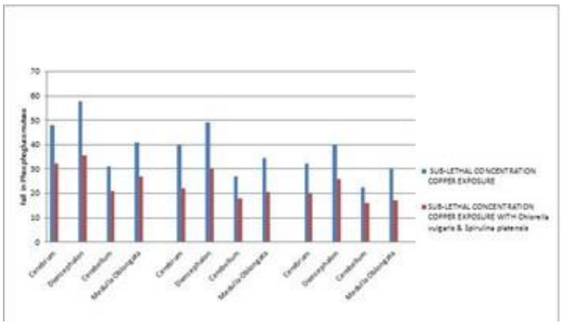

Figure 2: Combined influence of *Chlorella vulgaris* and *Spirulina platensis* on copper metal (sub-lethal) caused toxicity in three fresh water teleosts Phosphoglucomutase-chronic studies.

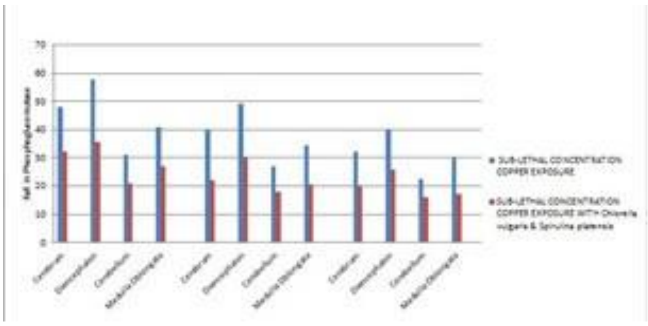

Figure 3: Combined influence of *Chlorella vulgaris* and *Spirulina platensis* on copper metal (sub-lethal) caused toxicity in three fresh water teleosts Phosphoglucomutase-chronic studies.

visualized as aquatic autotrophs has been used in industrial but also in domestic uses like water treatment as they are capable of removing waste to a great extent.

The uptake of copper and zinc by aquatic autotrophs used in present investigation has been realized that the aquatic autotrophs have an initial rapid stage and a slower stage. During rapid phase the metal ions are absorbed on the surface and transport them across the cell membrane and it is the first symptom of cell damage and deterioration of membranes [1-4].

It is further observed that there is an increase in the number of polyphosphate bodies with heavy metal toxicity in cyanobacteria as polyphosphate bodies have been working as indicators of metal absorption in cyanobacteria. The strong negative surface charge of polyphosphate in the phosphate bodies may help in absorbing the metal. The polyphosphate bodies may contain magnesium, sodium, iron and phosphorous. The polyphosphate bodies may not function in storage of polyphosphate but also help in detoxification mechanism [1,5].

The cyanobacteria further contain cyanophin granules that act as storage in the cell. Perhaps these bodies may participate in the cell internal detoxification process. The pH of the media may also influence the toxicity of copper and zinc by altering the form/ nature of heavy metals [6].

Hydrogen ions may also play a vital role to check the toxic impact of copper and zinc as the metal binding sight on the cell surface binds with a proton reflects. Those protons will compete with metal ions for the binding sight. Change in gases ratio in aquatic system may change the temperature of the media by that aquatic autotrophs may absorb heavy metals [7,8].

The above mentioned episodes are not totally/partially rules out even in the present investigation and the fall in phosphoglucomutase, hexokinase, phosphoglucoisomerase and phosphofructokinase in cerebrum, diencephalons and medulla oblongata in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* with direct sub-lethal and lethal metal exposure and in presence of aquatic autotrophs and their cell organization bound mechanism of' detoxification to neutralize the sub-¬lethal and lethal copper and zinc concentrations affect the acute and chronic studies prominently reflect that without the possibilities of the above mentioned and discussed processes it was not possible for *Chlorella vulgaris* and *Spirulina platensis* to detoxify the metal caused toxicity on phosphoglucomutase, hexokinase, phosphoglucoisomerase and phosphofructokinase in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* on a comparative basis from a tropical habitat .

The finding may help to understand the microbe-metal interaction and sub sequent detoxification of the metal to a less extent in a better way. The sub-cellular regions of Cyanobacteria and Anabaena cylindrica could trap the lead through its phosphate and precipitates in the form of lead phosphate on the cell wall inside the cell [9].

The following mechanisms are used for microbial bioremediation

Sequestration of toxic metals by cell wall components or by intracellular metal binding proteins and peptides such as Metallothioneins (MT) and phytochelatins along with compounds such as bacterial siderophores which are mostly catecholates, compared to fungi that produce hydroxamate siderophores.

Alteration of biochemical pathways to block metal uptake.

Conversion of metals to innocuous forms by enzymes.

Reduction of intracellular concentration of metals using precise efflux systems.

Mechanisms of removal of heavy metals from contaminated soils by microorganims through the processes of precipitation, biosorption via sequestration by intracellular metal binding proteins (metallothioneins) and conversion of metals to innocuous forms by enzymes (enzymatic transformation). The heavy metal removal significantly affected by the pH in the solution as hydrogen ions plays an important role in multicomponent absorption system. The increase in heavy metal uptake by autotroph *Spirulina platensis* and cynobacteria with the increasing pH. A pH dependence of ion generally occurs when heavy metal binding site on cell surface binds with proton. This indicate that the protons will compete with metal ions for the binding site. Hence most ions are absorbed at a highest pH in a better way due to lower competition with protons. This indicates that heavy metals were smartly absorbed in a pH range of 4-8 [10].

The potential negative surface charge of the poly-phosphate in the polyphosphate bodies will assist to absorb metal. Increase in the exposure time of autotrophs to heavy metals further increase the number of polyphosphate bodies and also composed of other materials such as magnesium, sodium, potassium, iron and. Such bodies not only function in polyphosphate storage and further functions as a detoxification process such a mechanism is not rule out even in the present investigation and the fall of phosphoglucomutase, hexokinase, phosphoglucoisomerase and phosphof\ructokinase with the metal exposure directly on one side and metal exposure in presence of *Spirulina* in *Labeo rohita*, *Clarias batrachus* and *Channa punctatus* on both side educates that the presence of the aquatic autotroph significantly checked the fall off the enzymes in different brain regions of the above saidfish species is quite innovative and need further investigation on a large scale for the application in the aquatic system and to check the menace of pollution.

CONCLUSION

This investigation further helps that aquatic autotrophs can be used to remove heavy metals from aquatic system over a wide range of pH. Such events might have taken place even in the present investigation and the less fall in phosphoglucomutase, hexokinase, phosphoglucoisomerase and phosphofructokinase in different brain regions of *Labeo rohita*, *Clarias batrachus* and *Channapunctatu* might be to a less degree in microbe presence than directexposure to heavy metals.

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