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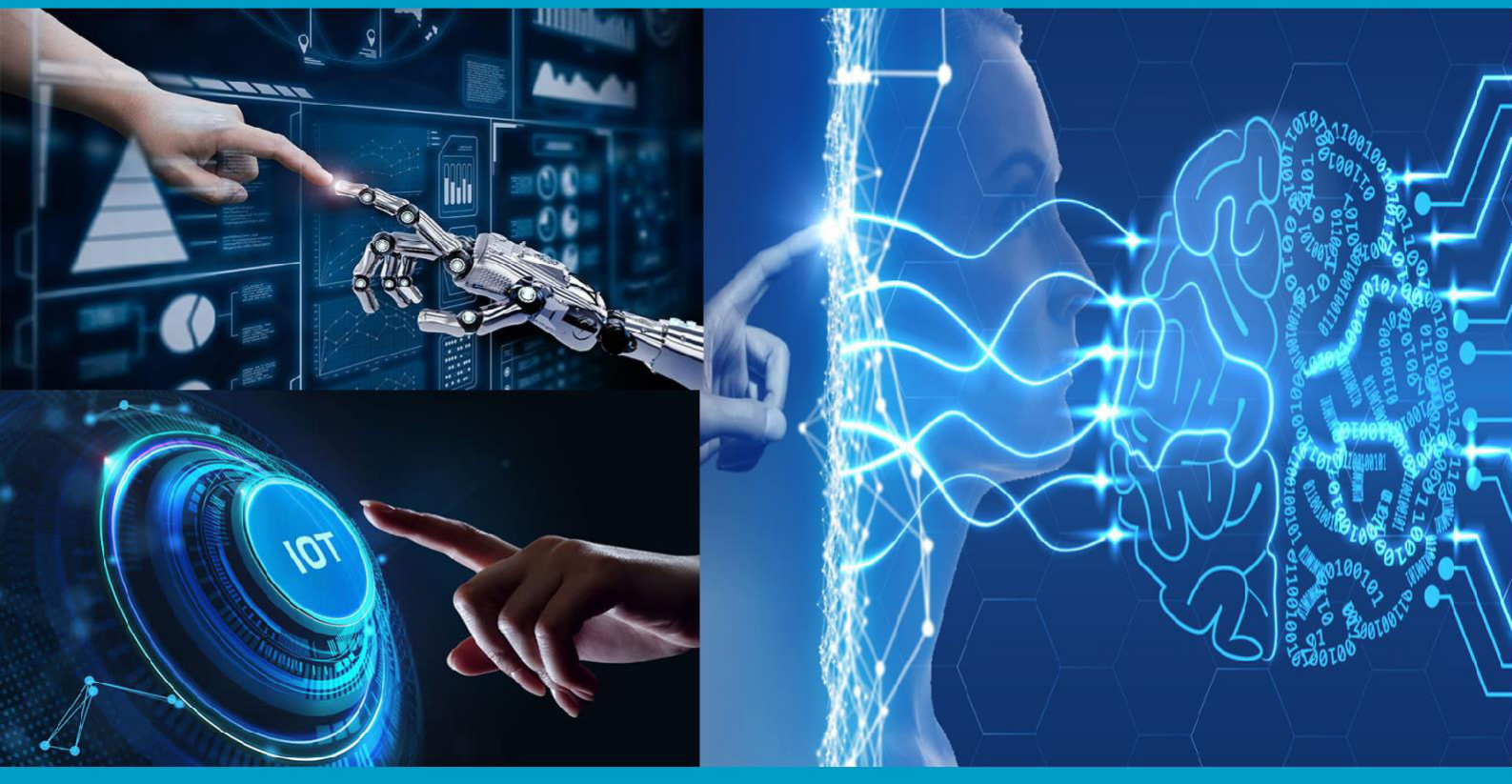

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Effects of Heavy Metals on Cyanobacteria

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ABSTRACT: Cyanobacteria also known as Cyanophyta, are a phylum of Gram-negative bacteria that obtain energy via photosynthesis. The name cyanobacteria refers to their color (from Ancient Greek κυανός (kuanós) 'blue'), which similarly forms the basis of cyanobacteria's common name, blue-green algae. They appear to have originated in a freshwater or terrestrial environment. Sericytochromatia, the proposed name of the paraphyletic and most basal group, is the ancestor of both the non-photosynthetic group Melainabacteria and the photosynthetic cyanobacteria, also called Oxyphotobacteria.Cyanobacteria use photosynthetic pigments, such as carotenoids, phycobilins, and various forms of chlorophyll, which absorb energy from light. Unlike heterotrophic prokaryotes, cyanobacteria have internal membranes. These are flattened sacs called thylakoids where photosynthesis is performed. Phototrophic eukaryotes such as green plants perform photosynthesis in plastids that are thought to have their ancestry in cyanobacteria, acquired long ago via a process called endosymbiosis. These endosymbiotic cyanobacteria in eukaryotes then evolved and differentiated into specialized organelles such as chloroplasts, chromoplasts, etioplasts, and leucoplasts, collectively known as plastids.Cyanobacteria are the first organisms known to have produced oxygen. By producing and releasing oxygen as a byproduct of photosynthesis, cyanobacteria are thought to have converted the early oxygen-poor, reducing atmosphere into an oxidizing one, causing the Great Oxidation Event and the "rusting of the Earth", which dramatically changed the composition of the Earth's life forms.The cyanobacteria Synechocystis and Cyanothece are important model organisms with potential applications in biotechnology for bioethanol production, food colorings, as a source of human and animal food, dietary supplements and raw materials. Cyanobacteria produce a range of toxins known as cyanotoxins that can pose a danger to humans and animals. This review dela with the effects of heavy metals on cyanobacteria.

KEYWORDS: heavy metals, cyanobacteria, effect, photosynthetic, cyanotoxins, production, oxygen

I. INTRODUCTION

Freshwater resources is seriously threatened by heavy metals pollution. Thus developing an effective screening method for the presence of heavy metals is required. In this paper, the potential of using cyanobacteria for heavy metals detection in water bodies is determined. The cyanobacteria *Anabaena cylindrica* had been cultured, immobilized, and exposed to Cu, Pb, and Cd with the concentrations of 0.01 – 10.00 mg/L. The responses of the cells to the heavy metals were measured for 60 min. The effect of the cell culture age and cell density were determined as well. The cells with 7 days of culture age, and the amount of cell with density of OD= 0.5 A (measured at 700 nm wavelength) were found to produce highest photosynthetic fluorescence for the detection for heavy metals. The cells were capable to provide detectable fluorescence emission within 10 min of exposure. Thus the cyanobacteria was identified as a good candidate to serve as bioindicator for the heavy metals in water. Non-diazotrophic cyanobacteria are unable to fix atmospheric nitrogen and rely on combined nitrogen for growth and development. In the absence of combined nitrogen sources, most non-diazotrophic cyanobacteria, e.g., *Synechocystis* sp. PCC 6803 or *Synechococcus elongatus* PCC 7942, enter a dormant stage called chlorosis. The chlorosis process involves switching off photosynthetic activities and downregulating protein biosynthesis. Addition of a combined nitrogen source induces the regeneration of chlorotic cells in a process called resuscitation. As heavy metals are ubiquitous in the cyanobacterial biosphere, their influence on the vegetative growth of cyanobacterial cells has been extensively studied. However, the effect of heavy metal stress on chlorotic cyanobacterial cells remains elusive. To simulate the natural conditions, we investigated the effects of long-term exposure of *S. elongatus* PCC 7942 cells to both heavy metal stress and nitrogen starvation. We were able to show that elevated heavy metal concentrations, especially for Ni^{2+}, Cd^{2+}, Cu^{2+} and Zn^{2+}, are highly toxic to nitrogen starved cells. In particular, cells exposed to elevated concentrations of Cd^{2+} or Ni^{2+} were not able to properly enter chlorosis as they failed to degrade phycobiliproteins and chlorophyll a and remained greenish. In resuscitation assays, these cells were unable to recover from the simultaneous nitrogen starvation and Cd^{2+} or Ni^{2+} stress. The elevated

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toxicity of Cd^{2+} or Ni^{2+} presumably occurs due to their interference with the onset of chlorosis in nitrogen-starved cells, eventually leading to cell death.[1,2]

The influence of two metals, copper and cadmium, was studied on the growth and ultrastructures of cyanobacterium *Anabaena flos-aquae* grown at three different temperatures: 10°C, 20°C, and 30°C. The highest concentration of chlorophyll *a* was observed at 20°C and the lowest at 10°C. Both toxic metal ions, Cu^{2+} and Cd^{2+}, inhibited growth of the tested cyanobacterium. Chlorophyll *a* concentration decreased with the increase of metal concentration. A 50% decrease in the growth of *A. flos-aquae* population, compared with the control, was reached at 0.61 mg l^{-1} cadmium and at 0.35 mg l^{-1} copper (at 20°C). Copper at all temperatures tested was proven to be more toxic than cadmium. At 3 mg l^{-1}, the lysis and distortion of cells was observed; however, after incubation at 9 mg l^{-1} cadmium, most of the cells were still intact, and only intrathylakoidal spaces started to appear. Copper caused considerably greater changes in the protein system of *A. flos-aquae* than did cadmium; in this case, not only phycobilins but also total proteins were destructed. The aim of this study was also to identify the place of metal accumulation and sorption in the tested cyanobacterium. Analysis of the energy-dispersion spectra of the characteristic x-ray radiation of trichomes and their sheaths showed that cadmium was completely accumulated in cells but was not found in the sheath. Spectrum of the isolated sheath after treatment with copper exhibited only traces of the metal, but isolated cells without a sheath showed a high peak of copper.

Many factors affect cell growth,metabolite accumulation and extracellular polymeric substances (EPSs) including nutrients, such as phosphate and nitrogen, temperature, light intensity, aeration rate, and mixotrophic condition, in microalgal cultures .Although the presence of EPSs are extremely preserved among cyanobacteria, there is not much information about factors that maximize thebiosynthesis of EPSs and affects the biosorption capacity . Several trace elements such as copper, cobalt, and nickel are essential cofactors in cyanobacteria strains. Unlike organic contaminants, heavy metals such as copper [3,4] and lead are the main pollutants of freshwater due to persistent, toxicity, recalcitrant, and non-biodegradable nature .On the other hand, heavy metal ions concentration at low concentrations are known to be toxic to the organisms because they inhibit many enzymes irreversibly. Heavy metal uptake capacity of algal biomass has proved to be the highest due to the presence of polymers containing functional groups (which can act as binding sites for metals) such as amino, hydroxyl, carboxyl, and sulfate.Polysaccharides, proteins, or lipids on the cell wall surface which are good examples for these polymers .Several studies have been conducted on phytoremediation investigation and several authors have established the fact that treatment of wastewaters using algae, and particularly microalgae decrease organic and inorganic nutrients, such as toxic chemicals remarkably . Our previous study indicated that cell growth and the production of EPSs are highly culture conditions dependent. There is no correlation between cell growth and the production of EPSs in cultures with different sources of nitrogen. In contrast, light intensity and cell growth in mixotrophic conditions have had a highly positive effect on the production of EPSs. In salt-grown cultures, thick layers of ASN_M strain supports the cells from NaCl stress hence its growth is maintained without the NaCl stress inhibition[5,6] .

II. DISCUSSION

Heavy metals constitute toxic, non-biodegradable and persistent environment pollutants which adversely affect all life forms, including humans, and cause ecological damage. The detrimental effects of heavy metals on living organisms are attributable to their action on a number of cellular and biochemical processes, biomolecules and structures in living organisms, including human beings. In humans, they are known to cause various patho-physiological disorders of hepatic, renal, respiratory and gastrointestinal system. The biotoxicity of heavy metals depends on their concentration, bioavailability, chemical forms and duration of exposure. Globally, the ever-increasing contamination of aquatic bodies and soil by heavy metals (e.g. Cd, Hg, Ag, As, Pb, Ni, Cr, Cu, Zn) owing to various anthropogenic activities is an issue of serious concern and challenge. Bioremediation of heavy metals, employing various microorganisms, including cyanobacteria (bluegreen algae), has been recognized as a cheaper, more effective and an eco-friendly alternative to the conventional physico-chemical remediation methods. Because of their tremendous adaptability and effective protective mechanisms against various abiotic stresses, cyanobacteria colonize and inhabit diverse terrestrial and aquatic habitats, including extreme and polluted ones. Various cyanobacterial species possess efficient heavy metal removal capabilities from aqueous solutions. They produce metal-binding proteins (metallothioneins) and metal-sequestering agents (e.g. exopolysaccharides). The bioremoval of heavy metals by cyanobacteria is mediated by biosorption and bioaccumulation. Cyanobacteria, because of their ubiquity, abundance, rapid growth rate, simple growth requirements,

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heavy metal tolerance and removal, and amenability to controlled laboratory culture and immobilization are the promising candidates for the bioremediation of heavy metal pollutants. [7,8]

There was a significant variation of residual Cd values among the different initial Cd concentrations considered. As shown in Fig. 1, the residual Cd tends to stabilize after day 12 for all initial concentrations. *N. muscorum* achieved a terminal residual Cd value of 0.033, 0.175 and 0.51 mg L^{-1} for the initial concentration of 0.5, 1 and 2 mg L^{-1}, respectively, translating to heavy metal removal efficiency of 93.4, 82.5 and 74.5%, respectively. Terminal residual Cd values achieved by *T. variabilis* were slightly higher at 0.054, 0.26 and 0.632 mg L^{-1} for the initial concentration of 0.5, 1 and 2 mg L^{-1}, respectively, reflecting removal efficiency values of 89.13, 74.00 and 68.38%, respectively (Fig. 1a). Cadmium was released again into the contaminated water as a result of the algae's sorption decline related to the toxic effect of Cd, and so the residual Cd marginally increased after 16 and 12 days for the initial concentrations of 1 and 2 mg L^{-1}, respectively.

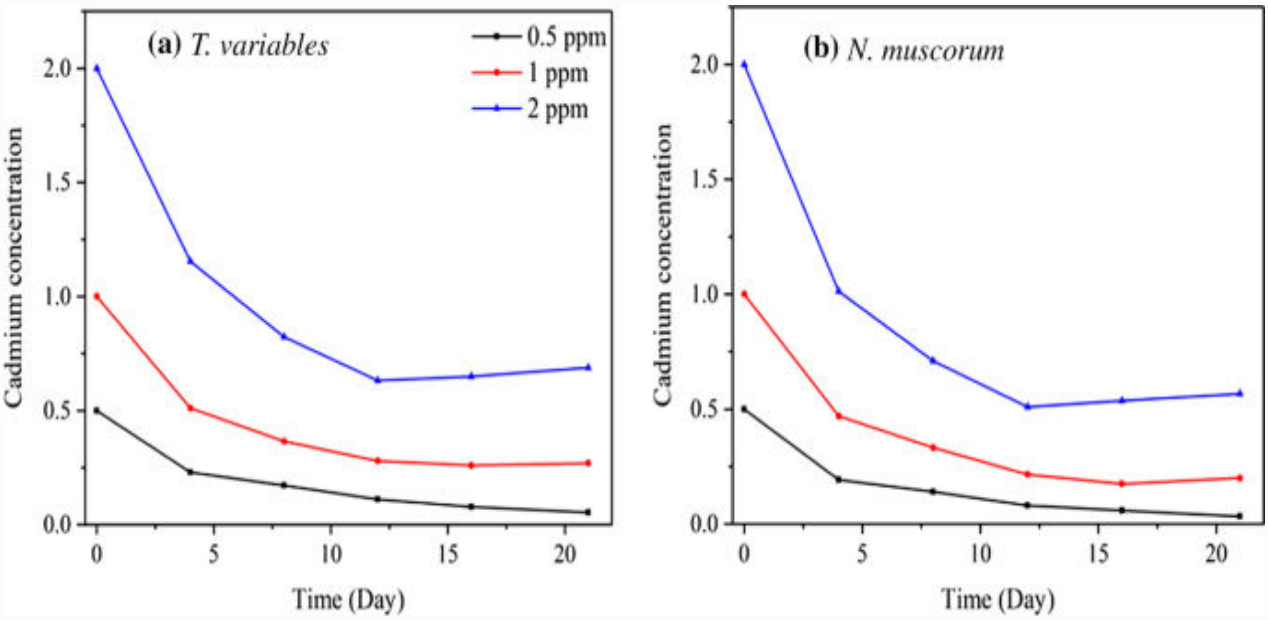

Fig. 1a Residual concentration of Cd treated by *T. variabilis* (a) and *N. muscorum* (b) for three different initial concentrations

III. RESULTS

Cyanobacteria exhibit an extraordinary resistance to many environmental factors including metal pollution. The present study was conducted to explore the possibility of using cyanobacteria for bioremediation of Co2+ and Zn2+ as essential nutrient elements for the growth of cyanobacteria which detoxify these metals. *Anabaena oryzae* and *Tolypothrix tenuis* cells collected from paddy fields expressed different degrees of tolerance to metal(s) stress due to cobalt, zinc, copper and mercury. The tolerance of these species under different concentrations (1, 10 and 100 ppm) of heavy metals was determined. [9,10]Observations were made on every 2nd day for the period of 12 days. Among the organisms studied, *T. tenuis* was more sensitive to the metals than *A. oryzae.* Copper at 1 ppm on the 8th day gave maximum carotenoid content (6.652 µg mL-1) in *A. oryzae*. The mercury treated cells showed lethality at (1, 10 and 100 ppm). There was gradual increase of carotenoid content after 12 days, especially at (1 and 10 ppm) of Co2+ and Zn2+ in *A. oryzae*. This indicated the possibility of application of this species for detoxification of effluents.

Effect of different concentrations of cobalt (III), zinc (II), copper (II) and mercury (II) on the carotenoid content in *A. oryzae* and *T. tenuis* was significantly varied. In control, the amount of carotenoid content

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in *A. oryzae* have shown (0.967, 1.934, 2.663, 3.392, 2.687 and 1.982 µg mL^{-1}) carotenoid content at various durations (2nd, 4th, 6th, 8th, 10th and 12th day).

Effect of Co^{2+} on the 2nd, 4th, 6th and 8th day at concentrations 1, 10 and 100 ppm on the carotenoid content was observed (Fig. 1b). There was decrease in carotenoid content on 12th day at 100 ppm. Zinc showed gradual increase in the carotenoid content at 1 ppm on every alternate day form 2nd day to 10th day. Similarly, at 10 ppm on 2nd, 4th and 6th day and at 100 ppm on the 2nd and 4th day carotenoid content was higher than control. Relatively, higher concentration of 100 ppm on the 6th day and for 10 and 100 ppm on the 8th day and 10th day and at all the test concentrations on the 12th day 1, 10 and 100 ppm the carotenoid content decreased. Copper on the 2nd, 4th, 6th and 8th at 1, 10 and 100 ppm stimulated more carotenoid content than the control. 10th day at 10 and 100 ppm and 12th day at 1, 10 and 100 ppm the carotenoid content was negligible of all the metals studied. Copper at 1 ppm on the 8th day showed maximum carotenoid content (6.652 µg mL^{-1}) nearly double than the control (3.392 µg mL^{-1}). Mercury caused less carotenoid content than the control.

In control, the amount of carotenoid content in *T. tenuis* varied from 1.540, 1, 1.647, 0.955, 0.818, 0.127 and 0.481 µg mL^{-1}, respectively, at 2nd, 4th, 6th, 8th, 10th and 12th day.[11,12]

Effect of cobalt Co^{2+}, Zn^{2+} and Cu^{2+} caused decrease in carotenoid content. Mercury at 1 ppm on the 2nd and 6th day caused more carotenoid content than at 10 and 100 ppm (Fig. 2).

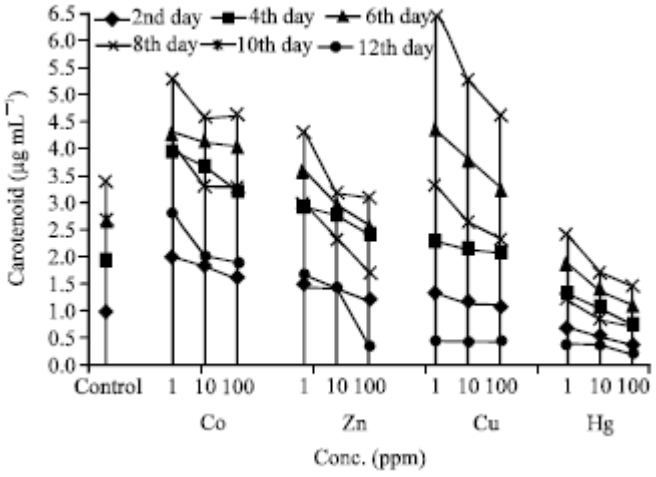

Fig. 1b: Effect of heavy metals on carotenoid content of *Anabaena oryzae*

Statistical analysis: The statistical data that will give information about goodness or fitness of model is regression (R^2), the coefficient of determination is a statistical measurement of how the regression line approximately equals to the real data points. Almost all the metal treated samples showed inverse linear relationship with the control in the two species studied. It was observed in the analysis that direct linear regression line positively and perfectly fits the data in Co^{2+} and Cu^{2+} treated samples (correlation), and there is perfect inverse linear relationship with Zn^{2+} as r^2 (t-test) equals to 1 (Fig. 3). In Co^{2+} and Zn^{2+} samples there is high degree of inverse linear relationship as the Linest (R^2) is equals to 1 (Fig. 4).

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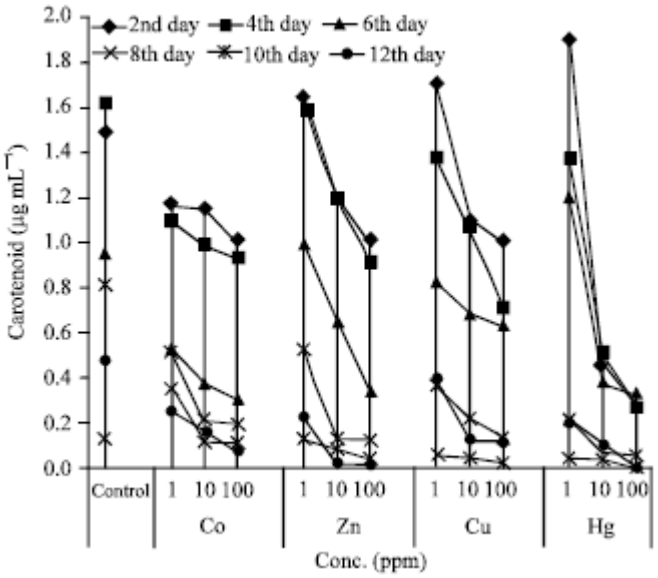
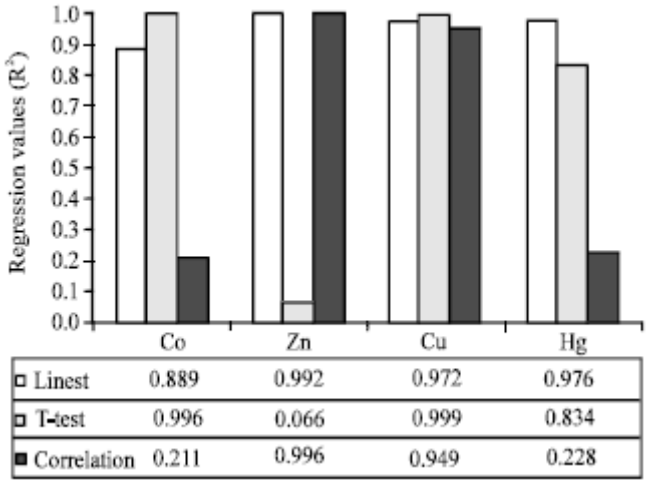


Fig. 2: Effect of heavy metals on carotenoid content of *Tolypothrix tenuis*



	Co	Zn	Cu	Hg
Linest	0.889	0.992	0.972	0.976
T-test	0.996	0.066	0.999	0.834
Correlation	0.211	0.996	0.949	0.228

Fig. 3: Simple Linear regression, t-test and correlation statistics of *Anabaena oryzae*

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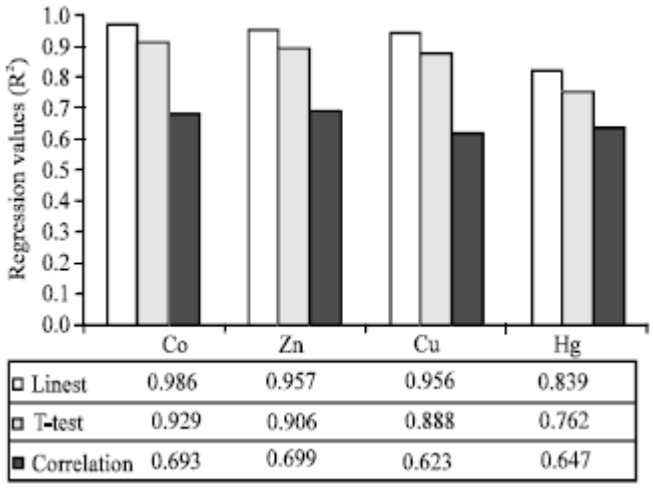

Fig. 4: Simple Linear regression, t-test and correlation statistics of *Tolypothrix tenuis*

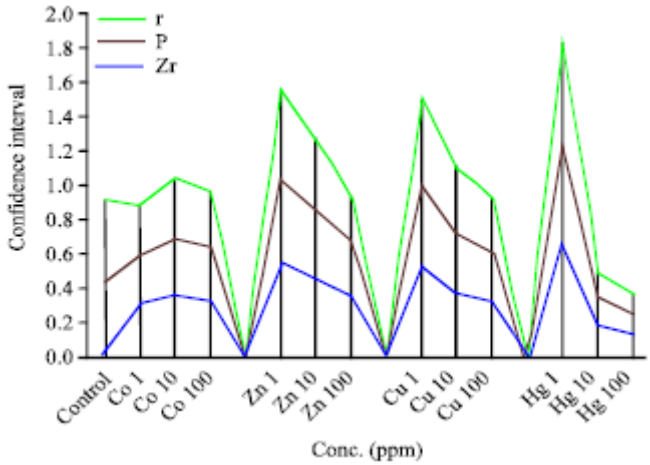

Fig. 5: Confidence interval for P in *Anabaena oryzae*, r: Sample confidence interval, P: Population confidence interval, Zr: Fisher transformation

It can be inferred that the use of simple linear regression analysis t-statistic computed is appropriate to test null hypothesis, when population p = 0. From (Fig. 5, 6) in case of P0, R^2 (correlation coefficient) is transformed to Zr (Fisher transformation). So, in order to find the confidence interval for P (control) the data range of P should be in between r (sample confidence observations) and Zr (Fisher transformation). Then we will not reject the hypothesis if P is in the confidence interval and our observations regarding the test organisms have 95% confidence.[13,14]

IV. CONCLUSIONS

In another study, the bioaccumulation of metals and its effects on pigments of Nostoc muscorum and Synechococcus PCC 7942 were assessed. The culture was grown in BG 11 liquid medium supplied with different metals like mercury (Hg), lead (Pb), and cadmium (Cd) and incubated (μM 20 concentrations) for 10 days under optimal conditions. The accumulated amounts of metals were determined by atomic absorption spectroscopy (AAS). The stress effects on photosynthetic pigment [15] chlorophyll a (Chl a) were monitored by laser-induced fluorescence (LIF). Bio-concentration factor (BCF) reached a peak in cells on the 2nd day of incubation followed by a gradual reduction. The highest reduction in the pigment concentrations (Chl a and β carotene) was observed at 20 μM L^{-1} Hg treatment. The

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results indicate that, cyanobacteria may serve as both potential species to be used as a biosensor and used to clean up heavy metals from contaminated water. These changes were analyzed with the long-term goal of exploiting cyanobacterial cells as biosensors.[16]

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