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A Study on the Impact of Most Commonly used Pesticides on Growth and Biochemical Parameters of Some Species of Cyanobacteria

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Abstract - The goal is to investigate the effects of common pesticides on the growth and biochemical parameters of some Cyanobacteria species, isolate, purify, and identify cyanobacterial strains, growth kinetics of purified samples, and biochemical parameters under selected metal stress, as well as the frequency of heterocyst and akinete under control and treated conditions. The Subarnarekha River basin, which runs through Ranchi district in India's Jharkhand state, served as the study area. PASW-SPSS-18 was used to calculate one way ANOVA and multiple co-relations to investigate the probability distribution pattern and significance of the collected data. Higher amounts of MDA accumulation under treatment conditions might be the consequence of photosynthetic membrane rupture, which causes a brief rise in SOD activity in the test organism. MDA An increase in SOD activity might be used as a biomarker for metal exposure. When Nostoc carneum blooms in water, it provides more surface area for the metal binding abilities of the other two species, potentially making it a highly effective heavy metal removal agent. Furthermore, it is cost-effective, ecologically friendly, and can be mass-produced.

Keywords - Cyanobacteria species, pesticides, Growth, Biochemical parameter

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INTRODUCTION

To address the rising demand for food in developing nations, synthetic pesticides are used in agricultural areas. Soil pH and salinity, fertility levels, and mechanical procedures have all been abandoned as a consequence of this. Despite European efforts to minimise the use of herbicides, pesticide usage has increased quickly over the previous two decades and lingers as organic pollutants in agricultural soils, groundwater, and surface waterways. According to EU data, annual consumption did not decrease between 1992 and 2003 (1).

Many beneficial microorganisms, even those that are not directly targeted by pesticides, have been reduced as a consequence of extensive application of these pesticides. This has resulted in major environmental pollution and ecological imbalance (2) Non-target species become a big worry when large quantities of chemicals are ingested in our environment (> 100.000 on an industrial scale). Using pesticides on a regular basis to control pests on target plants is also a concern.Thus the balance of the trophic food chain is seriously disturbed when pollutants are introduced (3).

These chemicals are routinely used in agricultural activities to control weeds and are regularly discharged into aquatic environments via surface runoff and atmospheric deposition. On farms, pesticides were sprayed, and the resulting leakage affected the ecosystems in the surrounding areas. In addition, the effects on ecological life elsewhere may be jeopardised owing to drift and volatilization (4). As a result, pesticide bioaccumulation in ecosystem primary producers and subsequent spread up the food chain are key environmental issues about pesticide contamination (5). On the other hand, the subsequent dispersion of these agrochemical chemicals and/or the breakdown products they produce has negative environmental effects (6, 7).

Cyanobacteria's evolutionary history may be traced back more than 3.45 billion years, assuming the fossil record from that period is genuine (8). One of the most important metabolic processes taking place in Earth's atmosphere is oxygenic photosynthesis, which generates and consumes energy in the form of ATP molecules (9) These fossilised biomarkers (molecular fossils) represent the earliest evidence for oxygenic photosynthesis and are found in the Pilbara Craton in Australia. It was previously considered that ancient cyanobacteria were the first photosynthetic organisms capable of exploiting water as the final source of electrons for the creation of photosynthesis reductant. Historically, the simultaneous release of free oxygen was an important event on our planet's surface. Allowing for the development of aerobic and

heterotrophic metabolism in the living world, it has progressively changed the atmosphere. In order to harness the sun's rays, oxygenic photosynthesis is the most important strategy. Oxygenic photosynthesis and nitrogen fixation are two of the most essential biological functions performed by Cyanobacteria, a diverse group of single- and multicellular prokaryotic germs (10). The chemically bonded energy and reductant generated in light reactions are used for carbon dioxide fixation. In order to maintain the atmosphere's essential gaseous composition, oxygenic photosynthesis transforms carbon dioxide into oxygen on a periodic basis (11). The carbon pool in paddy fields may be exploited by heterotrophic nitrogen fixers since cyanobacteria make up about half of the carbon fixed worldwide (12).

RESEARCH METHODOLOGY

Sampling site

Sampling was conducted out in several industrial belts of and Ranchi Jharkhand throughout the rainy season from mid-July to August and after the rainy season from October to November.

Water sample collection

Water sample was collected from the point where effluents are directly discharged. Few rocks and pebbles from the sites was also collect to examine the adhering cyanobacteria. Effluents or waste water were collected in plastic bottles from each sites randomly. Water samples were collected in 250 ml air tight plastic bottles from each site. Reference water sample was taken from a pond which was not directly contaminated by industrial effluents.

Physico-chemical analysis of water samples

According to the APHA, 1998 guidelines, water physicochemical analysis is adequate.

Isolation and purification of algal strains

Samples collected were first examined under microscope for the presence of any algal strains. Sample was isolated, purifies and identified and physico-chemical parameters analysis of water samples will do. Also growth kinetics of purified samples and biochemical parameters under selected metal stress was and find the heterocyst and akinete frequency under control and treated condition.

Identification

Isolated cyanobacterial strains were observed under microscope MLX-TR. Shape and colour of filament, position and number of heterocysts and akinetes were recorded. Identification of algal strains were performed following the keys given by Desikachary (1959).

Culture vessels

Conical flasks (Borosil) of 250 ml capacity plugged with non-absorbent cotton were used as the algal culture vessels. The volume of the medium inthe vessels was adjusted to about 100 ml to **avoid its** contact with the cotton plug while shaking. Hard glass test tubes of 20 ml capacity with non-absorbent cotton plug were used as experimentaltube.

Growth medium

There are a variety of culture medium for developing BGA. To cultivate nitrogen-fixing BGA, BG-11 media (Stanier et al., 1971) without nitrogen is often employed. The algal culture media utilised in this investigation was BG-1 I. All of the substances described below are produced as stock solutions and stored in glass reagent vials. For one litre of the medium, the volume of each component was calculated. A magnetic stirrer was used to mix the contents in the solution. Solid media was created by mixing 1 percent agar-agar with water and was used to make agar slants.

Heavy metal

Heavy metal salts, such as lead nitrate (Ph NO) for lead (Pb), cadmium (Cl), chloride (Cr), nickel chloride (CI) for Ni' (nickel), and potassium di chromate (K, Cr, O7) for chromate, were obtained and employed in the experiment (Cr). These heavy metal stock solutions (in ppm) are freshly made using sterile distilled water.

Pre paration-of metalsolution

In sterile OG-l I medium, metal solutions were produced. Various metal concentrations (ppm) were created from this stock solution.

Sterilization

To avoid microbiological contamination, culture media, culture containers, glasswares, and other accessories were autoclaved at 15 lb pressure for 20 minutes and then irradiated with UV radiation for 15 minutes before use.

Chemicals

All of the chemicals used in the experiment were purchased from Himedia, Qualigens, and Merck India Ltd and were of AR grade.

Inoculation

In the culture room, inoculation was done in an inoculation chamber. When inoculating cultures, aseptic measures were always used. The materials were homogenised in a glass hand-homogenizer, then filtered through a thin, sterile net of cheese cloth to remove clumps from the solution, and then

studied under a microscope to check whether homogenization had caused any harm to the cells. Log-phage filaments were always injected, either for culture maintenance or for experimental cultures.

Propagation and maintenance of cultures

Axenic culture was employed in all of the tests. The experimental algal 2 cultures were grown on culture racks within a culture room with light (7.5W/m) and temperature (260.5°C). To minimise clumping of the cells and their adherence to the vessel walls, the culture flasks and tubes were manually shook three times a day.

Experimental setup

The experiment employed axenic cultures of Anabaena variabilis, Fischerella sp., and Nostoc carneum. In sterilised nitrogen-free BG-11 medium, different concentrations (ppm) of heavy metal solutions were produced, and the following treatments were performed.

Measurement of growth

The development of cyanobacteria was monitored using a light scattering approach. A glass hand homogenizer was used to homogenise the experimental algae samples. The samples were shook vigorously. Individual sample absorbance (both control and heavy metal treatment) was measured in a UV-visible spectrophotometer at 760 nm at 3-day intervals up to the 15th day of incubation.

Estimation of protein

Protein quantitation was performed at 100°C for 5 minutes according to Lowry et al., 1951. Finally, a clear supernatant was obtained by centrifugation. 0.5 mL Folin's reagent was quickly added to the supernatant, shaken, and stored at room temperature for 30 minutes. Absorbanee was measured at 750 nm in comparison to a blank. Various concentrations of bovine serum albumin were used to create a standard curve (BSA). (tg of protein per ml of algal solution) was used to compute the protein content.

Pigment analysis

By measuring the biomass of the culture, growth (which is the addition of organic material to the cell) may be directly measured. Biomass may be measured using a variety of cellular components such as carbon, lipids, proteins, and plant pigments. The pigment content of these cellular components is used as a growth and development indicator. Chl-a is the primary light harvesting pigment in blue green algae, with Car and phycobilins serving as secondary pigments.

Estimation of metal accumulation in samples

After treatment, the accumulation of heavy metals in the cyanobacterial strains was measured using an atomic absorption spectrometer (AAS) according to Skoog's 1998

Statistical analysis

Data obtained from the laboratory experiment was calculated and graphically represented using MS-Excel-2013. To study the probability distribution pattern and significance of the obtained data one way ANOVA and multiple co-relations was compute using PASW-SPSS-18.

RESULT AND DISCUSSION

- **Physico-chemical analysis of water samples**

Water samples were examined for physicochemical characteristics. The research looked at parameters including pH, TSS, TDS, DO, BOD, and COD, and the results are shown in Table. Sample 10 was used as a baseline for all of the locations. All of the locations had a pH range of 7.1 to 8.2, with sample 8 having the greatest alkalinity. The concentration of dissolved oxygen (DO) at all sampling sites varied from 5.2 to 8.8, with the highest concentration in sample 1. 6. Total Suspended Solids (TSS) ranged from 22 to 364, with sample 5 having the highest concentration. TDS levels ranged from 132 to 840, with the highest in the sample. 5. The biological oxygen demand (BOD) ranged from 0.4 to 1.8, with sample 1 having the highest BOD. The chemical oxygen demand (COD) of the locations ranged from 8.1-20.4, with sample 1 having the highest COD. Sample 1 was high in Ni and Cd, according to heavy metals analyses. Zn, Ni, Cr, and Pb were abundant at Site 2. Zn, Ni, Cr, Pb, and Cd were abundant in Sample 4. Mn and Pb were abundant in sample 6. Cd, Cr, and Mn were abundant at site 5.

Table 1: Physico chemical analysis of water samples

Sample	PH	TSS (Mg/l)	TDS (mg/l)	DO (Mg/1)	BOD (Mg/l)	COD (Mg/l)	Zo (mg/l)	Mn (Mg/l)	Ni (Mg/l)	Cr (mg/1)	Pb (Mg/1)	Cd (Mg/1)
Sample 1	8.12	312	562	6.2	1.8	20.4	4.5	3.6	5.9	8.5	11.1	11. 8
Sample 2	7.13	32.0	174	8.4	0.6	10.2	4.9	4.5	6.5	9.5	20.2	7.3
Sample 3	7.22	68	150	7.5	0.6	13.2	5.6	4.6	5.7	7.3	13.8	8.9
Sample 4	7.51	41.0	132.0	9.1	0.4	8.1	6.9.	5.4	7.6	**11.8**	14.0	10.8
Sample 5	7.92	364.0	840.0	5.2	1.6	18.6	4.5	6.5	5.5	11.5	12.3	11.3
Sample 6	7.32	22.0	160.0	8.8	0.4	9.3	4.3	7.8	2.6	8.2	14.5	8.2
Sample 7	7.91	180.0	241.0	7.2	0.6	8.2	4.1	3.2	2.4	5.6	8.5	7.2
Sample 8	8.21	62.0	188	8.0	0.9	10.4	2.5	3.6	2.8	5.3	7.8	6.5
Sample 9	7.98	170.0	364.0	7.7	0.4	9.2	2.3	3.1	1.3	3.2	6.4	5.3
Sample 10	7.2	26	80	80	0.2	2.5	1.5	0.5	0.05	0.03	0.03	0.01

- **Isolation, purification, and maintenance of algal strains**

1 mL of water was placed into 25 mL of sterilised BG-11 media in petridishes and incubated at 250.5°C in a culture chamber under 7.5 W/m2 light

intensity. Algal colonies that appeared on the agar plates after 10-12 days of incubation were isolated and distributed on new agar plates. Colonies growing on agar plates were inspected microscopically and transferred to agar slants after roughly a week of development. This procedure was continued until individual pure colonies devoid of contamination were produced.

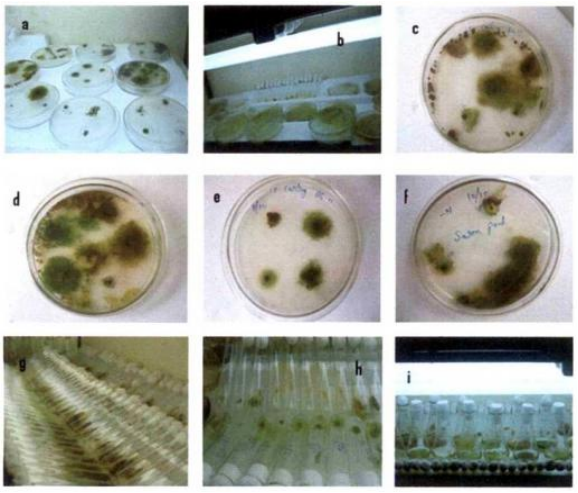

Figure 1: (a- f)Water samples were collected and stored in petriplates with BG-11 media in the culture chamber, with the appearance of algal patches growing in the petriplates; g. Patches of cyanobacteria were transplanted to Agar slants; h. On the plant culture room, purified cyanobacterial samples are growing in agar slants; i. Purified strains of cyanobacterial species were cultured in broth.

- **Changes in growth**

Growth behavior of Nostoc carneum under metal stress

The highest growth of Nostoc carneum in lead-treated conditions (Fig.2) is 2 ppm on the 15th day, 5 ppm on the 5th day, and 10 ppm on the 12th day of incubation. In comparison to their control, the organism exhibited 76.71 percent, 50.18 percent, and 30.68 percent on the 9th day, 74 percent, 50.22 percent, and 31.2 percent on the 12th day, and 76.75 percent, 50 percent, 28.75 percent on the 15th day (100 percent). Fig.4.5 shows the growth dynamics of N. carneum under chromium stress. On the 12th day, the growth rate was greater in 5 and 10 ppm, and on the 9th day, it was higher in 15 ppm, but the pace was slower than the control.

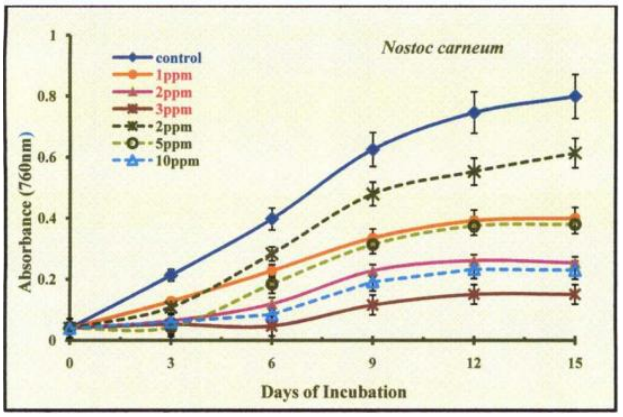

Figure 2: Effect of different concentration of cadmium (___solid lines) and lead (-— lines) on growth of Nostoc carneum cultured under laboratory condition.

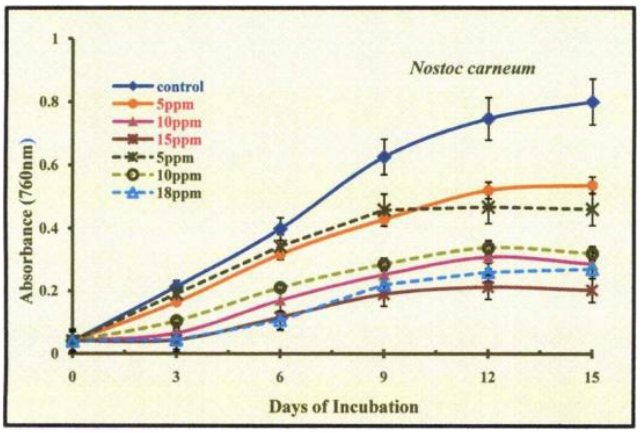

Figure 3: Effect of different concentration of chromium (___lines) and nickel (-----lines) on growth of Nostoc carneum cultured under laboratory condition.

Growth kinetics of Anabaena variabilis under metal stress

The highest growth of Anabaena variabilis under lead-treated conditions (Fig.3) occurs on the 15th day at 2 ppm, the 12th day at 5 ppm, and the 9th day at 8 ppm. The growth rates were 75.74 percent, 51.67 percent, and 38.64 percent on the 9th day, 79.27 percent, 56.75 percent, and 27.84 percent on the 12th day, and 79.08 percent, 47.92 percent, and 26.07 percent on the 15th day, in 2, 5, and 8 ppm, respectively, as compared to their respective controls (100 percent). Under chromium stress (Figure 4), the growth rate was greater on the 15th day in 5 ppm, on the 12th day in 10 ppm, and on the 15th day in 12 ppm. In comparison to control, growth was 74.75 percent, 54.43 percent, and 31.35 percent on the 9th day, 75.31 percent, 59.62 percent, and 27.49 percent on the 12th day, and 81.2 percent, 56.47 percent, and 25.44 percent on the 15th day (100 percent).

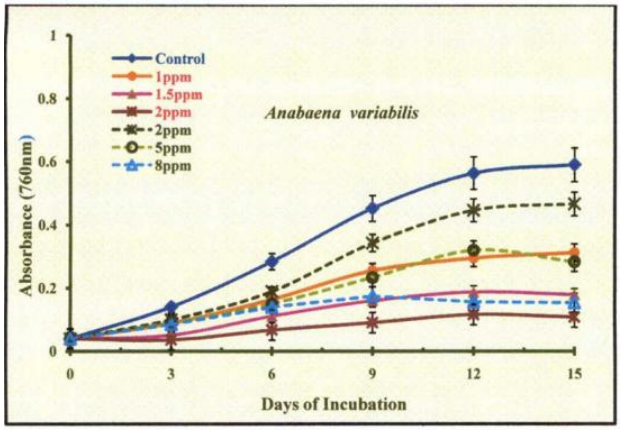

Figure 3: Effect of different concentration of cadmium (____lines) and lead (-------lines) on growth of Anabaena variabilis cultured under laboratory condition.

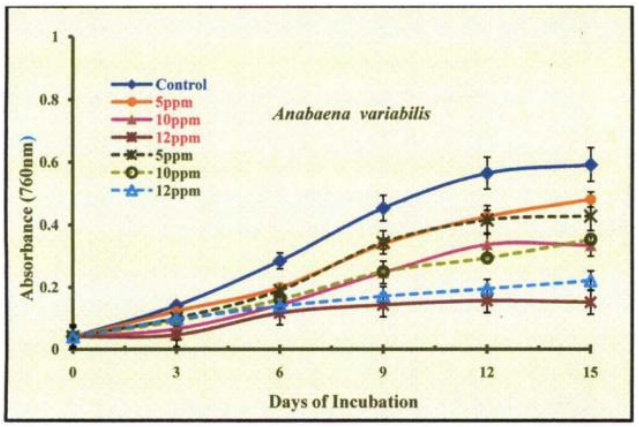

Figure 4: Effect of different concentration of chromium (___lines) and nickel (------lines) on growth of Anabaena variabilis cultured under laboratory condition.

- **Studies of heavy metal on biochemical parameter**

Changes in total protein Content

Protein content of Nostoc carneum under metal stress

On the 15th day in control and 2 ppm, on the 12th day in 5 ppm, and on the 9th day in 10 ppm, Nostoc carneum in lead treated conditions (Fig.5) achieved its maximum. Protein content reached its maximum at 5 ppm on the 12th day, while at 10 ppm, cadmium has a severe impact on protein content, with no significant growth seen and a maximum value on the 9th day. When compared to their control group, the protein content was 71.49 percent, 51.31 percent, and 45.17 percent on the 9th day, 70.22 percent, 56.87 percent, and 40.07 percent on the 12th day, and 66.66 percent, 31.91 percent, and 25.53 percent on the 15th day (100 percent). The total protein level was highest on the 9th day in 5, 10, and 15 ppm under chromium stress (Figure 6). No adverse effects of chromium were seen at 5 and 10 ppm, and the protein content followed a similar pattern, but at 15 ppm, the protein content dropped to a considerably lower level. In comparison to their control, the protein content was 92.98 percent, 74.56 percent, and 33.33 percent on the 9th day, 83.9 percent, 65.28 percent, and 35.49 percent on the 12th day, and 79 percent, 65.09 percent, 45.39 percent on the 15th day (100 percent).

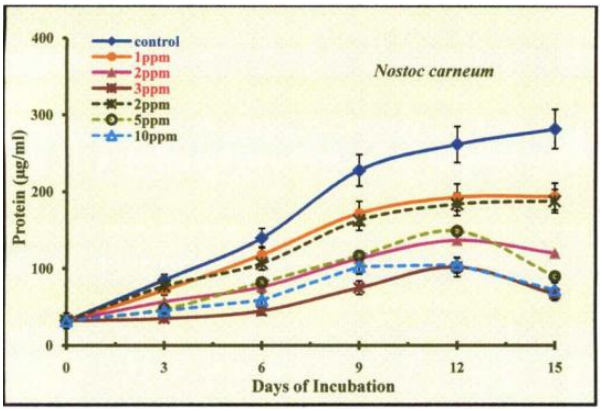

Figure 5: Effect of different concentration of cadmium (___lines) and lead (— lines) on protein content ofNostoc carneum cultured under laboratory condition.

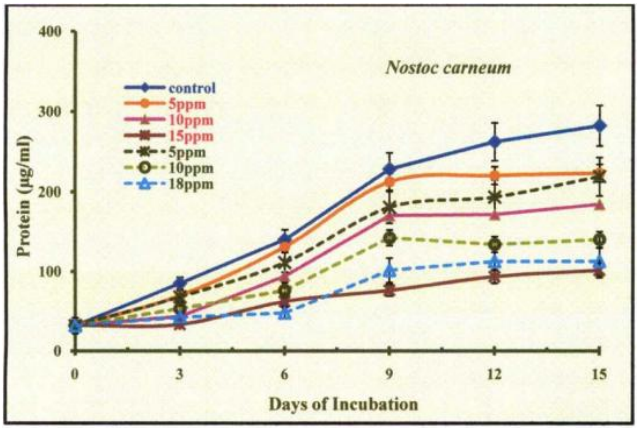

Figure 6: Effect of different concentration of chromium (___ lines) and nickel (— lines) on protein content ofNostoc carneum cultured under laboratory condition.

Changes in Chlorophyll Content

Chi a content of Nostoc carneum under metal stress

The greatest Chi a concentration in Nostoc carneum under lead treated conditions (Fig.7) is found on the 9th day in 2 and 10 ppm, and on the 12th day in 5 ppm. In comparison to their control group, the chi a content was 75.26 percent, 54.35 percent, and 45.29 percent on the 9th day, 62.87 percent, 52.69 percent, and 26.9 percent on the 12th day, and 51.82 percent, 42.68 percent, and 17.68 percent on the 15th day (100 percent). The chi a content was greater on the 12th day in control, on the 15th day in 5 ppm, and on the 12th day in 10 and 15 ppm under chromium stress. When compared to control, the

chlorophyll content on the 9th day was 66.2 percent, 50.17 percent, and 36.9%, on the 12th day it was 68.56 percent, 49.4 percent, and 35.02 percent, and on the 15th day it was 73.17 percent, 42.68 percent, and 29.87 percent, in 5,10 and 15 ppm, respectively (100 percent). The highest chi an accumulation in Nostoc carneum under nickel treated conditions (Fig.8) occurs on the 15th day in 5 ppm, the 12th day in 10 ppm, and the 18th day in 18 ppm. Up to 15 days of incubation, the 5ppm chi a concentration exhibits a progressive rise. When exposed to nickel, the chlorophyll content was 78.04 percent, 56.09 percent, and 44.94 percent on the 9th day, 74.25 percent, 57.18 percent, and 44.61 percent on the 12th day, and 80.79 percent, 53.35 percent, 35.67 percent on the 15th day, in 5, 10, and 18 ppm, respectively, as compared to control (100 percent).

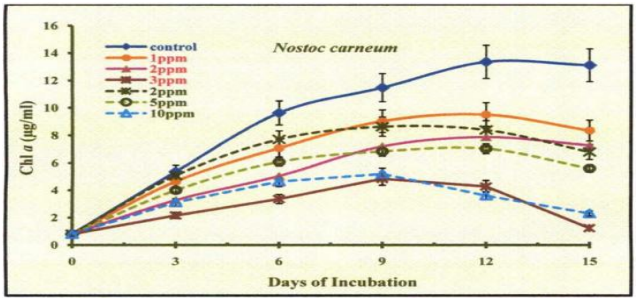

Figure 7: Effect of different concentration of cadmium (___ lines) and lead (— lines) on Chi a ofNostoc carneum cultured under laboratory condition.

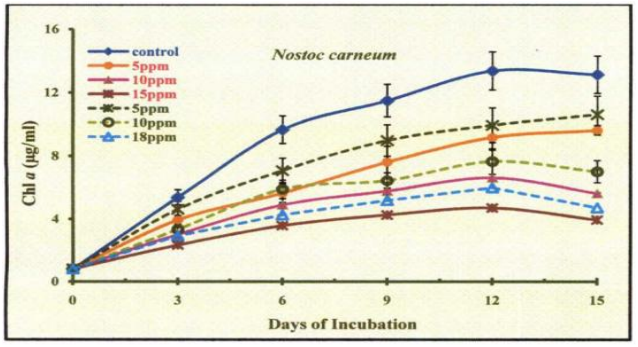

Figure 8: Effect of different concentration of chromium (___ lines) and nickel (— lines) on Chi a ofNostoc carneum cultured under laboratory condition.

- **Statistical analysis**

All of the data from the lab experiments was analysed in SPSS 18 and found to have a normal distribution pattern. Growth, Chi a, carotenoid, phycobilin, photosynthetic efficiency, protein, carbohydrate content, lipid content, exopolysaccharide content, SOD activity, MDA buildup, heterocyst frequency, and akinete frequency all reveal a significant difference at p 0.05.

Table 2: Multiple correlation among metal concentration, Growth, Protein,Chl a, Carotenoid, Phycobilin, Carbohydrate, Lipid, Exopolysacchaaride, PEA, SOD and MDA of Nostoc carneum treated with cadmium

	Days	Conc	Growth	Protein	Chl a	Car	PBS	Carbo Lipid Exop	SOD	MDA
Days	1									
Conc	0	1			*Nostoc carneum* during cadmium stress					
Growt	0.628	-0.633	1							
Protein	0.701	-0.575	0.978	1						
Chl a	0.639	-0.581	0.933	0.957	1					
Car	0.548	-0.668	0.941	0.962	0.952	1				
PBS	0.402	-0.788	0.938	0.911	0.892	0.938	1			
Carbo	0.705	0.720	0.445	0.736	0.946	-0.694	-0.896	1		
Lipid	0.654	0.878	-0.571	-0.552	-0.729	-0.399	-0.411	0.843		
Exop	0.562	0.754	-0.444	-0.169	-0.541	-0.265	-0.271	0.775		
PEA	0.987	-0.813	0.870	0.737	0.924	0.844	0.908	-0.361	-	
SOD	0.726	0.859	-0.702	-0.713	-0.995	-0.440	-0.677	0.886	1	
MDA	0.745	0.855	-0.754	-0.950	-0.783	-0.093	-0.252	0.883	0.98	1

CONCLUSION

Alkalinity has a negative impact on water quality. Phosphate and nitrates of calcium and magnesium were found to be the most common components of TSS at Site 5, followed by carbonates, bicarbonates, chlorides, magnesium chlorides, sodium chlorides, manganese nitrates, and miscellaneous particles. Only “Chi-a” is present in cyanobacteria, the primary photosynthetic pigment. Cellular growth and physiological condition may be determined by the quantity of chlorophyll in the photosynthetic unit of cyanobacterial cells.

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