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Comparative toxicity of trivalent and hexavalent chromium on a marine diatom

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Abstract

The response of a marine diatom *Nitzschia clausii* (Hantzsch) Hustedt to trivalent and hexavalent chromium at micro level was studied. A short term experimental study was made on the changes in growth, net production, respiration and photosynthetic pigments at sublethal level. The metal accumulation on the diatom was also determined. It was found that hexavalent chromium was more toxic than trivalent chromium in diatom.

Chromium is an important constituent of industrial and domestic wastes. Yet there exists little information regarding its effects on fresh water, estuarine and marine phytoplankton. Major sources of chromium are the electroplating wastes, factory wastes, industrial and textile dyes which have high amounts of chromium. Textile and leather tanning industries are the major sources of hexavalent chromium pollution. In the chrome alum wastes chromium exist in trivalent state. Toxicity experiments of various metals had proved that it was not the total metal concentration but the particular physical and chemical form of the metal that affected the organism. In the case of toxicity also chromium exists in the valency state. The speciation studies are aimed at understanding the metal interaction in aquatic ecosystem. Dissolved chromium occurs as trivalent or hexavalent with the latter normally predominating. The hexavalent form seems to be the most toxic and toxicity varies from species to species (Fukai 1967; Cranston and Murray 1978; Anderson 1981; Babitch *et al.*, 1982). Smille *et al.* (1981) reported that the two valency states such as trivalent and hexavalent chromium are interchangeable. They reported that bacterially produced hydrogen sulphide present in the tannery waste converts hexavalent chromium present in the tannery waste converts hexavalent chromium present in the marine environment to trivalent chromium. The behaviour of both the valency states of chromium in the aquatic environment was highlighted by Bartlett and Kimble (1976) and Meisch and Beckmann(1979).

The present study is based on the effect of two valency state of chromium such as trivalent and hexavalent chromium on the parameters of productivity on a marine diatom- isolated from Cochin backwaters.

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Material and methods

Nitzschia clausii (Hantzch) Hustedt, a marine diatom belonging to the class *Bacillariophyceae* was isolated from Cochin backwaters. Miquel's medium modified by Ketchum and Redfield (1938) was found suitable for the growth of the diatom. EDTA and Vitamin B_{12} was excluded from the medium as they form complexes with the metal thus reducing the actual toxicity. Since silica was essential for the better growth of diatoms, it was added to the medium in the form of Sodium meta silicate. *Nitzschia clausii* grows better at a salinity of 13-17‰. Hence, throughout the experiment salinity was maintained between 14 to 15‰.

The experiment was conducted in triplicate in twenty number of two litre 'Borosil' flasks plugged with sterile cotton, each flask containing one litre medium. The culture was maintained at 27.5°C±C. A 10 hour light and 14 hour dark cycle was provided and light intensity of 2400 lux was maintained throughout the experiment. Cultures under experimental growth phase were used as inoculum. For the uniformity of experiments a cell density of 5.2 x 10^4 of cells *Nitzschia clausii* were maintained. By preliminary experiments the growth phase was determined and it was 10 days for *N. clausii.*

The metals selected for study, the trivalent and hexavalent chromium were added to the medium as aqueous solutions of $K_2Cr_2O_7$ and Crk (SO4)2 respectively. Growth parameters selected for study included biomass, production, respiration and photosynthetic pigments. Biomass was determined on the basis of the number of cells and it was expressed as number of cells per ml. Rates of production and respiration were determined by modified winkler method from fourth day onwards on alternate days. Various photosynthetic pigments such as chlorophyll - *a*, chlorophyll - *c* and carotenoids were estimated by spectrophotometric analysis using Hitachi - U- 2000 spectrophotometer. For quantifying the pigments equations of Lorenzen (1967) for chlorophyll - *a* and chlorophyll-*c*, Jeffery and Humphrey (1975) and Strickland and Parsons (1968) for carotenoids were used. Pigment concentration were expressed as mg/litre. Atomic absorption spectrophotometry was used for quantifying the trace elements in the treated algal samples.

Results and discussion

The preliminary experiments to study the effect of chromium were based on biomass, visual changes such as colour, clumping of cells and adhesion to the bottom surfaces. From priliminary tests a fine screening test was carried out to determine the toxicity range. Total lack in growth was noticed at 0.7 ppm for hexavalent and 0.8 ppm for Cr^{3+}. The sublethal concentration of chromium for the present study was 0.4, 0.6 and 0.8 ppm for trivalent chromium and 0.4, 0.6 and 0.7 ppm for hexavalent chromium.

It was observed that in *N. clausii*, hexavalent chromium was not toxic at 0.6 ppm whereas trivalent chromium was not so toxic even at 0.8 ppm. Ajmal *et. al.*, (1984) reported that hexavalent chromium was more toxic than trivalent chromium in their studies of the effect of trivalent

and hexavalent chromium on micro organisms.

The cells of *N. clausii* grown in 0.6 ppm Cr^{6+} became attached to the culture flask and did not enter into the medium, when the flasks were shaken, resulting in the apparent decrease in the cell number. In some cases there was clumping of cells and they were found at the bottom of the culture flask as a mass. When the cells were examined under a microscope empty frustules were not noticed suggesting that cell disruption did not occur. There was yellowing of cells and the culture turned yellow in color.

In the case of *N. clausii* the growth was stimulated by hexavalent chromium in 0.4 ppm. In 0.6 and 0.7 ppm a well marked decrease was noticed (Fig. 5). However, in trivalent chromium samples such marked decrease was not observed even in 0.8 ppm. Though there was an initial decrease in growth, an increase was observed at the end of the growth phase (Fig. 1). Thus the toxic effect of the metal

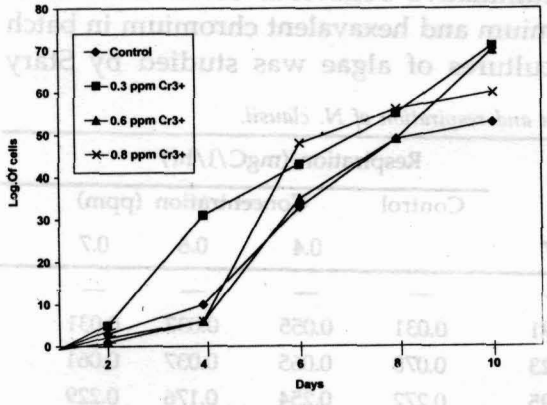

Fig. 1. *Effect of trivalent chromium on the biomass in N. clausii.*

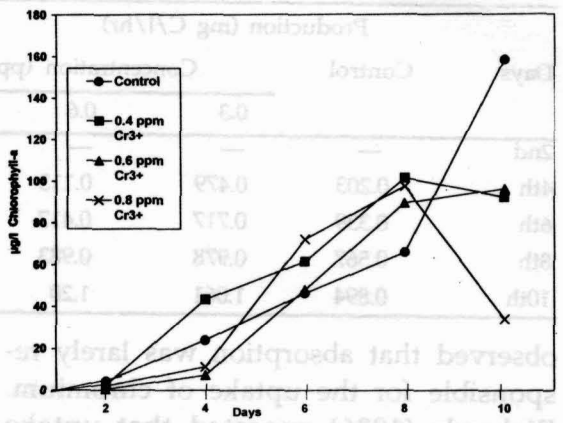

Fig. 2. *Effect of trivalent chromium on chlorophyll - a in N. clausii.*

noticed at the initial stage was reversed in the later stage.

This may be due to the fact that in laboratory cultures at high concentration, chromium may be absorbed on the cells, to reduce its concentration significantly.

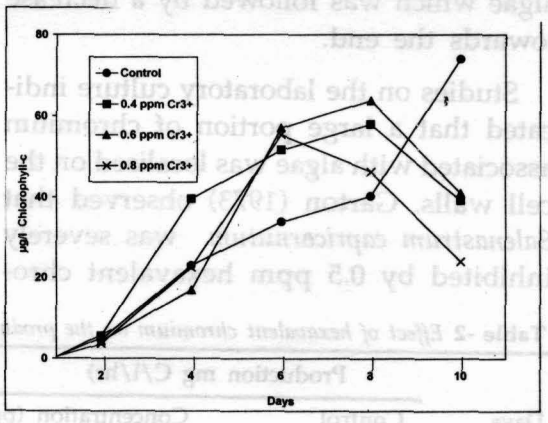

Fig. 3. *Effect of trivalent chromium on chlorophyllc in N. clausii.*

This allows some surviving cells to grow, multiply and reestablish the population. This is the "Sacrificial lamp effect" reported by Mangi *et al.* (1978). They

Table 1. *Effect of trivalent chromium on the production and respiration in N. clausii.*

Days	Production (mg C/l/hr)				Respiration (mg C/l/hr)			
	Control	Concentration (ppm)			Control	Concentration (ppm)		
		0.3	0.6	0.8		0.3	0.6	0.8
2nd	—	—	—	—	—	—	—	—
4th	0.203	0.479	0.113	0.176	0.031	0.060	0.035	0.024
6th	0.339	0.717	0.417	0.577	0.078	0.151	0.065	0.112
8th	0.567	0.978	0.983	0.782	0.272	0.339	0.254	0.324
10th	0.894	1.061	1.20	0.737	0.494	0.241	0.237	0.339

observed that absorption was larely responsible for the uptake of chromium. Richards (1936) sugested that uptake occurred through the cell wall. It is apparent that a stimulatory effect (Hormesis) by chromium was observed in the case of production and respiration. Such heightened uptake due to the effects of toxicants has been noted by several researchers. Laughlin and Guard (1981) found that low doses of mixed hydrocarbons (jet fueld) initially enhanced the growth of algae which was followed by a decrease towards the end.

Studies on the laboratory culture indicated that a large portion of chromium associated with algae was localised on the cell walls. Garton (1973) observed that *Salenastrum capricornutum* was severely inhibited by 0.5 ppm hexavalent chromium led low growth and yield on the diatom *Thalassiosira aestivalis*.

In *N. clausii* high concentration of hexavalent chromium (0.7 ppm Cr^{6+}) reduced the respiration (Table 2). However, respiration and production increased in 0.6 ppm trivalent chromium. Unlike hexavalent chromium, a clear cut toxicity was not noticed by trivalent chromium (Table 1).

This may be because trivalent was being rapidly cumulated in all algae, whereas hexavalent chromium was practically not cumulated in algae. It may be concluded that cumulation of trivalent chromium was predominantly due to chemical absorption on the surface of the algal cells. The cumulative behaviour of trivalent chromium and hexavalent chromium in batch cultures of algae was studied by Stary

Table -2 *Effect of hexavalent chromium on the production and respiration of N. clausii.*

Days	Production mg C/l/hr				Respiration (mgC/l/hr)			
	Control	Concentration (ppm)			Control	Concentration (ppm)		
		0.4	0.6	0.7		0.4	0.6	0.7
2nd	—	—	—	—	—	—	—	—
4th	0.204	0.386	0.045	0.091	0.031	0.055	0.037	0.031
6th	0.339	0.358	0.281	0.423	0.078	0.065	0.037	0.061
8th	0.567	0.497	0.295	0.795	0.272	0.254	0.176	0.229
10th	0.894	0.517	0.716	0.920	0.494	0.344	0.091	0.337

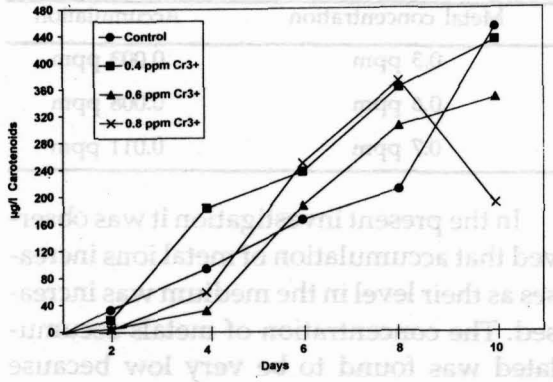

Fig. 4. Effect of trivalent chromium on the carotenoid pigment in N. clausii.

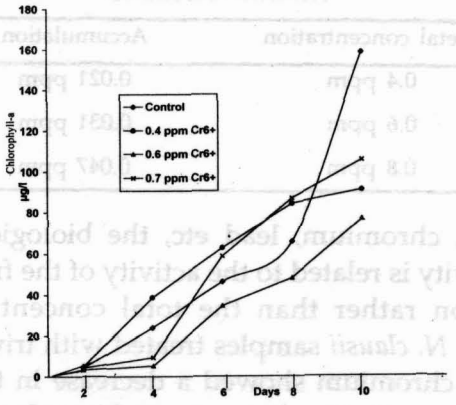

Fig. 6. *Effect of hexavalent chromium on chlorophyll- a in N. clausii*

and Florance (1987). Leland *et al.*, (1979) in their studies on toxicity of heavy metals and its bio-accumulation showed that concentration necessary to inhibit growth, metabolic purposes such as photosynthesis varies widely and depend on factors such as degree of chelation, concentration of cells, pH, nutrients, physiological state of cells, salinity and temperature.

There was variation in different pigment content by the effect of hexavalent chromium in *N. clausii* even though a general decrease was noticed at the end of the growth phase. On the 4th, 6th, 8th day it was observed that 0.4 ppm enhanced chlorophyll - *a* (Fig 6) and carotenoids (Fig - 8) but it was not reflected in chlorophyll -*c*. (Fig. 7). The chemistry and toxicological mechanism of chromium is found to be quite different from that of the other metals (Anderson and Morel, 1978). In the cationic metals such as nickel,

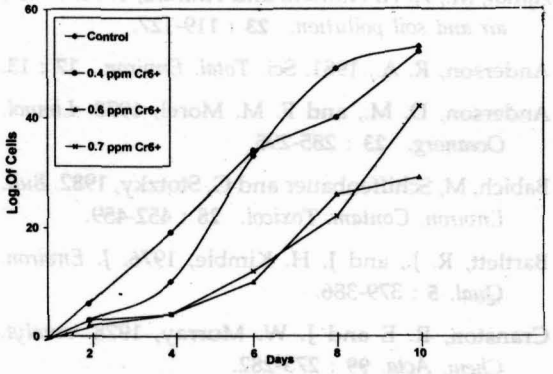

Fig. 5. *Effect of hexavalent chromium on the biomass in N. clausii.*

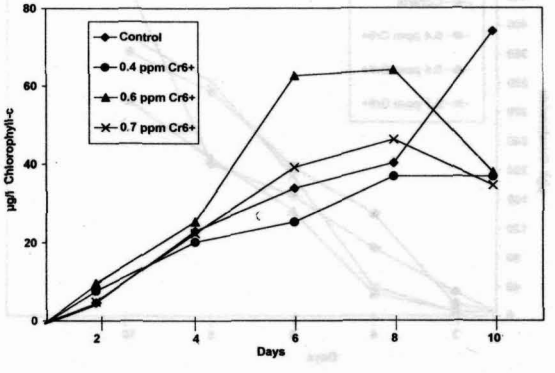

Fig. 7. *Effect of hexavalent chromium on chlorophyll-c in N. clausii.*

Table 3. *Uptake of chromium in N. clausii as determined by AAS (µg/100 mg dry wt. of algae).*

Trivalent Chromium		Hexavalent Chromium	
Metal concentration	Accumulation	Metal concentration	Accumulation
0.4 ppm	0.021 ppm	0.3 ppm	0.003 ppm
0.6 ppm	0.031 ppm	0.6 ppm	0.008 ppm
0.8 ppm	0.047 ppm	0.7 ppm	0.011 ppm

iron, chromium, lead etc, the biological activity is related to the activity of the free cation rather than the total concentration. *N. clausii* samples treated with trivalent chromium showed a decrease in the photosynthetic pigments at the end and early stage of growth (Fig. 2, 3, 4). Saraiva (1976) reported that the decrease may be due to intitial absorption of chromium on the cell wall followed by acting on the cell membrane.

The toxic effect was more dominant for hexavalent chromium in the parameters selected for study. Though trivalent chromium was toxic at 0.8 ppm, the morphological and physiological changes noticed by the effect of hexavalent chromium was not seen.

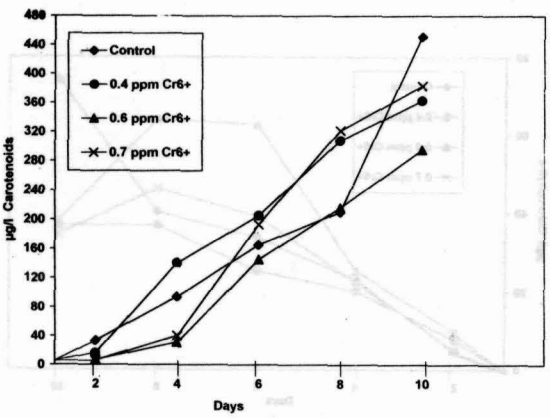

Fig. 8. *Effect of hexavalent chromium on the carotenoid pigments in N. clausii.*

In the present investigation it was observed that accumulation of metal ions increases as their level in the medium was increased. The concentration of metals accumulated was found to be very low because very low concentration was added to the test medium (Table 3). Skaar *et al.*, (1974) and Eide *et. al.*, (1979) reported that concentration of nickel in the cells of *Phaeodactylum tricornutum* was proportional to that in the medium upto a level of 750 ppb. The metal absorption generally decreases with the age of the culture.

Thus it was concluded that toxicity of metals was strongly dependent on the compostion of the culture medium, density of culture and metal losses through absorption, precipitation or volatilization.

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