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Tolerance of a Cyanobacterium *Phormidium fragile*, to strontium in presence or absence of other heavy metals

Cyanobacterium, *Phormidium fragile'* nin ağır metal içeren veya içermeyen ortamda stronsiyum' a dayanıklılığı

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Abstract:

This study was conducted to elucidate the effects of different concentrations of strontium and its interaction with certain metals upon some physiological aspects of *Phormidium fragile*.

The results revealed that dry weight, protein and carbohydrate contents as well as acid phosphatase activity decreased with increased strontium concentration. Low doses of strontium hardly affected lipids or DNA content as well as nitrate reductase and GOT activities. In the meantime, it increased GPT and alkaline phosphatase activities. Low concentrations of strontium decreased RNA content, but larger doses hardly affected its accumulation.

Calcium nullified the harmful effects of strontium on dry weight, protein, carbohydrates and RNA contents but could not counteract strontium effects on the other parameters.

Antimony hardly affected the influence of strontium on dry weight gain and DNA content, slightly decreased the carbohydrate content and alkaline phosphatase activity but slightly increased the protein content. Antimony nullified strontium effects on RNA or lipids levels, nitrate reductase, GOT, GPT and acid phosphatase activities. The addition of cadmium, singly or jointly with calcium and antimony to strontium-containing media as deleterious to all growth criteria. Pigment contents were severely attenuated by strontium alone or combined with other metals.

Keyword: Phromidium fragile, strontium, heavy metals

Introduction

Strontium is one of the alkaline earth metals that attracted the attention of several investigators. Its content, in different soils, was tentatively determined by many authors (Romney *et al.*, 1983; Wietholter and Corey , 1994). Strontium was also considered as a probable natural pollutant of air (Schroeder, 1970), water (Abdallah and Mostafa, 1980) and of soil (Gravel *et al.*, 1994).

A considerable accumulation of strontium was detected in several plants, e.g. *Chladophora fracta* and *Elodea candensis* (Piskunov and Treiger, 1980), the thallus of brown algae *Macrocystis integrifolia* and *Nereocystis luetkeana* (Rosell and Srivastava, 1984). Paribok *et al.* (1989) reported that strontium content in the leaves of 14 plants collected from the urban was higher than that from the ruler areas.

Following the Chernobyl reactor accident in Ukraine, considerable environmental radionuclide contamination was observed, including ^{137}Cs and ^{90}Sr, in Germany (Kaserouni *et al.*, 1988), in Austria (Artner *et al.*, 1990) an in Finland (Paasikallio *et al.*, 1994).

Calcium is a macronutrient for algae in general (Becker and Brand, 1982) and is involved in a large variety of physiological processes in plants (Marmé, 1983)). Calcium is known to alleviate heavy metal toxicity (Jayaraj *et al.*, 1992). According to the similar geochemical behaviour between strontium and calcium, the former was reported to substitute the latter causing similar reduction and/or activation of the processes in which it is enrolled (Kutsky and Weiss, 1982) and it is therefore, possible to estimate the calcium content in different media using natural strontium isotopes (Aberg *et al.*, 1990).

Cadmium is generally considered as one of the most dangerous pollutants. It can enter the environment as a by-product form zinc refining, coal combustion, mine wastes, fertilizers and pesticides (Hutton, 1983). It is known to inhibit vital metabolic activities of cyanobacteria (Singh and Yadava, 1983; Takamura *et al.*, 1989).Antimony is a non-essential element for plants (Parisis and Van Den Heede, 1992). Skeine *et al.*, (1992) reported that coal combustion could be a major source of antimony in the environment. Antimony smelting alkali wastes are highly toxic to aquatic organisms (Gao and Xiu, 1991).

Through precipitation and runoff, algae, the primary producers in the food chain, become exposed to heavy metals. The removal of strontium from low level radioactive streams can be achieved using *Pandorina morum* (Miller and Wilke, 1972). Skipnes *et al.*, (1975) reported that brown algae might be used to indicate pollution with strontium isotopes. Gogate *et al.*, (19759 reported that brown algae accumulated strontium in preference to calcium from sea water.

While the individual effects of heavy metals on algae have been widely studies exist concerning the effects of two or more metals in combination (Rai and Raizada, 1985; Khalil, 1994). Therefore, the combined impact of contaminating

metals should be determined by examining the individual and interactive and interactive effects of these metals.

The objectives of this study are to quantify the effect of different concentrations of strontium, using a common cyanobacterium, *Phormidium fragile*. The interactive effects of cadmium, calcium and antimony, singly or combined, with selected concentration of strontium were also studied.

Material and Methods

In this study, axenic log-phase cells of the non-hetero-cystous cyanobacterium, *Phormidium fragile*, were used as the source of inoculum. The organism was identified by Prof.G.S. Venkatarman, Microbiology Division, Indian Agricultural Research Institute, New Delhi, 1970.

Different concentrations of strontium (as chloride salt) were added to 250 ml Erlenmeyer flasks containing 50 ml of the fresh sterilised calcium-free nutrient medium (Shakkeeb, 1975) to give a final concentration of 0, 50, 100, 200, 300, 400, 500 and 600 mg/L. Interaction studies were performed using the non-inhibitory concentration (10 ppm) of calcium (Khalil, 1994), cadmium and antimony (Khalil, 1966). The three metals were added as chloride salts to some selected concentrations of strontium of prominent effects on the algae growth. The lower concentration of strontium (50 mg/L) was selected for its low toxicity to the alga; the intermediate and high levels (300 and 500 mg/L) were harmful but not lethal. These metals, either singly or combined, were added to the cultures at the beginning of the growth period. Control samples were maintained in fresh calcium-free nutrient medium.

Control and the experimental culture flasks were inoculated with 5 ml inocula. All the cultures were grown at 25°±3°C under continuos illumination (4.000 lux) for 7 days. At the expiry of the experimental period, the exponentially growing algae were collected by centrifugation, washed several times with distilled water. The biomasses of one set were dried at 110°C for 4 hours to determine the dry weight gain following the various treatments. Another set ture of fresh biomasses was extracted with ethalol- petroleum ether mixture (10:3 v/v) then filtered and evaporated. The residue was taken up with 85% acetone and its optical density was measured at 663, 644 and 452.5 nm to determine chlorophyll a and carotenoids contents (Metzner *et al.*, 1965). Another set of fresh algae biomasses was extracted using phosphate buffer (pH 6.8) to determine the phycobilins, according to Bryant *et al.*, (1979). The total carbohydrate content was determined colorimetrically using glucose as a standard (Dubois *et al.*, 1956). The procedure of Lowry *et al.*, (1951) was adopted for the estimation of protein in the differently treated dry biomasses, using bovine albumin as standard. For lipid estimation, the low molecular weight components were first removed from the cell pellets using ice-cold 2N perchloric acid. The pellets were further extracted several times with chloroform-methanol (2:1 v/v). The lipid content was estimated in this extract using the method of Kochert (1978). A known weight of the dried biomasses of

the different treatments was used to determine the nucleic acids content (Ogur and Rosen, 1971).

Enzymes were extracted and obtained from the supernatant fraction as follows: the whole biomass of each flask was homogenized with acid-washed sand and few ml of cold distilled water. The homogenate was centrifuged for 20 minutes at 18.000 rpm at 2°C. Glutamic-oxalacetic and glutamic-pyruvic transaminases (GOT, E.C.2.6.1.1., GPT, E.C.2.6.1.2.) and alkaline phosphatase (E.C.3.1.3.1.) and acid phosphatase (E.C3.1.3.2.) were estimated according to Bergmeyer (1974). An aliquot of the enzyme extract was mixed with the proper substrate and incubated at 37°C for 60 minutes (GOT), or 30 minutes (GPT). The enzymatic reaction was stopped by addition of dinitrophenylhyrazine, allowed to stand for 20 minutes at room temperature before addition of 5 ml of 0.4 N sodium hydroxide and determining the optical density of the reaction mixture against a blank at 510 nm.

Alkaline phosphatase fraction was determined in the fresh enzyme extract using sodium carbonate buffer (pH 10) while the acid phosphatase was determined using citric acid-sodium citrate buffer (pH 4.9). After neutralization, both alkaline and acid phosphatases were determined colorimetrically at 700 nm by the sulphite-metol method (Burton and Riley, 1956). Nitrate reductase (E.C-1.6.6.1) was estimated according to Harper (1972). The experimental cultures were prepared in triplicates and the results were analyzed statistically for significance between different treatments .

Results and Discussion

Table 1 shows the dry biomass gain by *Ph. fragile* in calcium - free media containing different concentrations of strontium which were added to the culture media at the beginning of the growth period . The dry weight decreased significantly with increasing the metal concentration . The drop was prominent and highly significant at 100 to 600 mg/L strontium concentration . This indicated that strontium had interfered with the metabolic pathways during the biosynthesis of dry matter.

It is also obvious that *Ph. fragile* showed a tolerance to strontium since it could survive 600 mg/L of the metal. Eleiwa and Naguib (1987) reported that 10^{-5} M strontium chloride was less suppressive to dry weight gain by soybean than to fresh weight. On the other hand, Weiss *et al.,* (1976) reported that the addition of strontium to calcium-deficient media enabled the cells to divide and calcification to take place in the coccolithophorid *Cricosphaera carterae.* These effects increasing strontium concentration. Kulich *et al.*, (1993) reported that 30 mg strontium Per kilogram soil did not reduce biomass formation in maize, *Vicia faba* or sunflower.

Table 1 further shows that both protein and carbohydrate contents of the organism significantly decreased with increasing metal concentration. This drop was more pronounced and highly significant at the larger doses of strontium. On

the other hand, the lipids content increased with increasing metal concentration. The lipids were highest at 300-400 mg/l strontium.

Different concentrations of strontium hardly affected DNA content (Table 1) except at 400 mg/l where a slight significant increase was observed. On the other hand, low concentrations of strontium (50-300 mg/L) significantly reduced the RNA content. However, higher concentrations hardly affected the accumulation of this nucleic acid. Such reduced RNA content might indicate lower biosynthetic activities leading to low dry weight. At large strontium doses, the drop in dry weight gain might be attributed to the toxic action of such doses to the rate of cell division and/or differentiation. The increased lipid accumulation might be attributed to their enhanced biosynthesis and thus depleted the organism of its carbohydrate content.

In this connection, Naguib and Barakat (1989) reported that strontium decreased RNA and DNA contents of *Vicia faba* leaves at the early growth stages. According to Briquet and Wiaux (1967), the mechanism by which toxic substances prevented or reduced plant development was intimately associated with RNA synthesis which controlled the synthesis of enzyme proteins.

Table 2 shows that chlorophyll a and carotenoids highly significantly decreased with increasing strontium concentrations. In the meantime, strontium severely suppressed the phycobiliproteins. The drop in the content of these three accessory pigments was highly significant at all concentrations of the metal. This might give a clew, apart from excessive lipid accumulation, to the reduced accumulation of carbohydrates in the strontium-treated alga.Eleiwa and Naguib (1987) reported that strontium stimulated pigmentation with gradual decline to the control level or less when reaching 10^{-4} M.

Table 3 shows the effect of different concentrations of strontium on the activity of some enzymes. Nitrate reductase, GOT and GPT activity increased with increasing metal concentration. This increase was prominent and highly significant at low concentrations (50-300 mg/l) in case of GTP activity and at higher levels (300-600 mg/L) in the case of nitrate reductase and GOT. While the activity of acid phosphatase decreased with increasing metal concentration, the reverse was the case with alkaline phosphatase activity up to 400 mg/L above which the activity dropped.

These observations lead to the suggestion that the first stages of nitrate assimilation were enhanced by the presence of strontium in the culture media but the shortage of energy supply (derived from photosynthetic activities) and/or intrinsic effect on enzymatic pathways lead to the drop in protein content and deviation toward lipid accumulation. Still, replacement of essential elements by large doses of strontium has its impact on the metabolic pathways.

Strontium induced diverse effects on enzymes ranging from null (Hammerschlag and Bobinski, 1981), activating (Wernett *et al.*, 1981) to inhibitory effects (Pandakova *et al.*, 1981). Although 10^{-6} M strontium, in vitro, had no effect, yet 10^{-3} M reduced alkaline and acid phosphatase activities of rat tissues. Reduced

alkaline and acid phosphatases in liver, small intestine and kidneys with their increase in bone were the result of feeding rat with stable strontium (Kshirsagar, 1976. It has been demonstrated that carbohydrate metabolism of plants may be indirectly controlled by the availability of inorganic phosphate which, in turn, is regulated by acid phosphatase activity (Malik and Singh, 1980).

The protective role of calcium is highly apparent by the observed increase of algae dry biomass compared with the cultures exposed to strontium alone, more prominently at the large doses of the latter (Table 4). In the meantime, the addition of 10 ppm cadmium to the same concentrations of strontium resulted in a drastic effect on the biomass gain. Addition of cadmium to 50 mg/L strontium completely arrested the algae growth. At 300 or 500 mg/L strontium the inhibitory effect of cadmium is slightly counteracted, but the dry weight gain was significantly ($P<0.001$) lower than the control value or strontium-fed samples alone. This indicates that strontium could not replace calcium as a protective element against the toxicity of heavy metals such as cadmium.

The addition of antimony to strontium-containing media hardly affected the dry weight gain of the organism. However, a slight increase at the highest strontium concentration (500 mg/L) could be observed indication that small doses of antimony could partially counteract the inhibitory effects of large doses of strontium.

Coupling the three elements with strontium completely inhibited the algae growth at 50 mg/L strontium. At higher concentrations of strontium, the organism could survive and the dry weight gain was intermediate between that of the presence of calcium or antimony alone in the 300 mg/L strontium. On the other hand, the addition of calcium and antimony to the media containing 500 mg/L strontium and 10 ppm cadmium hardly, if at all, affected the dry weight in the presence of the latter mixture.

This indicates that in the presence of low concentrations of strontium, cadmium (at 10 ppm concentration) was a potent toxic element to *Ph. fragile*; a response that was not alleviated by addition of calcium and antimony, at the same low dose. On the other hand, increasing strontium level in such mixture to 300 mg/L seemed to help in counteracting (antagonizing) the inhibitory effects of cadmium when the four elements were coupled together. Further increase in strontium to 500 mg/L seemed to hinder the protective action of antimony and/or calcium against cadmium in such mixture.

Several investigations emphasized the importance of calcium for the growth of algae (Marmé, 1983; Khalil, 1994). Cadmium is a potent toxicant to micro-organisms specially algae (Rebhun and Ben Amotz, 1984; Trevors, 1986). The literature seemed to be free of reports on antimony toxicity to plants (Parisis and Van Den Heede, 1992). However, the toxicity of a particular metal to an aquatic organism may be affected by the presence of another metal or chemical substance in the environment.

Aberg *et al.*, (1990) reported that strontium and calcium exhibited similar geochemical behaviour and therefore it si possible for strontium to replace calcium in many active sites. However, Rahimian (1972) hypothesized that calcium is required, in particular, for activating enzymes involved in cell wall formation of Golenkinia minustissima and cell division as well as for maintaining membrane integrity. Fuller and Hardcastle (1967) reported that uptake of strontium and calcium by cartain algae always favoured calcium over strontium when the two metals were present together in the nutrient media, thereby modifying the hazards of strontium entering the food chain.

Calcium is known to antagonize cadmium toxicity in *Anacystis nidulans* (Sing and Yadava, 1984) owing to a competition between the two metals for the same uptake system due to very similar ionic radii (Gipps and Coller, 1982) or their homogeneous chemistry (Norris and Kelly, 1977). Raizada and Rai (1985) suggested that calcium played a role in ameliorating heavy metal toxicity to *Nostoc muscorum*. The author, in unpublished data, (Khalil, 1986) reported that calcium nullified the stimulatory effect of small doses of antimony and partially counteracted the inhibitory effects of cadmium on the dry weight gain by *Ph. fragile*

Table 4 further shows that calcium seemed to have little, if at all, effect on protein or carbohydrate contents of *Ph. fragile* supplemented with 50 mg/L strontium. On the other hand, it counteracted the inhibitory effect of strontium when the latter was supplemented at 300 or 500 mg/L.

The toxicity of cadmium was further extended to the carbohydrates accumulation, where a sharp drop in their level (almost half the control level) was observed when cadmium was added to the media containing 300 or 500 mg/L strontium. In the meantime, cadmium slightly furthered protein accumulation by the strontium-treated samples, more prominently at 300 than 500 mg/L strontium.

These observations indicate that, in presence of strontium, cadmium was more effective on the carbohydrate than the nitrogen metabolism.

In this connection, Kremer and Markham (1982) noticed that the primary effect of cadmium is the inhibition of de novo protein biosynthesis which leads to enzyme deficiency and a resulting inhibition of photosynthesis, growth and other cell processes.

Although antimony slightly, if at all, affected the dry weight gain by *Ph. fragile* supplemented with a range of 50 to 500 mg/L strontium, yet it counteracted the inhibitory effects of strontium on protein accumulation, raising its level above that of strontium-treated samples, more prominently at the largest applied dose. On the other hand, antimony furthered the suppressive effects of strontium on the carbohydrate accumulation by the alga, almost to the same extent regardless of the concentration of the latter, an indication that antimony exerted its effect on one and the same target in the pathway of carbohydrate accumulation. In the meantime, it was able to counteract the suppressive effects of strontium was extremely deleterious to carbohydrate or protein accumulation.

Table 4 also shows that calcium was slightly suppressive to lipid accumulation when coupled with strontium, only at the latter's highest concentration. In the meantime, cadmium severely attenuated the lipid content of the alga, to a larger extent when coupled with 300 than 500 mg/L strontium. On the other hand, lipids significantly increased when antimony was coupled with 50 mg/L strontium, a response that faded with increased concentration of the latter. Such increase suggests that the apparent drop in the carbohydrate level (under such treatment) may be attributed to enhanced lipid accumulation. The presence of cadmium and calcium in such media had insignificant effect on the trend of lipid accumulation, an indication that both elements nullified each other's effect on lipid metabolism.

Table 4 further shows that calcium stimulated the accumulation of RNA by strontium-supplemented alga; a response that was furthered by 300 mg/L strontium and alleviated by 500 mg/L strontium. This indicates that the ratio between these two elements played a significant role in the antagonistic phenomenon between both of them, a response that was also observed between strontium and cadmium where the complete arrest of growth at the low strontium level was partially and progressively abolished by raising the strontium level up to 500 mg/L.

The results further show that the metabolic pathways leading to the accumulation of differed in their response to heavy metal application. This is most apparent is case of RNA accumulation which was most sensitive to cadmium when coupled with strontium where traces of RNA accumulated even at 300 mg/L strontium which hardly affected RNA accumulation when supplied singly, whereas when coupled with 500 mg/L strontium, almost 30% of RNA accumulated, compared with the latter when supplied singly. The same applies when calcium and antimony were supplemented to this mixture suggesting that te the antagonism between these latter elements did not augment the effect of cadmium.

The stimulatory effect of antimony on RNA accumulation was only apparent when coupled with 300 mg/L strontium. Higher levels of strontium abolished or even reversed such effect.

The response of DNA differed from that of RNA. Calcium or antimony hardly affected the DNA content of 50 mg/L strontium-fed samples. DNA content of 300 mg/L strontium-fed samples was attenuated whereas that of 500 mg/L strontium-fed samples was slightly increased by the presence than absence of these two elements. This indicates that 10 ppm of either calcium or antimony seemed to have similar effects on DNA accumulation. Their effect, in the presence of 50 mg/L strontium was negligible but it seems that they were able to replace the latter element at its larger doses thus attenuating its stimulator (at 300 mg/L strontium) or inhibitory (at 500 mg/L strontium) effect on DNA accumulation. The toxic effects of cadmium were still apparent and remained as such or furthered by addition of calcium and antimony to the strontium-cadmium media.

Table 5 clearly shows that all the combinations of calcium, cadmium or antimony with strontium could not nullify or counteract the inhibitory effects of the latter on chlorophyll a, carotenoids or phycobiliproteins. On the contrary, calcium, which is known for its protective role toward pigments synthesis (Pellegrini *et al.*, 1993), furthered the drop in chlorophyll a and carotenoids content. In the meantime, its presence in 50 mg/L strontium-media reversed the suppressive effect of the latter on phycobiliproteins but its coupling with the larger doses of strontium could not change the inhibitory effect of strontium on the accumulation of these pigments. Furthermore, the results lead to the conclusion that large doses of strontium blocked one and the same stage(s) in the biosynthesis of these phycobiliproteins since it was observed that the rise of strontium level from 300 to 500 mg/L did not alter the level of these pigments. In the meantime, calcium could not augment these suppressive effects, possibly due to its comparatively low ratio in the strontium media.

The lower chlorophyll a or carotenoids when cadmium was supplemented to 300 than 500 mg/L strontium media might be attributed to the lower efficacy of the former than the latter strontium concentration to abolish the toxicity of cadmium. In general, neither cadmium nor antimony was able to counteract the suppressive effects of strontium on phycobiliproteins, carotenoids or chlorophyll a, even in the presence of calcium.

Gotsis (1982) observed different responses of *Dunaliella minuta* to different rations of selenium/mercury and selenium/copper. The author (1996) reported that 10 mg/L antimony decreased the content of both chlorophyll a and carotenoids whereas calcium/cadmium combination counteracted this effect of antimony on either pigments.

Table 6 shows that the addition of calcium to strontium media hardly affected the activity of GOT, GPT and acid phosphatase in the presence of strontium alone. On the other hand, calcium counteracted the stimulatory effects of strontium on nitrate reductase activity but stimulated alkaline phosphatase activity. The latter's response to 500 mg/L strontium was not affected by calcium.

In this connection, Bergareche *et al.*, (1994) suggested that calcium might act as an intermediate in the sequence of events leading to new synthesis of nitrate reductase in cotyledons of cucumber seedling.

The addition of cadmium alone or coupled with calcium and antimony, to the strontium media lowered the activity of the tested enzymes. Lee (1976) indicated that acid phosphatase activity increased in cadmium- treated soybean leaf.

Antimony-strontium combinations slightly decreased the alkaline phosphatase activity at 50 and 300 mg/L strontium. At 500 mg/L strontium, antimony seemed without effect on the activity of this enzyme. Antimony alone, counteracted the stimulatory effect of strontium (at 50 mg/L) on GPT ; a response that faded with increased of GOT in strontium level. On the other hand, antimony increased the activity of GOT in strontium-treated samples; a response that was furthered by increasing strontium level to 300 mg/L then dropped at 500 mg/L. Antimony-

strontium combinations decreased the activity of nitrate reductase but hardly affected (at low concentration of strontium) or increased (at higher levels of strontium) the acid phosphatase activity.

These observations confirm the previous postulation that the interaction between the heavy metals largely depends on their relative concentrations. In this connection, Rai *et al.*, (1981) reported that one of the most characteristic features of heavy metal toxicity is the poisoning and inactivation of enzyme systems.

Table 1 : EFFECT OF DIFFERENT CONCENTRATIONS OF STRONTIUM ON SOME GROWTH PARAMETERS OF PHORMIDIUM FRAGILE AFTER 7 DAYS GROWTH PERIOD

Treatment	Dry Weight (mg)	Proteins µg per mg Dry Weight	Carbohydrates	Lipids	Nucleic Acids µg / g D. Wt.	
					R N A	D N A
Control	52.5	320.3	410.21	3.87	4.43	2.73
50 mg/l	48.2 a	285.6 a	399.22	4.12	2.48 b	2.79
100 mg/l	42.0 c	271.4 b	385.66 b	4.25	2.82 b	2.95
200 mg/l	39.3 c	258.5 b	380.88 c	4.83 b	3.20 b	2.98
300 mg/l	37.3 c	246.7 c	365.95 c	5.72 c	3.80 b	3.09
400 mg/l	31.4 c	217.2 c	356.50 c	5.57 c	4.46	3.47 a
500 mg/l	29.8 c	207.9 c	349.53 c	5.05 b	4.61	2.55
600 mg/l	27.7 c	194.6 c	325.38 c	4.85 b	4.43	2.30

a : Significantly different from the control value at $P < 0.05$

b : Significantly different from the control value at $P < 0.01$

c : Significantly different from the control value at $P < 0.001$

Table 2 : EFFECT OF DIFFERENT CONCENTRATIONS OF STRONTIUM ON THE PIGMENTS CONTENT OF PHORMIDIUM FRAGILE AFTER 7 DAYS GROWTH PERIOD

Treatment	Chl. a µg / mg	Crotenoids D. Wt.	Phycobilip roteins (µg/g D. Wt.)		
			CPC	APC	CPE
Control	5.11	2.20	39.96	39.08	15.99
50 mg/l	4.44 a	1.83 a	13.20 c	26.79 c	7.62 c
100 mg/l	3.79 c	1.61 c	10.76 c	26.95 c	4.62 c
200 mg/l	3.25 c	1.26 c	8.82 c	13.56 c	3.72 c
300 mg/l	2.73 c	1.07 c	3.79 c	11.93 c	3.27 c
400 mg/l	2.26 c	0.99 c	3.64 c	11.10 c	3.18 c
500 mg/l	2.03 c	0.90 c	3.60 c	7.18 c	2.96 c
600 mg/l	1.10 c	0.62 c	3.11 c	5.90 c	2.31 c

Chl. a : Chlorophyll a

CPC : c-phycocyanin

APC : Allophycocyanin

CPE : c-phycoerythrin

a : Significantly different at $p < 0.05$

b : Significantly different at $P < 0.01$

c : Significantly different at $P < 0.001$

Table 3 : EFFECT OF DIFFERENT CONCENTRATIONS OF STRONTIUM ON SOME ENZYMES OF PHORMIDIUM FRAGILE

Treatment	Nitrate Reductase **	G O T µM Pyruvate/ml/min.	G P T	Phosphatases Alkaline µg P / ml / min.	Acid
Control	0.961	0.123	0.213	5.20	2.85
50 mg/l	1.069	0.126	0.358 b	6.15 a	2.58 a
100 mg/l	1.082	0.131	0.316 b	6.20 a	2.30 b
200 mg/l	1.084	0.138	0.310 a	6.30 a	2.20 c
300 mg/l	1.130 a	0.146 a	0.291 a	6.70 b	2.16 c
400 mg/l	1.192 b	0.161 b	0.280	7.20 c	2.08 c
500 mg/l	1.264 c	0.168 b	0.229	5.20	1.96 c
600 mg/l	1.337 c	0.200 c	0.209	4.20 a	1.73 c

** : Nitrite-N /ml.min. For presentation , the data were multiplied by 10^3

a : Significantly different at $P < 0.05$
b : Significantly different at $P < 0.01$
c : Significantly different at $P < 0.001$

Table 4 : INTERACTIVE EFFECTS OF CALCIUM , CADMIUM AND ANTIMONY (10 mg/l) WITH DIFFERENT CONCENTRATIONS OF STRONTIUM ON SOME GROWTH CRITERIA OF PHORMIDIUM FRAGILE AFTER 7 DAYS GROWTH PERIOD

Treatment	Dry Weight (mg)	Protein µg / mg	Carbohydrates Dry Weight	Lipids	R N A µg/g Dry Weight	D N A
Control	58.0	297.50	394.01	4.21	3.62	2.59
50 mg/l Sr	52.5 b	253.13 b	379.20	4.43	2.38 a	2.48
+ Ca	55.0	257.43 b	375.27	4.29	4.30 a	2.73
+ Cd	----	------	------	----	----	----
+ Sb	52.0 b	276.93 a	323.34 c	5.51 c	3.81	2.44
+Ca+Cd+Sb	----	------	------	----	----	----
300 mg/l Sr	43.6 c	230.40 c	351.98 b	5.53 c	3.59	3.22 b
+ Ca	45.8 c	258.23 b	396.41	4.50	6.49 c	2.96 a
+ Cd	18.8 c	181.43 c	192.81 c	2.10 c	traces	1.72 c
+ Sb	43.1 c	249.68 b	287.50 c	4.77 a	5.24 b	2.45
+Ca+Cd+Sb	24.0 c	182.10 c	160.47 c	4.22	traces	1.29 c
500 mg/l Sr	32.7 c	189.8 c	291.81 c	4.23	4.98 b	2.41
+ Ca	46.0 c	242.55 c	383.60	3.83 a	4.75 b	2.77
+ Cd	31.3 c	194.85 c	180.92 c	3.33 b	1.39 c	1.96 c
+ Sb	39.9 c	231.15 c	272.98 c	3.81 a	2.95 a	2.63
+Ca+Cd+Sb	30.5 c	133.28 c	155.00 c	3.72 a	1.35 c	1.96 c

a : Significantly different from the control value at $P < 0.05$
b : Significantly different from the control value at $P < 0.01$
c : Significantly different from the control value at $P < 0.001$

Table 5 : INTERACTIVE EFFECTS OF CALCIUM , CADMIUM AND ANTIMONY WITH DIFFERENT CONCENTRATIONS OF STRONTIUM ON THE PIGMENTS CONTENT OF PHORMIDIUM FRAGILE AFTER 7 DAYS GROWTH PERIOD

Treatment	Chl. a	Carotenoids	Phycobiliproteins (µg/g D. Wt.)		
	µg / mg D. Wt.		CPC	APC	CPE
Control	4.64	2.47	42.20	36.00	16.08
150 mg/l Sr	4.11	1.69 c	16.11 c	28.25 a	8.24 c
+ Ca	3.65 a	1.52 c	23.04 c	31.78	10.54 c
+ Cd	----	----	-----	-----	-----
+ Sb	4.23	1.79 c	22.07 c	8.58 c	7.18 c
+Ca+Cd+Sb	----	----	-----	----	-----
300 mg/l Sr	2.69 c	0.93 c	3.22 c	10.30 c	2.69 c
+ Ca	2.05 c	0.84 c	3.02 c	3.78 c	2.78 c
+ Cd	0.40 c	0.12 c	4.16 c	5.20 c	2.99 c
+ Sb	2.09 c	0.94 c	4.67 c	7.58 c	3.17 c
+Ca+Cd+Sb	0.28 c	0.10 c	1.90 c	5.78 c	2.42 c
500 mg.l Sr	2.17 c	1.02 c	2.37 c	4.90 c	2.53 c
+ Ca	1.62 c	0.69 c	2.57 c	3.73 c	2.89 c
+ Cd	1.26 c	0.62 c	4.34 c	4.65 c	2.38 c
+ Sb	2.17 c	0.99 c	2.20 c	3.73 c	1.98 c
+Ca+Cd+Sb	2.50 c	1.03 c	3.39 c	4.95 c	2.53 c

Chl. a : Chlorophyll a
CPC : c-phycocyanin
APC : Allphycocyanin
CPE : c-phycoerythrin

a : Significantly different at $p < 0.05$
b : Significantly different at $P < 0.01$
c : Significantly different at $P < 0.001$

Table 6 : INTERACTIVE EFFECTS OF CALCIUM , CADMIUM , AND ANTIMONY (10 mg/l) WITH DIFFERENT CONCENTRATIONS OF STRONTIUM ON THE ACTIVITY OF SOME ENZYMES OF PHORMIDIUM FRAGILE

Treatment	Nitrate Reductase **	G O T µM Pyruvate/ml/min.	G P T	Phosphatases Alkaline µg P / ml / min.	Acid
Control	0.926	0.154	0.213	5.52	2.71
50 mg/l Sr	1.041	0.113	0.316 c	5.29	2.19 c
+ Ca	0.809	0.120	0.348 c	6.37	2.24 b
+ Cd	-----	-----	-----	----	----
+ Sb	0.876	0.204	0.240	4.23 a	2.18 c
+Ca+Cd+Sb	-----	-----	-----	----	----
300 mg/l Sr	1.104 a	0.133	0.216	6.06	2.12 c
+ Ca	0.835	0.132	0.220	7.18 a	2.24 a
+ Cd	0.746 a	0.054 c	0.068 c	4.29 a	1.68 c
+ Sb	0.868	0.177	0.240	4.61	2.50
+Ca+Cd+Sb	0.766 a	0.074 b	0.134 c	2.84 c	1.48 c
500 mg/l Sr	1.341 c	0.147	0.206	5.52	2.10 c
+ Ca	0.926	0.148	0.197	5.59	1.99 c
+ Cd	0.463 c	0.074 b	0.132 c	4.08 a	2.21 b
+ Sb	0.797	0.114	0.214	5.47	2.55
+Ca+Cd+Sb	0.688 b	0.087 a	0.156 b	4.08 a	1.59 c

** : Nitrite-N /ml/min. For presentation , the data were multiplied by 10^3

a : Significantly different at $P < 0.05$
b : Significantly different at $P < 0.01$
c : Significantly different at $P < 0.001$

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