Effect of cobalt on growth, pigments and the photosynthetic electron transport in *Monoraphidium minutum* and *Nitzchia perminuta*

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The unicellular green alga *Monoraphidium minutum* and the diatom *Nitzschia perminuta* were cultured under different concentrations of Co^{2+}. Growth and pigment content were slightly increased at low concentrations and inhibited by high Co^{2+} concentrations. The results concerning the effect of different concentrations of Co^{2+} on photosynthetic O_2 evolution showed a reduction in the amount of O_2 evolved by each alga in response to increasing Co^{2+} concentrations. However, an increase in O_2 evolution for both *M. minutum* and *N. perminuta* was observed at relatively low Co^{2+} concentrations. Photosynthetic electron transport in *M. minutum* was more sensitive to Co^{2+} toxicity than in *N. perminuta*. On the other hand, the effect of the heavy metal on respiration showed that higher Co^{2+} concentrations were inhibitory to O_2 uptake by the two algal species. Low Co^{2+} concentrations stimulated O_2 uptake by *M. minutum* throughout the experimental period. However, in *N. perminuta*, different concentrations of Co^{2+} led to a reduction of O_2 uptake. To localize the action site of Co^{2+} in the photosynthetic electron transport chain, the fluorescence induction technique was carried out. According to the results obtained, the inhibitory action of Co^{2+} is located on the acceptor side of PSII for both *M. minutum* and *N. perminuta.*

Key words: cobalt, diatoms, green algae, pigments, photosynthesis.

Efeito do cobalto no crescimento, pigmentos e transporte de elétrons na fotossíntese em *Monoraphidium minutum* e *Nitzchia perminuta*: A alga unicelular verde *Monoraphidium minutum* e a diatomácea *Nitzschia perminuta* foram cultivadas sob diferentes concentrações de Co^{2+}. O crescimento e o conteúdo de pigmentos foram pouco aumentados em baixas e inibidos em altas concentrações de Co^{2+}. Para ambas as algas, os resultados referentes às concentrações de Co^{2+} na liberação de O_2 fotossintético mostraram redução na quantidade de O_2 liberado à medida que se aumentou a concentração de Co^{2+}. No entanto, aumento na liberação de O_2 foi observado também para as duas algas em baixas concentrações de Co^{2+}. O transporte de elétrons na fotossíntese em *M. minutum* foi mais sensível à toxicidade causada por Co^{2+} que em *N. perminuta*. Por outro lado, o efeito desse metal pesado sobre a respiração mostrou que maiores concentrações de Co^{2+} foram inibitórias para a absorção de O_2 nas duas algas. Baixas concentrações de Co^{2+} estimularam a absorção de O_2 em *M. minutum*. Porém, diferentes concentrações de Co^{2+} levaram à redução da absorção de O_2 em *N. perminuta*. Para identificar o local de ação de Co^{2+} na cadeia de transporte de elétrons na fotossíntese, utilizou-se a técnica de indução de fluorescência. De acordo com os resultados, a ação inibitória do Co^{2+} está localizada no lado aceptor do PSII para ambas as algas.

Palavras-chave: alga verde, cobalto, diatomácea, fotossíntese, pigmentos.

INTRODUCTION

Heavy metals are prevalent in municipal and industrial effluents; they modify the structure and productivity of aquatic ecosystems (Magdaleno et al., 1997). Irrigated agriculture and industrial activity where a lack of conditions prevails for the control and safe disposal of wastes are two important sources of pollution (Custodio, 1992). From a biological point of view, heavy metals can be divided into two categories: essential

and non-essential (Reddy and Prasad, 1990). However, essential heavy metals have even been reported to be toxic at high concentrations. For example, some heavy metals including copper, zinc, nickel and chromium, are essential for growth at very low concentrations but toxic at slightly levels (Gadd and Griffiths, 1978; Reed and Gadd, 1989). As indicated by Czerpak et al. (1994), the concentration 5 x 10^{-6} - 10^{-5} mol.L^{-1} Co^{2+} exerted maximal stimulatory effect on *Chlorella pyrenoidosa* cells at the exponential growth phase in terms of fresh weight (150-160 % increase), dry weight (50-60 % increase), chlorophylls *a* and *b* (45-65 % increase), total carotenoids (55-65 % increase), water-soluble proteins (19-20 % increase) and monosaccharides content (55-60 % increase), when compared to the control culture. Lustigman et al. (1995) studied the effect of Co^{2+} on *Chlamydomonas reinhardtii*. They observed reduction of growth at 10 ppm Co^{2+} and without change in the morphology of the cells or pH. At 20 ppm Co^{2+}, on the other hand, growth was considerably reduced compared to the control and the colour of the organism became paler and the cells clumped. In addition, the pH value was lower compared to the pH of the control at the end of experimental period. Lu et al. (2000) demonstrated that chlorophyll fluorescence analysis could be a useful physiological tool to assess early stages of change in photosynthetic performance of algae in response to heavy metal pollution.

Attempts have been made to identify the site of inhibition for Co^{2+} in the PSII driven electron transport chain. Co^{2+} seems to have a direct effect on P680 (Tripathy et al., 1981, 1983; El-Sheekh and Hammouda, 1992). Miyachi et al. (1996) measured quantum requirements of photosynthetic O_2 evolution at 679 nm, fluorescence emission spectra at liquid nitrogen temperature and fluorescence induction kinetics in the presence of DCMU, in the cyanobacteria *Anabaena variabilis* M3, *Anabaena variabilis* ATCC and *Anacystis nidulans* R2, each grown under low or high Co^{2+} conditions. Low Co^{2+} grown cells of the cyanobacteria showed a higher quantum requirement of photosynthetic O_2 evolution together with a higher ratio of F710-740 to F680-700 fluorescence and a lower variable fluorescence in the presence of DCMU compared with high Co^{2+} grown cells. These findings indicate a change in excitation energy distribution in favour of photosystem I. This might also suggest an enhancement in ATP formation caused by cyclic electron flow, which in turn provokes dissolved inorganic carbon accumulation in these low Co^{2+} grown cells.

Unlike higher plants, cyanobacteria can adapt to changes in the nutritional status and other environmental changes quite readily (Reuter and Muller, 1993). Growth of *Synechocystis* PCC 6803 cells in 10 mmol.L^{-1} $CoCl_2$ stimulates the PSII electron transport rates (Tiwari and Mohanty, 1993). Tiwari and Mohanty (1996) suggested that supplementation of 10 mmol.L^{-1} $CoCl_2$ to the normal growth medium of *Synechocystis* PCC 6803 causes multiple changes involving a small increase in PSII to PSI ratio, enhanced funneling of energy to PSII and an increase in PSI electron transport, together with decreased PSI cross section and a reduction in the intersystem pool size. The cumulative effects of these alterations cause stimulation in electron transport and O_2 evolution. This report concerns a study of the effect of Co^{2+} on growth, pigment content, and photosynthesis of the fresh water algae *Monoraphidium minutum* and *Nitzschia perminuta* as well as the localization of the site of Co^{2+} inhibition in photosynthetic electron transport chain.

MATERIAL AND METHODS

Isolation and purification of the algae: *M. minutum* and *N. perminuta* were isolated from fresh water samples collected from the River Nile. One single cell from each colony was isolated, transferred to fresh solid medium and subjected to repeated subculturing on fresh solid media before transfer to sterilized liquid nutrient media.

Nutrient solution and Culture technique: Kuhl medium (Kuhl, 1962) was used for cultivation of *M. minutum* and Allen's and Stanier (1968) medium used for growth of *N. perminuta.* The culture illumination was provided by fluorescent tube lamps giving a light intensity of 120 watts.m^{-2}. The cultures were supplied with sterilized dry air (97%) and CO_2 (97 % : 3 %, v/v).

Determination of the optical density: Growth was followed by measuring the optical density of the green algal suspension at 560 nm, as recommended by Wetherel (1961). On the other hand, the optical density of *N. perminuta* was measured at 750 nm as cited in Sriharan et al. (1990).

Estimation of pigments: The spectrophotometric method recommended by McKinney (1941), which is suitable for microalgae, was used to estimate chlororphyll *a*, chlorophyll *b* and carotenoids.

Measurements of photosynthetic activity (O_2 evolution) and respiration (O_2 uptake): The photosynthetic activity was measured polarographically as O_2 evolution using a Clark-type electrode (YSI, model 53). The actinic white light was obtained from a 150 W tungsten lamp.

Fluorescence measurements: Algal cells equivalent to 5 $mg.ml^{-1}$ of chlororphyll *a* were centrifuged at 6,000 g_n for 10 min and were dark adapted for 30 min with different concentrations of Co^{2+} prior to fluorescence measurements. In the case of DCMU-treated cells, DCMU (5 $mmol.L^{-1}$) was added and the cells incubated for 1.5 min before the measurements. Fluorescence spectra were recorded with spectroflurophotometer (Shimadzu, RF-510) at room temperature with a 10 nm excitation slit and 5 nm emission slit. The excitation light was monochromatic light (440 nm) to excite chlororphyll *a* (Gupta and Singhal, 1996).

RESULTS

Effect of Co^{2+} on the growth of M. minutum: Low concentrations of Co^{2+} led to an increase in the growth of *M. minutum.* Thus, 8 and 13 % stimulation were observed in the cultures treated with 0.1 and 0.5 ppm, respectively, after 10 days of incubation. On the other hand, higher concentrations exerted an inhibitory effect on algal growth. Thus 5, 17 and 42 % reductions were observed in the cultures treated with 1, 2 and 3 ppm, respectively, after 10 days of incubation (figure 1).

Effect of cobalt on the growth of N. perminuta: Low concentrations of Co^{2+} (0.5 and 1.5 ppm) showed a slight increase in the growth of *N. perminuta* with increase of about 5 and 9 % respectively as shown in figure 2. On the other hand, considerable reduction in growth was observed with higher concentrations. The reductions were 14, 18 and 36 % below the control value after 7 days of incubation in cultures treated with 2.5, 3.5 and 5 ppm Co^{2+}, respectively.

Effect of cobalt on the pigments content of M. minutum: Data presented in table 1 show that application of 0.1 and 0.5 ppm Co^{2+} increased Chlorophyll *a* content whereas higher Co^{2+} concentrations (1, 2 and 3 ppm) led to significant reductions in chlorophyll *a* biosynthesis, with values of 25, 38 and 46 % below the control level, respectively. A similar effect of Co^{2+} on chlorophyll *b* biosynthesis was exhibited. Thus, low Co^{2+} concentrations increased the biosynthesis of chlorophyll *b*, while high concentrations led to reduction in its content. With

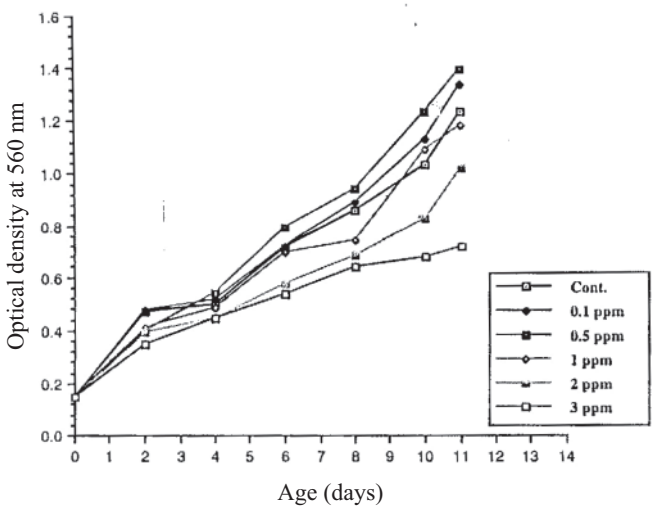

Figure 1. Effect of different Co^{2+} concentrations (ppm) on the growth of *M. minutum* as measured by optical density at 560 nm.

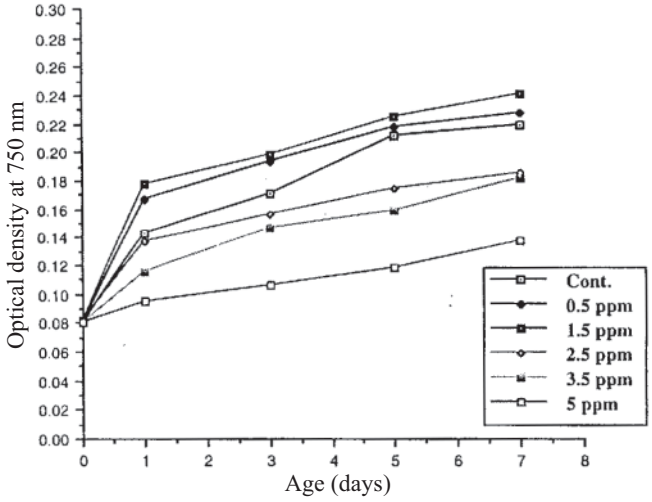

Figure2. Effect of different Co^{2+} concentrations (ppm) on the growth of *N. perminuta* as measured by optical density at 750 nm.

Table 1. Effect of different concentrations of Co^{2+} on the pigment content of *M. minutum* after the incubation for 11 days ($\mu g.ml^{-1}$ algal suspension).

Co^{2+} (ppm)	Chlorophylls		Carotenoids	Chlorophylls	
	a	*b*		*a+b*	*a/b*
0.0	8.02	7.05	2.82	15.07	1.14
0.1	8.57	7.68	2.91	16.25	1.12
0.5	10.35	9.04	3.02	19.39	1.14
1.0	6.03	5.37	2.66	11.40	1.12
2.0	4.98	4.28	2.40	9.26	1.16
3.0	4.35	3.74	1.71	8.09	1.16

chl.a:F=2.0+02*** ; carotenoids: F=2.7+03***; $p \leq 0.001$

respect to carotenoids, 0.1 and 0.5 ppm Co^{2+} stimulated their biosynthesis by 3 and 7 % above the control level after 11 days of incubation, respectively. In addition, *M. minutum* was more resistant, at least to some extent, to the toxicity of Co^{2+}. Thus 1, 2 and 3 ppm Co^{2+} resulted in 6, 15 and 39 % reductions in carotenoid content, respectively, compared with the control level. Moreover, 0.1 and 0.5 ppm Co^{2+} increased the total chlorophyll (*a*+*b*) of *M. minutum* by 8 and 29 % above the control value, respectively. On the other hand, a remarkable decrease in the total chlorophyll levels occurred, with reductions of 24, 39 and 46 % for cultures treated with 1, 2 and 3 ppm Co^{2+}, respectively after 11 days of incubation.

Effect of Co^{2+} on the pigments content of N. perminuta: The results shown in table 2 reveal that application of 0.5 and 1.5 ppm Co^{2+} increased chlorophyll *a* content by 4 and 12 % above the control level, respectively. However, chlorophyll *a* decreased significantly with the increase in Co^{2+} concentrations. Thus, 2.5, 3.5 and 5 ppm led to 4, 29 and 36% reductions below the control level, respectively, at the end of the incubation period. With respect to carotenoids they appeared to be more resistant to Co^{2+} phytotoxicity than chlorophyll *a*. Thus, the lower Co^{2+} concentrations (0.5 and 1.5 ppm) stimulated the biosynthesis of carotenoids. On the other hand, higher Co^{2+} concentrations resulted in lower reductions in carotenoids compared with chlorophyll *a*, except for 2.5 ppm which led to a higher reduction in carotenoids than in chlorophyll *a*. The magnitude of reduction was 16 and 28 % for the cultures treated with 3.5 and 5 ppm Co^{2+}, respectively.

Table 2. Effect of different concentrations of Co^{2+} on the pigment content of *N. perminuta* after the incubation for 7 days (μg.ml^{-1} algal suspension).

Co^{2+} (ppm)	Chl. *a*	Carotenoids	Chl.*a*/carotenoids
0.0	2.50	3.08	0.81
0.5	2.61	3.18	0.82
1.5	2.81	3.57	0.79
2.5	2.40	2.77	0.87
3.5	1.78	2.59	0.69
5.0	1.60	2.22	0.72

chl.a:F=1.2^{+02}*** ; carotenoids: F=6.3***; $p \leq 0.001$

Effect of different concentrations of Co^{2+} on the photosynthetic O_2 evolution and respiration of M. minutum: Table 3 shows that low concentrations of Co^{2+} (0.1 and 0.5 ppm) generally stimulated O_2 evolution and dark respiration throughout the experimental period (11 d). The maximum stimulations in O_2 evolution (59 %) and dark respiration (76 %) were recorded on the 10th and 11th days, respectively, for cultures treated with 0.5 ppm Co^{2+}, in comparison with the control. Photosynthetic activity of *M. minutum* showed progressive reductions in response to treatment with higher cobalt concentrations (1, 2 and 3 ppm) during the cultivation period except in the case of the 10th day of the culture treated with 1 ppm Co^{2+}, which produced the same amount of O_2 evolved as control culture (table 3). The greatest reduction in O_2 evolution was 58 %, recorded in the culture treated with 3 ppm Co^{2+}, on the 8th day of incubation. With regard to respiration, higher Co^{2+} concentrations (1, 2 and 3 ppm) led to greater reductions compared with O_2 evolution. The maximum inhibition value was recorded on the 11th day in the culture treated with 3 ppm Co^{2+}, with a value of 63 % below the control level.

Effect of different concentrations of Co^{2+} on the photosynthetic O_2 evolution and respiration of N. perminuta: Data presented in table 4 show that low concentrations of Co^{2+} (0.5 and 1.5 ppm) stimulated the O_2 evolution of *N. perminuta.* The most pronounced stimulation of O_2 evolution (51 %) was recorded on the 3rd day in cultures treated with 1.5 ppm Co^{2+}. On the other hand, application of higher Co^{2+} concentrations (2.5, 3.5 and 5 ppm) resulted in pronounced reductions in O_2 evolution. The maximum reduction was recorded in culture treated with 5 ppm Co^{2+} on the 5th day of treatment, with a value of 54 % below the control level.

With regard to respiration, *N. perminuta* generally showed a progressive reduction of O_2 uptake in response to the increase in Co^{2+} concentrations, except on the 1st day of the incubation period, where O_2 uptake decreased with increasing Co^{2+} concentrations up to 2.5 ppm. However, O_2 uptake was at the control level in the case of 3.5 and 5 ppm Co^{2+}. The maximum reduction value, 38 %, was recorded on the 5th day for the culture treated with 5 ppm Co^{2+}. High concentrations of Co^{2+} had a more inhibitory effect on O_2 evolution than respiration.

Effect of Co^{2+} on chlorophyll a a fluorescence emission spectrum of algal cells: The cells of *M. minutum* treated with 0.1 ppm Co^{2+} exhibited a slight (5.5 %) increase in fluorescence intensity. On the other hand, cultures treated with 3 ppm Co^{2+} showed a decrease in fluorescence intensity of 9.2 % (figure 3). In the presence of 5 mmol.L^{-1} DCMU, the fluorescence emission increased markedly. However, cultures treated with

Table 3. Effect of different Co^{2+} concentrations on photosynthetic activity (O_2 evolution calculated as µmol O_2.mg chlorophyll^{-1}.h^{-1}) and dark respiration (O_2 uptake calculated as µmol O_2.h^{-1}) of *M. minutum*.

	Co^{2+} (ppm)											
	Control		0.1		0.5		1.0		2.0		3.0	
Days	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.
2	267	1.04	294	1.04	359	1.19	245	0.89	151	0.89	163	0.89
4	245	1.19	275	1.48	301	1.78	218	1.04	197	1.04	125	0.89
6	215	0.89	245	1.04	319	1.19	190	0.59	165	0.44	109	0.44
8	210	0.89	240	0.89	240	1.19	180	0.59	156	0.59	89	0.44
10	191	1.04	247	1.19	303	1.48	191	0.89	160	0.59	97	0.59
11	175	0.59	203	0.89	225	1.04	162	0.44	117	0.44	93	0.22

*** P≤0.001

F-value:	(O_2 evolution)	*F*-value:	(O_2 uptake)
Day	1.7^{+02***}	Day	2.8^{+03***}
Cobalt	8.2^{+02***}	Cobalt	4.8^{+02***}
Day x Cobalt	1.2^{+01***}	Day x Cobalt	3.0^{+01***}

Table 4. Effect of different Co^{2+} concentrations on photosynthetic activity (O_2 evolution calculated as µmol O_2.mg chlorophyll^{-1}.h^{-1}) and dark respiration (O_2 uptake calculated as µmol O_2.h^{-1}) of *N. perminuta*.

	Co^{2+} (ppm)											
	Control		0.5		1.5		2.5		3.5		5.0	
Days	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.	O2 evol.	O2 upt.
1	1242	0.89	1608	0.60	1788	0.60	1214	0.74	1157	0.89	939	0.89
3	1376	2.07	1625	1.93	2086	2.07	1342	1.78	1213	1.78	944	1.63
5	1481	2.37	1503	2.22	1531	2.22	939	2.07	827	2.07	687	1.48
7	933	2.96	939	2.50	1039	2.82	822	2.37	687	2.37	617	1.93

*** P≤0.001

F-value:	(O_2 evolution)	*F*-value:	(O_2 uptake)
Day	2.3^{+05***}	Day	5.6^{+04***}
Cobalt	1.7^{+05***}	Cobalt	1.8^{+04***}
Day x Cobalt	1.2^{+04***}	Day x Cobalt	2.5^{+03***}

DCMU and 0.1 ppm Co^{2+} showed only a slight increase above the value of the DCMU-treated cells. On the other hand, culture treated with 3 ppm Co^{2+} and DCMU showed no noteworthy change in the fluorescence emission spectra compared with that of the control culture treated with DCMU alone. The cells of *N. perminuta* treated with 0.5 and 5 ppm Co^{2+} showed progressive increases in the emission spectra (14.3 and 19 %, respectively) compared with the control. In the presence of DCMU, cultures treated with 0.5 and 5 ppm Co^{2+} exhibited an increase in the emission spectra of chlorophyll *a* (8.7 and 40.6 %, respectively) compared with the culture treated with DCMU alone (figure 4).

DISCUSSION

The present results are in agreement with those obtained by Lustigman et al. (1995) who reported that 10 and 20 ppm Co^{2+} resulted in partial inhibition of growth of *C. reinhardtii*, while concentrations of 30 ppm or higher completely prevented algal growth. In addition, El-Naggar et al. (1999) found that a lower Co^{2+} concentration (0.01 ppm) stimulated growth of *Nostoc muscorum*, while it showed a non-significant effect on *Calothrix fusca* growth. However, higher Co^{2+} concentrations were inhibitory for both organisms. On the other hand, growth promotion at low Co^{2+} concentrations may be

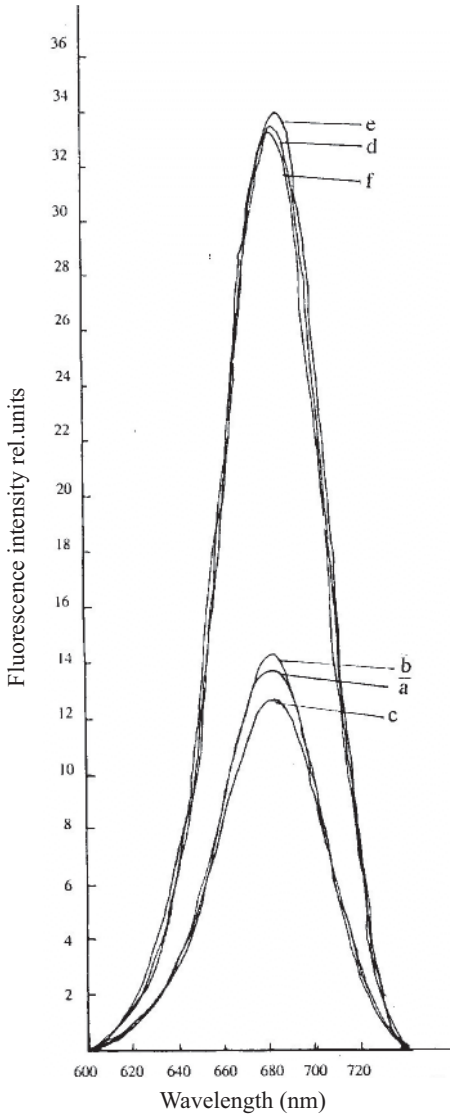

Figure 3. Fluorescence emission spectra of *M. minutum* treated with different cobalt concentrations for 15 min: (a) control, (b) 0.1 ppm Co^{2+}, (c) 3 ppm Co^{2+}, (d) DCMU alone, (e) DCMU + 0.1 ppm Co^{2+}, (f) DCMU + 3 ppm Co^{2+}.

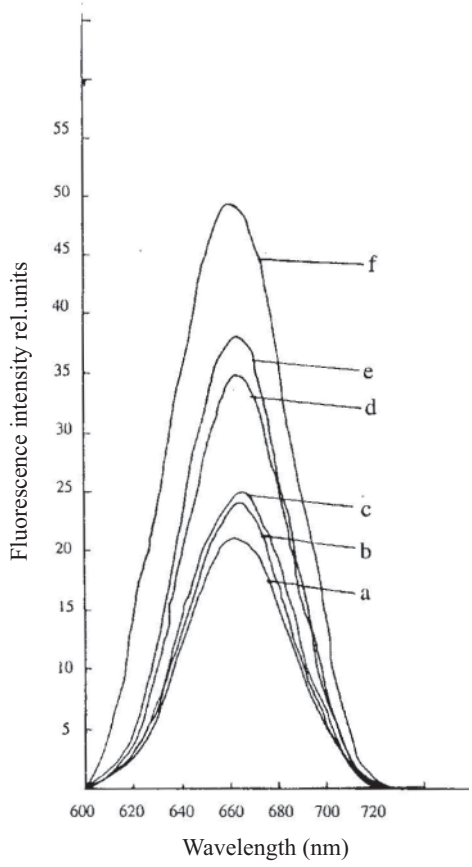

Figure 4. Fluorescence emission spectra of *N. perminuta* treated with different cobalt concentrations for 15 min: (a) control, (b) 0.5 ppm Co^{2+}, (c) 5 ppm Co^{2+}, (d) DCMU alone, (e) DCMU + 0.5 ppm Co^{2+}, (f) DCMU + 5 ppm Co^{2+}.

due to Co^{2+} substitution for Zn^{2+} in some metalloenzymes *in vitro* and *in vivo* as reported by Price and Morel (1990).

The present study indicated that application of low Co^{2+} concentrations (0.1 and 0.5 ppm) to *M. minutum* cultures led to significant increases in different pigment fractions (chlorophylls *a* and *b*, and carotenoids) reaching maximum values at the end of the incubation period for both organisms. On the other hand, progressive increases in Co^{2+}concentration (1, 2 and 3 ppm) for *M. minutum* caused reduction in the pigment content in a concentration-dependent fashion. Carotenoids appeared to be more resistant to Co^{2+} toxicity than chlorophylls *a* and *b*. Compared with the controls a higher chlorophyll *a*/*b* ratio was observed in *M. minutum* treated with high Co^{2+} concentrations due to the greater sensitivity chlorophyll *b* compared with chlorophyll *a* as the Co^{2+} concentration increased.

Our results show that lower Co^{2+} concentrations (0.5 and 1.5 ppm) led to a significant increase in chlorophyll *a* and carotenoids of *N. perminuta*. In contrast, higher Co^{2+} concentrations (2.5, 3.5 and 5 ppm) were associated with progressive reductions in pigment content. However, higher Co^{2+} concentrations (3.5 and 5 ppm) showed a greater inhibitory effect on chlorophyll *a* compared with carotenoids at the end of the incubation period. These results are in agreement with those obtained by Csatorday et al. (1984), who reported inhibition of chlorophyll biosynthesis as a result of Co^{2+} treatment. The mechanism proposed for this inhibition is the replacement of magnesium in the chlorophyll molecule. Consequently cells accumulate protoporphyrin and synthesis of chlorophyll is blocked. In this regard, De Filippis et al. (1981) reported that reduction of chlorophyll a content is a common symptom of heavy metals toxicity. This may be attributed to

inhibition of reduction steps in the biosynthetic pathways of the pigment.

In the present study the effect of different concentrations of Co^{2+} on photosynthetic O_2 evolution showed a tendency towards reducing the amount of O_2 evolved by each alga in response to Co^{2+}. However, an increase in O_2 evolution by *M. minutum* and *N. perminuta* was observed at relatively low Co^{2+} concentrations throughout the incubation period. The magnitude of the inhibitory action was found to increase with higher metal concentrations.

At 2 and 3 ppm Co^{2+} the degree of inhibition of O_2 evolution for *M. minutum* was greater than for *N. perminuta*, indicating that photosynthetic electron transport in *M. minutum* is more sensitive to Co^{2+} toxicity. These results are in agreement with those of El-Naggar et al. (1999) who found that low Co^{2+} concentration increased both O_2 evolution and dark respiration in two cyanobacterial species, *C. fusca* and *N. muscorum*, whereas higher concentrations were inhibitory. Further confirmation of our results can be found in the data of Tiwari and Mohanty (1996) who reported that *Synechocystis* PCC 6803 cell growth in a medium containing 10 $mmol.L^{-1}$ $CoCl_2$ exhibited a large stimulation (50 %) of O_2 evolution and an increase (~30 %) in photosynthetic electron transport. With regard to the observed inhibition as a result of the addition of higher concentrations of Co^{2+}, El-Sheekh and Hammouda (1992) reported that 25 and 50 $mmol.L^{-1}$ Co^{2+} inhibited O_2 evolution of *Chlorella minutissima* by 58.3 and 70.3 %, respectively.

With regard to the effects of various levels of Co^{2+} on respiration, the results obtained show that higher Co^{2+} concentrations have an inhibitory effect on O_2 uptake by the two algal species. Low Co^{2+} concentrations stimulated O_2 uptake of *M. minutum* throughout the experimental period. With respect to *N. perminuta*, different concentrations of Co^{2+} caused inhibition of O_2 uptake throughout the incubation period. From the above-mentioned results, it is noteworthy that the effect of heavy metals on respiration is concentration and species dependent.

Changes in chlorophyll *a* fluorescence intensity at room temperature are intimately associated with PSII activity and they reflect the redox states of Q, the primary acceptor of PSII (Goedheer, 1972; Renger and Schreiber, 1986). These changes play an important role in localizing the sites of primary damage of PSII (Schmidt et al., 1990). With regard to *M. minutum* and *N. perminuta,* the results show that low concentrations of Co^{2+} increased the fluorescence intensity in DCMU-treated and untreated cells. This increase was accompanied by increasing O_2 evolution in the case of DCMU-untreated cells. These results suggest that low concentrations of Co^{2+} stimulate electron transport at the donor side of PSII. Our results are in agreement with those reported by Tiwari and Mohanty (1996) who observed that *Synechocystis* cells grown in a medium containing 10 $mmol.L^{-1}$ $CoCl_2$ showed a large stimulation (50 %) of O_2 evolution and an overall increase (~30 %) in the rate of photosynthetic electron transport resulting from a small increase (15-20 %) in the number of PSII units in Co^{2+}-grown cells.

Further increase in Co^{2+} concentration increased the fluorescence intensity of *M. minutum* and *N. perminuta* cells. These results indicate that high concentrations of Co^{2+} inhibit the electron transport at the acceptor side of PSII in *M. minutum* and *N. perminuta*. The results concerning the effect of different concentrations of Co^{2+} on the electron transport of the two algae studied here are in agreement with those of Baker et al. (1982) and Mohanty et al. (1989), who reported that the effect of Co^{2+}, Ni^{2+} or Zn^{2+} is possibly due to their role in modifying the function of Q_B, thereby impairing the PSII activity. Co^{2+}, Ni^{2+} or Zn^{2+} can impair the Q_B function in three possible ways. Interruption of electron flow between Q_A and Q_B directs modification of Q_B or alteration of components beyond Q_B, which leads to the impairment of PSII activity at the Q_B site.

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