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GROWTH AND PIGMENT BIOSYNTHESIS OF *SPIRULINA PLATENSIS* AS AFFECTED BY Pb^{2+} CONCENTRATIONS

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Abstract

Growth and pigment biosynthesis of *Spirulina platensis* Geitler, an edible cyanobacterium were stimulated at low concentration (1 mg/l) of Pb^{2+}. The pigment biosynthesis was found to decrease with increasing Pb^{2+}concentrations. The ratio between chlorophyll *a* and β carotene was constant regardless of the Pb^{2+}concentration in the medium indicating that both are equally affected by Pb^{2+}.

It is believed that Pb^{2+} would be toxic to most plants, because its excess amount may alter several physiological and biochemical processes (Mesmar and Jaber 1991). *Spirulina* (*Arthrospira*), an edible cyanobacterium, is globally considered as a valuable source of food supplement (Lee 1997, Li and Qi 1997) as it contains some compounds like essential fatty- and amino acids, antioxidants vitamins and minerals, at relatively high concentrations (Richmond *et al*. 1980, Roughan 1989, Cohen *et al.*1995) and thus has been cultured commercially in China (Zhang 1998, Michael 1999) as well as in some other countries. The cell wall components of *Spirulina*, such as peptydoglycan, teichuronic acid, teichoic acid, polysaccharides and proteins (Schiewer and Wong 2000) which display mainly carboxylic, hydroxyl and phosphate groups (Aksu 2002) may give the algal wall a binding property inducing bioaccumulation of heavy metals. Under this background, the present study involves an assessment on growth and pigment biosynthesis of *Spirulina platensis*, as affected by Pb^{2+} concentrations.

Spirulina platensis (S_{6-1}) culture maintained in the laboratory at College of Marine Life Sciences, Ocean University of China, was used in this study. It was grown at 25 ± 2 °C in Zarrouk liquid medium (Parada *et al*. 1998), for eight-ten days under white fluorescent light (90 μmol photon $m^{-2}s^{-1}$) with 14 h illumination. At the exponential growth phase, the culture was filtered and used for Pb^{2+} (as $Pb(NO_3)_2$) treatment. Deionized water was used to prepare all solutions. All the chemicals were of analytical grade. Optical density was measured at 560 nm using a Spectrophotometer UV - 2102. An ultrasonic liquid processor (SONICS) was used to break cells for pigment analysis. Chlorophyll *a* and β carotene were extracted in 90 % acetone and assayed according to Bwn-Amotz and Avron (1983). All the procedures were performed under aseptic conditions using three triplicates.

Effect of Pb^{2+} on growth: The cultures treated with 1, 2 and 3 mg/l, resulted 10, 5 and 4% increased growth, respectively over the control after two days of incubation. On the other hand, a slight inhibitory effect on its growth was observed at higher concentration of Pb^{2+} (Table 1). El-Naggar *et al*. (1999) reported that lower concentrations of heavy metal (Co^{2+}) stimulate growth of *Nostoc muscorum*, followed by inhibition at higher concentrations. At low concentrations, substitution of Pb^{2+} for Zn^{2+} in some metabolloenzymes *in vitro* and *in vivo* may result in growth promotion (El-Sheekh *et al*. 2003). Growth reduction at higher metal concentrations could result

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from the inhibition of enzyme systems, photosynthesis, respiration, protein and nucleic acid synthesis. Torzillo (1998) mentioned that environmental stress affects the functioning of photosystem II in *Spirulina* directly or indirectly causing growth reduction.

Table1. Effect of Pb^{2+} concentrations (mg/l) on the growth of *S. platensis*.

Culture time (Days)	Growth (O.D.) Pb^{2+} concentrations (mg/l)					
	0	1	2	3	4	5
2	0.134 ± 0.01	0.148 ± 0.01	0.141 ± 0.01	0.140 ± 0.02	0.136 ± 0.02	0.134 ± 0.01
4	0.182 ± 0.01	0.203 ± 0.03	0.193 ± 0.01	0.192 ± 0.02	0.191 ± 0.03	0.191 ± 0.04
6	0.294 ± 0.05	0.301± 0.06	0.293 ± 0.05	0.291 ± 0.07	0.284 ± 0.04	0.281 ± 0.05
8	0.351 ± 0.06	0.372 ± 0.02	0.372 ± 0.07	0.364 ± 0.06	0.341 ± 0.04	0.341 ± 0.05
10	0.464 ± 0.05	0.482 ± 0.04	0.484 ± 0.05	0.474 ± 0.05	0.461 ± 0.07	0.454 ± 0.08

95% confidence interval was used to determine error.

Effect of Pb^{2+} on pigments: Low concentration of Pb^{2+} (1 mg/l) shows a slight increase in chlorophyll *a* content, whereas cultures treated with 3, 4 and 5 mg/l Pb^{2+} reduced the amount by 4, 11 and 15 % respectively, compared to control. β carotene increased (9 %) at lower concentrations (1 mg/l) and decreased by 6, 7 and 7% at 3, 4 and 5 mg/l Pb^{2+}, respectively. However, the ratio between chlorophyll *a* and β carotene remained constant (3.8) regardless of the Pb^{2+}concentration in the medium (Fig. 1) indicating that chlorophyll *a* and β carotene are equally affected by Pb^{2+} concentrations.

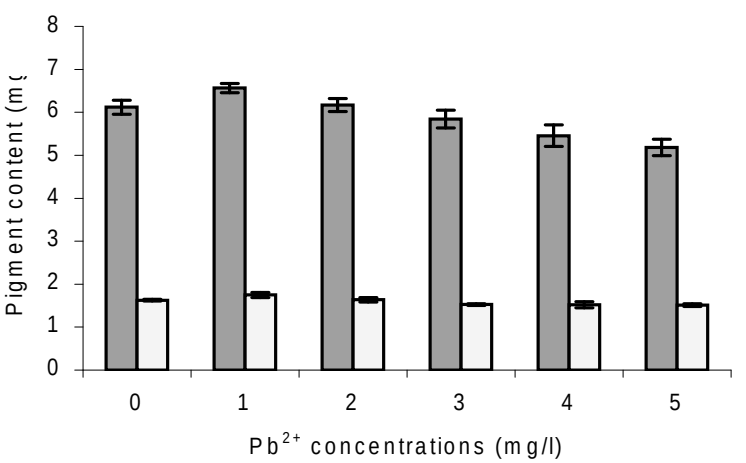

Fig. 1. Effect of Pb^{2+} concentrations on pigment content of *S. platensis*. ▭ Chl. a (mg/l), ▭ β car. (mg/l).

De Filippis *et al*. (1981) reported that the reduction in chlorophyll *a* content is a common symptom of heavy metal toxicity. Csatorday *et al*. (1984) reported in microalgae an inhibition of chlorophyll biosynthesis due to high Co^{2+} treatments. Heavy metals like Arsenic (As) at 100 µg/l or above have been found to affect N_2-fixing activity of symbiotic cyanobacteria *Anabaena azollae* (Aziz 2001). He also observed negative effect of As on chlorophyll *a* and *b* synthesis and

increased anthocyanin formation in *Azolla filiculoides* as the concentration of As is increased. The present reductions in chlorophyll *a* and β carotene at higher concentrations are in agreement with previous reports.

It appears that at 1 mg/l Pb^{2+} the growth and pigment synthesis were stimulated, but at 3 mg/l or above those were reduced.

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