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Zinc Effect on Chlorophyll a, Total Carbohydrate, Total Protein Contents and Biomass of Cyanobacterial Species

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Abstract

The present study aimed to investigate the effects of various zinc concentrations (2.5 mg/l to 80 mg/l) on biomass, chlorophyll a, total carbohydrate and total protein contents of 10 cyanobacterial species. Biomass, chlorophyll a and carbohydrate content in all of cyanobacterial species were measured by spectrophotometric method. Zinc is toxic to these organisms causing severe inhibition of such physiological process as growth, chlorophyll a, carbohydrate and protein contents at concentrations less than 10 mg/l. *Anabaena* sp. GO1 and *Gloeothece* sp. GO9 were partly stimulated at lower zinc concntration (2.5 mg/l). In the high concentrations of zinc is more than 10 mg/L, biomass and other parameters of all cultures were completely reduced, except *Anabaena* sp. GO2 species.

Key words: Cyanobacteria, Metal tolerance, Growth, Chlorophyll, Total carbohydrate, Total protein.

INTRODUCTION

Heavy metal pollution of water through the discharge of industrial wastewater has become one of the most serious environmental concerns threatening the ecosystem of water bodies. Heavy metal levels in environment are constantly increasing because of their enhanced utilization in various industrial activities [1]. Heavy metals pose a serious threat to the environment, animals and humans because of their toxicity [2,3].

Zinc may also increase the acidity of waters. Some fish can accumulate zinc in their bodies, when they live in zinc-contaminated waterways. When zinc enters the bodies of these fish it is able to bio magnify up the food chain. Therefore, there is significant interest regarding zinc removal from wastewater [4,5] and its toxicity for humans at levels of 100–500 mg/day [72]. WHO recommended the maximum acceptable concentration of zinc in drinking water as 5.0 mg/l [6]. When the soils of farmland are polluted with zinc, animals will absorb concentrations that are damaging to their health. On zinc-rich soils only a limited number of plants has a chance of survival. Finally, zinc can interrupt the activity in soils, as it negatively influences the activity of microorganisms and earthworms. The breakdown of organic matter may seriously slow down because of this [73]. Metals can be distinguished from other toxic pollutants, since are non-biodegradable and can accumulate in the living tissues, thus becoming concentrated throughout the food chain. Zinc, one of the metal is important pollutant. The main sources of Zn2+ in the environment are zinc fertilizers, sewage sludges and mining and smelting [7].

The main techniques utilized for treatment of zinc bearing waste streams include evaporation, adsorption by activated carbon, ion exchange, membran processing, solvent extraction, precipiation, reverse osmosis etc. These methods have been found to be limited, because they often involved high capital and operational cost and may also be associated with the generation of toxic secondary waste which further present treatment problem [8]. The processes are sometimes neither effective nor selective and most of them are expensive too. Environmentally friendly processes need to be developed to clean up the environment without creating harmful waste products [9]. Therefore economically viable and eco-friendly technologies such as biosorption and/or bioaccumulation are required to reduce heavy metal concentrations to acceptable environmental levels [2,3]. Biosorption and bioaccumulation belong to the group of biological methods suitable for heavy metal removal from wastewater [10]. Different biomass have been used to adsorb metal ions from the environment. In this respect, the search for a new ecofriendly, economical and effective metal adsorbent is focused on biomaterials such as bacteria, fungi, cyanobacteria, yeast, algae, and plants [11,12].

Cyanobacteria commonly used microorganisms for biosorption. Cyanobacteria are a diverse group of prokaryotes widespread in different habitats [13]. These prokaryotic autotrophic organisms quickly respond and adapt to stress conditions in general and heavy metals in particular [14,15,16]. Cyanobacteria are increasingly being exploited for environmental protection, bioremediation and bioreclamation purposes because of several advantages like large biomass

production, simple nutrient requirements and non-toxic nature. These organisms detoxify metals via metal-binding proteins, evolutionarily strongly conserved proteins of low molecular weight and high cysteine and metal contents [17]. Cyanobacteria are suggested to have some added advantages over other microorganisms because of their large surface area, greater mucilage volume with high binding affinity and simple nutrient requirements [18].

When microorganisms are used to biosorption and bioaccumulation of heavy metals, it is important to know the tolerance of each species to each metal and how stable is this tolerance [19]. In metal tolerance studies, a lot of characteristics were investigated on cyanobacteria. These characteristics are including; growth [3,20-23], growth rate [24], dry weight [25,26], chlorophyll content [25,27-32], carotenes [30], phycocyanin and phycoerythrin [32], respirate rate [26,28], photosynthetic rate [21,24,25], total protein [30], total ATP content [33], DNA content [31], phosphatase [26], sulphatase [26], dehidrogenase activity and alkaline phosphatase [34].

The goal of this study was to determine the effect of zinc on the biomass, chlorophyll a, total carbohydrate and total protein content of various cyanobacteria.

MATERIALS AND METHODS

Materials

The unicelular and filamentous cyanobacteria used in this study. Cyanobacterial strains were obtained from previous studies of Dr. Gulten Okmen, Department of Biology, University of Mugla, Turkey. These included, *Anabaena* sp. GO1, *Anabaena* sp. GO2, *Anabaena* sp. GO3, *Anabaena* sp. GO4, *Anabaena* sp. GO5, *Anabaena* sp. GO6, *Anabaena* sp. GO7, *Synechocystis* sp. GO8, *Gloeothece* sp. GO9, *Anabaena* sp. GO10.

Stock cultures were grown in the N-free BG-11 medium [35]. Temperature was maintained at 20 ± 2°C and cultures were grown under a cool white light. Cells logarithmic phase of growth used as inocula for experiments. Experiments were conducted in batch cultures by using 10ml of inoculated medium in 25 ml serum bottles. Serum bottles were enclosed with cotton plugs. Culture media were adjusted accordingly pH8 with 1N NaOH and 1N HCl. Illumination was supplied with 600 lux cool white light.

Cultivation

The influence of different concentrations of $ZnCl_2$ (2,5 – 80 mg/l) on the biomass, total carbohydrate, total protein and chlorophyll a content was also tested on ten isolates. Cultures in log phase were used in this study. The experimental cultures were grown in 25 ml serum bottles containing 10ml N-free BG-11 medium under the same conditions as described below. The cultures were grown in a liquid sterilized medium at 20 ± 2°C under light (600 lux) for 35 days. All experiments were performed is triplicate and parallel conditions.

Analysis the effect of the metal stress on biomass

Cultures harvested by centrifuge (Hettich, EBA 12; Germany). Then the cultures were washed with distilled water three times and dried to constant weight at 70°C for 12 hours. Dry weights were measured.

Analysis the effect of the metal stress on total carbohydrate contents

Total carbohydrate contents were mesured using the phenol-sulfuric acid assay and using glucose as a standard. The harvested cells were hydrolyzed in sulfuric acid (1 N) in a boiling water batch for one hour (Memmert, WNB14, England) cell debris was removed by centrifugation and total carbohydrates were determined in the supernatant by the phenol-sulfuric acid method [36].

Analysis the effect of the metal stress on total protein contents

Using the method described by Gornall et al. (1949), total protein was colorimetrically measured at 540nm against blank using spectrophotometer. A calibration curve was constructed using BSA (bovine serum albumin) [37].

Analysis the effect of the metal stress on chlorophyll a content

The spectrophotometric method (Shimadzu, UV-1201V, Japan) recommended by Porra et al., (1989) was used for determination [38].

Statistical treatment

All experiments were performed in 3 replicates. Data presented in this study are presented in means ± standard deviation (SD).

RESULTS

The some characteristics of 10 cyanobacteria treated with different concentrations of zinc under 600 lux light intensity are listed in Table 1. All of species were affected from zinc stress.

The biomass of *Anabaena* sp. GO1 was affected differently by zinc stresses. The biomass of *Anabaena* sp. GO1 has been stimulated at 2.5 mg/l Zn concentration, whereas the biomass of this strain has been repressed at 5 mg/l zinc concentration. The chlorophyll a, total carbohydrate and total protein contents of this strain were stimulated by 2.5 mg/l Zn concentration.

In the same way, the biomass of *Gloeothece* sp. GO9 was affected differently by zinc concentrations. The biomass of *Gloeothece sp.* GO9 has been increased at 2.5 and 5 mg/l zinc concentration. This strain has been supressed at 10 mg/l Zn concentration. The chlorophyll a, carbohydrate and protein contents of this strain were stimulated at 2.5 and 5 mg/l Zn concentrations.

Therefore, the biomasses of *Anabaena* sp. GO4 and GO5 were not sharply repressed by 2.5 mg/l Zn concentration (2.2 and 1.3 mg/ml). Whereas, the biomasses of other strains were sharply inhibited. However, chlorophyll a, carbohydrate and protein contents of the other strains were supressed at increasing zinc concentrations.

The growth of *Anabaena* sp. GO2 was rapidly influenced by increasing zinc concentration, but this strain has been improved up to 20 ppm Zn concentration. Whereas, the biomasses of the other six species were rapidly inhibited (*Anabaena* sp. GO2, GO3, GO6, GO7, GO10 and *Synechocystis* sp. GO8). Accordingly, the chlorophyll a, carbohydrate and protein contents of the this six species were repressed by increasing zinc concetrations (Table 1).

Table 1. The some characteristics of 10 cyanobacteria treated with different concentrations of zinc under 600 lux light intensity.

Microorganisms	Concentrations (mg/l)	Dry weight (mg/ml)	Chlorophyll a content (mg/ml)	Total carbohydrate content (mg/ml)	Total protein content (mg/ml)
Anabaena sp. GO1	Control	2.4 ±0.8	9.2 ± 0.3	4.0 ±1.38	1.6 ±0.5
	2.5	3.4 ±0.02	13.1 ± 0.12	5.7 ±0.3	2.3 ±0.6
	5	0.005 ±0.003	0.02 ± 0.001	0.01 ±0.006	0.0039 ±0.0002
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Anabaena sp. GO2	Control	2.1 ±0.5	1.76 ± 0.4	3.8 ±0.9	1.2 ±0.3
	2.5	0.017 ±0.003	0.01 ± 0.0006	0.031 ±0	0.010 ±0.001
	5	0.009 ±0.001	0.007 ± 0.001	0.017 ±0.002	0.0058 ±0.0001
	10	0.0079 ±0.001	0.006 ± 0.001	0.017 ±0.004	0.0056 ±0.001
	20	0.0002 ±0.00001	0.0001 ±0.000002	0.0003 ±0.000018	0.0001 ±0.000006
	40	0	0	0	0
	80	0	0	0	0
Anabaena sp. GO3	Control	1.7 ±0.1	2.4 ± 0.17	25 ±1.8	0.76 ±0.05
	2.5	0.008 ±0.001	0.01 ± 0.0009	0.1 ±0.01	0.0036 ±0.0002
	5	0.003 ±0.001	0.004 ± 0.0001	0.048 ±0.005	0.0014 ±0.0003
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Anabaena sp. GO4	Control	3.0 ±0.4	8.6 ± 0.13	4.9 ±0.69	1.9 ±0.2
	2,5	2.2 ±0.2	6.3 ± 0.7	3.5 ±0.39	1.4 ±0.15
	5	0.094 ±0.002	0.26 ± 0.01	0.15 ±0.01	0.06 ±0.01
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Anabaena sp. GO5	Control	1.6 ±0.3	4.7 ± 0.89	19.76 ±0.758	0.73 ±0.13
	2.5	1.3 ±0.07	3.3 ± 0.19	15.01 ±0.162	0.51 ±0.02
	5	0.013 ±0.002	0.03 ± 0.006	0.19 ±5.65	0.0052 ±0.001
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Anabaena sp. GO6	Control	2.5 ±0.3	3.1 ± 0.46	3.5 ±0.52	1.3 ±0.19
	2.5	0.18 ±0.01	0.22 ± 0.13	0.25 ±0.14	0.096 ±0.05
	5	0.008 ±0.003	0.01 ± 0.004	0.013 ±0.004	0.0048 ±0.001
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Anabaena sp. GO7	Control	0.64 ±0.06	1.18 ± 0.12	2.6 ±0.27	1.8 ±0.18
	2.5	0.43 ±0.1	0.79 ± 0.26	1.7 ±0.59	1.2 ±0.4
	5	0.006 ±0.0004	0.01 ± 0.0008	0.027 ±0.001	0.018 ±0.001
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Synechocystis sp. GO8	Control	0.51 ±0.1	3.73 ± 0.8	10.4 ±2.25	0.81 ±0.1
	2.5	0.1 ±0.005	0.79 ± 0.04	2.2 ±0.12	0.17 ±0.009
	5	0.061 ±0.002	0,44 ± 0.017	1.2 ±0.1	0.096 ±0.008
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Gloeothece sp. GO9	Control	1.7 ±0.2	2.40 ± 039	3.3 ±0.53	1.1 ±0.18
	2.5	2.5 ±0.4	3.58 ± 0.59	4.9 ±0.8	1.6 ±0.2
	5	2.3 ±0.3	3.28 ± 0.15	4.0 ±0.9	1.5 ±0.3
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0
Anabaena sp. GO10	Control	1.2 ±0.1	2.28 ± 0.28	2.9 ±0.36	0.85 ±0.1
	2.5	0.56 ±0.07	1.02 ± 0.13	1.3 ±0.16	0.38 ±0.04
	5	0.22 ±0.01	0.4 ± 0.19	0.5 ±0.24	0.14 ±0.06
	10	0	0	0	0
	20	0	0	0	0
	40	0	0	0	0
	80	0	0	0	0

DISCUSSION

Algae and cyanobacteria are a key part of aquatic ecosystems, in which they play a role of primary producents. These organisms are very important for ecotoxicological assessment. Therefore, this study of potential stresses in batch cultures of cyanobacteria strains aimed to elucidate the influence of zinc on protein, chlorophyll and carbohydrate contents, as related to aspects of cell physiology reflected in biomass and carbon fixation capacity.

Metal resistance is widespread in microorganisms [39,40]. Most studies indicated that acclimation or adaptation to higher metal concentrations is accompanied by the potential for increased tolerance by adjusting physiological or biochemical mechanisms [41,42]. Cyanobacteria are affected from different environmental factors. Variation in growth conditions influenced the growths and other activities of cyanobacteria. Metal stresses are important factors that affect the cyanobacterial growth. The occurrence of some algae, including cyanobacteria, in metal contaminated aquatic bodies may lead us to conclude that these organisms are able to resist/tolerate metal toxicity [43]. For example, Audholia et al (1993) reported that *Phormidium uncinatum* was grown well at high concentration of Zn^{+2} (50 mg/l) [15].

In this study, all of species were affected from zinc stress, but the biomasses of four species were affected differently by zinc stress, these are *Anabaena* sp. GO4, GO5, GO6 and *Gloeothece* sp. GO9. In this study, only the biomass of *Gloeothece* sp. GO9 and *Anabaena* sp. GO1 were increased whereas, the dry weights of the other species were rapidly inhibited. The chlorophyll a content of *Gloeothece* sp. GO9 has been stimulated at 2.5 mg/l Zn concentration (3.580 mg/ml). The total carbohydrate contents of *Gloeothece* sp. GO9 and *Anabaena* sp. GO1 strains were observed to be stimulated by initial concentration of zinc (2.5 mg/l). The total protein contents of all strains were rapidly inhibited, except for *Anabaena* sp. GO1 and *Gloeothece* sp. GO9.

At the end of this study it was determined that all of cultures, showed a tolerance of 5 mg/l zinc concentration. In addition growth, total carbohydrate, chlorophyll a and total protein contents of all cultures were completely inhibited at 10 ppm zinc concentration which is similar to the reports of Okmen et al. (2007) [44]. According to the literature, zinc markedly inhibited the growths and nitrogenase activity of *Anabaena* and *Nodularia* sp. Otherwise, under 10ppm zinc concentration, the growths and nitrogenase activities of all cultures were completely reduced. Damage of cell membrane at high metal concentration will lead to uncontrolled efflux/influx of electrolytes or even other vital ions [45], which may be responsible for inhibition of growth.

The growth of *Anabaena* sp. GO2 was rapidly influenced by increasing zinc concentration, but this strain has been improved up to 20 mg/l Zn concentration. Mallick and Rai (1989) reported that active intracellular sequestration to prevent exposure to essential cellular components is one of possible mechanisms for heavy metal resistance [46], and many reports have been published showing metal inclusions into the cell body [47].

Chlorophyll is the most important pigment in algal and cyanobacterial cells for collecting solar energy for photosynthesis [48,49]. Otherwise, photosynthesis is generally inhibited by heavy metals [50]. Earlier Rosko and Rachlin (1977) explained some of the reasons i.e. [51] (1) destruction of light harvesting pigments [52], (2) inhibition of key enzymes of CO_2-fixation cycle [53], and (3) destruction of photosynthetic membrane [46,54]. Since ATP and NADPH are the primary requirements for CO_2-fixation, it can be inferred that any reduction in ATP synthesis may lead to inhibition of $^{14}CO_2$ incorporation [55,56].

The total chlorophyll contents of all the strains were found to be inhibited rapidly, except for three species (*Anabaena* sp. GO4, GO5 and *Gloeothece* sp. GO9) (Table 1). Takamura et al. (1989) reported that the Cd and Zn concentrations which inhibited photosynthesis were a higher than those observed in nature [19]. Some investigations found that heavy metals generally inhibit overall physiological processes with marked effects on chlorophyll a and b [57,58]. Prasad and Prasad (1987) reported that heavy metals inhibit the enzyme that is responsible for the chlorophyll synthesis [59]. Only chlorophyll a content of *Gloeothece* sp. GO9 has been stimulated at 2.5 mg/l zinc concentration. As indicated from the literature, low zinc concentrations (1.5 mg/l for *Scenedesmus obliquus*, and 0.5 mg/l for *S. quadricauda*) induced an increase in pigment contents [60].

The total carbohydrate contents of *Anabaena* sp. GO1 and *Gloeothece* sp. GO9 strains were observed to be stimulated by initial (2.5 mg/l) concentration of zinc. Most studies indicated that acclimation or adaptation to higher metal concentrations is accompanied by the potential for increased tolerance by adjusting physiological or biochemical mechanisms [41,42]. Earlier studies showed that metal efflux was an important mechanism to regulate the intracellular metal content in bacteria [61,62]. Furthermore, the internal metals were found to be partitioned in different subcellular compartments, which may affect the tolerance capability [63,64].

The protein content of *Anabaena* sp. GO1 and *Gloeothece* sp. GO9 have been stimulated at 2.5 mg/l zinc concentration. It has been shown that heavy metal tolerance in cyanobacteria may be due to one or more mechanisms like compartmentalization [65], antioxidant enzymes [66], or metal-binding proteins [67]. Omar (2002) reported an increase of arginine, proline and cystine at low concentration of zinc [68]. Similarly, Kobbia et al. (1985) reported an accumulation of cystine and arginine in *Chlorella fusca* in response to Ni^{2+} treatment [69]. The increase in proline content may be attributed to one of following mechanism: zinc may promote synthesis of proline from glutamate; it may decrease the rate of proline oxidation, and/or inhibit the incorporation of proline into protein. Buhl and Stewart (1983) reported that lower Zn^{2+} concentration had pronounced effect on raising the proportion of aminoacids in both tested microorganisms [70].

The results of this study show that, there is a species dependant differentiation in the metal sensitivity. Premuzic et al. (1991) established that chemical and structural characteristics of cell membranes vary with species and should therefore influence the capacity for uptake of metals by different microorganisms [71].

Generally *Gloeothece* sp. GO9 had the best optimal performance of growths in zinc stress conditions, making it a suitable candidate for bioaccumulation and biosorption. More studies will be needed in order to determine the characteristics of metal sensitivities of all cyanobacterial species.

From the above data, *Gloeothece* sp. GO9 seems to be tolerant to heavy metal (Zn) and is able to accumulate this metal by adsorption on the pellets and / or through sequestration via metal binding protein. Therefore, it can be recommended it to be employed in the purification of waste contamined with this metal.

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