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Effect of zinc and different nutrition conditions on chlorophyll *a*, biochemical structure and activity of some enzymes in *Microcystis aeruginosa*

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ABSTRACT

Experiments were carried out under laboratory culture conditions to investigate the effect of modification in the main nutrients nitrogen and phosphorus (N and P) of BG11 synthetic medium on biological parameters of *Microcystis aeruginosa*, and evaluate the effect of zinc, not only on biochemical structure, but also on the activity of catalase and alkaline phosphatase enzymes. Low metal dose (0.05mg/l) stimulates the algal growth through increasing chlorophyll *a*, dry weight and biochemical contents (proteins, carbohydrates and lipids), while high doses inhibit the algal growth. Zn showed significant linear regression ($P<0.05$) with the biochemical parameters where r^2 for chl. *a*, dry weight, proteins, carbohydrates and lipids = 0.87, 0.847, 0.584, 0.584 and 0.830, respectively. The inhibitory effects of higher doses of metal on the tested algae reflected the fact that the toxicity of Zn is greatly dose-dependent. Catalase activity of the tested algae showed notable increase to 0.02 and 0.028 U/L at low metal concentrations 0.05 and 0.1 mg/l. While the enzymatic activity decreased gradually in other zinc concentrations reaching their lowest value (0.0032 U/L) at the higher dose (3 mg/l). Zn caused a marked and gradual decrease in the activity of the alkaine phosphatase enzyme of *M. aeruginosa* and the lowest value of enzyme activity (0.0041 U/L) was observed at higher Zn addition (3mg/l). There was a positive significant regression between Zn and both catalase and alkaline phosphatase (r^2= 0.503 and 0.835). Chlorophyll *a* and the organic matter content expressed in dry weight showed a preference for higher phosphorus levels at which their maximum values; 892.38 and 0.0013 mg/l were obtained with the highest phosphorus concentration 175% (70 µg/l), whereas the lowest results recorded at the starvation state 25% (10 µg/l) of the nutrient. Similar tendency observed for protein, carbohydrate and lipid contents, where their values were gradually decreased with the deficiency of the phosphorous in the medium. Data also elucidate how chl. *a*, dry weight and the biochemical contents of the tested algae increased in response to N supplementation. There was a significant regression ($P<0.05$) under variable nitrogen concentration with chlorophyll *a*, dry weight, proteins and carbohydrates where r^2= 0.66, 0.769, 0.715 and 0.773, respectively. A positive significant regression (r^2= 0.878) of alkaline phosphatase was detected under variable P concentrations.

1. INTRODUCTION

Pollution of heavy metals resulted from agricultural and industrial effluents have become one of the most serious environmental problems.

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Usage of biomaterials such as algae, bacteria, yeast and fungi in biosorption of metals is regarded as effective biotechnology for the treatment of wastewater containing heavy metals (Wang and Chen, 2006). Some evidence has indicated that algae have the capability to sorb metals either on the outer cell surface or inside its cells. Carboxyl group has a great role in metal binding process (Mehta and Gaur, 2005). Zinc is an essential micronutrient for growth of the organisms at low concentration, which is also potentially hazardous to organisms when present at higher concentrations (Nan *et al*., 2002; Rainbow, 2002). The main sources of Zn^{2+} in the environment are zinc fertilizers, sewage sludges and mining and smelting (Bradl, 2005).

Blue green algae are a common group used for metal binding due to their high metal sorption characteristics (Li *et al*., 2004; Baptista and Vasconcelos, 2006; Cain *et al*., 2008; Zeng *et al*., 2009). *Microcystis* are widely occurred as dominant species in the eutrophic freshwater lakes. It was proved that *Microcystis* is potentially effective biosorbent of trace metals from various effluents in the aquatic environment (Chen *et al*., 2005). Biosorbents of trace metals by *Microcystis* affected greatly on metal cycling in the aquatic habitats (Morel and Price, 2003). *Microcystis aeruginosa* has higher bioaccumulation capacity for cadmium (Cd) and zinc (Zn) in bioassay experiments (Zeng *et al*., 2012).

In nature the toxic elements may present the synergistic or antagonistic effect on the enzymatic activity of different algal species (Awasthi, 2012). Catalase enzyme protects algae from O_2 toxic products (Ajayan and Selvaraju, 2012). Alkaline phosphatase activity is considered an important marker of phosphate status in phytoplankton communities (Dyhrman and Palenik, 1999; Nicholson *et al*., 2006). Phytoplankton is widely used in the assessment of risk development of environmental regulations by metals (Levy *et al*., 2007).

2. AIM of WORK

The previous studies on the effects of zinc on *Microcystis aeruginosa* described the changes in chlorophyll *a* and biochemical contents (Drábková *et al*., 2007; Hong *et al*., 2008; Pan *et al*., 2008). So, the objective of the present work is to investigate the effect of modification in the main nutrients (nitrogen and phosphorus) of BG11 synthetic medium on the tested *M. aeruginosa* and evaluate the effect of Zn not only on the above-mentioned biological parameters, but also on the activity of catalase and alkaline phosphatase enzymes.

3. MATERIALS AND METHODS

3.1 Experimental design

3.1. I. Algae strains and culture conditions

The tested algal species used in this study was of a pure culture of *Microcystis aeruginosa*, was isolated from flourishing phytoplankton in some Khors of Lake Nasser. Cultures were grown in BG11 medium (Allen, 1973), at room temperature $25\pm1\ ^0C$ under fluorescence light intensity of ≈2500 Lux with 14:10 h light/dark cycle. All experiments were performed in triplicate and parallel conditions. The following experiments were carried out:

3.1. II. Zinc effect on *Microcystis aeruginosa*

The stock solution of $ZnSO_4 \cdot 7H_2O$ was prepared. Metal concentrations were chosen to represent low and high values of natural water. Zn was tested at concentration of 0.05, 0.1, 0.5, 1, 2 and 3 mg/l.

3.1. III. Effect of modifications in nitrogen and phosphorus on the growth of *Microcystis aeruginosa*

Experiments were conducted to evaluate the performance of *Microcystis aeruginosa* when it grows with some modifications in N and P concentrations of the standard culture BG11 medium. Cells were cultured in BG11 medium with altered concentrations of nitrogen and phosphorus (N and P) ratio either by increasing or reducing. The experiment culture was then incubated at room temperature for six days.

3.1. III. a) Constant nitrogen concentration with variable phosphorus values

Constant nitrogen concentration, standard concentration, and changed phosphorus (K_2PO_4. $3H_2O$) in culture medium. phosphorus supplementations were 175%, 150 % and 125% with concentrations 70, 60 and 50 µg/l, while phosphorus deficient are 75%, 50% and 25% with concentrations 30, 20 and 10 µg/l, respectively of the standard formula where control =40 µg/l.

3.1. III. b) Constant phosphorus concentration with variable nitrogen values

The medium was prepared with constant phosphorus concentration, standard concentration, and changed nitrogen ($NaNO_3$). Nitrogen supplementations were 175%, 150 % and 125% with concentrations 2.625, 2.25 and 1.875 g/l. Whereas nitrogen deficient were 75%, 50% and 25% with concentrations 1.125, 0.75 and 0.375 g/l of the standard concentration (control= 1.5 g/l).

3. 2 The biological parameters

In every experiment the following parameters were measured: Chlorophyll *a* (Chl. *a*) estimation obtained from the Trichromatric equation (APHA, 1998). Dry weight of algal culture was measured using nucleopore filter paper of 25-mm diameter and 0.45 µm-pore size. Proteins were estimated following Biuret method (David and Hazel, 1993). Algal carbohydrate contents were measured according to phenol-sulphoric acid method (Dubois *et al*., 1956), while lipid contents were determined by the sulphophosphovanillin procedure (Chabrol and Castellano, 1961). Catalase activity was measured by spectrophotometric method based on the decomposition of H_2O_2 (Fossati, *et al*., 1980; Aebi, 1984). Colorimetric determination of alkaline phosphatase activity based on liberation of phenol which measured calorimetrically in the presence of 4-aminophenazone and potassium ferricyanide (Belfield and Goldberg, 1971).

3. 3 Data analysis

Symbols and error bars represent average and standard deviation of replicate cultures are represented at each figure. The regression was done to determine the relationship between the measured parameters and both Zn and nutrients (N and P) using SPSS version 14.

4. RESULTS AND DISCUSSION

The data obtained showed that *Microcystis aeruginosa* was affected by Zn exposure according to its dose. The low metal concentration (0.05mg/l) stimulated the algal growth as represented by chlorophyll *a*, dry weight and biochemical contents (proteins, carbohydrates and lipids) as shown in Figure 1. Lue *et al*. (2000) demonstrated that chlorophyll analysis could be useful physiological tool to determine early stages of change in photosynthetic performance of algae in response to heavy metal exposure. Heavy metals can induce algal cell stress and synthesis of biocompounds as pigments, lipids, exopolymers, peptides and phytohormones (Miazek *et al*., 2015). While, higher Zn doses caused an inhibition in *M. aeruginosa* growth as compared with the control (Fig. 1). Thus, although low concentrations of some metals are metabolically important to many living organisms, at higher levels they can potentially be toxic (Phillips, 1995; Sunda and Huntsman, 1998; Pinto *et al*., 2003). The inhibitory effects of higher doses of metal on the tested algae reflecting the fact that the toxicity of Zn is greatly dose-dependent (Baumann *et al*., 2009). Also, the inhibition in growth rate of the tested algae may be due to the use of energy in adaptation and repair of damage caused by zinc stress (Santos *et al*., 2012). Heavy metals induce algae to produce reactive oxygen species (ROS), which in turn changes in molecular structure including lipids, proteins and nucleic acids (Collén *et al*., 2003). Zn showed significant linear regression ($P<0.05$) with the biochemical parameters (chl. *a* $r^2= 0.87$, dry weight $r^2= 0.847$, protein $r^2= 0.584$, carbohydrate $r^2= 0.584$ and lipid $r^2= 0.830$). A significantly positive relationship ($r^2= 0.760$) was observed between growth rate of *M. aeruginosa* and protein contents (Li *et al*., 2014).

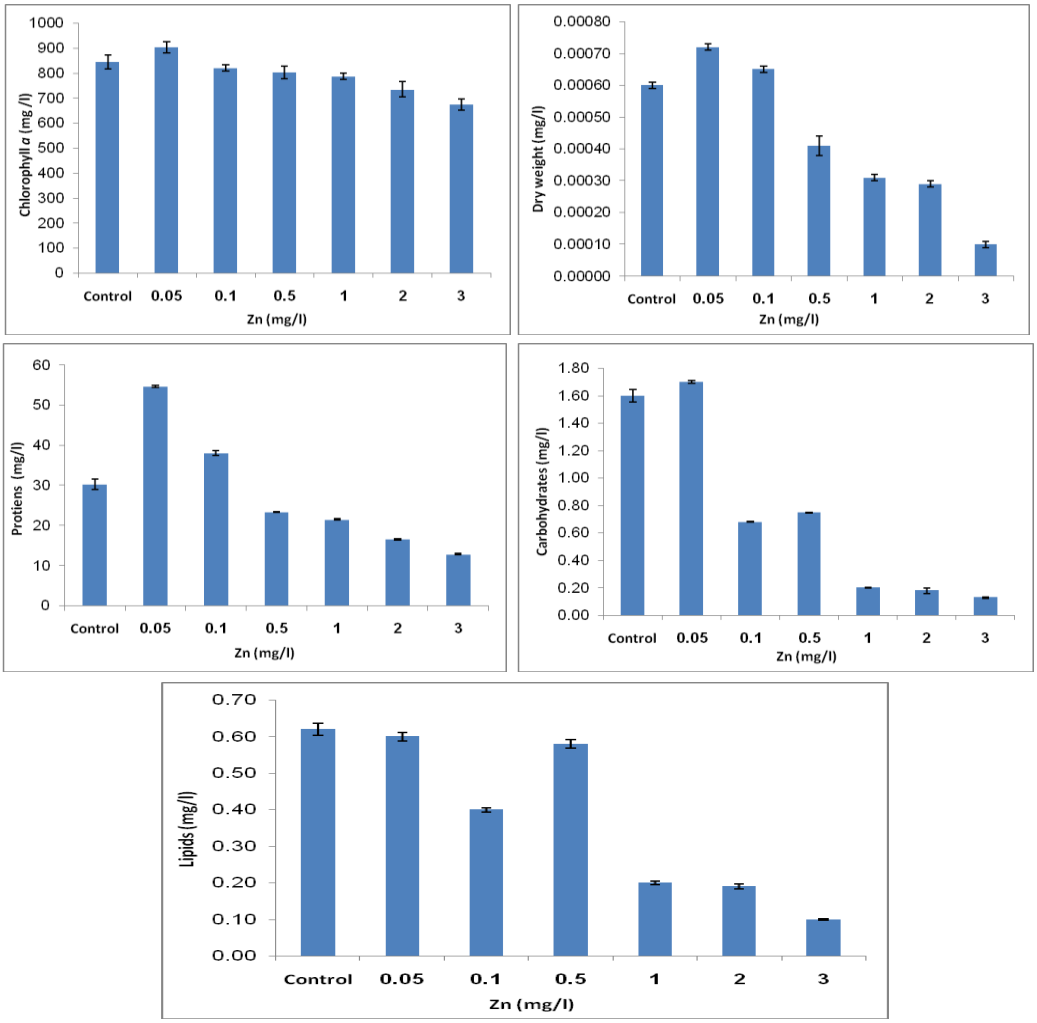

Fig. 1: Effect of various concentrations of zinc on chlorophyll *a*, dry weight and biochemical contents of *Microcystis aeruginosa* (Vertical bars indicate SE, n = 3)**.**

The catalase activity of the tested alga showed notable increase at low metal concentrations 0.05 and 0.1 mg/l to 0.02 and 0.028 U/L (Fig. 2). While the enzymatic activity decreased gradually in other zinc concentrations reaching their lowest value 0.0032 U/L at higher dose 3 mg/l. Zn showed significant linear regression with catalase (r^2= 0.503). Zinc induced the activation of the antioxidant enzyme catalase at lower concentration and there was a significant correlation between metal concentration and catalase activity (Soto *et al*., 2011).

It was detected that different Zn concentrations caused a marked and gradual decrease in the activity of the alkaline phosphatase enzyme of the tested alga (Fig. 2). The lowest value in the enzyme activity (0.0041 U/L) was observed at higher Zn addition (3mg/l). There was a positive significant regression between Zn and alkaline phosphatase (r^2= 0.835). Metals at low concentrations are essential for microalgae cells to perform cellular functions, as Zn is important for alkaline phosphatase activity for phosphorus acquisition (Miazek *et al*., 2015).

Chlorophyll *a* and the organic matter content expressed in dry weight showed a preference for higher phosphorus levels at which their maximum values 892.38 and 0.0013 mg/l were obtained with the highest phosphorus concentration 175% (70 µg/l), whereas the lowest results recorded at the starvation state 25% (10 µg/l) of the nutrient (Fig. 3).

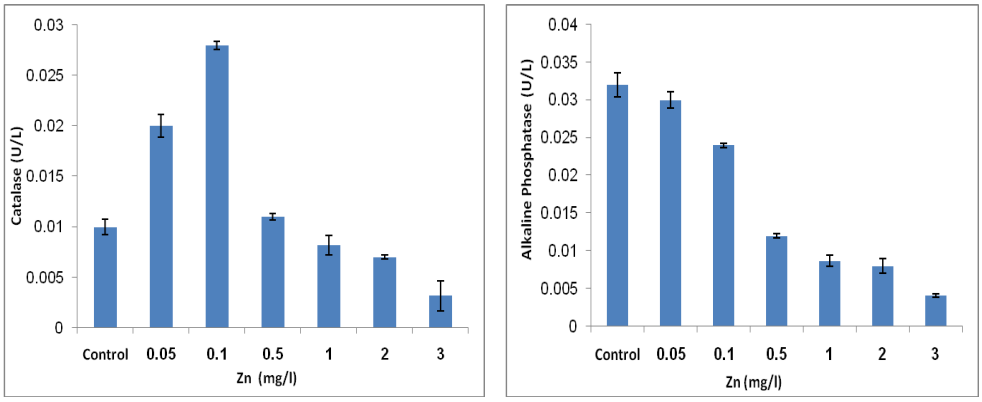

Fig. 2: Effect of various concentrations of zinc on catalase and alkaline phosphatase activity of *Microcystis aeruginosa* (Vertical bars indicate SE, n = 3)**.**

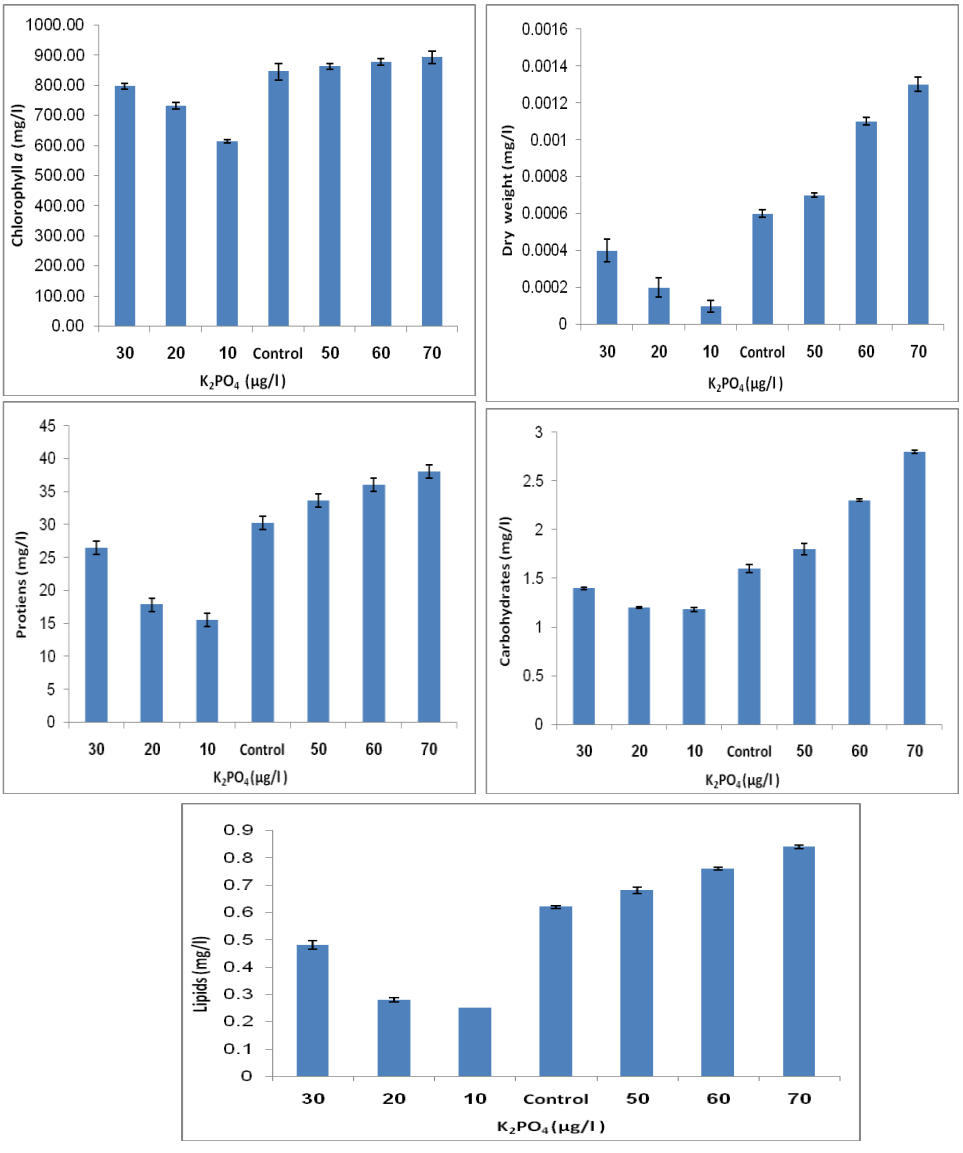

Fig. 3: Effect of various phosphorus concentrations on chlorophyll *a*, dry weight and biochemical contents of *Microcystis aeruginosa* (Vertical bars indicate SE, n = 3)**.**

Also, protein exhibited its higher concentration (38.098 mg/l) as compared to the control with P supplementations (70 µg/l) and its content decreased gradually when the phosphorous content diminished. Similar tendency observed for both carbohydrate and

lipid contents, where their values were gradually decreased with the deficiency of the phosphorous in the media (Fig. 3).

Generally the growth of *M. aeruginosa* was increased with increasing the concentrations of phosphorus and decreased continuously with decreasing of P level below the control. Bioassay experiment demonstrated that high densities of *M. aeruginosa* were associated with low nitrogen to phosphorus (N:P) ratio (Jacoby, 2000; Downing *et al*., 2005; Zaher , 2012). Data analyses indicated that there was a significant regression between P and the measured protein, carbohydrate and dry weight (r^2= 0.553, 0.661 and 0.664, respectively). Low phosphorus concentration is a restricting factor for the growth and reproduction of *M. aeruginosa* (Qin *et al*., 2013).

Changes in *M. aeruginosa* biochemical content were investigated at fixed phosphorus level and variable nitrogen concentrations of the BG11 medium. Chlorophyll *a* level showed an increasing manner in its values with the elevation N concentrations from 125% to 175%, and with the gradual decrease in N level there was a decline in Chl. *a* results. Data also elucidate how dry weight and the determined biochemical contents of the tested alga increased in response to N supplementations (Fig. 4).

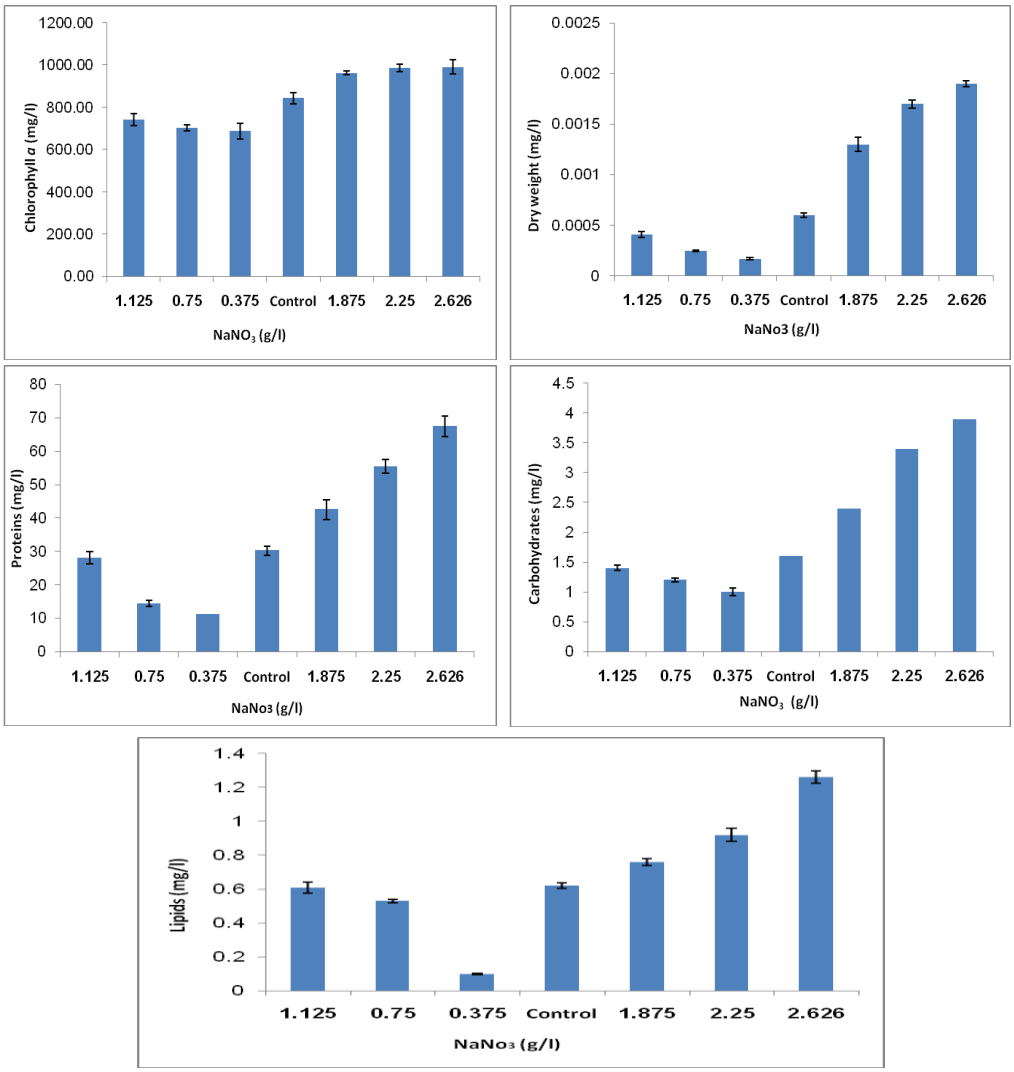

Fig. 4: Effect of various nitrogen concentrations on chlorophyll *a*, dry weight and biochemical contents of *Microcystis aeruginosa* (Vertical bars indicate SE, n = 3)**.**

There was a significant regression ($P<0.05$) under variable nitrogen concentration with chlorophyll *a*, dry weight, proteins and carbohydrates where $r^2= 0.66$, 0.769, 0.715 and 0.773, respectively. Abiotic factors and nutrients (especially nitrogen) are affected polysaccharides and protein synthesis, which in turn affect the growth rate of *Microcystis aeruginosa* (Li *et al.*, 2014).

Modifications in nitrogen and phosphorus concentrations in the BG11 synthetic medium lead to an increase in catalase activity for the tested alga to 0.021 and 0.023 U/L at 125% concentration for both N (1.875 g/l) and P (50 µg/l), respectively (Fig. 5). The activity of catalase was stimulated to protect *Microcystis aeruginosa* cell from oxidative damage at nitrogen level of 0.5 mg/l (Liu, 2015).

On the other hand, the results elucidated that alkaline phosphatase activity was more affected to changes in nutrient concentrations than the tested metal. The leakage in P to 25% (10 µg/l) lead to the increase in enzyme activity to its maximum value 0.04 U/L and the lowest value 0.013 and 0.018 U/L were at concentration 25% for N and 175% for P (Fig. 5). A positive significant regression ($r^2= 0.878$) of alkaline phosphatase was detected under variable P concentrations. *Microcystis* has relatively high capacity for acquisition phosphorus that could allow it to persist under stress conditions (Olsen, 1989). Phytoplankton can produce alkaline phosphatase under phosphorus shortage, to hydrolyze organic phosphorus, compensating P deficiency in their environment (Gillor *et al.*, 2002; Young *et al.*, 2010). The filamentous cyanobacteria *Cylindrospermopsis raciborskii* is able to acclimate to an environment with phosphorus concentration below 0.05 mgL^{-1}, through a decrease in the growth rate, photosynthetic procedure and increase in the activity of alkaline phosphatase and catalase (Wu *et al.*, 2012).

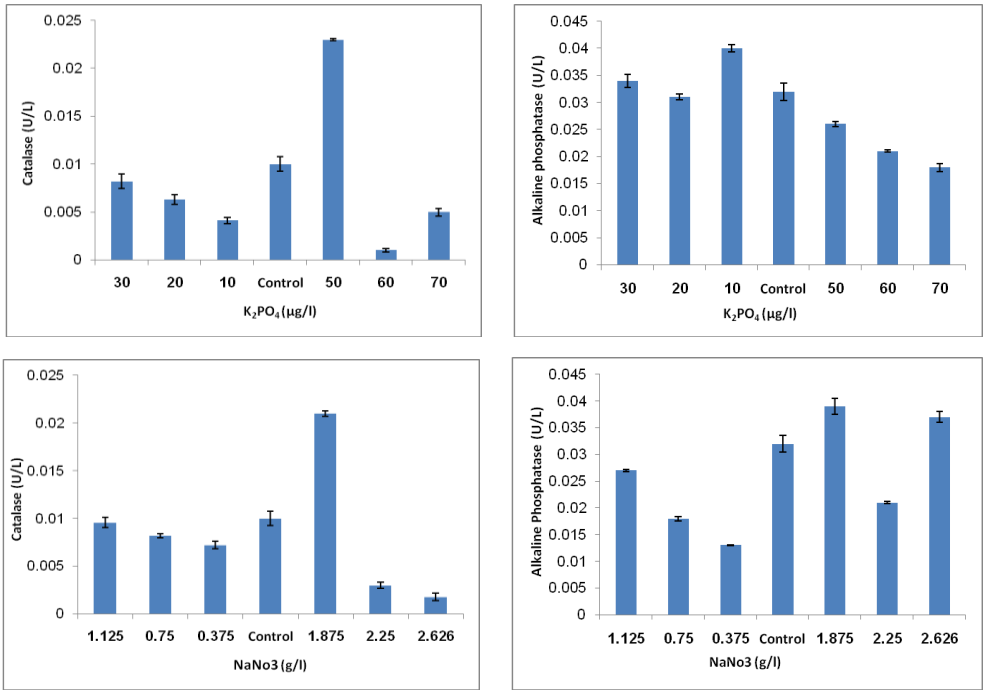

Fig. 5: Effect of various concentrations of nitrogen and phosphorus on catalase and alkaline phosphatase activity of *Microcystis aeruginosa* (Vertical bars indicate SE, n = 3)**.**

5. CONCLUSION AND RECOMMENDATIONS

The inhibitory and stimulatory effects of zinc on algal catalase or alkaline phosphatase depend on metal concentration. The exposure of *Microcystis aeruginosa* to up to 0.05 mg/l of Zn and lower nutrients inhibited its pigment and biochemical structure. Further investigations are needed to define the suitable dosages of metal and nutrients on algal biological process, which is the key to develop efficacious microalgae production systems.

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