HIGH NITRATE SUPPLY INDUCES CHLOROPHYLL DEGRADATION *CHLORELLA* SP.

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Abstract
The effects of high nitrate concentrations on chlorophyll degradation in *Chlorella* sp. were investigated. The decrease in the ratio of chlorophyll a/b with enrichment of high nitrate concentration (0.5 and 1 mM $NaNO_3$) was also caused by a decrease in chlorophyll a and an increase in chlorophyll b concentration in *Chlorella* sps. cultures.
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Introduction - Nutrient concentrations play an important role in the growth of phytoplankton, and the nitrogen sources considered most important for the growth of phytoplankton are nitrate and ammonium. Much higher nitrate concentrations have been found in aquatic ecosystems which were strongly contaminated by agricultural and urban activities [3], [1]. Enrichment of nitrogen and phosporus in aquatic enviroment can lead blooming of algae. Previously studies have also reported that nitrate could affect photosynthesis, growth and cellular toxicity of phytoplankton and metal toxicity to phytoplankton [3], [4], et [5]. Previosuly studied demostrated that excessive nitrogen fertilization causes osmotic stress, in which reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and hydroxyl radical (•OH) are produced [6]. ROS are highly toxic and can highly damage normal metabolism of lipids, proteins and nucleic acids and then inhibit plant and algae growth. Although, they found high nitrate concentration lead to inhibiton of growth of phytoplankton, effects of high nitrate stress on chlorophyll content, chlorophyll degradation and lipid peroxidation is still unexplored. In order to gain some insight into toxicity of nitrate in green algae, this study examined the effects of nitrate stress on chlorophyll degradation.

Material and Method - *Cultures and materials : Chlorella sp.* was obtained from EGEMAC culture collection, University of Ege, Izmir, Turkey. Eight flasks of 100 ml *Chlorella sp.* were used for the experiment. *Chlorella sp.* culture was grown photoautotrophically in Rudic Medium (RD) at 31°C in under continuous illumination (Table 1). Illumination provided by daylight fluorescence tubes at 20 µmol photons $m^{-2}s^{-1}$. Chlorella cells were harvested by centrifugation and transferred to a fresh medium, grown under the same conditions for 1 day; 0.05, 0.5 and 1 mM $NaNO_3$ were then added to the nitrate stress groups, respectively. The cultures were sampled 1, 3, 18, 24 and 44 h by removing 30 ml of the culture each time. All the experiments were repeated three times. Cell density was mesaured by spectrophotometer at 663 nm. About 20 mg cells was extracted in the dark for 1 h at 65 °C in 3 ml dimethyl sulfoxide (DMSO) in presence of polyvinylpolyrrolidone to minimize chlorophyll degradation. To assess chlorophylls, absorbance of the extracts was read at 665.1, and 649.1 nm. Statistical analysis was performed with one-way analysis of variance (ANOVA)(SPSS for Windows version 11.0).

Results and Discussion - Chlorophyll intermediate molecules are also potential chloroplast signals that could regulate photosynthetic gene expression, growth rates, and cell-death processes [7]. Our present results showed growth rate decrease closely correlated with decreasing chlorophyll a/b ratio following to supplemental different $NaNO_3$ concentration (Figure 1). Chlorophyll b is formed from chlorophyll a by the oxidation of the methyl group on ring II to the aldehyde and the ratio of chlorophyll a/b is more sensitive to modification than chlorophyll a+b. In green plants, antenna size is determined by the amount of light-harvesting chlorophyll a/b protein complex that is associated with the photosystems [7]. Conversion of chlorophyll b to chlorophyll a not only impacts the chlorophyll a/b ratio but also is the first step of chlorophyll degradation. In this study, on nutritional *Chlorella* sp. media and supplement 0.05 mM nitrate media, pigment levels were not significantly different (Table 1), but they did differ between groups in the presence of different nitrate concentration. The chlorophyll a/b ratio was significantly decreased in both 0.5 and 1 mM nitrate enrichment groups (Table 1). Our present results confirmed that supplemental $NaNO_3$ causes a significantly increase in chlorophyll b and a concomitant decrease in chlorophyll a, consistent with accelerated conversion of one to the other (Tab. 4). In conclusion, the levels of nitrate concentrations used in the present study ranged from 0.05 to 1 mM. According to the present study, these high nitrate concentrations caused chlorophyll degradation. Therefore, further research is required to investigate the effect of high nitrate concentrations on antioxidant mechanism in green algae. Table 1 shows Chlorophyll *a* /Chlorophyll *b* (Chl*a*/*b*) ratio of the alga Chlorella sp. cultivated with a nutritional level of nitrate (control) and nitrate supplement of 0.05, 0.5, and 1 mM.

Tab. 1. Values in bold are significantly different from control samples. Significance of differences ($p<0.05$) was checked by one-way analysis of variance (ANOVA). n=number of replicates, x=mean values, SD=standard deviations

Chla/b	n	1 h	3 h	18 h	24 h	44 h
		X±SD	X±SD	X±SD	X±SD	X±SD
Control	3	3,12±0.12	3,55±0.31	3,23±0.05	3,08±0.14	3,03±0.04
0.05 mM $NaNO_3$	3	3,84±0.27	3,30±0.13	2,97±0.24	2,83±0.34	3.32±0.23
0.5 mM $NaNO_3$	3	3,79±0.13	2,72±0.1	1,95±0.12	1,42±0.01	0.95±0.01
1 mM $NaNO_3$	3	3,60±0.12	2,47±0.27	0,97±0.06	0,88±0.02	0.53±0.13
ANOVA						
F probability		0.06	0.0003	0.0001	0.0001	0.0001

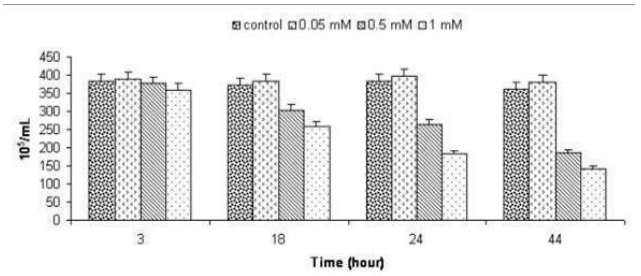

Fig. 1. Cell density of the alga Chlorella sp. cultivated with a nutritional level of nitrate (control) and nitrate supplement of 0.05, 0.5, and 1 mM.

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