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# Investigation of Physiological Indices of Cryptocoryne Fisch. ex Wydler in the Tropospheric Ozone

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**ABSTRACT:** Due to the increase in $O_3$ gas in the troposphere layer, which is caused by industrial pollution, can cause devastating effects on crops. Contamination through this gas, causing changes in metabolic and physiological systems in plants, and thereby causing negative effects on growth and products produced. Absorption of $O_3$, depending on plant type, condition and plant resistance is different. Ozone is a gas which its increasing in the atmosphere, causing changes on the plant such as crystals (irregular spots less than a millimeter in diameter, yellowish-brown), spots (areas of black pigment, small diameter 2-4 mm), bronzing, and reddening, is the effect of this gas can cause harmful effects on plants. Ozone impacts on aquatic plants, has not been studied much, so in this 60-day study, with Ozone 0.5 milligrams per liter against the control plant, the treatment was began and according to the results obtained, the control plants in compared with treated plants with Ozone, 0.5 milligrams per liter, have had better growth and biochemical parameters, finally, the adverse effect of ozone on these parameters, indicators were recorded.
***Keywords:*** Ozone, Cryptocoryne, Physiological indicators

## INTRODUCTION

### *Effect of ozone on plants*

Troposphere ozone has impact on the health of the man, as well as causes greenhouse gases in the environment (Bryan J. Bloomer et al/2010). In the absence of sufficient moisture in the soil, due to stomatal control to prevent excessive sweating, troposphere ozone also reduces ozone destruction (Delatorre/2012). Ozone causes long-term physiology changes in plant (Krupa and Manning, 1988). Ozone due to gas exchange, through the stomata enters leaf, and as a strong oxidant causes few symptoms such as chlorosis and necrosis. The expression of this issue is important because, leaf chlorosis and necrosis in the farm by normal aging ozone in the plant is created. In addition, in the plants affected by Ozone, several other indications has created that these symptoms may include: crystals (irregular spots, less than 1 millimeter in diameter, yellowish-brown), spots (the small black pigment areas with diameter of 2-4 mm), bronzing, and reddening... Ozone symptoms usually between veins of the upper level of the older leaves, is created. However, it may occur in both leaf surfaces, in certain areas. The type and severity of damage depends on several factors, such as time, concentration and amount of the Ozone, climate and plant geneticist. One or all of the symptoms at all areas, under specific conditions can occur. Special symptoms on different parts of the plant are another signs. With continued exposure to $O_3$, daily, classical signs (stippling, flecking, bronzing, and reddening) gradually with chlorosis and necrosis are observed.

## MATERIALS AND METHODS

In this study, in order to the ecological and botanical studies, examples of macroscopic Cryptocoryne algae, in the Environmental Research Laboratory, School of Biological Sciences, University of Kharazmi, in good conditions for growing this plant, were planted in aquarium with $25^{o}$c conditions, and then the 60-day period, treated samples with Ozone (Fig.1) were collected, and for comparison of the effects of Ozone, control samples were also collected and studied. In this research, measuring morphological measurements (growth), the physiology indictors of healthy and treated plants, were studied. Measured morphological indictors that are

indicative of plant growing, including the measurement of plant length, leaf area[1] and leaf dry weight[2] and leaf fresh weight in control and treated plants, which after completion of treatment, at a specified time range for all samples were measured (Fig. 2).


Figure 1. A view of the ozone generator machine

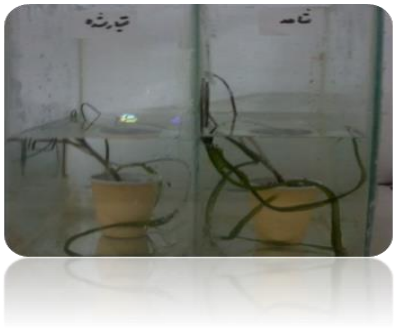

Figure 2. View of the treated samples observed. The sample control: Cryptocoryne Macroscopic algae

***Length measurement***

The plant length, which is one of the growth parameters, was studied. The length of the control plant and treated plant in a specific time range for all samples was measured. So that from each plant, randomly leaves were selected, leaf length per centimeter by ruler, from petiole to the tip of the leaf were calculated, were recorded.

***Measurement of fresh and dry weight***

After the end of treatment, at a specified time range, detached leaves were selected randomly, and with a sensitive digital scale model EK-I, the fresh weight of leaves immediately after being removed from the petiole, were measured, and for measuring the dry weight of the leaves, the same leaves in Avon with a temperature of 75 ° C for 48 h were placed, then with a sensitive scale model EK-I, leaves dry weight were measured, and achieved numbers were recorded (Watson / 1952).

***Sensing pigment content***

To check the contents of pigments, including chlorophyll b / a, total and carotenoids, leaves after weighing by the sensitive digital scale model EK-I, with acetone 80% in porcelain mortar became uniform, the absorbance of the extract after passing the Paper filter, using Visible and Ultraviolet spectroscopy model cintracol GBC at wavelengths 661.6 -644.8 and 470 nm was read, and then using the formula, pigments content in terms of mg / gr wet weight were calculated. 80% acetone as a control solution was used, to set zero absorbance a spectrophotometer was used (Linchtenthaler / 1987).

Chlorophyll a =[11/24(A661.6-20.04(A644.8) ] + V/1000X dilution factor
Chlorophyll b=[20.3(A 644.8)-4/19(A 661.6)] + V/1000X dilution factor
Total Chlorophyll: [7.05(A 661.6)] – [18.09 (A 644.8)] +V/1000X dilution factor
A = the amount of light absorbed
W = Wet weight of sample in gram
V = volume of extract extracted per ml

***Measurement of leaf area***

To this end, among the second split leaves per plant, four leaves were randomly selected, and then they were placed on graph paper, based on the area occupied by the square of the leaves on the graph paper, leaf area per square millimeter ($mm^2$), was calculated.

The results of the treatment of ozone on plants
Results of measurements of leaf length

[1] LA
[2] LDW

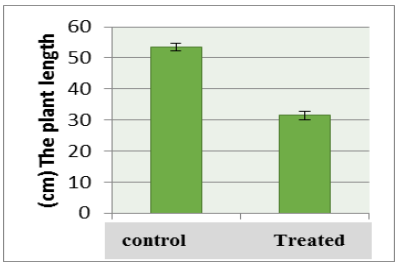


Figure 3. The length of leaves of control and treated plants in terms of centimeters (cm)

Table 3. Variance of data obtained from the treatment on leaf length

| The source of the Changes | The sum of squares (SS) | Degrees of freedom (Df) | Mean-square (MS) | Variance (F) | Significance level (P) |
|---|---|---|---|---|---|
| Between groups | 968.000 | 1 | 968.000 | 152.842 | *** |
| Within groups | 38.000 | 6 | 6.333 | | |
| Total | 1006.000 | 7 | | | |

In general, the UV-B effect on cell division is not only enlarging it, this ray also can cause delay in seedling emergence, decrease of the height, decrease of elongation rate of the main stem and branches. Decrease of the plant length mainly occurs due to the shortening of internode, and reducing the length between occurs due to reduction in cell number rather than cell length. Another reason for the length of the stem is the auxin hormone decomposition. Due to the results of this experiment, the leaf length of the treated plant, 31.5 cm compared with the control plant, 53.5 cm was seen (Figure 3).

***Results of measurements of plant fresh weight***

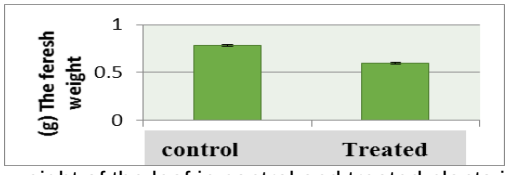


Figure 4. The fresh weight of the leaf in control and treated plants in terms of gram (g)

Table 4. Variance of data resulting from treatment on the fresh weight of leaf

| The source of the Changes | The sum of squares (SS) | Degrees of freedom (Df) | Mean-square (MS) | Variance (F) | Significance level (P) |
|---|---|---|---|---|---|
| Between groups | 0.067 | 1 | 0.067 | 175.681 | *** |
| Within groups | 0.002 | 6 | 0.000 | | |
| Total | 0.069 | 7 | | | |

Ozone has specifications, such as plant cell and tissue damage, has adverse effects on plant growth, especially in plant weight. In this experiment, applying Ozone, plant fresh weight equivalent to 0.60 g in compared with the control sample, which has shown a weight equivalent to 0.79, was observed (**Figure 4 ).**

***Results of measurements of leaf dry weight***

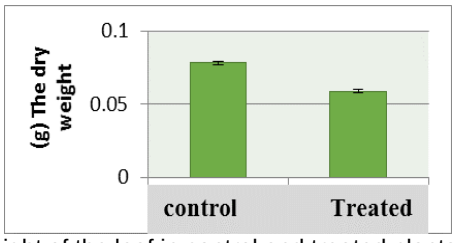


Figure 5. The dry weight of the leaf in control and treated plants in terms of gram (g)

Table5. Variance of data obtained from treatment on the dry weight of leaf

| The source of the Changes | The sum of squares (SS) | Degrees of freedom (Df) | Mean-square (MS) | Variance (F) | Significance level (P) |
|---|---|---|---|---|---|
| Between groups | 0.001 | 1 | 0.001 | 99.369 | *** |
| Within groups | 0.000 | 6 | 0.000 | | |
| Total | 0.001 | 7 | | | |

As in Figure 5 shown the dry weight is normal by 0.07 g and the treatment sample show the 0.055 g by weight.

Measurement results of the sensing pigment content in the control and treatment plants were calculated as follows.

***The results obtained from chlorophyll a***

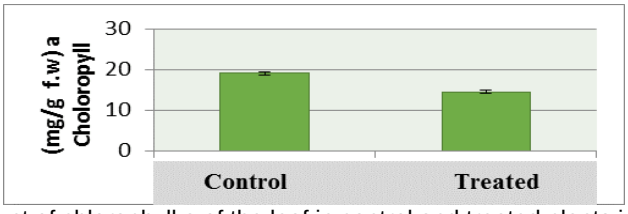


Figure 6. The amount of chlorophyll a of the leaf in control and treated plants in terms of gram (g)

Table 6. Variance of data obtained from treatment on the amount of the chlorophyll a of the leaf

| The source of the Changes | The sum of squares (SS) | Degrees of freedom (Df) | Mean-square (MS) | Variance (F) | Significance level (P) |
|---|---|---|---|---|---|
| Between groups | 41.223 | 1 | 41.223 | 47.650 | *** |
| Within groups | 5.191 | 6 | 0.865 | | |
| Total | 46.414 | 7 | | | |

UV Rays cause some molecular damages, because UV absorption by aromatic amino acids, and nucleotides, can lead to non-functioning of nucleic acids and relevant proteins (2001, Casti P et al). Therefore, in this experiment, due to the degradation performance ozone on chlorophyll, according to Figure 6, the amount of chlorophyll a of the control plants, 19, and treated plants 14.9 was observed.

***The results obtained from measurement of the chlorophyll b content***

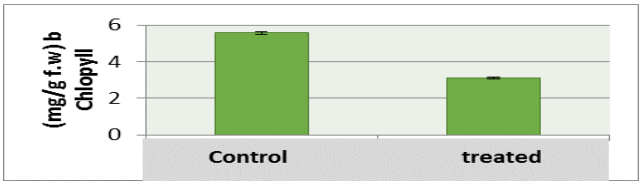


Figure 7. The amount of chlorophyll b of the leaf in control and treated plants in terms of gram (mg/g f.w)

Table 7. Variance of data obtained from treatment on the amount of the chlorophyll b of the leaf

| The source of the Changes | The sum of squares (SS) | Degrees of freedom (Df) | Mean-square (MS) | Variance (F) | Significance level (P) |
|---|---|---|---|---|---|
| Between groups | 12.054 | 1 | 12.054 | 1.032 | *** |
| Within groups | 0.070 | 6 | 0.012 | | |
| Total | 12.124 | 7 | | | |

Chlorophyll b usually is seen in higher plants. While in green - blue, brown and red algae, there is not this chlorophyll. According to Figure 7, the chlorophyll b in the control plants,5, and in the treated plants, 3.1 was observed.

***The results obtained from measurement of the total chlorophyll***

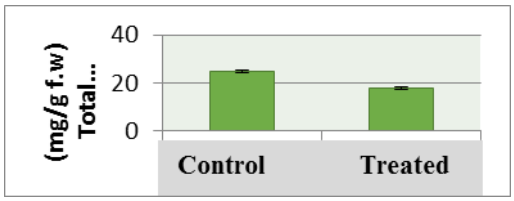


Figure 8. The amount of total chlorophyll in control and treated plants in terms of gram (mg/g f.w)

Table 8. Variance of data obtained from treatment on the amount of the total chlorophyll of the leaf

| The source of the Changes | The sum of squares (SS) | Degrees of freedom (Df) | Mean-square (MS) | Variance (F) | Significance level (P) |
|---|---|---|---|---|---|
| Between groups | 105.779 | 1 | 105.779 | 93.371 | *** |
| Within groups | 6.797 | 6 | 1.133 | | |
| Total | 112.576 | 7 | | | |

According to Figure 8, the total chlorophyll content in plants treated 26 and in control plants 18.5 was observed.

***Results obtained from measurements of leaf area***

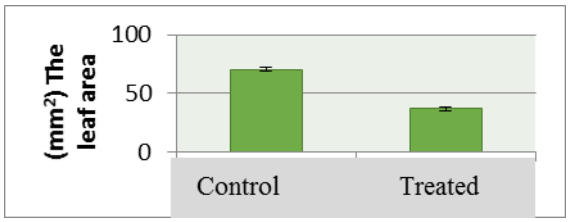


Figure 9. The amount of leaf area in plants treated versus control plants ($mm^2$)

Table9. Variance of data obtained from treatment on the leaf area

| The source of the Changes | The sum of squares (SS) | Degrees of freedom (Df) | Mean-square (MS) | Variance (F) | Significance level (P) |
|---|---|---|---|---|---|
| Between groups | 2244.500 | 1 | 2244.500 | 184.479 | *** |
| Within groups | 73.000 | 6 | 12.167 | | |
| Total | 2317.500 | 7 | | | |

UV Ray reduces growth .In most cases, the leaves when are exposed to the radiation, become thicker, and gain more weight per unit area., This would not necessarily mean that prevent growth, but this may be a change in the pattern of cell division and elongation, which will lead to create a thicker leaf with more cell layer (Kafi and Damghani, 2002). According to Figure 9, the leaf area in the control plants, 72, and in the treated plants, 38, was observed.

## CONCLUSIONS

In this study it was found that if the plants are exposed to $O_3$, irreparable damage to the plant will be imported, it has a negative effect on the indicators of growth such as length, weight and leaf area. Ozone increases the activity of peroxidase, catalase, superoxide dismutase enzymes and leaf electrolytic and reduces carbon fixation (Reduction of the Rubisco activity) and finally guard cell homeostasis is impaired (Edwin et al., 2005). When plants are exposed to Ozone, photosynthesis, growth and ultimately crop yield will be reduced. The factor that help greater influence of ozone from plant stomata, and open stomata, will increase the

sensitivity of plants to Ozone. Ozone, as a secondary pollutant enters the leaf through the stomata, and publishes in Apoplast. (Bathia et al., 2012). Both heat and drought factors, by closing stomata to conserve water, reduce Ozone received (Emberson et al., 2012). All living organisms, especially plants, have mechanisms and reactions to cope or adapt to environmental stress (Stapleton, 1992), which can be noted to molecular changes molecular and phytochemical changes in this regard (Reddy et al., 2004). Many studies on UV effects and other stresses on plants have been conducted (Singh et al., 1996). (Biggs et al. 1975) and (Krizek et al. 1997), in their study show the reduction of the chlorophyll, complexity and waxy leaves, reduction of the leaf area (Balakrishnan et al. 1997), increase of flavonoids in UV treated plants in compared with controls. Also, (Mazza et al. 1999), (Mackerness et al., 2001) examined creating oxidative stress regarding this ray. Flavonoids are a class of polyphenolic compounds in plants with different actions. Increase of metabolism of phenylpropanoids and phenolic compounds could be observed under stress conditions and environmental factors. Isoflavones Synthesis and some other flavonoids, when plants are infected or injured, or are under low temperature conditions and nutritional deficiencies, are induced. Plants, flavonoids of the UV absorbers, and other phenolic compounds mainly accumulate in vacuols of the epidermal cells. In recent years, the antioxidant properties of flavonoids are further considered ( Diaz J *et al.2001).*

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