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Interaction effects of zinc chloride and crude aqueous extract of *Bouhinia variegata* mixtures upon photosynthsis and respiration of *Chlorella vulgaris* Beijer cultures

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Introduction

Heavy metals belonging to the most dangerous environmental stress factors, as oxidative stresses, they inhibited the basic physical functions of plants (Clijsters and Van Assche, 1985; Van Assche and Clijsters, 1990; Meharg, 1994 and Gallego, *et. al*., 1996). It has been previously shown that different heavy metals can interfere with various step of the photosynthetic electron transport chain (Tuba and Csintalan, 1992 and Krupa and Baszyski, 1995). Inhibition effects were found on both donor and acceptors sides of photosystem II (PS II), at the cytochrome b/f complex and also in photosystem I (PSI) (Droppa and Horvàth, 1990; Krupa and Baszynski, 1995 and Baroń, *et al*.1995). On the other hand, heavy metals are the most toxic pollutants to living organisms in aquatic and terrestrial environments.

Zinc is one of the major industrial pollutants of terrestrial and aquatic environment (Foy, *et al*, 1978 and Freedman and Hutchinson, 1981). It is caused inhibition of photosynthetic CO_2 in both higher plants and algae (Van Assche and Clijsters, 1986, a; b). In the algal cells zinc caused inhibition of photosynthetic electron transport observed after zinc accumulation by organisms. The main site of action was on the level of the water-splitting system at the oxidizing side of photosystem II (Arndt, 1974 and De Filipps, *et al*., 1981). The most important mechanism of the toxic action of zinc to be the poisoning agent of enzymes (Bowen, 1966). Also, Szalontia, *et al*., 1999 reported that some heavy metals such as Cu, Pb and Zn strongly inhibited photosystem II activity. Therefore, the damaging effects of poisonous heavy metals are element-specific; for example Cu, Pb and Zn interact directly with thyalkoid membrane of the photosynthetic apparatus. In accordance with this, De Filippis, *et al*., (1981a) found that Zinc caused inhibition of NADP-oxidoreductase and they have significantly effect upon the cells' supply of NADPH2.

GS/MS analysis of Crude aqueous extract (CAE) of *Bouhinia variegata* showed a presence of many chemical natural products as Myo-Inositol, 4-C-methyl ($C_7H_{14}O_6$), Octadecanedioic acid ($C_{18}H_{34}O_4$), Cyclopropanecarbonitril ($C_{18}H_{17}N_2Cl$) and 2,6-Diphenyl-4, 4-dihydropyridine-3, 5-dicarbonitril ($C_{21}H1_7\ N_3$).

The main goal of the present study aims to obtain detail informations about the functional structural interaction effects between $ZnCl_2$ and CAE of *B. variegata* mixture upon photosynthesis and respiration of *Chlorella vulgaris* Beijer (Chlorophyceae) as a unicellular green fresh water alga. Therefore, in the further we can use that nature materials in the purification of water by increase the level of oxygen evolution in water,

which leads to decrease of anaerobic pathogenic microorganism's growth. That effect leads to make water more fresh and healthy. As we know the treatment of water by natural plant materials are environmentally safe.

Materials and Methods

Tested alga: *Chlorella vulgaris* Beijer was collected from the Nile River and used as a tested organism. Beijerinck's nutritive culture was used as medium for enrichment and growth of the tested alga (Stein, 1966; Ahmed *et al.*, 1985; 1989).
Treatments: *Chlorella vulgaris* Beijer cultures were subjected to different concentrations mixtures (20,40,60,80 and 100/ 2, 4, 6, 8 and 10 ppm) of $ZnCl_2$/CAE of *B. variegata* for 7 days. The treated cultures with 00.00 $ZnCl_2$ / ACE of *B. variegata* was used as absolute control cultures.

Analytical Method:
1-Phytochemical screening of CAE of *B. variegata*: Using GC/MS [Finnigan MAT SSQ 7000 mass spectrometer coupled with varian 3400 gas chromatography] according to Sharaf, *et al*. (2000).
2- Oxygen evolution: Using the site module 97.08 dissolved oxygen electrode as a polargraphic device, which described by Clark oxygen electrode (Lessler *et al*., 1956). Electrode separated from the magnetic stirred assay medium by a Teflon membrane (Oxygen membrane/electrolyte). The data of the oxygen evolution in the present study were calculated as µ mole O_2 ml^{-1}algal suspension hr^{-1}.
3- Oxygen uptake: Using oxygen uptake in the dark conditions as indicator. The system mentioned above was used for the determination of dark respiration. At the end of oxygen evolution measurements, all the lights were switched off and the flasks were wrapped tightly in aluminum foil for complete darkness. The results of oxygen uptake were calculated as µ mole O2 ml^{-1} algal suspension hr^{-1}. Two replicates of each treatment by $ZnCl_2$/CAE of *B. variegata* were carried out.

Results

The results concerned with the interaction effect between $ZnCl_2$ and CAE of *Bouhinia variegata* upon photosynthesis and respiration of *Chlorella vulgaris* Beijer cultures for 7days were recorded.
Oxygen evolution was markedly increased under the lower concentration of $ZnCl_2$ (20 and 40 ppm). Otherwise, under the higher concentrations of $ZnCl_2$ (60,80 and 100 ppm) the oxygen evolution was decreased. The maximum value of oxygen evolution is 199% when treated with 40 ppm of $ZnCl_2$. The minimum value of oxygen evolution reached to 50% when treated with 100ppm of $ZnCl_2$. The oxygen uptake was markedly increased under the concentrations of 20 and 40 ppm of $ZnCl_2$. Under the relatively higher concentrations of $ZnCl_2$ (60, 80 and 100 ppm) the oxygen uptake was reduced.
Oxygen evolution rate was gradually increased under the different concentrations of $ZnCl_2$/CAE of *B. variegata*. The maximum value of oxygen evolution (361%) was occurred under the effect of (40/2 ppm) of $ZnCl_2$/CAE of *B. variegata.* The minimum value of oxygen evolution (93%) was occurred under the effect of (100/2 ppm) of $ZnCl_2$/CAE of *B. variegata*. The photosynthesis contents of *Chlorella vulgaris* Beijer

cultures have increased when subjected to various concentrations of (20,40,60, 80 and 100/4,6 and 8 ppm) of $ZnCl_2$/CAE of *B. variegata*. The maximum value of oxygen evolution is 533% under the effect of (60/8 ppm) of $ZnCl_2$/CAE of *B. variegata*. However, the minimum content of oxygen evolution is 100% under the effect of higher concentrations of (100/6 and 8 ppm) of $ZnCl_2$/CAE of *B. variegata*. The content of oxygen evolution was markedly reduced under the effect of (20,40,60,80 and 100/10 ppm) of $ZnCl_2$/CAE of *B. variegata*.

On the other hand, the oxygen uptake rate was markedly increased under the effect of (20,40 and 80/4, 6, 8 and 10 ppm) of $ZnCl_2$/CAE of *B. variegata*. The maximum value of oxygen uptake content is 330 % under the effect of $ZnCl_2$/ACE (20/4 ppm), while the minimum content of oxygen uptake is 36 % under the effect of (100/2 ppm) of $ZnCl_2$/CAE of *B. variegata*. The previously obtained results were compared to absolute control 00.00 of $ZnCl_2$/CAE of *B. variegata.*

Discussion

The adverse effects of Zinc chloride upon plants may causes disturbances in plant metabolism, which consequently lead to a reduction of photosynthesis and some related activities (Kojima, *et al*., 1987 and Rai, *et al*., 1990 & 1994). Many trials have been made to help the plants to overcome that disturbances using variable treatments of crude aqueous extract (CAE) of *B. variegata* in the laboratory aiming to apply this results in future in a commercial area.

In accordance with this, in some algae, the inhibition of photosynthetic electron transport was clearly observed after Zinc accumulation by organisms. These action due to increasing the level of water-splitting system at the oxidizing of photosystem II (Arndt, 1974, De Filippis, *et al*., 1981, a, Kojima, *et al*., 1987 and Rai, *et al*., 1990 & 1994).

Recently, Hangovan, *et al*., 1998 and Szalontia, *et al*., 1999 suggested that the Pb, Cu and Zn are strongly inhibited photosynthetic electron transport related to their ability to effect the lipid-protein interactions and it's ability to change the protein conformations in the chloroplast thylakoids.

The exogenously addition of crude aqueous extracted (CAE) of *B. variegata* to stressed Chlorella vulgaris Beijer by $ZnCl_2$ for 7 days causes stimulation of photosynthesis and some related metabolic activities.

In conclusion, from the obtained results we can declare that, the addition of crude aqueous extract of *B. variegata* to various concentrations of $ZnCl_2$ upon *Chlorella vulgaris* cultures caused counteract to the adverse effects of $ZnCl_2$ on photosynthesis and respiration. So, we can say that the deciduous of some plant leaves like *B. variegata* in rivers or lakes. Or addition of CAE of *B. variegata* to the water may be play an important role in counteracts of the adverse effect of some heavy metals, which may pollute water and then make it more fresh and reliable.

References

Ahmed, A., Radi, A., Heikal, M. and Abdel-Basset, R. (1989): *J. Plant Physiol*. **(135)** 175-178.

Ahmed, A., Mohammed, A., Heikal, M. and Mohamed, R. (1985): *Acta Hydrobiol*. **(27)** 25-32.

Arndt, U. (1974): *Environ. Pollut*. **(6)** 181–194.
Baroń, M, Arellano, J. and Lopez-Gorgé, J. (1995): *Physiol. plant* **(94)** 174-180.
Baszynski,T.(1986): *Folia Physiol. Cyto. Gen*. **(1)** 7- 27.
Bowen, H. (1966): Trace elements in biochemistry. Academic Press, London.
Clijsters, H. and Van Assche, F. (1985): *Photosynth. Res.* **(7)** 381-401
Clijsters , H. and Van Assche F.(1990): *Photosynth.* **(7)** 31- 40.
De Filppis, L., Hampp, R. and Ziegler, H. (1981): *Arch. Microbiol*. **(128)** 407 – 411.
Droppa, M. and Horvàth, G. (1990): *Crit. Rev. Plant Sci*. **(9)** 111-123.
Foy, C., Chaney, R. and White, M. (1978): *Ann. Rev. Plant Physiol*. **(29)** 551 – 566.
Freedman, B. and Hutchinson, T. (1981): Sources of metal and elemental contamination of terrestrial environments. In: Lepp, N. (ed.): The effect of Heavy Metal pollution on plants Vol. II, 35-94. Applied Science Publishers. London.
Gallego, S., Benavides, M. and Tomaro, M. (1996): *Plant Sci*. **(121)** 151-159
Gross, R., Puno, P. and Dugger, W. (1970): *Plant Physiol*. **(46)** 184 – 185.
Hangovan, K., Canizares, R., Gonzalez, S. and Voltolina, D. (1998): *Bull. Envio. Contom Toxicol*. **(60)** 936– 943.
Kojima, Y., Hiyama, A. and Sakurai, H. (1987): Effect of mercurials on iron sulphur centers of PSI of Anacystis nidulans In: Progress in photosynthesis Research. Biggins. J. (ed.) Nijhoff. Junk, The Hague, PP. 57 – 60.
Krupa, Z. and Basznski, A.(1995): *Acta Physiol. Plant* **(17)** 177-190.
Lessler, M., Malloy, E. and Schwab, C. (1956): *Fed. Proc*. **(24)** 336.
Meharg, A. (1994): *Plant Cell Environ.* **(17)** 989-993.
Morel, N., Rueter, J. and Morel, F. (1978): *J. Phycol*. **(14)** 43–48.
Rai, L., Singh, A. and Mallick, N., (1991): *J. Plant Physiol.* **(137)** 419 – 424.
Rai, P., Mallick, N. and Rai, L. (1994): *Biomed. Environ. Sci.* **(7)** 56– 67.
Sharaf, M., Nagat, S. and El-Ansari, A.(2000): *Biochem. system. and Ecol*. **(28)** 291-293.
Stein, J. (1966): *J. Phycol*. **(2)** 23–28.
Szalontai, I., Horvàth, M., Madolna, D. and Gàbor, H (1999): Photosynthesis Res. **(61)** 241-252.
Tuba, Z. and Csintalan, Z. (1992): The effect of pollution on the physiological processes in plants In: Kovàces , M.Podani, J.Tuba,Z., Turcsàny, G., Csintatan, Z. and Meenks JLD (eds) Biological Indicators in Environmental Protection, PP. 169-191. Ellis Horwood, Chichester.
Van Assche, F. and Clijsters, H. (1990): *Plant Cell Environ*. **(13)** 195-206.
Van Assche, F. and clijsters, H. (1986 a): *Physiol. Plant.* **(66)** 717–721.
Van Assche, F. and Clijsters, H. (1986 b): *J. Plant Physiol*. **(125)** 355 – 360.