RESEARCH ARTICLE **(Open Access)**

Malondialdehyde as a Biomarker of Oxidative Stress in Common Reed (*Phragmites australis*) in the Albanian Part of Lake Ohrid

ENIS DALO [*1,2], RIGERTA SADIKAJ [2], HAZBIJE SAHITI [1]

[1]Department of Biology, University of Prishtina, Faculty of Mathematics and Natural Sciences, Prishtina, Kosovo

[2]Departmentof Biotechnology, University of Tirana, Faculty of Natural Science, Tirana, Albania

*Corresponding author; E-mail: enisd8@gmail.com

Abstract

Aquatic plants are constantly exposed to various environmental factors that cause reactive oxygen species (ROS) production. One of these factors that endanger the Ohrid Lake's plants is the accumulation of heavy metals by plants. Various metals like nickel, chromium increase ROS production and with it the malondialdehyde (MDA) values in the plants. The purpose of this research was to evaluate oxidative stress in common reed by measuring the values of MDA and to see the possibility of using of MDA as a biomarker for the assessment of pollution in the Lake Ohrid. Plant samples were analyzed by Health Packer spectrophotometric method. The research conducted on three points at the entrance of Pogradec, at the former factory of ferro nickel and at the village Lin. Although not significant the greater values of MDA (4.83 ± 0.57 µmol/g wet tissue) in stem of common reed had in the factory point. Our results showed that a further study on MDA as a biomarker of lipid peroxidation on various parts of the plant and even in various aquatic plants should be continued in the Albanian part of Lake Ohrid.

Keywords: Ohrid; MDA; plant; oxidative stress; common reed.

1. Introduction

Oxidative stress is one of the negative effects of environmental stress factors on high sessile plants. This stress caused by the overproduction of reactive oxygen species (ROS) may be the result of exposure to drought, salinity, heavy metals, nutritional disorders or radioactivity. In ROS are present the superoxide radical ($O_2^{\cdot-}$), the singlet oxygen ($^1O_2$), the hydroxyl radical ($OH^{\cdot}$), the hydroperoxyl radical ($HO_2^{\cdot}$), the hydrogen peroxide (H_2O_2) like that. These highly reactive and partially reduced oxygen particles can be produced in different parts of the plant such as chloroplasts, mitochondria, peroxisomes, plasma membranes, apoplasts, endoplasmic reticulum, and cell-wall. Their production occurs both during the normal metabolism of the plant and under the induction of environmental factors. Exposure to environmental stress factors increases the overproduction of ROS and this increase oxidative stress. Oxidative stress, which is defined as the imbalance of ROS production and antioxidant protection, causes damage in different parts of the cell such as proteins, lipids, carbohydrates and DNA [17, 5, 4, 6, 18]. One of the environmental stress factors that causes ROS overproduction in plants are heavy metals [18]. In moderate concentrations, heavy metals do not affect plant growth, but if the tolerance threshold passes, this may lead to the death of the plant. In their tissues aquatic macrophytes can accumulate heavy metals in considerable quantities [10, 21]. A typical aquatic aquatic plant that can accumulate heavy metals in different tissues in different parts of it like roots, stems, and leaves is common reed (*Phragmites australis*) [3]. This plant is quite widespread along the shore of the Albanian part of Lake Ohrid. According to Albrecht et al. the macrophytes of this lake can grow in four zones of the so-called Clodophora zone, Chara zone, Phragmites australis zone, Potamogeton zone. From this point of view, the purpose of this study was to evaluate the oxidative stress of the *Phragmites australis* stem in three points of the lake and to predict whether MDA could be a parameter of lake plants that could serve as a biomarker for evaluation of pollution in the lake.

2. Material and Methods

2.1. Study area

The research was conducted in Ohrid Lake, which is one of the oldest lakes in the world located in Balkan

*Corresponding author: Enis Dalo; E-mail: enisd8@gmail.com
(Accepted for publication 30.09.2020)
***ISSN**: 2218-2020,*

Peninsula [15]. Lake Ohrid is a transboundary lake shared between the Former Yugoslav Republic of Macedonia (FYROM) and the Republic of Albania (Fig. 1). The lake is located at 693.5m above sea level and has a maximum length of 30.4 km (N–S), a maximum width of 14.7 km (W–E), surface area of 358 km^2, and a tub-shaped bathymetry with a maximum water depth of 293 m, a mean water depth of ∼151 m, and a total volume of 50.7 km3 [23]. Despite the fact that the Lake Ohrid is under the influence of the anthropogenic factor this lake is still considered as an oligotrophic lake with high amounts of dissolved oxygen in the deep waters [13]. Another special characteristic of this ecosystem is high biodiversity and extremely high endemicity rates, Ohrid Lake hosts various endemic species from the entire food chain [1, 19].

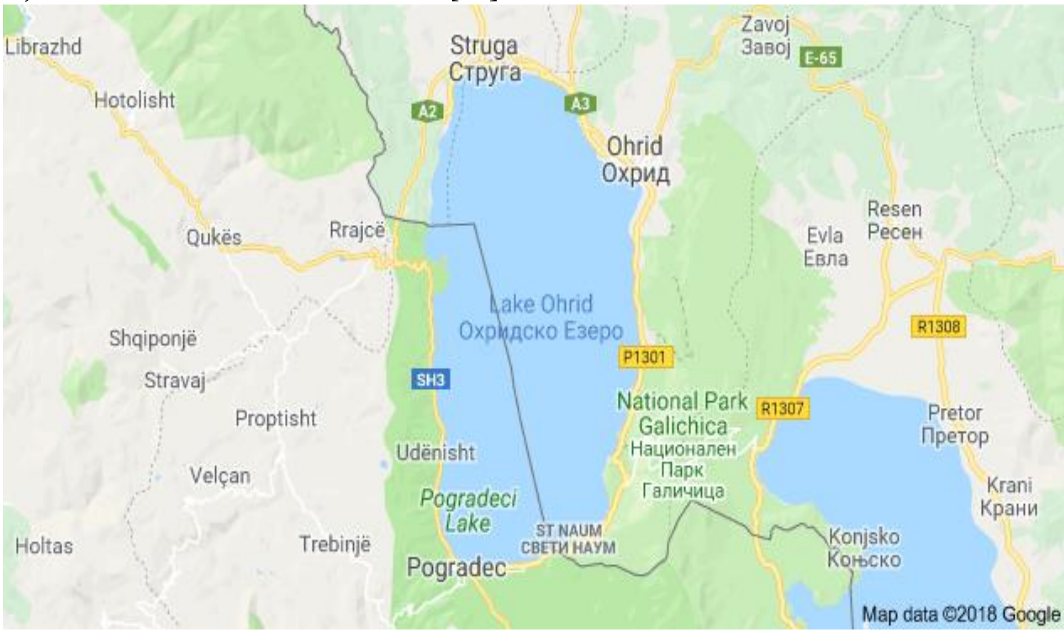

Figure 1. Map of Lake Ohrid (https://www.google.com/maps/place/Lake+Ohrid)

2.2. Sampling

The research was conducted in the spring of 2015. The sampling was done at three points in Lake Ohrid on the side that belongs to Albania, precisely at the entrance of Pogradec (area occupied by *Phragmites australis*), near the former Fe-Ni mine and in the village of Lin. Lin village has served as a reference point due to its distance from the mine. The macrophytes samples have been collected by hand from the shore of the lake, then the leaves have been removed and only the stems are placed in sterile glasses filled with the water taken from the lake. The prepared samples are brought to the laboratory in the ice containers at a temperature of about 5°C, then part of the stemsare dried at room temperature while the rest stalk is placed at a temperature of -20°C.

2.3. Determination of malondialdehyde

Malondialdehyde (MDA) was determined according to Heath and Packer 1968 with some modifications. The principle of this method is based on the reaction of MDA with thiobarbituric acid, whereby a colored complex is formed. Firstly, frozen samples of plant stems with countermeasure 0.5 g are homogenized with 10% trichloroacetic acid (TCA). Then the homogenate was centrifuged at 7000 X g for 10 minutes. One ml homogenate is mixed with two ml of 0.5% thiobarbituric acid dissolved in 10% TCA. This mixture is placed at 95°C for 45 minutes and then cooled to room temperature. After centrifugation, the formed color is read at 532 nm in the spectrophotometer. Calculation of the MDA value is done with extinction coefficient 1.56×10^5 M^{-1} cm^{-1} expressed as μmol MDA per gram of wet tissue.

2.4. Statistical analysis

Differences between the points are expressed using ANOVA and a Post Hoc Turkey test. Data were expressed as means ± standard error (SE) for each samples (n=3) for MDA. P values of $p \leq 0.01$ were considered to be significant.

3. Results and Discussion

Although not significant the greater values of MDA (4.83 ± 0.57μmol/g wet tissue) in stem of common reed had in the factory point. (Table 1).

Table 1. MDA values in stem of common reed (*Phragmites australis*) at three sample points on the Albanian part of Lake Ohrid

	Entry of Pogradec	Former Fe-Ni mine	Lin village
µmol MDA/g wet tissue	3.94 ± 0.39	4.83 ± 0.57	3.55 ± 0.73

(The results are presented as Mean ± SD, significant to the reference point of Lin ** p <0.01 and *p<0.05) (n=3)

Lake Ohrid is endangered by pollution. The presence of old mining waste around the lake shore causes heavy metals such as chromium, nickel, molybdenum, and iron to be transported to the lake. They are mainly transferred to the lake after the rain. In addition to metals and agriculture, the inadequacy of water treatment at certain points of the lake threatens it with pollution [2]. Previous works has shown high values of chromium, nickel, iron and molybdenum in sediment [11, 22]. According to Malaj et al. mining points have had exceptionally high values of a number of metals in sediment compared to Lin and the points that were far from the mines. Also some macrophytes grown in Lake Ohrid as Chara tomentosa have accumulated higher amounts of some heavy metals especially Fe and Ni compared to other metals such as Cr and Cu, whereas accumulation of Pb and Cd has been small. The accumulation was larger at 5 m depth than at 10 m depth except for Pb and Cd that showed no significant difference at these two depths [20].

The presence of metal in high amounts of sediment causes them to accumulate from aquatic plants of roots. Their accumulation varies from plant to plant and their main accumulation is in the root [3]. One of these plants that grow in the lake Ohrid is common reed (*Phragmites australis*) [1]. Heavy metals are considered as environmental pollutants that cause physiological reactions to plants. One of the risks that can cause plants is ROS production. ROS changes normal cellular physiology by degrading proteins, lipids, nucleic acids and enzymes [14]. As a marker that evaluates the degree of lipid peroxidation is malondialdehyde (MDA). There are numerous researches carried out on plants especially on leaves of plant during which MDA is estimated [7,9,12,16]. Malar et al., investigated the impact of lead on MDA in water hyacinths by treating it with different lead doses of 100 to 100 mg/L with a determination that there is an increase in MDA values up to 400 mg/L. But then at higher doses there was a decrease in MDA [12]. The study realized by Jyknus et al. analyzed the impacts of some heavy metals like Cu, Zn, Ni, Cr, Pb, Cd on oxidative stress and growth of spring barley. These metals induced MDA levels with increased metal concentration. In addition to the MDA increase, there has been a reduction of the dry biomass. A moderate increase in MDA has been the case with chromium and nickel treatment. Since Cr has been continuously increasing with dose increase, the highest dose of 1000 micromol has been shown to decrease [9]. A research conducted by Rahman et al. has shown that chromium treatment with different concentrations of 0.5 to 3 mg/L in mangrove seedlings has been continuously increasing MDA and the maximum value reached in 3 mg/L. The change was not significant only in the lowest dosage of treatment compared to the control [16]. Treatment with Co, Ni, Cd (as SO_4), Cr (as dichromate) and Pb (as nitrate) at the rate of 0.25 mM has caused significant increase in MDA in the case of Cr, Co, Cd and Pb [7].

4. Conclusions

Our results showed that a further study on MDA as a biomarker of oxidative stress on various parts of the plant and even in various aquatic plants should be continued in the Albanian part of Lake Ohrid.

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