ISSN 1454 - 8003
Proceedings of 2023 International Conference on Hydraulics and Pneumatics - HERVEX
November 8-10, Băile Govora, Romania

MEASURING CATALASE ACTIVITY IN LI TREATED AQUATIC PLANT TISSUES

Anamaria Iulia TÖRÖK [1], Eniko KOVACS [1,2,*], Erika Andrea LEVEI[1]

[1] INCDO-INOE 2000, Research Institute for Analytical Instrumentation, 67 Donath Street, 400293 Cluj-Napoca, Romania

[2] Faculty of Horticulture, University of Agricultural Sciences and Veterinary Medicine, 3-5 Manastur Street, 400372 Cluj-Napoca, Romania

* eniko.kovacs@icia.ro

Abstract: *Living organism's metabolism can respond to environmental stress through their antioxidant system. The catalase enzyme regulates the H_2O_2 levels protecting the cells against oxidative damage caused by abiotic stress. Lemna minor are ideal test plants for the detection of metal pollution. Therefore, in the present study Lemna minor plant tissue exposed to various lithium concentrations (C_{Li} = 0, 1, 3, 5 mg/L) was used to assess the changes in the catalase enzyme activity. Moreover, a quantification method for Catalase antioxidant activity using a nano UV-Vis spectrophotometer determination was developed. It was observed that high lithium concentrations (5 mg/L) can negatively affect the catalase enzyme activity, decreasing the activity by 71% compared with the control plants (C_{Li} = 0 mg/L). It has been shown that quantification of the catalase enzyme activity can be successfully used as an important signalling biomarker to the induced lithium stress.*

Keywords: *Catalase enzyme, Lemna minor, lithium, enzyme extraction, defense mechanism*

1. Introduction

Catalase (CAT, EC 1.11.1.6) is an antioxidant enzyme found in all living organisms in the cellular peroxisome compartment [1]. Living cells developed several antioxidant mechanisms to regulate the reactive oxygen species (ROS) during various oxidative stress, such as heat, cold, inorganic and organic pollutants [2]. The CAT enzyme plays an essential role in the dissociation of hydrogen peroxide (H_2O_2) into molecular oxygen (O_2) and water (H_2O) [2]. CAT is linked to peroxidases and helps to protect cells from oxidative damage caused by free radicals. Under stress conditions, plants trigger de novo synthesis of H_2O_2 through xanthine and NADPH dependent oxidase, which has been identified as a signalling molecule involved in essential and complex mechanisms of redox homeostasis [3]. Generally, plants, such as peas, beans, rice, and cabbage, show a reduced CAT activity following exposure to Zn, Cr, Cd, As or other metals [1].
The most common CAT activity quantification method is based on the UV-Vis spectrophotometric technique which involves tracking the H_2O_2 consumption in time by measuring the absorbance at 240 nm [4]. The absorbance differences per unit of time define the CAT activity [5].
The nano spectrophotometers are ideal for small volume samples and have capillary-type or sample plate containers to measure low or microvolume samples ranging from 0.5 to 5 µl, as well as the possibility to measure using a quartz cuvette and measure absorbance with a high degree of accuracy and reproductivity
Aquatic plants are emerging biorenewable energy sources due to their aggressive growing and regeneration characteristics, are considered sustainable, have high clean energy potentials, can generate sugar and starch contents can be generated, and the biomass is easily convertible into renewable energy [6]. Duckweed, also known as *Lemna minor* from the Lemnaceae family, is a freshwater aquatic plant highly used for monitoring and toxicity test procedures due to its morphological characteristics and high adaptation capacity to various environmental conditions [7]. Duckweed has a high rate of growth and biomass production, as well as high amino acids and protein content [7, 8]. *Similarly, to other aquatic plants, Lemna minor* have also applications in

phytoremediation (bioaccumulation and biodegradation) of toxic organic and inorganic pollutants, biomass feedstock for biogas, biofuels, biorefineries, and as animal feed [6, 9].

Lithium (Li) is a naturally occurring trace element which can be found in water, soil, rocks and certain minerals (such as amblygonite, petalite, lepidolite, spodumene) [10]. Furthermore, the Li concertation is highly dependent on a region's geology, topography, and hydrogeology. It can be easily washed out after a rainfall and it can be detected in ground water as a result of mineral weathering. Generally, the drinking water Li concentrations can range from <1 to 219 µg/L [10].

Different plant species can absorb a high amount of Li and positively affect growth and development [11]. However, plant species have Li uptake capacities with specific toxicity symptoms [12]. Several studies are focused on the investigation of the role of Li, modifications, and toxicity signalling in the living organism in contact with various Li concentrations. A low level of Li can improve plant productivity by increasing the growth yield, hastening maturation, or increasing disease resistance [13]. This research aimed to optimize and develop a quantification method for CAT antioxidant activity using a nano spectrophotometer in order to assess the changes in the CAT enzyme activity following exposure of *Lemna minor* to various lithium concentrations (Li, C_{Li} = 0, 1, 3, 5 mg/L).

2. Material and methods

2.1 Plant Material and Growth Conditions

Lemna minor aquatic plants were grown in Hoagland's nutrient solution containing 1.25 mM KNO_3, 1.25 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.25 mM KH_2PO_4, 10 µM FeEDTA, 11.6 µM H_3BO_3, 4.5 µM $MnCl_2 \cdot 4H_2O$, 0.19 µM $ZnSO_4 \cdot 7H_2O$, 0.12 µM $Na_2MoO_4 \cdot 2H_2O$, and 0.08 µM $CuSO_4 \cdot 5H_2O$, the pH of the medium was adjusted to 5.5 (±0.05) with 0.01 M KOH. A ten-hour photoperiod and a day/night temperature of 20 ±2°C was used during the experiments. After a growth period of 30 days the plants were transplanted in smaller pots. After two days of adaptation, the nutrient medium was enriched with Li at final concentrations of 0, 1, 3 and 5 mg/L Li (m = 4 g plant, v = 400 mL) prepared from Li_2SO_4. After 7 days of exposure to Li, the plants were harvested and frozen for further analysis.

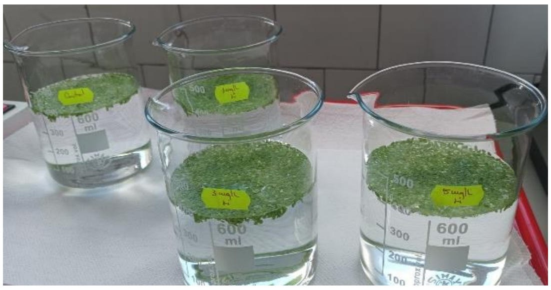

Fig. 1. *Lemna minor* aquatic plant exposed to 0, 1, 3, 5 mg/L Li

2.2 Antioxidant enzyme extraction

The frozen plant tissues were ground, then 0.5 g of ground tissues were homogenized in 5 mL of 100 mM potassium phosphate buffer (pH 7.5), containing 1 mM EDTA. The extract was mixed well, centrifuged for 30 min at 10,000 x g at 4°C, then the supernatant was used as a source of crude enzyme for the assay of CAT activities. The supernatant was stored at -20 °C until the CAT activity assays were performed.

2.3 Antioxidant enzyme activity assay

The plant extracts CAT activity was determined using the Nanodrop One Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) UV-Vis spectrophotometer, at a wavelength of 240 nm by monitoring the variation in H_2O_2 absorption, according to the suggestions of Aebi (1984) [5], Sarker et al. (2018) [14] Barbosa et al. (2019) [15] and Senthilkumar et al. (2021) [16]. A volume of 0.5 mL of 75 mH H_2O_2 with 1.5 ml of 100 mM phosphate buffer (pH 7.00) were used as reaction mixtures for each plant extracts. The reaction mixtures were without the plant extract was used as blank sample. A volume of 1 ml of plant enzyme extract was added to the reaction mixture to obtain a 3 mL final volume. The reaction started after adding the plant extracts to the reaction mixtures, and the absorbance change was monitored for 2 min. The total CAT activities were expressed as μmol min⁻1 mg⁻1 protein, calculated by the following formula Eq (1); where A_0 and A_{180} is the initial and final absorbance, V_t is the final volume of the reaction mixture (3 mL), ε_{240} is the molar extinction coefficient for H_2O_2 (34.9 mol^{-1} cm^{-1}), and d is the optical path of the cuvette (1 cm), V_s is the volume of the sample (1 mL), C_t is the total protein concentration in the samples, and 0.01 is the absorbance change that caused by 1 U (one unit) of enzyme per min at 240 nm.

$$U/mg = (A_0 - A_{180}) \times V_t/\varepsilon_{240} \times d \times V_s \times C_t \times 0.001 \quad (1)$$

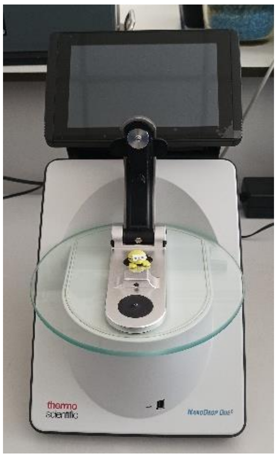

Fig. 2. Nanodrop One Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), UV-Vis Spectrophotometer

3. Results and discussion

In general, the CAT enzyme activity quantification is a low-cost and rapid method used to evaluate the effect of oxidative stress on metal-exposed plants. In the first step, the plant tissues are homogenized in an ice-cold buffer devoid of other antioxidants [17]. The CAT enzyme activity quantification was performed at 20°C in the current study, however, it can be determined between 0-37°C, as the temperature differences in this range do not affect the assay [16]. In the present study, the CAT enzyme assay was optimized for the quantification with a spectrophotometer for aquatic plant tissues. The extraction method was performed with a high repeatability and accuracy rate.

The Li exposure (0, 3, 5 mg/L) induced the decrease of CAT antioxidants (Fig. 1). In the present study, the highest CAT activity was observed in the case of the control plants (0 mg/L Li). By increasing the Li concentrations in the Hoagland's nutrient solution, the exposed plants showed lower CAT activity with 44% in the case of 1 mg/L Li, 61% at 3 mg/L Li and 71% at 5 mg/L Li. In contrast with our results, Zn acts as a biostimulator and activates the CAT enzyme activity as the Zn levels are increased to a maximum 1.5 mg/L concentration [18]. Moreover, Parlack et al. (2012) found that *L. gibba*, *L. minor* and *S. polyrrhiza* plant can adapt to Zn toxicity through the antioxidant defence system [18].

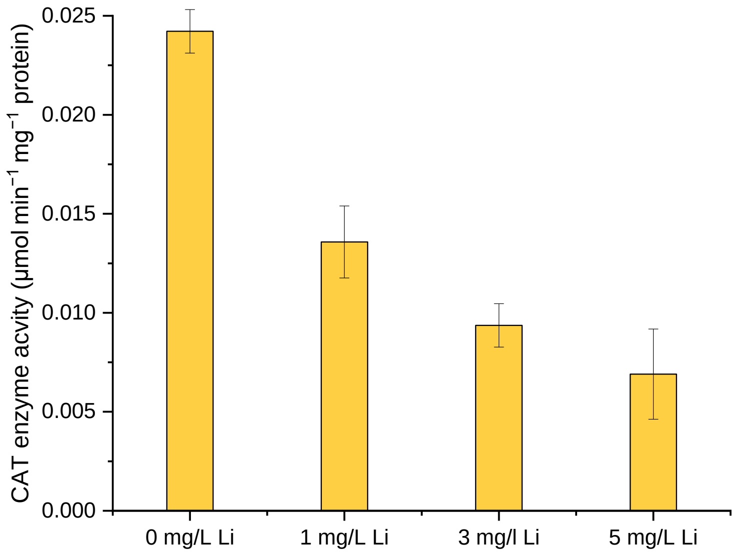

Fig. 3. Catalase enzyme activity of the *Lemna minor* aquatic plants exposed to 0, 1, 3 and 5 mg/L Li

4. Conclusions

Lemna minor aquatic plant tissue was utilized for the catalase enzyme extraction to assess the impact of lithium exposure. The catalase activity was measured after seven days of exposure to concentrations of 0, 1, 3 and 5 mg/L Li. The catalase activity of the plant extracts was measured by a nano UV-Vis spectrophotometer at a wavelength of 240 nm by determining the variation in H_2O_2 absorption. Lithium had a negatively affected the antioxidant defence mechanism of *Lemna minor*.

Acknowledgments

This research was funded by the Ministry of Research, Innovation and Digitization through Program 1—Development of the national research & development system, Subprogram 1.2—Institutional Performance—Projects that finance RDI excellence, Contract No. 18PFE/30.12.2021 and Core Program within the National Research Development and Innovation Plan 2022–2027, carried out with the support of MCID, project No. PN 23 05.

References

[1] Gangwar, Savita, Vijay Pratap Singh, Durgesh Kumar Tripathi, Devendra Kumar Chauhan, Sheo Mohan Prasad, and Jagat Narayan Maurya. "Chapter 10 - Plant Responses to Metal Stress: The Emerging Role of Plant Growth Hormones in Toxicity Alleviation." In *Emerging Technologies and Management of Crop Stress Tolerance*, edited by Parvaiz Ahmad and Saiema Rasool, 215-48. San Diego, Academic Press, 2014.
[2] Olson, K. R., Y. Gao, E. R. DeLeon, M. Arif, F. Arif, N. Arora, and K. D. Straub. "Catalase as a Sulfide-Sulfur Oxido-Reductase: An Ancient (and Modern?) Regulator of Reactive Sulfur Species (Rss)." *Redox Biol* 12 (August 2017): 325-39.
[3] Montavon, P., K. R. Kukic, and K. Bortlik. "A Simple Method to Measure Effective Catalase Activities: Optimization, Validation, and Application in Green Coffee." *Anal Biochem* 360, no. 2 (January 2007): 207-15.
[4] Hadwan, Mahmoud Hussein. "Simple Spectrophotometric Assay for Measuring Catalase Activity in Biological Tissues." *BMC Biochemistry* 19, no. 1 (August 2018): 7.
[5] Aebi, H. "Catalase in Vitro." *Methods Enzymol* 105 (1984): 121-6.
[6] Arefin, Md Arman, Fazlur Rashid, and Amirul Islam. "A Review of Biofuel Production from Floating Aquatic Plants: An Emerging Source of Bio-Renewable Energy." *Biofuels, Bioproducts and Biorefining* 15, no. 2 (2021): 574-91.

[7] Pagliuso, Débora, Adriana Grandis, Janaina S. Fortirer, Plinio Camargo, Eny I. Floh, and Marcos S. Buckeridge. "Duckweeds as Promising Food Feedstocks Globally." *Agronomy* 12, 4 (2022): 796.
[8] Mohedano, Rodrigo A., Rejane H. R. Costa, Flávia A. Tavares, and Paulo Belli Filho. "High Nutrient Removal Rate from Swine Wastes and Protein Biomass Production by Full-Scale Duckweed Ponds." *Bioresource Technology* 112 (May 2012): 98-104.
[9] Krzywonos, Małgorzata, Zdzisława Romanowska-Duda, Przemysław Seruga, Beata Messyasz, and Stanisław Mec. "The Use of Plants from the Lemnaceae Family for Biofuel Production—a Bibliometric and in-Depth Content Analysis." *Energies* 16, no. 4 (2023): 2058.
[10] Barjasteh-Askari, F., M. Davoudi, H. Amini, M. Ghorbani, M. Yaseri, M. Yunesian, A. H. Mahvi, and D. Lester. "Relationship between Suicide Mortality and Lithium in Drinking Water: A Systematic Review and Meta-Analysis." *J Affect Disord* 264 (March 2020): 234-41.
[11] Shahzad, B., M. Tanveer, W. Hassan, A. N. Shah, S. A. Anjum, S. A. Cheema, and I. Ali. "Lithium Toxicity in Plants: Reasons, Mechanisms and Remediation Possibilities - a Review." *Plant Physiol Biochem* 107 (October 2016): 104-15.
[12] Kuloğlu, Selin Sipahi, Emine Yalçin, Kültiğin Çavuşoğlu, and Ali Acar. "Dose-Dependent Toxicity Profile and Genotoxicity Mechanism of Lithium Carbonate." *Sci Rep* 12, no. 1 (August 2022): 13504.
[13] Shahzad, Babar, Mohsin Tanveer, Waseem Hassan, Adnan Noor Shah, Shakeel Ahmad Anjum, Sardar Alam Cheema, and Iftikhar Ali. "Lithium Toxicity in Plants: Reasons, Mechanisms and Remediation Possibilities – a Review." *Plant Physiology and Biochemistry* 107 (October 2016): 104-15.
[14] Sarker, U., and S. Oba. "Catalase, Superoxide Dismutase and Ascorbate-Glutathione Cycle Enzymes Confer Drought Tolerance of Amaranthus Tricolor." *Sci Rep* 8, no. 1 (November 2018): 16496.
[15] Barbosa Neto, A. G., M. B. Morais, E. D. Dutra, and T. Calsa Junior. "Biological Diversity of Lemna Aequinoctialis Welw. Isolates Influences Biomass Production and Wastewater Phytoremediation." *Bioresource Technology Reports* 6 (June 2019): 251-59.
[16] Senthilkumar, M., N. Amaresan, and A. Sankaranarayanan. "Estimation of Catalase." In *Plant-Microbe Interactions: Laboratory Techniques*, edited by M. Senthilkumar, N. Amaresan, and A. Sankaranarayanan, 113-15. New York, Springer US, 2021.
[17] Gagné, François. "Chapter 6 - Oxidative Stress." In *Biochemical Ecotoxicology*, edited by François Gagné, 103-15. Oxford: Academic Press, 2014.
[18] Uruç Parlak, Kadiriye, and Dilek Demirezen Yilmaz. "Response of Antioxidant Defences to Zn Stress in Three Duckweed Species." *Ecotoxicology and Environmental Safety* 85 (November 2012): 52-58.