30th International Conference Ecological Truth & Environmental Research 2023

Proceedings

Editor
Prof. Dr Snežana Šerbula

University of Belgrade, Technical Faculty in Bor

30

ECOTER

30th International Conference Ecological Truth & Environmental Research 2023

Proceedings

Editor
Prof. Dr Snežana Šerbula

PROCEEDINGS

30th INTERNATIONAL CONFERENCE

ECOLOGICAL TRUTH AND ENVIRONMENTAL RESEARCH – EcoTER'23

Editor:

Prof. Dr Snežana Šerbula

University of Belgrade, Technical Faculty in Bor

Editor of Student section:

Prof. Dr Maja Nujkić

University of Belgrade, Technical Faculty in Bor

Technical editors:

Jelena Milosavljević, PhD, University of Belgrade, Technical Faculty in Bor

Asst. prof. Dr Ana Radojević, University of Belgrade, Technical Faculty in Bor

Sonja Stanković, **MSc**, University of Belgrade, Technical Faculty in Bor

Cover design:

Aleksandar Cvetković, **BSc**, University of Belgrade, Technical Faculty in Bor

Publisher: University of Belgrade, Technical Faculty in Bor

For the publisher: Prof. Dr Dejan Tanikić, Dean

Printed: University of Belgrade, Technical Faculty in Bor, 100 copies, electronic edition

Year of publication: 2023

ISBN 978-86-6305-137-9

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

502/504(082)(0.034.2)
574(082)(0.034.2)

INTERNATIONAL Conference Ecological Truth & Environmental Research (30 ; 2023)
Proceedings [Elektronski izvor] / 30th International Conference Ecological Truth & Environmental Research - EcoTER'23, 20-23 June 2023, Serbia ; organized by University of Belgrade, Technical faculty in Bor (Serbia) ; co-organizers University of Banja Luka, Faculty of Technology – Banja Luka (B&H) ... [et al.] ; [editor Snežana Šerbula]. - Bor : University of Belgrade, Technical faculty, 2023 (Bor : University of Belgrade, Technical faculty). - 1 elektronski optički disk (CD-ROM) ; 12 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Preface / Snežana Šerbula. - Tiraž 100. - Bibliografija uz svaki rad.

ISBN 978-86-6305-137-9

а) Животна средина -- Зборници б) Екологија – Зборници

COBISS.SR-ID 118723849

30th International Conference
Ecological Truth and Environmental Research – EcoTER'23

is organized by:

UNIVERSITY OF BELGRADE
TECHNICAL FACULTY IN BOR (SERBIA)

Co-organizers of the Conference:

**University of Banja Luka, Faculty of Technology,
Banja Luka (B&H)**

**University of Montenegro, Faculty of Metallurgy and Technology,
Podgorica (Montenegro)**

University of Zagreb, Faculty of Metallurgy, Sisak (Croatia)

**University of Pristina, Faculty of Technical Sciences,
Kosovska Mitrovica**

Association of Young Researchers Bor (Serbia)

HONORARY COMMITTEE

Dr. Petar Paunović
(Zaječar, Serbia)
Prof. Dr Zvonimir Stanković
(Bor, Serbia)
Prof. Dr Velizar Stanković
(Bor, Serbia)
Prof. Dr Milan Antonijević
(Bor, Serbia)
Dragan Ranđelović, Association of Young Researchers Bor
(Bor, Serbia)
Toplica Marjanović, Association of Young Researchers Bor
(Bor, Serbia)
Mihajlo Stanković, Special Nature Reserve Zasavica
(Sremska Mitrovica, Serbia)

SCIENTIFIC COMMITTEE

Prof. Dr Snežana Šerbula, *President*

Prof. Dr Alok Mittal (India)
Prof. Dr Jan Bogaert (Belgium)
Prof. Dr Aleksandra Nadgórska-Socha (Poland)
Prof. Dr Luis A. Cisternas (Chile)
Prof. Dr Wenhong Fan (China)
Prof. Dr Martin Brtnický (Czech Republic)
Prof. Dr Isabel M. De Oliveira Abrantes (Portugal)
Prof. Dr Shengguo Xue (China)
Prof. Dr Tomáš Lošák (Czech Republic)
Prof. Dr Maurice Millet (France)
Prof. Dr Murray T. Brown (New Zealand)
Prof. Dr Xiaosan Luo (China)
Prof. Dr Daniel J. Bain (United States of America)
Prof. Dr Che Fauziah Binti Ishak (Malaysia)
Prof. Dr Richard Thornton Baker (United Kingdom)
Prof. Dr Mohamed Damak (Tunisia)
Prof. Dr Jyoti Mittal (India)
Prof. Dr Miriam Balaban (United States of America)
Prof. Dr Yeomin Yoon (United States of America)
Prof. Dr Chang-min Park (South Korea)
Prof. Dr Faramarz Doulati Ardejani (Iran)
Prof. Dr Ladislav Lazić (Croatia)
Prof. Dr Natalija Dolić (Croatia)
Prof. Dr Milutin Milosavljević (Kosovska Mitrovica)
Prof. Dr Nenad Stavretović (Serbia)
Prof. Dr Ivan Mihajlović (Serbia)
Prof. Dr Milovan Vuković (Serbia)
Prof. Dr Nada Blagojević (Montenegro)
Prof. Dr Darko Vuksanović (Montenegro)
Prof. Dr Irena Nikolić (Montenegro)
Prof. Dr Šefket Goletić (B&H)
Prof. Dr Džafer Dautbegović (B&H)
Prof. Dr Borislav Malinović (B&H)
Prof. Dr Slavica Sladojević (B&H)
Prof. Dr Nada Šumatić (B&H)
Prof. Dr Snežana Milić (Serbia)

ORGANIZING COMMITTEE

Prof. Dr Snežana Šerbula, *President*

Prof. Dr Snežana Milić, *Vice President*

Prof. Dr Đorđe Nikolić, *Vice President*

Prof. Dr Marija Petrović Mihajlović

Prof. Dr Milan Radovanović

Prof. Dr Milica Veličković

Prof. Dr Danijela Voza

Prof. Dr Maja Nujkić

Prof. Dr Žaklina Tasić

Dr Ana Simonović

Dr Tanja Kalinović

Dr Ana Radojević

Dr Jelena Kalinović

Dr Jelena Milosavljević

Sonja Stanković, MSc

Miljan Marković, MSc

Vladan Nedelkovski, MSc

Aleksandar Cvetković, BSc

EFFECTS OF SO_2 AND NO_2 ON THE PHOTOSYNTHETIC EFFICIENCY AND CATALASE ANTIOXIDATIVE ENZYME ACTIVITY IN *Betula pendula* Roth

Dragana Pavlović[1*], Marija Matić[1], Veljko Perović[1], Olga Kostić[1], Dimitrije Sekulić[1], Miroslava Mitrović[1], Pavle Pavlović[1]

[1]Department of Ecology, Institute for Biological Research 'Siniša Stanković' – National Institute of the Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, SERBIA

[*]*dragana.pavlovic@ibiss.bg.ac.rs*

Abstract

Air pollution in urban environment is one of the major stressors for vegetation. The aim of the present study was to investigate the levels of sulfur dioxide (SO_2) and nitrogen dioxide (NO_2) in the air in Belgrade and Smederevo and their effects on photosynthetic efficiency and catalase enzyme activity of Betula pendula Roth*. It was found that SO_2 and NO_2 concentrations increased from June to October at both studied sites but did not exceed the limits set by the national regulation. NO_2 concentrations above the limits were measured only in October in Belgrade. It was also found that the values of the parameter of photosynthetic efficiency (Fv/Fm) were within the optimal range determined for deciduous trees, except in June in Belgrade, indicating that birch has optimal photosynthetic efficiency. Average catalase activity increased during the course of season at both sites. The lowest enzyme activity was measured in June in Belgrade and the highest in October in Smederevo. It was be concluded that under the given environmental conditions, the birch trees show no signs of damage and that the basic physiological processes are running at an optimal level. The increase in photosynthetic efficiency and catalase activity in birch leaves in the second part of the season could represent some kind of adaptation mechanism that allows it to survive under unfavorable environmental conditions.*

Keywords: air pollution, photosynthetic efficiency, catalase enzyme activity, *Betula pendula* Roth.

INTRODUCTION

Air pollution is one of the most serious environmental problems, especially in the urban environment where most of the world's population lives. Urban air quality is continuously affected by emissions from stationary and mobile combustion sources, which generate a large number of pollutants whose chemical composition is extremely heterogeneous [1]. Sulphur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), and ozone (O_3) are the main hazardous air pollutants responsible for acid rain formation, crop yield decline, and ecological damage [2–4]. Sulphur dioxide is known as a primary pollutant that is produced in large quantities from the combustion of coal and other fuels in industry and households [4]. Unlike SO_2, NO_2 belongs to both primary and secondary pollutants and is a precursor of harmful secondary pollutants such as ozone. The major anthropogenic sources of NO_2 are industrial fossil fuel combustion, vehicle exhaust, biomass burning, and electricity generation [4]. At higher concentrations, SO_2 and NO_2 cause oxidative damage to biochemical and physiological

processes in plants. However, plants have several enzymatic and non-enzymatic antioxidant defense mechanisms to prevent oxidative damage [5,6]. Non-enzymatic antioxidants include glutathione, carotenoids, tocopherols, and flavonoids, while catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), etc. are antioxidant enzymes of plants [6,7].

In this research, the concentrations of SO_2 and NO_2 in the air in Belgrade and Smederevo in June and October were monitored in order to determine whether they exceed the limits set by the Regulation on Monitoring Conditions and Requirements for Air Quality [8] and how these pollutants affect the efficiency of photosynthesis and the activity of catalase enzyme activity in *Betula pendula* trees.

MATERIALS AND METHODS

Sampling and field research

Photosynthetic efficiency and collection of leaf samples of *Betula pendula* Roth for determination of catalase enzyme activity were carried out in urban parks in Belgrade and Smederevo, exposed to different sources of pollution, in June and October 2012. In Belgrade, the samples were taken in the park "Hall Pioneer", which is located near several main roads, where traffic exhaust is a main source of pollution, while in Smederevo the samples were taken in the park "Tri heroja", which is about 7 km away from the main source of pollution "Železara Smederevo".

The data describing SO_2 and NO_2 concentrations in Belgrade and Smederevo during 2012 were obtained from the Serbian Environmental Protection Agency, which summarises data from the network of local air quality monitoring stations in Belgrade and Smederevo.

Photosynthetic efficiency was measured using the method of induced chlorophyll fluorescence kinetics of photosystem II. Measurements were taken with the aid of a portable Plant Stress Meter (BioMonitor S.C.I. AB, Sweden), as described by Krause and Weis [9]. Chlorophyll was excited for 2 s by actinic light with a photon flux density of 200 and 400 μmol m^{-2} s^{-1}. Prior to measuring, samples were adapted to the dark for approximately 30 min. [10–12]. Measurements were performed in thirty repetitions (n=30).

Leaf extract preparation and enzyme activity assays

Birch leaves were placed in liquid nitrogen immediately after sampling and stored at a temperature of -80°C until analysis. For enzyme extraction, the frozen leaf tissue was homogenized in a mortar with pestle and liquid nitrogen and then extracted in ice-cold 0.1 M potassium phosphate extraction buffer (pH 6.5) containing 3% polyvinylpyrrolidine (PVP) and 5% phenylmethanesulfonyl fluoride (PMSF). After homogenization, the crude leaf extracts were centrifuged at 14,000 x g at 4°C for 20 min, and the supernatants obtained were aliquoted and used to measure protein content and enzyme activity. Protein content was determined according to Bradford [13], using bovine serum albumin (BSA) as a standard. Catalase activities were determined spectrophotometrically in duplicate at 20°C using a Shimadzu UV-160 spectrophotometer. Catalase activity was measured by adding 10 μl of the enzyme extract to 1 ml of a reaction mixture containing 50 mM K-phosphate buffer (pH 7)

and 30% H_2O_2 and measuring the changes in absorbance at 240 nm for 3 min [14]. The results of catalase activity were expressed in units per mg of protein (U mg^{-1}).

Statistical analysis

The data from this research was analyzed using statistical analysis (ANOVA) and means were separated with a Bonferroni test at a level of significance of $p<0.05$, using the Statistica software package [15].

RESULTS AND DISCUSSION

Concentrations of SO_2 and NO_2 in Belgrade and Smederevo in June and October 2012 are shown in Figure 1.

Concentrations of SO_2 and NO_2 increased at both study sites during the season. The highest SO_2 concentrations were measured in Belgrade in October (23.69 μg m^{-3}) and did not exceed the limits set by the Regulation on Monitoring Conditions and Requirements for Air Quality (50 μg m^{-3}) [8]. NO_2 concentrations ranged from 10.60 μg m^{-3} in June in Smederevo to 42.56 μg m^{-3} in October in Belgrade, where the limit values (40 μg m^{-3}) [8] were only exceeded during the course of this research. It is clear that the studied birch individuals were exposed to lower concentrations of gaseous pollutants (SO_2 and NO_2) at the beginning of the growing season in relation to at the end of the season. Throughout the season, individuals from Belgrade were significantly more affected than those from Smederevo ($p<0.001$).

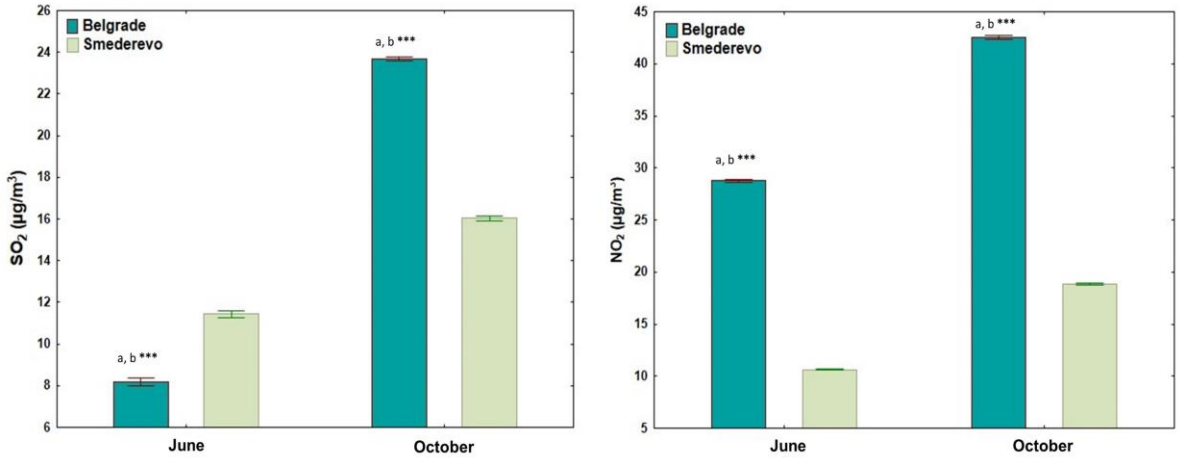

Figure 1 *SO_2 and NO_2 concentrations in Belgrade and Smederevo in June and October of 2012 (Source: Serbian Environmental Protection Agency)*

The variation of photosynthetic efficiency and catalase activity in samples of birch leaves in Belgrade and Smederevo are shown in Figure 2.

The analysis of the parameter Fv/Fm in birch showed a regularity in the seasonal dynamics, with the minimum values measured in June and the maximum in October at both study sites. It was also found that the Fv/Fm values in birch from Belgrade (0.731–0.781) and from Smederevo (0.773–0.783) were within the optimal range for deciduous trees (0.750–0.850) [16], except in June in Belgrade. This result indicates that SO_2 and NO_2 may have negative effects on birch, but not to an extent that would seriously threaten its

functioning. Nevertheless, the higher SO_2 and NO_2 concentrations in Belgrade affected photosynthetic efficiency, resulting in lower Fv/Fm in Belgrade compared to Smederevo in both seasonal periods, whereas this difference was significant only in June ($p<0.001$), Figure 2. Photosynthesis is one of the first processes affected by high SO_2 concentration [17], but when plants are exposed to low SO_2 concentrations, the absorbed SO_2 is oxidized and used to synthesize proteins. NO_2 is also known as a toxic gaseous pollutant that reduces net photosynthetic rate and chlorophyll content and inhibits photosynthesis at elevated concentrations. However, some relevant studies have found that plants exposed to moderate levels of atmospheric NO_2 for extended periods of time exhibit increased absorption and metabolism of nutrients used by plants for growth and development [18–20]. In general, whether SO_2 and NO_2 have adverse effects on plants under certain conditions is species-specific and depends on their concentration, the time period the plant is exposed to them, and the rate at which the plant takes them up [17,20,21].

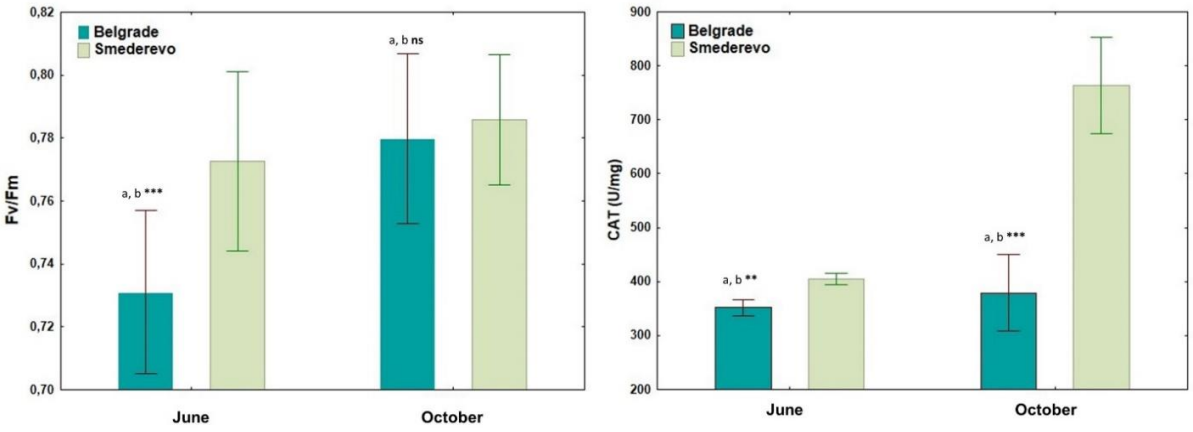

Figure 2 *The variation of photosinthetic efficiency (Fv/Fm) and catalase enzyme activity (CAT) in birch leaves in Belgrade and Smederevo in June and October 2012*

Average catalase enzyme activity increased over the season at both sites. The lowest enzyme activity was measured in June in Belgrade (351.71 U mg^{-1}) and the highest in October in Smederevo (763.55 U mg^{-1}). The increase in enzyme activity during the season can be associated with the increase in SO_2 and NO_2 concentrations. Similar effects of pollution on enzymatic activity of catalase were found by Ghorbanli *et al.* [22] who discovered that the activity level of catalase enzyme was increased in *Nerium oleander* and *Robinia pseudo acacia* leaves collected from the contaminated sampling site compared to the control site, but that increase was not statistically significant. However, from the results presented here, it is also evident that catalase activity is higher in birch trees from Smederevo, although SO_2 and NO_2 levels were lower than in Belgrade. Such result is not unusual, considering that the mechanisms of plant resistance under stress conditions are very complex and include several physiological and biochemical strategies aimed at mitigating the negative effects of various environmental stress factors through their joint action [23]. Prysedskyj [24] studied the influence of the type of complex compounds nitrogen and sulfur on catalase activity of selected tree and shrub species and concluded that the influence of pollutants on catalase activity depends on plant resistance, structure, and duration of pollutant effectiveness. He

found that in contrast to less resistant plants, species resistant to pollution are characterized by the absence of changes in catalase activity, i.e. an increase in catalase function due to the short-term effect of pollutants [24].

CONCLUSION

From the obtained results, it can be concluded that under the given environmental conditions, the birch trees do not show any signs of morphological damage and the basic physiological processes are at an optimal level. The increase in the value of the photosynthetic efficiency parameter and catalase activity in the second part of the vegetation period could be considered as an adaptation mechanism of the birch that allows it to survive in unfavourable site conditions.

ACKNOWLEDGEMENT

The authors are grateful to the Ministry of Science, Technological Development and Innovation of the Republic of Serbia for financial support according to the contract with the registration number (451-03-47/2023-01/200007).

REFERENCES

[1] Fusaro L., Salvatori E., Winkler A., *et al*., Ecol. Indic. 126 (2021) 107707.

[2] Mukherjee A., Agrawal S. B., Agrawal M. Sci. Total Environ. 710 (2020) 136363.

[3] Aghajanzadeh T. A., Otaghsara S. H. T., Jafari N., *et al*., J. Plant Physiol. Breed. 11 (1) (2021) 49–62.

[4] Wang Y., Jin W., Che Y., *et al*., Forests 10 (2019) 312.

[5] Li L., Yi H., Plant Physiol. Biochem. 58 (2012) 46–53.

[6] Hasanuzzaman M., Bhuyan M. H. M. B., Zulfiqar F., *et al*., Antioxidants 9 (2020) 681.

[7] Dumanović J., Nepovimova E., Natić M., *et al*., Front. Plant Sci. 11 (2021) 552969.

[8] OGRS (2010/2013) Regulation on Monitoring Conditions and Requirements for Air Quality. Official Gazette of the Republic of Serbia. No. 11/10; 75/10; 63/13.

[9] Krause H.M., Wei E. Annu. Rev. Plant Physiol. 42 (1991) 313–349.

[10] Mitrović M., Pavlović P., Lakušić D., *et al*., Sci. Total Environ. 407 (2008) 338–347.

[11] Gajić G., Mitrović M., Pavlović P., *et al*., Ecotoxicol. Environ. Saf. 72 (2009) 1090–1101.

[12] Kostić O., Mitrović M., Knežević M., *et al*., Arch. Biol. Sci. 64 (1) (2012) 145–158.

[13] Bradford M.M. Anal. Biochem. 72 (1976) 248–254.

[14] Aebi H. Meth. Enzymol. 105 (1984) 121–126.

[15] SPSS (2007) SYSTAT Version 16: Statistics. SPSS, Chicago, IL.

[16] Björkman O., Demmig B. Planta 170 (1987) 489–504.

[17] Lee H.K., Khaine I., Kwak M. J., *et al*., Hortic. Environ. Biotechnol. 58 (6) (2017) 523–529.

[18] Takahashi M., Nakagawa M., Sakamoto A., *et al*., New Phytol. 168 (2005) 149–154.

[19] Adam S.E.H., Shigeto J., Sakamoto A., *et al*., Botany 86 (4) (2008) 442–442.

[20] Jiechen W., Yue W., Huihui Z., *et al*., J. Plant Interact. 16 (1) (2021) 422–431.

[21] Hu Y., Bellaloui N., Tigabu M., *et al*., Acta Physiol. Plantarum 37 (2015) 39.

[22] Ghorbanli M., Bakand Z., Bakhshi khaniki G., *et al*., Iran. J. Environ. Health Sci. Eng. 4 (3) (2007) 157–162.

[23] Skrypnik L., Maslennikov P., Feduraev P., *et al*., Plants 10 (2021) 1871.

[24] Prysedskyj Y., Biosyst. Divers. 24 (2) (2016) 295–301.