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Assessing Environmental Pollution in Kosovo's Industrial Areas Using Plant Bioindicators

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ABSTRACT

Environmental pollution is a global issue, and Kosovo is no exception, grappling with extensive mining activities. The research aims to assess the influence of industrial pollution in Drenas and Mitrovica using oxidative stress biomarkers (malondialdehyde – MDA, and the amount of glutathione transferase – GST) and biochemical indicators (total proteins) in three plant species (*Achillea millefolium*, *Hypericum perforatum* and *Plantago lanceolata*). Plants were collected within a one-kilometer radius around industrial complexes in Drenas and Mitrovica, with Peja serving as a control point. In the homogenate of plant leaves, total proteins were determined using the Lowry method, MDA levels were determined using the Health Packer method, and GST activity was assessed using Habig method. GST activity was higher in the three plant species collected in Drenas and Mitrovica compared to those in Peje, the control point. Apart from Hypericum perforatoum in Mitrovica, the study revealed a significant increase in MDA levels in the three plant species from industrial areas compared to those in the control point. Generally, plants collected in industrial areas exhibited lower total protein levels than those in Peja. A negative correlation was observed between MDA levels and total proteins of *Achillea millefolium* and *Plantago lanceolata*, but not in Hypericum perforatum. The findings underscore the impact of pollution on the three plant species investigated in the study.

Keywords: total protein, MDA, GST, *Achillea millefolium, Hypericum perforatum, Plantago lanceolata.*

INTRODUCTION

In the natural environment, plants are constantly exposed to abiotic stresses, including extreme temperatures, salt stress, drought and heavy metals. These factors significantly that have a huge impact global agriculture, leading to substantial economic losses. A comprehensive study by Chaki, Begara-Morales, and Barroso (2020) extensively explored the impact of abiotic stress on plant growth and development. Mining operations play a crucial role in driving economic growth and industrial development worldwide. However, the extraction and processing of minerals often result in adverse environmental consequences, giving rise to concerns about their impact on local ecosystems and human health. Kosovo, situated in the Balkan Peninsula, is rich in mineral resources and has a history of extensive mining activities. Drenas and Mitrovica are prominent regions significantly affected by mining operations, with extensive activities involving lead, zinc, and other ores (Melcher & Reichl, 2017). While mining contributes to regional economic progress (Hoti, 2014), concerns have been raised about possible environmental and ecological consequences, as well as risks to human well-being. Mining-related industrial activities contribute to the release of various pollutants into the surrounding environment, including heavy metals such as lead and zinc, along with other potentially toxic substances. These pollutants have been documented to exert detrimental effects on nearby ecosystems and organisms across different environmental compartments. Studies have specifically highlighted the adverse impacts of mining-related pollutants on the atmosphere, where their release into the air has been shown to have negative consequences (Paçarizi et al., 2021). Additionally, mining pollution can extend to food products, affecting their safety and quality. Research exploring the contamination of food items with mining-related pollutants includes studies by (Demaku et al., 2023; Gashi et al., 2020; Kastrati et al., 2021; Ozuni et al., 2010; Repetto et al., 2012). Common plant species found in the region of Drenas, Mitorvica and Peja include *Achillea millefolium*, *Hypericum perforatum, Plantago lanceolata* and various others. These plants are recognized for their ecological significance and their potential as bio indicators of environmental conditions (Glavač et al., 2017). According to Oztetik (2015) plants respond to pollution by increasing the production of reactive oxygen species and oxidative stress, including various defense mechanisms. Some parameters serving as biomarkers for antioxidant protectioninclude the activity of glutathione S-transferase (GST), malondialdehyde (MDA), and total proteins.

Glutathione S-transferases are antioxidant enzymes with multifaceted roles in biological systems. They actively participate in detoxification processes, shielding organisms from the detrimental consequences of reactive oxygen species (ROS) reactions, as demonstrated by Edwards, Dixon, and Walbot (2000). Additionally, GSTs exhibit involvement in hormone transport, as highlighted by (Gullner et al., 2018). In the realm of plants, GSTs constitute an extensive family of enzymes encoded by numerous gene families. They significantly contribute to the detoxification of harmful substances by binding with glutathione, thereby alleviating stress and aiding in hormone transport. These versatile enzymes also play a critical role in plant-pathogen interactions, as elucidated by (Kumar & Trivedi, 2018; Yohannes et al., 2022). Moreover, GSTs demonstrate peroxidase activity, serving as effective scavengers of ROS generated during oxidative stress. They play a vital role in facilitating the transport of essential hormones such as auxins and jasmonates across membranes, which is integral to plant growth and development. This hormone transport function has been well-documented by Kumar and Trivedi (2018), and Zhuge et al. (2020). In the context of environmental adaptation, GSTs are indispensable for plant survival, and their contribution to plant resilience in the face of diverse environmental stressors is underscored by Vaish et al. (2020).

While malondialdehyde serves as a well-established biomarker of lipid peroxidation, resulting from oxidative stress within cells, elevated MDA levels indicate increased oxidative damage to lipids, including those in cell membranes (Aralbaeva et al., 2017; Ma et al., 2015). Hence, the quantification of MDA levels holds significant scientific merit, serving as a valuable tool for assessing the oxidative stress status and evaluating the degree of damage inflicted by environmental pollutants. Assessing the MDA content in plants provides valuable insights into the degree of stress experienced by plant cells, allowing researchers to obtain a comprehensive understanding of how plants physiologically respond to environmental challenges, as highlighted by Grotto et al. (2009). The measurement of MDA content serves as an effective indicator of damage to plant membranes, reflecting the peroxidation of polyunsaturated fatty acids (PUFA) resulting from oxidative stress. Consequently, the elevation in MDA content serves as a reliable indicator for evaluating both oxidative stress and the toxicity of heavy metals, as demonstrated by Morales and Munné-Bosch (2019); Al-Fartosy et al. (2014).

Proteins, as essential macromolecules, play a pivotal role in the intricate functions of living organisms. In the context of plants, they exhibit a diverse array of structural and functional activities (Rasheed et al. 2020 ; Sarkar et al., 2020; Day 1996). At the same time sufficient protein intake from plant sources is crucial for maintaining these biological functions (Kumar and Trivedi 2018; Sarkar et al. 2020; Yohannes et al. 2022). Plants offer a unique repository of nutrients not found in other organisms and contribute significantly to the structural aspects of food through processes like emulsification, foaming, and gelation (Day, 2013). In response to the challenges posed by rapid population growth, food security has emerged as a critical concern within the agrifood industry. This has led an increased focus on plant-based foods due to their high protein content (Oztetik, 2015a; Sarkar et al., 2020). In addition to proteins, plant lipids play a pivotal role in cellular functions. They are the primary constituents of cell membranes and serve as vital signals and energy sources for seed germination. These lipids, synthesized within plastids, encompass various types, including triglycerides, phospholipids, galactolipids, and sphingolipids. Furthermore, lipids contribute significantly to processes such as cell membrane formation, protection, and cell division (Kim, 2020; Suh et al., 2015).

The study aims to investigate the impact of mining in Drenas and Mitrovica on GST, MDA, and total protein levels in *Achillea millefolium*, *Hypericum perforatum*, and *Plantago lanceolata*. By measuring these markers of oxidative stress and detoxification processes, the research seeks to enhance our understanding of the overall impact of mining activities on these plant species in the specified regions.

MATERIAL AND METHODS

The plant species *Achillea millefolium*, *Hypericum perforatum*, and *Plantago lanceolata* were collected from three localities in Kosovo (Drenas, Mitrovica and Peja), representing the four cardinal directions (north, south, east, west). It is noteworthy that Peja serves as a reference point in this study due to the absence of any mining activity in the area. Thus, 36 plant specimens were collected for each plant type in one locality, resulting in a total of 108 samples across the three localities, providing a comprehensive dataset for subsequent analyses and investigations. Sampling points were established starting from point 0, the center of the pollution source, and continuing at intervals of 0.5 km, 1 km. Data collection was carried out from May to July 2022 covering crucial growth stages. Plants in the field were frozen in liquid nitrogen and in the laboratory, they were stored at -20 °C.

Glutathione S-transferase analysis: For the Glutathione S-transferase enzyme activity, 0.5 g of plant sample was weighed and homogenized with 5 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100 and 1% (w/v) polyvinylpyrrolidone (PVP). In measuring GST activity, 10 µl of supernatant, 850 µl of 0.02M phosphate buffer with pH 6.5, 50 µl of CDNB 0.02M, 50 µl of GSH 0.02 M were used. Incubation then took place in a water bath at 37 ˚C for 10 minutes and sample was read at a wavelength of 340 nm (Habig et al., 1974). MDA analysis: For MDA analysis 0.5 g of plant sample was homogenized in 5 ml of 10% TCA (trichloroacetic acid) and centrifuged for 10 minutes. The supernatant was used for spectrophotometric measurement by Health Packer. In this method, 1ml of supernatant was mixed with 2ml of 0.5% TBA (thiobarbituric acid) dissolved in 10% TCA. The mixture was heated in a water bath at 95°C for 45 minutes, cooled to room temperature, and subsequently measured at the 532 nm. A blind test was also conducted using 1ml of distilled water and 2 ml of 0.5% TBA dissolved in 10% TCA. (Heath & Packer, 1968; Pise et al., 2013; Wills, 1969). Total Protein Analysis: For total protein analysis 0.5 g of plant sample was weighed and homogenized with 5 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100 and 1% (w/v) polyvinylpyrrolidone (PVP). Total proteins were determined according to the Lowry method, using standard stock solutions of bovine serum albumin to prepare standard curves. The absorbance of the colored sample was measured at a wavelength of 750 nm (Lowry et al., 1951).

STATISTICAL ANALYSIS

Statistical analysis of the acquired data was conducted using the mean ± standard deviation (SD) values. One-way analysis of variance (ANOVA) with Tuckey post hoc test was employed to establish statistical comparisons among the groups. Significance levels were set at a threshold of $p<0.05$ or $p<0.01$.

RESULTS ANS DISCUSSION

The GST values presented in Table 1 range from 0.049±0.004 mg/protein in Peje (*Hypericum perforatum*) to 0.25±0.008 mg/protein in Mitrovica (*Plantago lanceolata*). In *Achillea millefolium,* significantly higher GST values were recorded in Mitrovica (0.106±0.014 mg/protein), followed by Drenasi (0.067±0.007 mg/protein) and Peja (0.066±0.002 mg/protein). For *Hypericum perforatum* in Drenas, GST values are higher (0.077±0.011 mg/protein) compared to Mitrovica (0.063±0.004 mg/protein) and Peja (0.049±0.004 mg/protein). Among the three plant species, *Plantago lanceolata* exhibits the highest GST values recorded in Mitrovica (0.23±0.018 mg/protein), followed by Drenasi (0.22±0.034 mg/protein) and Peja (0.083±0.007 mg/protein). The plants collected in Peja, serving as a control point, showed lower GST values compared to those collected in

Mitrovica and Drenas. These two localities (Mitrovica and Drenasi) are industrial areas characterized by mining activity. Previous research has reported that the content of heavy metals in the soils of these two areas exceeds the maximum values allowed according to the standards of the World Health Organization WHO and EU standards (Sahiti et al., 2023; Zogaj et al., 2014; Zogaj & Düring, 2016). The increase in GST values of plants collected in Drenas and Mitrovica could be attributed to heavy metal pollution. GST in plants is recognized as a protective mechanism managing biotic and abiotic stress, including heavy metals (Kumar & Trivedi, 2018; Tie, 2012). GST expression, inducible by a various of biotic and abiotic factors, may contribute to tolerance to oxidative stress (Zagorchev et al., 2013). The ability of GST to conjugate GSH to hydrophobic and other xenobiotic compounds gives this enzyme have an ameliorating effect on damages caused by oxidative stress such as lipid peroxidation and DNA damage (Tie 2012; Vaish et al. 2020). Elevation of GST activity against heavy metal induced stress has also been reported by(Benhamdi et al., 2021; Yilmaz et al., 2017). In both cases, GST activity was higher in leaves than in roots.

The values of MDA presented in Table 2 indicate significantly higher amounts ($p<0.05$; $p<0.01$) in the three species of plants collected in Drenas and Mitrovica compared to thosein Peje. In all cases, the MDA levels for the three plant species follow the sequence: Drenas>Mitrovice>Peje. The highest MDA values were recorded in Plantago lanceolate in Drenas (8.875±1.40 /g protein), while the lowest was observed in *Achillea millefolium* in Peje (0.288±0.20/g protein). The elevated MDA values in plants collected in Drenas and Mitrovica suggest oxidative stress, like induced by heavy metals released into the environment through mining activities in these areas. Heavy metals are known to react with cellular components, leading to the generation of reactive oxygen spices (ROS), which, in turn, cause damage to cellular macromolecules and lipid peroxidation. . Therefore, the measurement of lipid peroxidation, represented by the amount of MDA, serve as an indicator of oxidative stress (Demidchik, 2015, p. 2; Viehweger, 2014). This correlation has been documented in numerous studies, where the amount of MDA has been reported to be linked to the levels of metals to which plants have been exposed (Azarakhsh et al., 2014; Benhamdi et al., 2021; Tie, 2012; Yilmaz et al., 2017). The use of malondialdehyde as a marker of lipid peroxidation, not only in plants and animals but also in medicine, is increasing, as evidence by recent research papers (Khoubnasabjafari & Jouyban, 2020).

In contrast to the values of GST and MDA, which were higher in the plants in Drenas and Mitrovica compared to Peja, the total proteins of the three plant species collected in these two localities showed lower values (Table 3) compared to those collected in Peja. Among the three plant species, in *Plantago lanceolata*, the lowest total protein values were recorded in the following order: in Mitrovica (2.49±0.26 mg/L), in Drenas (3.01±0.26 mg/L) and Peje (6.36 ±0.47 mg/L). *Hypericum perforatum* (Drenas 10.69±0.65 mg/L Mitrovice 9.35±0.53 mg/L Peje 11.24±0.61 mg/L) and *Achillea millefolim* (Drenas 8.86 ±0.43 mg/L, Mitirovice 6.82±0.78 and Peje 9.97±0.75 mg/L) showed the highest values of total proteins.

Table 1. Value of GST (mg/protein) in plants collected in three localities

The plant species	Drenas	Mitrovice	Peje
Achillea millefolium	0.067±0.007	0.106*±0.014	0.066±0.002
Hypericum perforatum	0.077*±0.011	0.063±0.004	0.049±0.004
Plantago lanceolata	0.22**±0.034	0.23**±0.018	0.083±0.007

Note: the results are presented as mean ± SD, *significant value $p < 0.05$; **significant value $p < 0.01$.

Table 2. Value of MDA (MDA/g protein) in plants collected in three localities

The plant species	Drenas	Mitrovice	Peje
Achillea millefolium	0.615**±0.031	0.486*±0.082	0.288±0.020
Hypericum perforatum	0.814**±0.059	0.608±0.053	0.546±0.036
Plantago lanceolata	8.875**±1.40	7.546*±1.23	3.406±0.44

Note: the results are presented as mean ± SD, *significant value $p < 0.05$; **significant value $p < 0.01$.

Table 3. Value of total protein mg/L in plants collected in three localities

The plant species	Drenas	Mitrovice	Peje
Achillea millefolium	8.86±0.43	6.82**±0.78	9.97±0.75
Hypericum perforatum	10.69±0.65	9.35±0.53	11.24±0.61
Plantago lanceolata	3.01**±0.29	2.49**±0.26	6.36±0.47

Note: the results are presented as mean±SD, *significant value $p<0.05$; **significant value $p<0.01$.

Table 4. Pearson's correlation between amount of MDA and total protein in plants

Achillea millefolium	*Hypericum perforatum*	*Plantago lanceolata*
-0.458	0.014	-0.936

According to (Buqaj et al., 2023; Šajn et al., 2013) the content of heavy metals in the soil (Pb, Cd, Hg, As, Zn, Cu and Cr in Mitrovica and Cr and Ni in Drenas) of these two areas exceeds the allowed limits. It seems that soil pollution with heavy metals has initiated oxidative stress, and consequently, the decrease of total proteins in plants collected in industrial areas in Mitrovica and Drenas can be attributed to the stressful condition caused by heavy metals. A decrease in the amount of total proteins in cases of exposure of plants to heavy metals has also been reported by many researches who have studied oxidative stress and changes in the biochemical content of plants. Aki & Yücel (2019) found a decrease in the amount of total proteins of *Solanum lycopersicum* mill in exposure to Cd and Cu. The same effect on the amount of total proteins was also reported in the Glycen max plant exposed to Cu, Cr and Ni by Duan et al. 2020. Reduction in the amount of total proteins was also reported by (Gutiérrez-Martínez et al., 2020; A. Kumar et al., 2019; Niyoifasha et al., 2023). The decrease in protein content in plants may be caused as a result of the increase in the activity of proteases induced by the oxidative stress caused by heavy metals. Another factor that may have influenced the fragmentation of proteins and consequently the decrease in the amount of total proteins is the peroxidation of lipids also caused by the exposure of plants to heavy metals (John et al., 2008; Solanki & Dhankar, 2011). Aldehyde radicals derived from lipid peroxidation are stable and show high reactivity with proteins (Carbone et al., 2005). MDA as a product of lipid oxidation can cause changes in proteins such as covalent modifications, breaking of the polypeptide chain and their carbonylation (Sun et al., 2022).

Pearson's correlation analysis (Table 4) reveals a strong negative correlation between the amount of MDA and total proteins in *Achillea millefolium* (-0.458) and *Plantago lanceolata* (-0.936) plants. Such a correlation was not observed in Hypericum perforatum, although a slight increase in the amount of total proteins is noted in Drenas and Mitrovica compared to Peja. In an in vitro study on the oxidation of walnut proteins caused by lipid peroxidation, using MDA as a product of lipid peroxidation, a drastic change in the structure and functionality of proteins due to the action of MDA was identified (Sun et al. 2022).

CONCLUSIONS

Research findings indicate that the three plant species (*Achille millefolium, Hypericum perforatum* and *Plantago lanceolata*) collected in industrial areas in Drenas and Mitrovica exhibit high activity of glutathione S-transferase (GST) compared to GST activity in Peja. Elevated MDA values in the three plant species were also observed in the plants collected in these two industrial areas compared to those collected in Peja. Additionally, Pearson's correlation analysis reveals a negative correlation observed between the amount of MDA and total proteins of *Achillea millefolium* and *Plantago lanceolata*. This alteration in biomarker values (GST, MDA and total protein) may result from the response to oxidative stress induced by mining activity in these industrial areas.

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