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Bioindicator potential and stress responses of *Achillea millefolium* to heavy metal contamination in Kosovo soils

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ABSTRACT

The Ferronikeli smelter, as one of the most well-known industrial areas in Kosovo, poses a very serious concern for the living world. The objective of the research study in this area was the evaluation of environmental pollution through the quantifying of the concentration of heavy metals (Ni, Pb, Zn) in the soil as well as in the vegetative parts (root, stem and leaves) of the *Achillea millefolium* plant, which the population uses for therapeutic purposes. Also, the assessment of the environmental condition was made through the parameters of oxidative stress (sulfhydryl groups and protein carbonylation) in the leaves of the same plant. The concentration of heavy metals was determined with ICPE9800 – plasma emission spectrometer, from which it was observed that Ni, and Zn revealed high values compared to Pb both in the soil as well as in the roots and leaves of the *Achillea millefolium* plant. Also, the values of biochemical parameters exhibited high values in this area, precisely due to mining activity. Therefore, it was recommended that this area and this plant species be systematically monitored, as it is a good indicator of the environmental condition.

Keywords: environmental, ferronickel, *Achillea millefolium*, heavy metals, bioindicator.

INTRODUCTION

Environmental pollution is one of the greatest problems confronting the entire globe. One of the main factors constituting to pollution is the processing and release of heavy metals, which pose a very serious issue in the environment-food-health chain (Ali et al., 2019; Spahić et al., 2018; Tchounwou et al., 2012). Kosovo has too experienced major pollution in recent decades. Sources of heavy metal pollution originate form geological, industrial, agricultural, pharmaceutical, domestic, environmental, technological and atmospheric sources (He et al., 2005). Taking into account the large use of medicinal plants as therapeutics, it has been considered essential to research and evaluate their impact on public health (Macnair, 2003) since these plants are used as an alternative form of medicine all over the world. Therefore, the high presence of heavy metals in plants has raised serious concerns for human health, requiring systematic monitoring (Maharia et al., 2010).

As emphasized by Gjorgieva et al. (2010), medicinal plants should be analyzed to assess the concentration of chemical elements, specifically heavy metals, both in soil and in plant tissues. Therefore, there has been a growing interest in these species recently, especially those plants that have phytoremediation capacity, such as *Achillea millefolium, Hypericum perforatum, Plantago lanceolata and Urtica dioica* (Ahatović et al.,

2020; Hussain et al., 2011; Nyakudya et al., 2020; Ruwali et al., 2013). Although most of these plants grow in different environments, the capacity to accumulate heavy metals varies according to the species, as well as according to age, vegetative organs and soil characteristics (Lajayer et al., 2017; Barthwal et al., 2008; Kos et al., 1996).

Many environmental factors such as extreme temperatures, dryness, salinity, toxic metals, ultraviolet rays, air pollution (Caverzan et al., 2016; Choudhury et al., 2013; Molassiotis et al., 2016), pesticide use, and pathogenic infections may induce oxidative stress in plants (Pacheco et al., 2017; Sabir et al., 2015). Toxic metals, by influencing the formation of reactive oxygen species, can lead to reduced plant growth, altered physiology and metabolism, as well as impaired plant cell integrity (SRO). In this context, assessing oxidative stress responses in plants such as A. millefolium provides critical insights into their physiological tolerance to environmental pollutants, especially in the areas with strong industrial activity.

Thiol groups are found primarily in cells as part of the side chain of the amino acid cysteine, the major product of plant sulfur assimilation (Pivato et al., 2014). These thiol (SH) groups on cysteine protein residues can undergo various oxidative modifications by reactive oxygen/nitrogen species. Reversible oxidation of cysteine, including S-nitrosylation, S-sulfenylation, S-glutathionylation, and disulfide formation, regulate numerous biological functions, such as enzymatic catalysis, antioxidants, and other signaling pathways (Li and Kast, 2017). Protein carbonylation represents the oxidation of proteins driven by reactive oxygen species, usually referring to the process that forms aldehydes and ketones, and is widely used as a marker for assessing oxidative stress (Suzuki et al., 2010). Protein carbonylation is commonly performed to determine the concentration of oxidative stress in the context of cellular damage, aging, and several age-related disorders (Akagawa, 2021; Li et al., 2024; Orgado et al., 2023; Wehr and Levine, 2013)

Therefore, the objective of this study was threefold: (1) to assess the concentration of heavy metals – nickel (Ni), lead (Pb) and zinc (Zn) – in the soil samples collected near the Ferronickel Smelter in Drenas; (2) to determine the degree of metal accumulation in different vegetative parts (roots, stems and leaves) of *Achillea millefolium*; and (3) to investigate the impact of metal exposure on oxidative stress levels using specific biochemical indicators. By integrating metal accumulation data with oxidative stress biomarkers, the study sought to advance to a better understanding of environmental pollution and its biological consequences, and to validate the use of *Achillea millefolium* as a reliable bioindicator species in mining-affected landscapes.

MATERIALS AND METHODS

Study area

The mining complex and smelter of Ferronikeli in Drenas represent an important component of the industrial economy of Kosovo, including a nickel processing plant that is supplied with raw materials from the mines of Çikatova and Glavica (New Co Ferronikeli. 2025). To evaluate the level of pollution in this area, 20 soil samples and 20 plant samples were collected from four industrial areas within the Drenas locality. Similarly, 20 such samples were taken as comparative samples in the Peja locality, serving as control points. Oxidative stress was also analyzed through biochemical parameters using sulfhydryl groups and protein carbonylation as bioindicators.

Soil and plant sampling

Soil samples were collected with a hand probe according to the random method at a depth of 0–15 cm. The samples were taken in 4 zones of the Drenas locality (Z1–Z4), each sample consisted of an average of 10 subsamples, including 1–2 kg per zone. The Peja locality served as a reference point. The samples were then stored for drying at ambient temperature for 6–8 weeks, organic residues were removed and sieved through a 2 mm diameter sieve.

The decomposition of soil samples was carried out using the aqua regia method ($HCl+HNO_3$, 3:1 v/v) according to this procedure: 0.3 g of sample (soil) was weighed on an analytical balance and placed in Teflon tubes in which 6 ml of HCl and 2 ml of HNO_3 were placed, closed well and placed in a MARS 6™ microwave for 50 minutes at 200 °C (Zogaj and Duering, 2015), after digestion the samples were cooled, diluted with distilled water to 50 ml and stored at 4 °C until reading. a dilution ratio of 1:20 for the Drenas samples and 1:10 for the Peja samples. The concentration of metals (Ni, Pb, Zn) was determined using a Shimazu

ICPE-9800 Plasma Atomic Emission Spectrophotometer. *Achillea millefolium* plant samples were collected at the same locations where the soil samples were taken and placed in 50 cm nylon bags, marked with numbers and coordinates. They were then placed for drying in well-ventilated rooms, without contact with sunlight, for a period of about six weeks. After drying, the vegetative organs were separated separately, finely ground using blender. Plant extraction was performed in the microwave using 0.5 g of sample (root, stem, leaves) weighed with an analytical balance, which were placed in Teflon tubes. Then, 5 ml H_2O, 5 ml HNO_3 and 3 ml H_2O_2 were added to each tube. The Teflon tubes were tightly closed and placed in a MARS 6™ microwave oven for 50 minutes at 200 °C (Czarnecki and Düring, 2014). After completion of the process, the tubes were cooled in a desiccator and the contents were transferred to 50 ml plastic tubes, where they were leveled with distilled water. The samples were analyzed on an ICPE 9800 (atomic emission plasma spectrometer) from SHIMADZU.

Assessment of biochemical parameters

For biochemical parameter measurements, plant material was stored in liquid nitrogen during field sample collection, then the samples were stored in a refrigerator at -20 °C until oxidative stress parameters were analyzed. For the assessment of oxidative stress parameters, only the leaves of the plants: Achillea millefolium, collected in Drenas and Peja as a control point, were analyzed. Plant extracts were measured at wavelengths depending on the working protocol in a spectrophotometer (Thermo Scientific™ GENESYS 10S UV-Vis).

Thiol (sulfhydryl) group determination

Thiol (sulfhydryl) groups were extracted by weighing 0.5 g of sample (fresh leaf) 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 turn, for reading in the spectrophotometer, 250 µl of plant extract, 750 µl of Tris-HCl pH 8.2, 3950 µl of methanol and 50 µl of DTNB were used, incubated in the dark for 15 minutes, centrifuged and read at a wavelength of 415 nm. A blank test was also prepared in a test tube, the same components were placed, only distilled H2O was placed instead of the sample (Sedlak and Lindsay, 1968).

Protein carbonylation analysis

Protein carbonylation was quantified using the 2.4 dinitrophenylhydrazine (DNPH) method. (Cvjetko et al., 2010; Yanar et al., 2011) 400 µl of supernatant (sample) was transfer in a test tube, 600 µl (10 mmol DNPH dissolved in 2 mol HCl) was added, incubated in the dark at room temperature for 1 hour (from time to time it was mixed in a vortex), then 500 µl of 10% TCA (cold) was added and for 10–15 minutes they were kept at -20 °C, centrifuged and washed three times with 1000 µl of ethanol acetyl-acetate (1:1); finally, 2 ml of urea dissolved in phosphate buffer pH= 2.4 was added and read at a wavelength of 370 nm.

Statistical analysis

Statistical analysis was conducted using the IBM SPSS statistics program (version 21). The mean, standard deviation were calculated, and one-way analysis of variance (ANOVA) was conducted to evaluate for significant differences between sample areas with p-values $p<0.05$ and $p<0.01$ considered statistically significant.

RESULTS AND DISCUSSION

The high concentration of Ni and Pb in Drenas pose a serious concern for both the environment and living organisms. Similar findings were reported by Zogaj and Düring, (2016) that the proximity of the industrial complex is associated high concentrations of Ni.

Assessment of soil contamination with nickel, lead and zinc in Drenas and Peja

The high concentration of heavy metals (Ni, Pb and Zn) in the soil samples at the Drenas and Peja localities are summarized in Table 1. In Drenas, a significant $p<0.01$ difference of Ni was detected at Z2 (1037.5±383.8 mg/kg) compared to Z3 (49.95±10.15mg/kg) and Z4 (157.6±29.74 mg/kg). Also, a $p<0.05$ difference was observed between Z2 (1037.5±383.8 mg/kg) and Z1 (401.1±312.5 mg/kg), indicating a localized accumulation of metals associated with the proximity of the smelter. Lead (Pb) also demonstrated significant differences $p<0.05$ in this locality and between areas Z4(82.39±40.10 mg/kg) and Z2(23.49±10.47 mg/kg), while Zn did not show any significant

differences between areas of Drenas, the values of which ranged from (36.34 to 54.56mg/kg). In general, the concentration of heavy metals in Drenas followed the order Ni>Zn>Pb, indicating a higher concentration of Ni in this industrial area. Peja, as a non-industrial or mining area, had a lower level of heavy metal concentration. However, the concentration of Zn was $p<0.05$ in Z4 (126±34.86 mg/kg) compared to Z1 (68.41±16.76mg/kg), highlighting a possible local source or some geochemical difference. Regarding the concentration of Ni and Pb, no significant difference was observed between the areas of the Peja locality, the values of which remain close to the permitted limits.

If the two sites are compared, Drenas revealed higher concentrations of the heavy metals Ni, Pb and Zn compared to the Peja site. These findings are also consistent with the previously reported research suggesting that industrial areas, especially mining and metallurgical ones, are a source of heavy metal pollution (Prathumratana et al., 2020; Šajn et al., 2013). Ni in particular exceeds the limits allowed by the Kosovo government, a concern that has also been reported by (Buqaj et al., 2023; Sahiti et al., 2023).The high concentration of Ni in Drenas points to the possible potential for heavy metals to enter the food chain as a result of mining activity, while the lower concentration in Peja indicates the possibility of heavy metal pollution as a result of anthropogenic factors.

Metal accumulation in vegetative organs of *Achillea millefolium* from the Drenas

A comparative analysis of Ni concentration within the four zones of the Drenas locality in the vegetative organs (roots, stems and leaves) of the *Achillea millefolium* plant is summarized in Table 2. The result indicate that the root system has a higher concentration of Ni (9.87±3.06; 27.88±16.18; 5.47±1.70; 19.81±8.47 mg/kg) compared to the stem and leaves. Regarding the root system, statistically significant ($p<0.05$) differences for Ni were detected in zones Z2 (27.88±16.18 mg/kg) with Z3 (5.47 ±1.70 mg/kg) which has a lower value, while, regarding the concentration of Ni in the stem and leaves, no statistically ($p>0.05$) significant difference was observed in any of the zones. In terms of the concentration of Pb in the Drenas in the three vegetative organs of the Achillea millefolium plant, significant differences were observed ($p<0.05$; $p<0.01$) were observed in the root system between zone Z4 (3.13±0.61 mg/kg) and zones Z1, Z2 and Z3 (0.52±0.28; 0.28±0.12; 1.42±0.32 mg/kg). In the context of the stem, no Pb values were evidenced in any of the analyzed zones ($p>0.05$). Likewise, in the leaves, the concentration of Pb exhibited no statistically significant differences between the zones within the Drenas site. Zinc (Zn) in Drenas, revealed a statistically significant difference ($p<0.05$; $p<0.01$) for the root system between Z4 (27.2±6.38 mg/kg) with Z1 (19.45±1.21 mg/kg) and Z3 (13.66±1.62 mg/kg), but also between Z2 (25.79±2.87 mg/kg) and Z3 (13.66±1.62 mg/kg). Regarding the stem, statistically significant differences ($p<0.05$) are observed between Z2 and Z3 (16.48±2.10; 10.50±0.52 mg/kg), but no differences are observed between the other zones. While regarding the leaves, the concentration of Zn does not show any significant difference in any of the zones. These findings confirm the ability of the root system to accumulate heavy metals, specifically Ni and Pb. This has been confirmed by previous studies showing that the root system possess the capacity to accumulate heavy metals due to the soil contaminated with these metals (Verma and Dubey, 2003). As for the lack of Pb in the stem, it is seen that the mobility of this element is limited in this plant tissue. According to the World Health Organization (WHO, 2007) the

Table 1. Concentration of heavy metals (mg/kg) in soil samples from Drenas and Peja regions

Soil	Z	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Drenas	Z_1	401.1±312.5[a]	44.13±4.45	53.39±14.64
	Z_2	1037±383.8[a,b]	23.49±10.47[a]	49.53±20.25
	Z_3	49.95±10.15[b]	44.57±18.66	36.34±13.22
	Z_4	157.6±29.74[b]	82.39±40.10[a]	54.56±38.72
Peja	Z_1	48.39±5.08	44.43±17.85	68.41±16.76[a]
	Z_2	82.39±31.33	62.13±4.03	87.57±22.08
	Z_3	48.73±8.37	52.28±17.68	76.86±21.32
	Z_4	76.30±21.70	73.37±16.04	126.63±34.84[a]

Note: data are expressed as mean ± standard deviation (SD), a significant value $p<0.05$; b significant value $p<0.01$.

Table 2. Concentration of heavy metals (mg/kg) in the vegetative organs in *Achillea millefolium* collected from Drenas

Achillea millefolium	Z	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Root	Z_1	9.87±3.06	0.52±0.28[b]	19.45±1.21[a]
	Z_2	27.88±16.18[a]	0.28±0.12[a]	25.79±2.87
	Z_3	5.47±1,70[a]	1.42±0.32[a,b]	13.66±1.62[b]
	Z_4	19.81±8.47	3.13±0.61[b]	27.20±6.38[a,b]
Stem	Z_1	3.74±1.31	NM	13.92±2.54
	Z_2	3.12±2.45	NM	16.48±2.10[a]
	Z_3	1.04±0.27	NM	10.50±0.52[a]
	Z_4	3.74±1.82	NM	12.91±4.39
Leaf	Z_1	14.79±3.65	0.92 ±0.23	31.79±12.48
	Z_2	13.02±2.90	0.97±0.40	32.11±11.85
	Z_3	5.23±1.17	1.15±0.17	21.66±1.94
	Z_4	13.75±6.67	1.22±0.29	42.90±31.13

Note: data are expressed as mean ± standard deviation (SD), NM- not measurable, a- significant value $p<0.05$; b- significant value $p<0.01$.

recommended maximum of heavy metals in medicinal plants are: 0.3 mg/kg for Ni, 0.1–0.5 mg/kg for Pb and 50-100mg/kg for Zn. The concentrations recorded in this study reached high levels of these metals which exceed the permitted limits in some parts of the plant, especially in the roots, raising concerns regarding the use of *Achillea millefolium* for medicinal purposes in industrial areas.

Metal accumulation in vegetativ organs of Achillea millefolium from the Peja (Control site)

The results present in Table 3 relate to the concentration of heavy metals in the Achillea millefolium plant collected in the Peja locality (control). Statistically significant differences ($p<0.05$) regarding the amount of Ni were recorded only in the roots of this plant, specifically between Z1 (6.73 ± 2.49 mg/kg) and Z3 (2.66 ± 0.23 mg/kg). These results support the previous findings that the root system exhibits heavy metal accumulation capabilities due to their direct contact with the soil and limited translocation to the aerial parts (Bashir et al., 2024; Verma and Dubey, 2003).

In turn, regarding the amount of Ni in the stem and leaves, no statistically significant difference was observed. Regarding the concentration of Pb in the four zones within the Peja locality, statistically significant differences ($p<0.01$)

Table 3. Concentration of heavy metals (mg/kg) in the vegetative organs in *Achillea millefolium* collected from Peja

Achillea millefolium	Z	Ni (mg/kg)	Pb (mg/kg)	Zn (mg/kg)
Root	Z_1	6.73±2.29[a]	3.31±1.41	22.50±2.49[b]
	Z_2	3.87±0.91	2.73±1.46	18.86±5.92[b]
	Z_3	2.66±0.23[a]	1.43±0.34	10.41±0.50[b]
	Z_4	4.60±1.62	0.73±0.25	10.58±3.17
Stem	Z_1	0.35±0.07	NM	9.53±5.46
	Z_2	0.41±0.19	NM	11.86±2.58
	Z_3	0.41±0.20	NM	10.04±1.74
	Z_4	0.59±0.30	NM	12.24±3.42
Leaf	Z_1	1.65±0.97	0.24±0.01[b]	26.73±3.83[a,b]
	Z_2	1.20±0.32	0.46±0.11[b]	21.12±1.92[a]
	Z_3	1.05±0.36	0.04±0.02[b]	17.08±1.14[b]
	Z_4	1.81±0.98	0.15±0.04[b]	15.63±3.11[a,b]

Note: data are expressed as mean ± standard deviation (SD), NM- not measurable, (a) significant value $p<0.05$; (b) significant value $p<0.01$.

were noted only in the leaves of this plant between Z2 (0.46 ± 0.11 mg/kg) with Z1, Z3, Z4 (0.24 ± 0.013 mg/kg; 0.04 ± 0.02 mg/kg; 0.15 ± 0.04 mg/kg). Regarding the stem, no Pb value was detected in this vegetative organ. No statistically significant changes were observed in the root system, despite the values in the table. From the data, statistically significant changes are observed in the concentration of Zn within the areas of the Peja locality. The root system presents significant differences ($p<0.01$) between Z1 (22.50 ± 2.49 mg/kg) with Z3 and Z4 (10.41 ± 0.50 mg/kg; 10.58 ± 3.17 mg/kg), as well as between Z2 (18.86 ± 5.92 mg/kg) with Z3 and Z4 (10.41 ± 0.50 mg/kg; 10.58 ± 3.17 mg/kg).

Regarding the stem of this plant species, no statistically significant differences are observed. However, in the leaves, statistically significant differences ($p<0.01$) of Zn were evidenced between Z1 (26.73 ± 3.83 mg/kg) with Z3 and Z4 (17.08 ± 1.14 mg/kg; 15.63 ± 3.11 mg/kg), as well as ($p<0.05$) between Z2 (21.12 ± 1.92 mg/kg) with Z1 and Z4 (26.73 ± 3.83 mg/kg; 15.63 ± 3.11 mg/kg). These findings underscore the high concentrations of heavy metals in the roots of the *Achillea millefolium* plant, consistent with the previous research showing that the root system generally serves as the main source of toxic metals due to adsorption on root surfaces and limited mobility towards aboveground parts (Lajayer et al., 2017; Barthwal et al., 2008). Furthermore although nickel plays an essential physiological role in plants by being involved in nitrogen metabolism and enzymatic function, high concentrations have been shown to inhibit chlorophyll biosynthesis, enzymatic activity, and photosynthetic electron transport (Genchi et al., 2020; Modarresi et al., 2024; Sreekanth et al., 2013). The absorption and mobility of heavy metals in plants is influenced by many factors including plant type, age, structure, soil pH and bioavailability of metals (Deng et al., 2025).The lowest concentrations of Ni and Pb in Peja as a control point indicated the possibility of reference in this locality, also the impact of anthropogenic factors in the industrial area in Drenas showed higher levels of heavy metals.

Biochemical markers of oxidative stress in Achillea mellefolium

It is known that heavy metals trigger oxidative stress in plants by inducing reactive oxygen species (ROS) which can alter the structure and function of proteins. This effect can be evaluated through biochemical parameters, such as sulfhydryl groups (SH) and protein carbonylation which have been used to indicate redox balance and oxidative damage (Baba and Bhatnagar, 2018; Bajra-Brahimaj et al., 2024; Hussain et al., 2024). The results in Table 4 report the concentration of sulfhydryl groups and protein carbonylation in the leaves of *Achillea millefolium* collected in Drenas as an industrial site and in Peja as a non-industrial site. The concentration of sulfhydryl groups (SH) as an oxidative damage to proteins and potential antioxidants exhibited markedly higher values in the samples taken in Drenas (41.01±24.99 µmol/mg proteins) compared to Peja (21.13±0.79 µmol/mg proteins). The high values of sulfhydryl groups may be attributed to mining activities, a fact also confirmed by Srivastava and Srivastava (2023).

However, the high standard deviation observed in Drenas implies considerable variability in the oxidative response between individual samples, perhaps reflecting heterogeneous exposure to pollutants and microenvironmental conditions. In contrast, the lower standard deviation in Peja implies more uniform environmental conditions and a stable physiological state among plants. Protein carbonylation, which serves as a marker of irreversible oxidative damage to proteins, revealed comparable mean values in both areas (7.55±2.93 µmol/mg) for Drenas and (7.27±2.94 µmol/mg) for Peja. This suggests that despite the differences and exposure to pollution, the overall level of protein oxidation does not change. Both have similar standard deviations, suggesting a local analysis of the values.

Table 4. Concentration of sulfhydryl groups (SH) and protein carbonylation in *Achillea millefolium* leaves from Drenas and Peja

Biochemical parameter	Drenas (µmol/mg protein)	Peja µmol/mg protein)
Sulfhidril grups (SH)	41.01±24.99[a]	21.13±10.79
Protein carbonylation	7.55±2.93	7.27±2.94

Note: data are expressed as mean ± standard deviation (SD), (a) significant value $p<0.05$; (b) significant value $p<0.01$.

These findings may reflect complex interactions between metal-induced ROS generation and the plant's antioxidant defenses. While elevated concentrations of metals such as Ni and Pb are known to promote ROS formation and subsequent protein oxidation (El-Amier et al., 2019; Fedorova et al., 2014; Salas-Moreno et al., 2019), the antioxidant capacity of *A. millefolium* particularly its thiol-mediated buffering systems may mitigate oxidative injury to some extent, thereby maintaining stable protein carbonyl levels even in contaminated environments.

CONCLUSIONS

This study provided important results on heavy metal pollution in the industrial area of Drenas by examining the concentrations of Ni, Pb and Zn in both soil and Achillea millefolium plant, together with oxidative stress parameters. The results clearly demonstrate that the soils in Drenas contain high levels of nickel and lead, especially in the areas located closer to the ferronickel smelter, while Peja as an area without industrial activity exhibited significantly lower concentrations of metals, confirming its suitability as a control.

Achillea millefolium showed the ability to absorb and retain heavy metals, especially zinc and nickel, with the highest concentrations consistently found in root tissues. Among the sampled areas, Z2 showed the highest accumulation of nickel in the roots, while Z4 was distinguished by high levels of zinc in both roots and leaves. Stem tissues generally showed lower levels of metal accumulation, suggesting selective mechanisms of translocation and partitioning. These patterns support the classification of *A. millefolium* as a potential bioaccumulating species capable of reflecting localized pollution gradients.

In addition to the metal-absorbing ability, *A. millefolium* exhibited physiological responses to environmental stress, as evidenced by changes in biochemical parameters. In particular, the increased concentration of thiol groups in the samples from Drenasi imply an increase in antioxidant defense mechanisms in response to heavy metal exposure. Protein carbonylation values, which indicate oxidative damage to proteins, were similar in both study sites, probably due to the species' natural defense mechanisms that mitigate the effects of ROS.

The research findings confirm the dual role of *Achillea millefolium* as a bioaccumulator and as a sensitive biological indicator of oxidative stress. Its ability to accumulate metals in specific organs and to respond to oxidative stress underscore its value for environmental monitoring. The continued use of this species in ecological assessments is strongly recommended, especially in the areas affected by mining and industrial activities, to better understand the distribution of pollutants and their biological consequences.

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