STUDY OF HEAVY METALS IN WILD *JUNIPERUS* PLANTS FROM KOSOVO

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Abstract

The aim of this study is to determine the environmental indicators and to evaluate the pollution presence in various regions of Kosovo. As an environmental indicator we selected two wild species of Junipers; *Juniperus communis* L. and *Juniperus oxycedrus* L. which are medical plants, useful for their therapeutic aspect. We intended to use these plants as environmental indicators, so we chose five areas in Kosovo territory as a combination of contamination and non-contamination areas. In Kosovo are different pollution sources, generated by Thermal Electric Plant, in Obiliq, and from other industrial processes in the Mitrovica region. Here was observed a strong contamination of soils, river waters and plants with heavy metals over the permissible norms. Such contamination presents a permanent risk to the environment as a result of uncontrolled releases of industrial wastes into river waters and in the lands nearby industrial zones. *Juniperus oxycedrus* L. samples had been collected in Vllahi – Shala e Bajgorës, close to mines, on the outskirts of Trepça, and in Mushtisht – Therandë. The sample of *Juniperus communis* L have been collected in Sllatinë - Fushë Kosovë, near Obiliq, and Novobërd – Artanë and Bajgore – Trepçe. We intend to evaluate the content of heavy metal elements like Cd, Cu, Fe, Ni, Pb and Zn using ICP-OES technique (EPA method 6010C:2007). In all five study areas it was observed that the soils had high levels of iron, lead, nickel and zin, which in most cases were observed also in leaves and berries.

Key words: *Juniperus communis* L., *Juniperus oxycedrus* L., heavy metal, contamination.

Përmbledhje

Qëllimi i këtij studimi është të përcaktojë treguesit mjedisorë dhe të vlerësojë praninë e ndotjes në rajone të ndryshme të Kosovës. Ne kemi zgjedhur si tregues mjedisor dy specie të egra të dëllinjës; *Juniperus communis* L. dhe *Juniperus oxycedrus* L., të cilat janë bimë mjekësore, të dobishme për aspektin e tyre terapeutik. Ne kishim për qëllim t'i përdorim këto bimë si tregues mjedisorë, kështu që zgjodhëm pesë zona në territorin e Kosovës si kombinim i zonave të ndotura dhe më pak të ndotura. Në Kosovë ekzistojnë burime të ndryshme të ndotjes, të gjeneruara nga Termocentrali në Obiliq dhe nga proceset industriale në rajonin e Mitrovicës. Këtu është venë re një ndotje e fortë e tokave, ujërave të lumenjve dhe bimëve me metale të rënda mbi normat e lejueshme. Një ndotje e tillë paraqet rrezik të përhershëm për mjedisin si rezultat i shkarkimeve të pakontrolluara të mbetjeve industriale në ujërat e lumenjve dhe në tokat përreth. Mostrat e *Juniperus oxycedrus* L. janë mbledhur në Vllahi - Shala e Bajgorës, afër minierave, në periferi të Trepçës dhe në Mushtisht - Therandë. Mostra e *Juniperus communis* L janë mbledhur në

Sllatinë - Fushë Kosovë, afër Obiliq, dhe gjithashtu në Novobërd - Artanë dhe Bajgore - Trepçe. Ne synojmë të vlerësojmë përmbajtjen e elementeve të metaleve të rëndë si Cd, Cu, Fe, Ni, Pb dhe Zn duke përdorur teknikën ICP-OES (metoda EPA 6010C: 2007). Në të pesë zonat e studimit u vu re se tokat kishin nivele të larta hekuri, plumbi, nikeli dhe zinku, të cilat në shumicën e rasteve ishin vërejtur edhe në gjethe dhe fruta.

Fjalë kyçe: *Juniperus communis* L., *Juniperus oxycedrus* L., metale të rëndë, ndotje.

Introduction

The genus *Juniperus* as part of the family *Cupressaceae*, is evergreen aromatic shrub or tree mostly distributed throughout the temperate regions of North Hemisphere. *Juniperus communis* L. and *Juniperus oxycedrus* L. as two specific species, occur in different habitats, from North Europe to Mediterranean regions, including Balkan Peninsula: Broome *et a*l. (2017); Farjon (2013). These plants are classified as one of the top 25 globally threatened species of European Green Belt; Shuka *et al*. (2010) and is included to Red List of Threatened Species: Farjon (2013); Allen *et al.* (2014); IUCN (2019). The reasons of erosion in wild plants are urban expansion, fruits collection without rules and regulations on their biological renovation, and fires that cause irreversible losses: Broome *et al.* (2017).

Juniperus communis L. and *Juniperus oxycedrus* L. are priority species in the framework of natural heritage conservation due to their distribution decline and absence of population viability and regeneration in Balkan regions. These spontaneous species widespread in Kosovo, it is considered to have economic potential.

Juniperus communis L. and *Juniperus oxycedrus L.* cover various regions in Kosovo and juniper plants are mainly used in distillery and for medical purposes. The matured berries collected in autumn and dried slowly in the shade could be used as diuretic, antiseptic, aromatic, stomachic and antirheumatism: Kandeel *et al.* (2015); Xhulieta *et al.* (2018).

Juniperus oxycedrus L. is distributed in the Mediterranean region and in Kosovo it grows in dry hilly areas on serpentine rock substrate. The essential oil yields depending on the population origins and ranged from 0.4 to 1.8%, based on dry weight: Hajdari *et al.* (2014). In Kosovo *Juniperus communis* L. is found in hilly and mountainous regions, in silicate fields, and rarely could be found in carbonate and serpentine soils. The chemical composition of cone essential oils has a large distribution and the yield of essential oil ranged from 0.4 to 3.8% based on dry weight of plant material: Hajdari *et al.* (2015). The main essential-oil components were α-pinene, β-myrcene, sabinene, and d-limonene.

During the last decades in different countries has been an increasing awareness of the potential adverse effects of soil pollution by trace heavy metals: Adriano (2001). Therefore, assessing soil metal contamination is of

major interest for governments and regulators who are concerned with public health and sustainable development policies. The metal toxicity in soils depends on complex geochemical and biological interactions: van Gestel (2008). Plants could be used to assess metal bioavailability, but the use of a single plant species is open to criticism. Thus, the indication of metal bioavailability by plants needs further investigation before being adopted in routine assessments of soil quality.

In this work, we propose performing heavy metal analyses and evaluation to judge the contamination of soils using *Juniperus* plants species, representative of local plant communities from five contaminated or no-contaminated sites.

Material and methods

Five areas have been selected in Kosovo territory to analyze soil and plant materials; i) Vllahi – Shala e Bajgorës, close to lead and zinc mines, on the outskirts of Trepça and ii) Mushtisht – Therandë are locations of *Juniperus oxicedrus* and iii) Novobërd-Artanë, iv) Bajgore-Trepçe and v) Sllatinë-Fushë Kosovë, near Thermopower Plant in Obiliq, are locations of *Juniperus communis*.

From this selection, two sites are near lead, zinc and nickel mines and one site is near an anthropogenic contamination source. The two other sites (Mushtisht – Therandë and Novobërd-Artanë) are relatively clean sites.

Juniper materials and soils were collected in 5 different areas of Kosovo during 2015 and all the samples were analyzed the following year for the heavy metal's contents.

It was important that the plucking and selection of biological material we must do throughout the growing season. It is important that leaf, flower and fruit materials were always taken from the same place preselected in advance. All plant materials and soils were collected from an area 150 m by 150 m, to guarantee a uniform material for preparation of homogeneous samples.

The set of plant materials, selected at designated areas, were washed with distillated water in order to remove the pollution like dust and other contaminants. Then they were dried at room temperature. Plant parts (needles and ripe berries) were isolated and oven-dried at 80 °C for 48 h and then 0.2 g was taken and transferred into Teflon vessels. After this, 6 mL of 65% (v/v) HNO_3 (Merck) and 2 mL of 30% (v/v) peroxide hydrogen (H_2O_2) were added.

Soil samples (about 500 g) were collected from a depth of about 10 cm with a stainless-steel shovel. They were oven-dried at 80 °C for 48 h and passed through a 2-mm sieve. After that, 0.3 g was weighed and 9 mL 65% (v/v) HNO_3, 3 mL 37% (v/v) HCl and 2 mL 48% (v/v) HF (Merck) were added.

Samples were mineralized in a microwave oven (Berghof) and after cooling, the samples were filtered by using Whatman filters and the volume was made up to 50 mL with ultrapure water in volumetric flasks.

Figure 1. Collected samples and their analysis in Inductively Coupled Plasma; ICP-OES Perkin Elmer Optima 2100 DV, according to method EPA 6010C, 2007

We planned to evaluate the content of elements like Cd, Cu, Fe, Ni, Pb and Zn in plant and soil samples. The elementary analysis was carried out using ICP-OES (Perkin Elmer, Optima 2100 DV) and the evaluation of the results was done according to EPA method. 6010C: 2007. The samples have been analyzed at the licensed and accredited laboratory Agrovet Laboratory in Fushe Kosova, Prishtine.

Material analysis and evaluation has been done referring to European Community reference data for soil, leaves and ripe berries: Angioni *et al.* (2003); Broome, (2003); Ceburnis *et al*. (2000).

Results and discussion

Heavy metal accumulation in plants was measured in leaf and berries samples taken from the representative of the plant collected on each site. We employed Inductively Coupled Plasma – Optic Emission Spectroscopy (ICP-OES) to assess the concentration of selected heavy metals (Cd, Cu, Fe, Ni, Pb and Zn) in soil and plants. Firstly, we present the sites where flourish wild *Juniperus communis* L. and after we continue with the sites of *Juniperus oxycedrus* L.

The mean concentration of Cd, Cu, Fe, Ni, Pb and Zn from surface soil, and from leaves and berries samples have been summarized in the following Tables.

Table 1. Heavy metal concentration of soil samples in the five sites during 2015 monitoring survey.

	Element [mg / kg]					
Soil	Cd	Cu	Fe	Ni	Pb	Zn

Juniperus oxicedrus Vllahi	0.00	46.90	24802.73	2265.01	1235.40	3854.55
Juniperus communis Novobërd	0.00	57.32	15464.11	41.84	65.55	67.94
Juniperus communis Bajgore	0.00	48.57	16731.70	268.04	96.35	118.43
Juniperus oxicedrus Mushtisht	0.00	18.88	19125.76	1592.48	18.76	31.12
Juniperus communis Sllatinë	0.00	13.82	12259.46	44.49	81.70	55.65

Table 2. Heavy metal concentration of berries samples in the five sites during 2015 monitoring survey

	Element [mg / kg]					
Berries	Cd	Cu	Fe	Ni	Pb	Zn
Juniperus oxicedrus Vllahi	0.00	6.45	128.94	30.95	1.19	14.28
Juniperus communis Novobërd	0.00	8.87	316.95	58.82	1.69	15.05
Juniperus communis Bajgore	0.00	2.49	69.48	6.66	3.38	7.06
Juniperus oxicedrus Mushtisht	0.00	4.74	74.70	22.63	0.99	7.61
Juniperus communis Sllatinë	0.00	4.84	70.77	4.64	2.86	8.09

Table 3. Heavy metal concentration of leaves samples in the five sites during 2015 monitoring survey

	Element [mg / kg]					
Leaves	Cd	Cu	Fe	Ni	Pb	Zn
Juniperus oxicedrus Vllahi	0.00	18.37	953.98	30.35	1.90	24.16

Juniperus communis Novobërd	0.00	3.61	135.24	0.00	0.00	13.79
Juniperus communis Bajgore	0.00	4.47	108.56	12.14	4.86	14.86
Juniperus oxicedrus Mushtisht	0.00	4.86	117.28	23.82	0.00	9.01
Juniperus communis Sllatinë	0.00	4.96	136.67	0.00	0.00	11.56

The levels of elements content (ppm values) in the soils of the Sllatina area, in Fushë Kosovë are presented in Fig. 2, where it is evident that the highest value belongs to iron - 12259.46 ppm (parts per million 10^{-6} kg). This value, referring to the target values of soils and permissible values of plants published by WHO: FAO/WHO (2011); Ruqia *et al* (2015) is extremely high.

The values of cupper, iron, nickel, lead and zinc are largely present in berries (fruits) and leaves, and that reflected the soil content. The values of cadmium are very low, and it is noticed that in berries and leaves are also extremely low levels <0.1 ppb, which shows that berries are not dangerous for consumption.

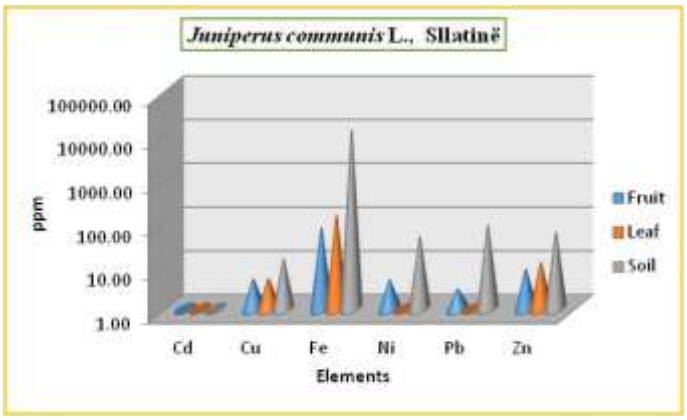


Figure 2. The values of heavy metals Cd, Cu, Fe, Ni, Pb, Zn in *Juniperus Communis* L., (fruits, leaves and soil) in the area of Sllatinë

In the graphics, it is clear that the heavy metal elements in soil, during vegetation period has been transported in berries of *Juniperus Communis* L., but in leaves they are at much lower levels.

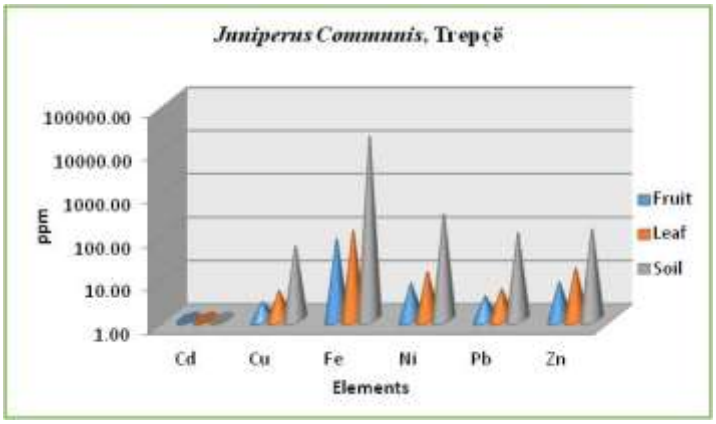
In the leaf material, iron content had the values of 136.67 ppm, much less than iron content in the soils analyzed, while in fruit it was at 74.7 ppm, much higher than the permissible norms: Ruqia Nazir *et al.* (2015); Afzal

Shah *et al.* (2013). The lead content in fruits was 2.86 ppm and in leaves was at extremely low levels < 1.0 ppb, below the permissible norms for plant materials. The same picture is for Cu, where its content was at lower levels both in soil and in plant materials than in those of permissible limits. The Ni content in soil, which was at 1592.48 ppm, above the target value of soil.

In Fig. 3 are presented the levels of heavy metal elements content (ppm values) in Bajgore-Trepçe and Novobërd - Artanë, locations of *Juniperus communis* L.

The results of the heavy metal analysis in soil, leaf and fruit samples of *Juniperus Communis* collected nearby the village of Bajgore, near Trepca mines in Mitrovica region, indicated high value of Fe content in soils of this area.

Fe content in the analyzed soils was 16731.7 ppm, above the permissible norm and this is reflected in the values of Fe in the fruits of *Juniperus Communis*, 69.48 ppm. It is known that this plant is not an accumulator of heavy metals, but again the Fe content is above the permissible values for plants: FAO/WHO (2011); Afzal Shah *et al.* (2013); Vodyanitskii (2016).



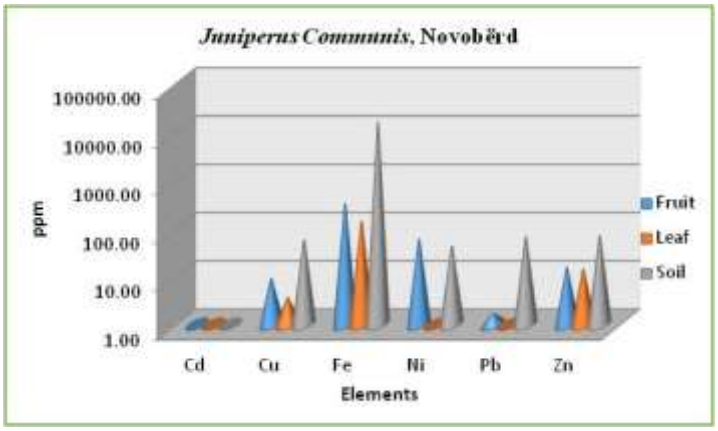
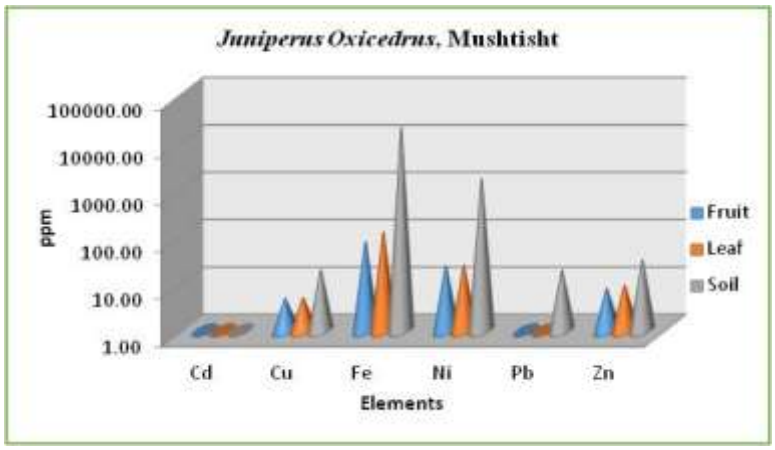


Figure 3 The values of heavy metals Cd, Cu, Fe, Ni, Pb, Zn in *Juniperus Communis* L., in the area of Bajgore - Trepçe and Novobërd – Artanë

In the site of Bajgore – Trepçe and Novobërd – Artanë, lead contents in soil samples were 96.35 ppm and 65.55 ppm respectively. In the two stations the lead content in soils had been transmitted differently in leaves and berries. In leaves the lead content were 4.86 ppm and < 1 ppm respectively. Concerning Pb contents in berries, it is recorded 3.38 ppm and 1.69 ppm respectively. It is obvious that in Novobërd – Artanë station, the lead content is 5 time less in leaves and 2 time les in berries, compare to Bajgore-Trepçe station, that belong to a polluted area.

It is noticed that the soils in these areas had very high iron levels 16731.7 ppm in Bajgore – Trepça and 15464.1 ppm in Novobërd – Medellak. The iron was transmitted to some extent and at high levels in fruits: 69.48 ppm and 316.95 ppm respectively for the two stations. When considering the permissible values issued by WHO, the recommended level of iron in plants is 20 mg / kg (ppm): Afzal Shah *et al*. (2013); Nema *et al*. (2014). From the analysis of other heavy metal elements, the copper content in soils, fruits and leaves were under permissible values recommended by WHO.



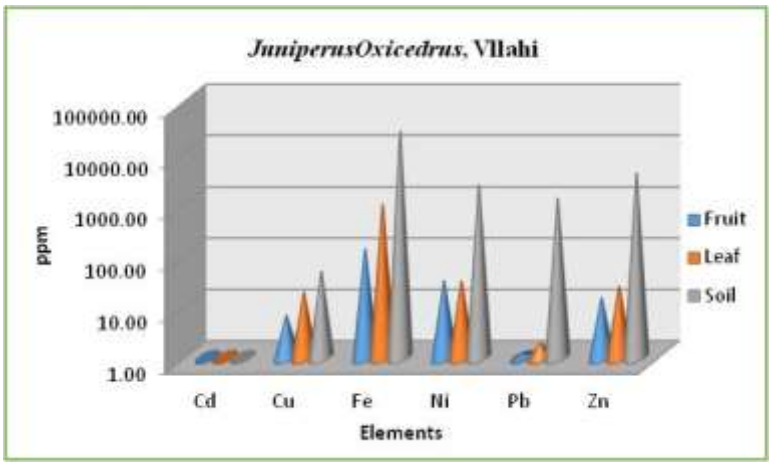


Figure 4 The values of heavy metals Cd, Cu, Fe, Ni, Pb, Zn in *Juniperus oxycedrus* L., in the area of Mushtisht – Therandë and Vllahi– Shala e Bajgorës

The heavy metal elements content of *Juniperus oxycedrus* L., in the area of Mushtisht – Therandë, in northeast of Prizren, near Sharri mauntain, and Vllahi – Shala e Bajgorës, in northeast of Mitrovica, near lead and zinc mines, are presented in the Figure 4.

In the areas of Mushtisht and Vllahi, the Fe levels analyzed in the soil materials were 19125.76 ppm and 24802.73 ppm respectively. This is related with the environment pollution, and therefore in red juniper plants (leaves and berries) the Fe contents in the two stations were relatively high; in leaves (needle shape) 117.28 ppm and 953.98 ppm respectively and in berries 74.7 ppm and 128.94 ppm. It was a large difference in Fe contents, above the maximum acceptable limit: Ceburnis and Steinnes (2000). Maybe this situation is related with polluted environment around the mines.

Likewise, the levels of Pb content in soil samples were 18.79 ppm and 1235.40 ppm respectively in Mushtisht and Vllahi stations, with very high contamination in Vllahi area. In this case, Pb content in leaves and barriers were 1.90 and 1.19 ppm, whereas in Mushtisht area Pb content in leaves and barriers were < 1 ppb and 0.99 ppm respectively. Lead contents are almost irrelevant in leaves and fruits; thus the lead was not transmitted in the consumable material of Juniper, but its content was above new WHO recommendations (berries and other small fruits - 0.2 mg/kg): FAO/WHO (2011); Salazar *et al.* (2014).

From the upper diagrams of Figure 4, the Ni and Zn contents in soil were higher in Vllahi – Shala e Bajgorës station; 2265 ppm and 3854 ppm respectively. In the two stations, Zn and Ni contents decreased gradually from soil to leaves and berries, and a large amount of Zn and Ni was transmitted to leaves and berries. In Mushtisht station the Ni content in soil, leaves and berries was: 1592.48, 23.82 and 22.63 ppm, where the permission

value for the whole plant is 10 mg/kg. The Zn content in soil, leaves and berries was: 31.12, 9.01 and 7.61 ppm, and the permission value for the whole plant is 0.6 mg/kg. Around Vllahi – Shala e Bajgorës station the situation was really more polluted; Ni and Zn contents in red berries were 30.95 ppm and 14.28 ppm respectively.

In these two contrast areas, Vllahi – Shala e Bajgorës, near the lead and zinc mines and Mushtisht – Therandë, near Sharri maintain, we observed a decrease of Zn and Fe contamination in berries; 2 time and 1.8 time respectively. The presence of Zn contamination is leaves and berries is strongly related with soil contamination from the lead and zinc mines.

Conclusions

In all five study areas it was noticed that the soils had high levels of iron, lead, nickel and zinc, which in most cases was observed also in leaves and fruits-berries of *Juniperus oxicedrus* L. and *Juniperus comunis* L.

In the case of *Juniperus oxicedrus* L. Fe, Ni and Zn contamination is evident for Vllahi – Shala e Bajgorës area, near the lead and zinc mines.

The two selected areas in northeast of Kosova, Bajgore – Trepça and Vllahi – Shala e Bajgorës, both located near lead and zinc mines had large contamination in leaves and berries with heavy metal elements.

As the black and red juniper plants are used in distillery and for medical purposes, it is necessary the analysis of heavy elements with appropriate experimental methods of ripe berried before use.

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