HEAVY METALS IN *PRIMULA VERIS* L., PRIMULACEAE IN DIFFERENT REGIONS OF KOSOVO

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ABSTRACT

Primula veris L., Primulaceae is a perennial plant with oval leaves and yellow flowers. It is widespread in different regions of Kosovo, in mountainous and hilly areas and in subalpine and alpine pastures. For years it is well known as a medicinal plant for pharmacological actions. In this study we intend to evaluate the heavy metal contents and their distributions in roots, leaves and flowers of *Primula veris*. were selected as sampling locations for investigation purposes as they are environmentally clean areas. During the biennial monitoring, plant materials from the *Primula veris* population have been collected and investigated. In addition, soil samples where these plants were grown have been collected. As this plant is used of medicinal purposes, its vegetative parts such as roots, stems, stalks and leaves and yellow flowers were investigated applying the ICP-OES technique based on the EPA method 6010C: 2007 for the Cd, Cu, Fe, Ni, Pb, Zn content. The heavy metals content in soil was compared with the heavy metal content in different parts of *Primula veris* plants. During the survey of 2015in Badovc area, Pb contamination in soil, leaves and flowers was 111.84 ppm, 2.86 ppm and 1.70 ppm, respectively, whereas in Novobërd - Artanë area the Pb content in soil, leaves and flowers was 520.34 ppm, 11.62 ppm and 0.79 ppm, respectively. So, only a small amount of lead was transferred to flowers, as the main consumable part. There was an equal distribution of heavy metals such Fe, Cu and Zn among the stalks and leaves, and flowers. The Cd concentration was found at a very low rate in both areas. As heavy metal contamination was found at a very low rate, the *Primula veris* flowers could be used for medicinal purposes.
Key words: *Primula veris*, medicinal plants, heavy metals, soil

1. INTRODUCTION

Kosovo has a great biological, ecological and landscape diversity. The flora of Kosovo is made up of 1,800 plant species. There are about 200 Balkan endemic plant species and 13 Kosovo endemic plant species that could be here found (Rexhepi 2003; Shuka *et al*., 2010; Mustafa *et al*., 2012;

Menczer *et al*., 2018;). The great number of endemic and stenoendemic plants are an added value to the biodiversity. Unfortunately, anthropogenic activities pose threats to these plants. River canyons, and subalpine and alpine zones are of great importance as they are rich in endemic-relict species, and medicinal and aromatic plants (MAP) and wild berries (WB) (Millaku 1999; Menczer *et al*., 2018). Sharri Mountains, Albanian Alps, Kamenica, Goollak regions (eastern part of Kosovo) and Mitrovica are rich in medicinal and aromatic plants as based on the surveys.

Primula veris L. are small, long-lived perennials from the family Primulaceae, growing wild in temperate Europe and western Asia (Millaku 2010). Cowslip grows on nutrient-poor grasslands, herb-rich meadows, and at the edges and in clearings of warm and bright woodlands. Cowslips produce a rosette of leaves and leafless flower stalks, up to 20–30 cm high. Cowslip flowers are fragrant, bright yellow with orange spots at the edge of each lobe. They are formed at the top of the stalks in an umbel-like inflorescence. It is in flower from April to May, and the seeds ripen from July to August (Menczer *et al*., 2018).

Primula veris L. is one of the most important aromatic and medicinal plants grown in the territory of Kosovo. Given the increasing demand of pharmaceutical industry for the use of this plant, efforts were made to cultivate some cowslip populations with a high production and yield of flowers and roots.

The cowslip (*Primula veris* L.) is one of the most important aromatic and medicinal plants, in Kosovo and of great importance for the pharmaceutical industry. Therefore, it has an increasing demand in the domestic and foreign markets (Millaku 2010; Luma *et al*., 2018).

Extracts from rhizomes, roots and flowers of *Primula veris* are components of many pharmaceutical products (Włodarczyk 2020). Until now, *Primula veris* L has been collected from wild habitats of SE Europe, endangering this species existence. In the future cultivation should be associated with controlled collection from wild habitats.

The characteristic constituents of flowers and roots of *Primula veris* L. are triterpene saponins, and phenolic compounds, including flavonoids (about 3% in flowers), phenolic acids, and phenolic glycosides. Saponins are responsible for the secretolytic and expectorant activity. In turn, phenolic compounds reveal antioxidant, antimicrobial, and cytostatic properties (Richards 2003; Luma *et al*., 2018).

There is a long history in the clinical use of medicinal plants. Chemical pollutants such as heavy metals and pesticide residues as common plant pollutants, pose serious risks to human health (Shaban *et al*., 2016). Medicinal plants can easily be contaminated with heavy metals through soil, water and air. Usually the soil is subject to pollution through atmospheric deposition of

heavy metals from various sources including industrial activities, precipitation, atmospheric dusts and products used for plant protection (Maobe *et al.*, 2012). Heavy metal pollution can occur due to factors including irrigation with polluted water, addition of metal-based fertilizers and pesticides, industrial emissions, transport, harvesting process, storage and sale (Radwan and Salama 2006; Duran *et al.* 2007; Tuzen and Soylak 2007).

The present study aims to determine the presence of heavy metals in the cowslip populations, and the way these elements were transferred from soil to roots, leaves and flowers.

Primula veris materials were collected from two different areas of Kosovo in 2015 and 2016 to investigate the heavy metals content as this plant is of great importance for the pharmaceutical industry.

2. MATERIAL AND METHODS

Figure 1 depicts the Novobërd - Artanë and Badovc - Graçanica regions, the sampling areas as they are ecologically clean. Plucking and selecting plant materials at the same period of blossoming is very important. The roots, stem, stalks and leaves and flowers were collected from the same preselected place. All plant materials and soils were collected in 2015 and 2016 in an area of 150m x 150m^2, for uniform materials to be used for homogeneous samples.

Fig. 1. Selected areas in Kosovo for collecting *Primula veris* plant materials

The collection of plant and soil materials for further heavy metal analysis occurred in April and May. The set of plant materials were selected at designated areas. Once selected, they were taken to laboratory to be washed with running distillated water to remove the pollution like dust, soil and other contaminants. Once cleaned, they were covered to dry up at room temperature, in the dark and with controlled ventilation.

Plant fragments (roots, stalks & leaves and yellow flowers) were isolated and oven-dried at 80 °C for 48 h, and then 0.25 g was taken and transferred into Teflon vessels. Once transferred into Teflon vessels, 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 at a depth of about 10 cm using a stainless-steel shovel. They were oven-dried at 80 °C for 48 h and passed through a 2-mm sieve. Subsequently, 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.

The extraction from plant materials is in line with the BS EN 13805: 2014. The samples were mineralized in a microwave oven (Berghof), and after cooling they were filtered via Whatman filters and the volume was made up to 50 mL with ultrapure water in volumetric flasks.

Fig. 2: Autosampler and Inductively Coupled Plasma ICP-OES, DV 2100, Perkin Elmer.

We planned to evaluate the content of metal 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 results were evaluated based on EPA method. 6010C: 2007. The samples have been analyzed at the Agrovet Laboratory in Fushe- Kosova, Prishtine, a licensed and accredited laboratory.

Materials were analysed and evaluated in line with the European

Community reference data for soil, roots, stalks, leaves and flowers: (Broome, 2000; Ceburnis *et al*., 2000; Angioni *et al.*, 2003).

3. RESULTS AND DISCUSSIONS

Heavy metals content in the roots, stalks, leaves and flowers samples of the plant is here investigated. The Inductively Coupled Plasma – Optic Emission Spectroscopy (ICP-OES) was used to assess the concentration of Cd, Cu, Fe, Ni, Pb and Zn in soil and plants, first for the Novobërd - Artanë region, situated in the east of Prishtina. The beech forests are found at an altitude of 800 - 1000 m. In addition, this region is mentioned for its medicinal and aromatic plants. *Primula veris* or cowslip has a wide distribution in this area.

The Figure 3a depicts the heavy metals content (ppm values) in soil for the Novobërd - Artanë region. As it could be clearly noted, Fe, Pb and Zn are found at the highest levels, 20695.8 ppm (parts per million - 10^{-6} kg), 520.3 ppm 231.0 ppm, respectively, i.e., at an exceeding rate as based on (FAO/WHO 2011; Ruqia *et al*., 2015).

The forthcoming paragraph reports about the relation between heavy metal content in soil and the roots of *Primula veris.* The heavy metal content in roots (Fig. 3b) is found as following: Fe – 525.1 ppm, 39 time less than in soil; Pd – 10.7 ppm, 48 time lass than in soil; Ni 9.98 ppm, 10 time less than in soil; Zn – 25.3 ppm, 9 time less than in soil.

The mobility of heavy metal as Cd, Pb, and Ni in soils and the ability of these elements to migrate from soil to other environmental components, including medicinal plants pose a significant health risk to humans and animals (Bezlova *et al*., 2012; Velicković *et al*., 2020). Existing studies prove bioaccumulation of heavy metals and arsenic in different above- and underground plant parts (Kabata-Pendias, 2000). From the elementary analysis cowslip is not an accumulative plant for lead and iron, but it had more affinity for Ni, Cu and Zn, as these elements belong to three consecutive boxes in periodic table.

As stems connect the roots to the leaves, they help transporting even the heavy metals to the stalks, leaves and the yellow flowers of the plant. The investigation of 2015 reported that the flowers had a lower heavy metals content than the stalks and leaves. The Pb content in flowers was 0.78 ppm, while in stalks and leaves it was 11.62 pp. The Ni content was 7.72 ppm in flowers and 15.79 ppm in leaves. Fe, Cu and Zn had a roughly equal distribution.

The Ni (101.87 ppm) and Pb content in soil was above the maximum permissible values, but these heavy metals content in the roots and flowers,

the main parts used for medicinal purposes, was within permissible limits (WHO 2011; Afzal Shah *et al*., 2013; Ruqia Nazir *et al*., 2015).

The Zn content in roots (25.30 ppm) was ten time lower than in soil, and its transport to different parts of the plant during blooming was equal — 17.60 ppm in stalks and leaves, and 17.43 ppm in flowers. Zn is one of the most important elements that play a vital role in the physiological and metabolic processes of many organisms.

The Cd content was very low (< 0.1 ppb), probably to the ecological quality of the areas.

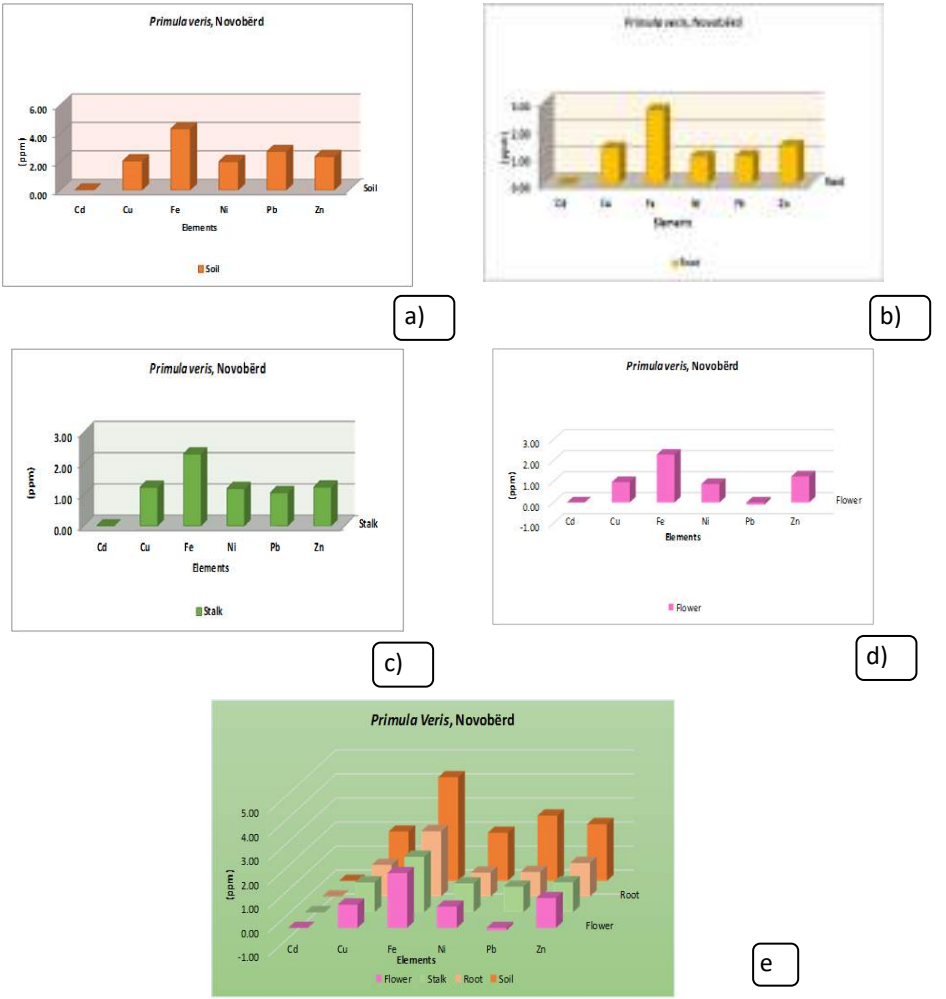

Fig.3: Heavy metals content of Cd, Cu, Fe, Ni, Pb and Zn in A - soil, B - roots, C – stalks, leaves, D – flowers and E- summery for *Primula veris***,** in Novobërd - Artanë area during 2015.

Regarding the Novobërd - Artanë area, all the heavy metals, except the Cd, are found at an exceeding level, and in most cases are also found in the plant material of the *Primula veris* (Diana *et al*., 2012).

The forthcoming paragraph reports about the heavy metals distribution in the Badovc area, near the Graçanica region, in the south-east of Prishtina. Soil samples and plant (roots, stems, stalks, leaves and flowers) were collected. The Figure 4 depicts the heavy metals content in soil, roots, stalks, leaves and flowers. Figure. 4e summarizes the heavy metals distribution.

Regarding the heavy metals content in soil, it was reported following: Fe – 19602.5 ppm, Ni – 1266.5 ppm, Pb – 111.8 ppm, Zn – 85.1 ppm and Cu – 29.7 ppm. Cd content was found within permissible limits — < 0.1 ppb (FAO/WHO 2011, Ruqia Nazir *et al*., 2015).

This accumulation of heavy metals in roots shows the ability of these elements to migrate from soil to plant components. The Figure 4b depicts the heavy metals content in the roots: Fe – 534.2 ppm, 38 time less than in soil; Pd – 44.2 ppm, 2.5 time lass than in soil; Ni 207.7 ppm, 6 time less than in soil; Zn – 42.8 ppm, 2 time less than in soil. The bioaccumulation of heavy metals is high for Pb, Ni and Zn.

The distribution of heavy elements among flowers, stalks and leaves is an important step in the mobility of heavy metals. There is a balanced distribution of Cu, Fe and Zn, alike for the *Primula veris* plant collected from the Novobërd - Artanë area. Pb and Ni showed a different situation.

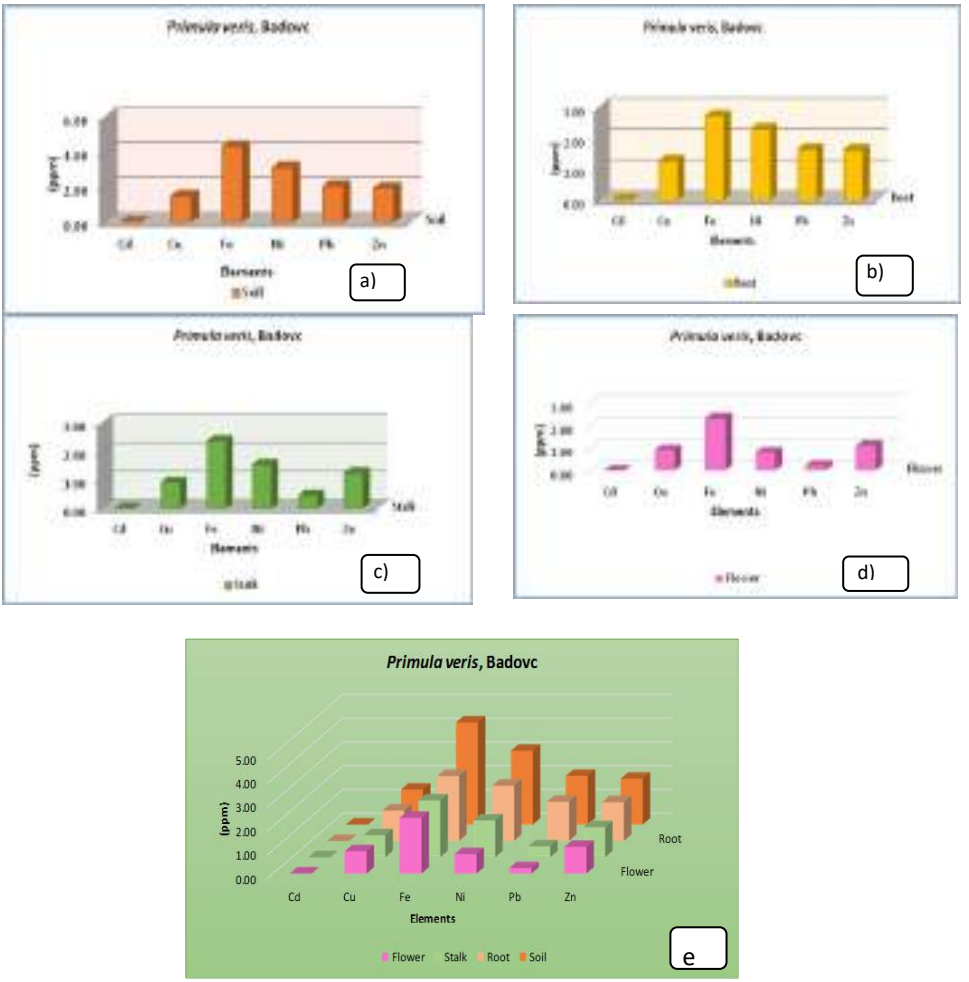

Fig. 4: Heavy metals content of Cd, Cu, Fe, Ni, Pb and Zn in a - soil, b - roots, c - stalks & leaves, d – flowers and e- summery for *Primula veris***,** in Badovc area during 2015.

The Pb content in flowers was 1.70 ppm, while in the stalks and leaves it was 2.86 ppm. Ni content in flowers was 6.59 ppm, while in the leaves it was 35.21 ppm. About the same situation was reported for the Novobërd - Artanë and Badovc areas.

The roots of *Primula veris* collected from Novobërd – Artanë area reported: Fe – 525.1 ppm, Zn – 25.3 ppm and Cu – 20.85 ppm, while for Badovc area it was reported: Fe – 534.2 ppm, Ni – 207.7 ppm, Pb – 44.2 ppm and Zn – 42.8 ppm— all exceeding the permissible limits.

As Cu, Fe, Pb and Zn were found at a high rate in the soil, roots of the *Primula veris* exhibited high levels of these metals (Fig.4e). Regarding the flowers, Cu, Fe, and Zn were also found at a higher rate than the permissible (Bezlova *et al*., 2012).

Cu content soil is below the permissible limits, while in the plant roots it is 18.74 ppm, i.e. exceeding the allowable limits. In flowers and leaves it is 8.58 ppm and 8.38 ppm, respectively, i.e. under the permissible limits (FAO/WHO 2011; Ruqia Nazir *et al*, 2015). These plant materials could be used as pure medicinal plants (Bezlova *et al*., 2012; Velicković *et al*., 2020).

In the soil and roots, Ni is 1266.5 ppm and 207.7 ppm, respectively, i.e. above the permissible limits. In flowers, it is 6.59 ppm, i.e. below the permissible limits as recommended by the WHO (10mg / kg = 10 ppm). Ni found to be helpful for human and animal health (Vodyanitskii 2016).

Pb was found at high values, 111.84 ppm, but in flowers, it was 1.70 ppm. In stalks and leaves, it was 2.86 ppm, i.e. below the permissible limits (FAO/WHO 2011; Shah *et al*., 2013; Ruqia Nazir *et al*., 2015). The *Primula veris* flowers had low contamination from heavy metal elements.

During the surveys, it was investigated whether the heavy metals distribution in *Primula veris* remained unchangeable for both areas (Fig. 5).

The data of 2016 reported this heavy metals content was found at the highest level in roots of *Primula veris* as following: the Novobërd – Artanë area: Fe – 966.76 ppm, Zn – 59.48 ppm and Cu – 33.63 ppm, the Badovc area: Fe – 686.47 ppm, Ni – 120.12 ppm, Pb – 28.17 ppm and Zn – 66.16 ppm. Therefore, these heavy metals content remains the same.

The distribution of the metals through stalks, leaves and yellow flowers is typical of heavy metals.

In Novobërd - Artanë area, in 2016, the data reported that the flowers had a lower Pb and Ni contents than in stalks and leaves. In flowers, it was 0.64 ppm, and in stalks and leaves, it was 13.78 ppm. In flowers and leaves, Ni was 3.08 ppm and 12.18 ppm, respectively. Fe, Cu and Zn had a roughly equal distribution of heavy metals among stalks, leaves and flowers.

This difference of Pb content in flowers and leaves in the Novobërd - Artanë area remains the same throughout the investigation.

Regarding the Badovc area, the data of 2016 reported that the flowers had a lower Pb and Ni contents than the stalks and leaves. Pb content in flower was 2.69 ppm, while in stalks and leaves, it was 4.45 ppm. Ni content in flowers was 3.56 ppm, while in leaves it was 20.30. Fe, Cu and Zn had a roughly equal distribution among stalks, leaves and flowers.

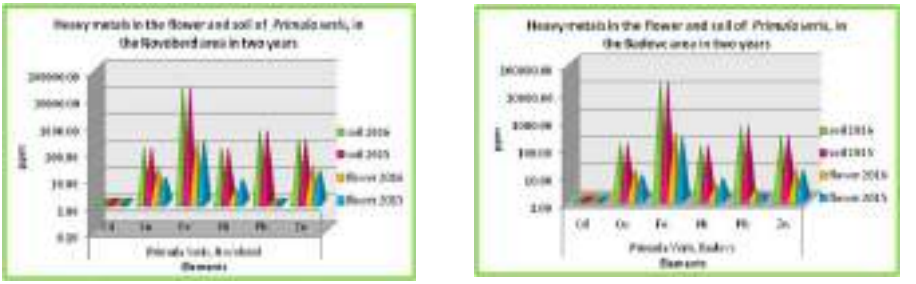

Fig. 5. Heavy metals content in flowers and soil of *Primula veris*, in the areas of Novobërd - Artanë and Badovc for 2015 and 2016.

The ratio of Ni content in leaves / flowers was 5.7 for 2016 and 5.3 for 2015, so closely the same value during two successive years for Badovc area.

In the Badovc area, the Pb content in flowers for 2015 was 1.70 ppm. In 2016 it was 2.69 ppm, i.e. above the permissible limits. In Novobërd - Artanë area, the Pb content in flowers was 0.79 ppm and 0.64 ppm, in 2015 and 2016, respectively, i.e. below the permissible limits, which means that Novobërd - Artanë area belong to a clean area.

Although *Primula veris L.* is not a heavy metal accumulator plant, some parts it reported heavy metal contamination above the permissible limits. Mainly, the roots and flowers of this plant are used in medicinal industry.

4. CONCLUSIONS

The presence Cd, Cu, Fe, Pb, Ni and Zn in soils and plant materials of *Primula veris* L (roots, leaves and flowers) is here investigated.

The results reported that the Fe content was found at a very high rate in soil. Consequently, it was found at the same rate in its roots and flowers.

There is an unequal distribution of Pb and Ni between flowers and leaves. Pb content was found at a low level in Novobërd - Artanë area. Ni content found at a low level in the Badovc - Gracanica area. Cu, Fe and Zn had approximately an equal distribution.

The main group of elements in roots, in the two selected area, remained unchanged throughout the investigation period.

Cu, Iron Fe, and Zn were found at a high rate in flowers. Pb and Ni were within the permissible limits.

Cd was found at a very low level proving that these areas are ecologically clean.

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