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DETERMINATION OF HEAVY METALS CONTENT IN *Achillea millefolium L.* AND RESPECTIVE INFUSIONS

SUMMARY

In this paper, the content of heavy metals (Cu, Mn, Fe, Co, Ni, Pb, Zn) was determined by Flame Atomic Absorption Spectrometry (FAAS) in the leaves of *Achillea millefolium L.*, as well as in herbal tea infusions prepared out of them. Samples are taken from selected locations in Bosnia and Herzegovina, including five urban and five rural areas. The content of analyzed metals in *Achillea millefolium* leaves in samples from urban areas was in the range: <LOD (Ni); <LOD – 0,132 mg/kg (Co); 0,684 – 3,92 mg/kg (Pb); 4,47 – 8,79 mg/kg (Zn); 7,42 – 10,14 mg/kg (Cu); 46,16 – 100 mg/kg (Mn); 82,14 – 143 (Fe). The content of analyzed metals in the leaves of *Achillea millefolium* in samples from rural areas was in the range: <LOD (Ni); <LOD – 0,435 mg/kg (Co); 5,84 – 11,54 mg/kg (Cu); 7,43 – 24,76 mg/kg (Zn); 1,24 – 77,72 mg/kg (Pb); 62,54 – 203 mg/kg (Mn); 52,14 – 246 mg/kg (Fe). The content of analyzed metals in tea infusions prepared from *Achillea millefolium* from all urban and rural locations ranged from: <LOD (Co); 0,172 – 0,659 mg/kg (Pb); 0,467 – 1,81 mg/kg (Ni); 0,865 – 1,95 mg/kg (Cu); 1,58 – 4,75 mg/kg (Zn); 1,81 – 5,67 mg/kg (Fe); 7,90 – 34,68 mg/kg (Mn). By comparing the content of heavy metals in the leaves of *Achillea millefolium* collected from urban and rural locations, a wide variation of the content was observed, depending on the sampling locations and the potential natural or anthropogenic sources of metals nearby. The results of metal content in herbal tea infusions of the *Achillea millefolium* suggested that the metals are mostly present in water-insoluble forms.

Keywords: *Achillea millefolium*, heavy metals, water infusion, urban and rural locations, FAAS

INTRODUCTION

Plants represent a direct or indirect source of minerals in the human diet. The species that are utilized to produce a variety of phytopreparations used in the

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pharmaceutical industry-which are available as single- or multi-component teas and are extensively used in traditional medicine-are especially significant. Wildcrafted plants (more than 200 species) and cultivated plant (around 30 species) are sources of medicinal raw materials (Živkov-Baloš et al., 2014). Herbal teas are the primary application for medicinal herbs. The complex chemical composition of plant includes flavonoids, alkaloids, enzymes, minerals, and trace elements (Ražić and Kuntić, 2011). Kabata Pendias (2000), in a study mentioned that medicinal plant can accumulate more heavy metals such as Cd, As, Pb, and Hg compared to other plants. Mineral elements like Ca, Cu, Fe, K, S, and Zn, which are vital to human physiology and some of which are frequently lacking or present in traces in human diets, can be found in tea plants. However, while vital elements like Cu, Fe, and Zn might influence various biochemical pathways based on the intracellular concentration, the presence of As, Cd, Ni, and Pb can be concerning due to their significant toxicity, even at low levels (Fernandes et al., 2022). Heavy metals enter the environment from natural and anthropogenic sources. Medicinal plants growing in nature can accumulate heavy metals depending on their individual properties, metal concentration in soil, air, and water, climatic factors, and other environmental factors. Metal accumulation in plants is mainly governed by two processes, which involve the uptake of metals into plant cells and their translocation from the roots to other parts of the plant. In hyperaccumulating plants, the highest percentage of heavy metals in plants is transported from the roots to the stem, so the concentration of metals in the aerial parts is higher than in the roots; however, in non-hyperaccumulating plants, heavy metals are usually not transported to the shoot, and the highest concentration is usually found in the roots. *Achillea millefolium* L. is a plant that has been characterized as a hyperaccumulator of heavy metals (Riyazuddin et al., 2021; Nikolova et al., 2018; Radanović et al., 2001).

The name "hyperaccumulator" describes numerous plants that are characterized by the ability to grow on soils rich in metals and the ability to accumulate extremely large amounts of heavy metals in shoots, far above the level in which they are found in most species, without phytotoxic effects. Three basic characteristics distinguish hyperaccumulators from related non-hyperaccumulator species: a markedly increased degree of absorption of heavy metals, faster translocation from roots to shoots, and a greater ability to detoxify and extract heavy metals in the leaves. The herbaceous perennial plant *Achillea millefolium* belongs to the *Asteraceae* family and is popularly known as yarrow. The genus *Achillea* includes over 130 perennial plant species native to the northern hemisphere from Europe to Asia and grows in temperate climates in arid or semi-arid habitats. Numerous studies done worldwide have found the plants of the *Asteraceae* family have strong antioxidant properties, antidiabetic activity, antimicrobial activity, anti-cancer activity, antifungal activity, antiviral and antihelminths activity. Because of their extremely complex chemical composition, species of the *Asteraceae* family show the many activities indicated. High levels of fiber and inulin, a complex carbohydrate, are characteristics of plants in the *Asteraceae* family. They also contain a variety of phenolic compounds, primarily phenolic acids, flavonols, anthocyanins, and sesquiterpene lactones, as well as important dietary phytochemicals, primarily carotenoids, tocopherols, and ascorbic acid. (Yanakieva et al., 2023; Kostić et al., 2020). Kostić et al., (2020). mentioned that more than 80 species belonging to the *Asteraceae* family are used extensively as edible plants in the Mediterranean and Balkan regions. Balijagić et al., 2021, mentioned that in Montenegro close to 300 plants are used in the pharmaceutical industry and in folk medicine; one of them *is Achillea millefolium. Achillea millefolium*, the best known and most widespread species, has been included among the most commonly used plant species in folk and conventional medicine for more than 3000 years (Mitich, 1990). *Achillea millefolium* is a perennial plant growing from 30 cm to 60 cm, with a single or sometimes forked stem. The leaves are from 5 cm to 20 cm long, finely pinnate, and the tips of the leaves end in tiny, prickly tips. The flowers are usually white and arranged at the top of the stem (Ali et al., 2017).

The content of heavy metals in plants helps us to assess environmental pollution. Metals such as iron (Fe), zinc (Zn), copper (Cu), cobalt (Co), and chromium (Cr) are essential nutrients for plants, which are toxic only in high concentrations. Heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg) are toxic metals that have no functional role in metabolism. One of the most serious environmental problems is the increasing presence of heavy metals in the environment, which causes plants to absorb them either from the soil or from the atmosphere through dry and wet deposition. This is associated with unsuitable agricultural practices, such as using soils that are heavily contaminated with metals or using excessive amounts of pesticides, herbicides, and fertilizers. Plants can further transfer the accumulated metals from the soil to the food chain (soil → plant → food), causing adverse effects on human health (Popescu et al., 2021). Many studies have been done on the chemical composition of *Achillea millefolium*, but in Bosnia and Herzegovina there is no systematic study of this plant considering its possible use in pharmacy, traditional medicine, and also for soil bioremediation. (Manojlović and Singh, 2012; Murtić et al., 2019; Murtić et al., 2021;

Based on everything previously mentioned, the aims of this research are: a) to determine the content of heavy metals in the leaves of *Achillea millefolium* sampled at five urban and five rural locations in Bosnia and Herzegovina; b) determine the content of heavy metals in tea infusions prepared from *Achillea millefolium;* c) to assess the level of environmental pollution with heavy metals and assess the exposure of the human body to heavy metals due to the consumption of tea infusion.

MATERIAL AND METHODS

Achillea millefolium samples were collected in October 2022 and May 2023 at ten selected locations in Bosnia and Herzegovina, including urban and rural areas. Sampling locations are shown in Figure 1. Fresh leaves of *Achillea millefolium* were collected during stable weather conditions, sunny weather, and no precipitation. About 500 g of the sample was collected in paper bags in order to prevent their contamination. The samples were cleaned of mechanical contamination, they were not washed so that the particles deposited on the surface of the leaf could be analyzed. The samples were dried in air and in a ventilated place for 3 weeks. The dried samples were crushed in the laboratory in a mortar using a pestle. About 1.0000 g (±0.0001 g) of the sample was weighed on an analytical balance, and all analysis was done in triplicate. Heavy metals were extracted from plant samples by acid digestion: 10 ml of 65% HNO3 was added to the sample, and it was heated at a moderate temperature until the evaporation of brown vapors stopped. After cooling to room temperature, 2.5 ml of 30% H2O2 was added, and heating was continued at the same temperature range until the solution became clear. The cooled solutions were filtered into a measuring vessel of 50 mL and diluted with ultrapure water (Figure 2).

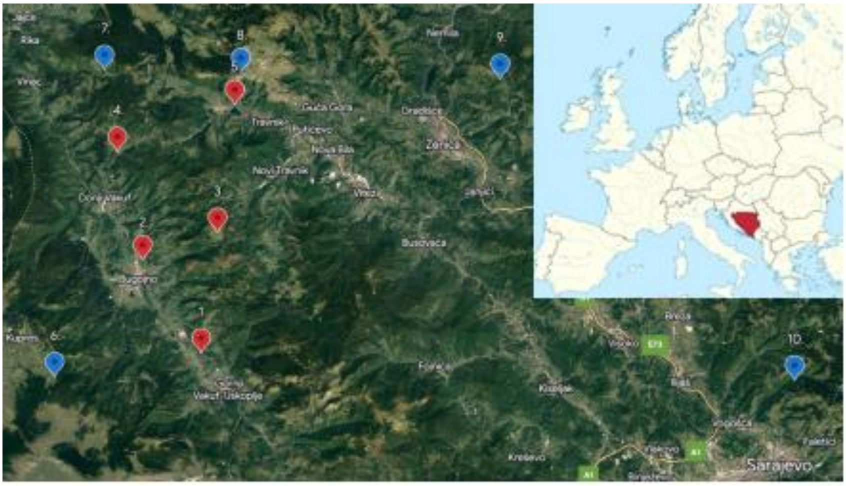


Figure 1. *Achillea millefolium* sampling location (Google Earth). Urban locations are marked in red, and rural locations in blue.

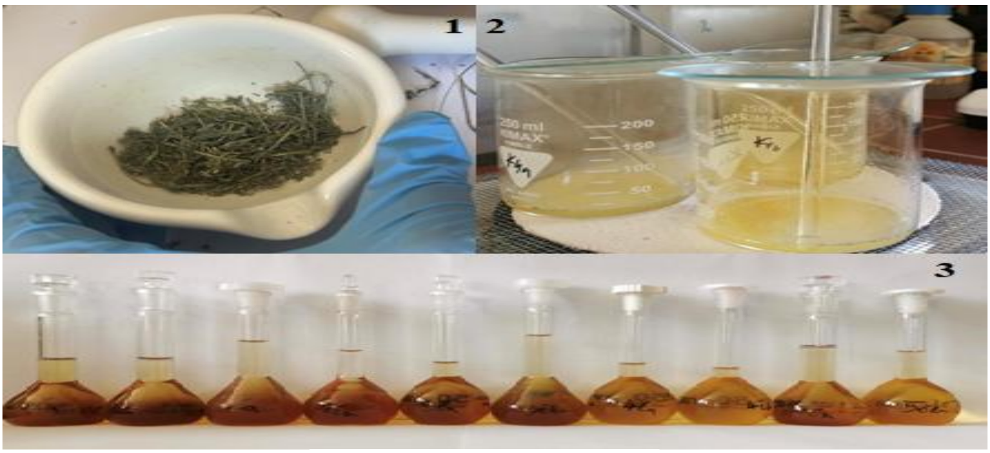


Figure 2. Sample preparation.
1-*Achillea millefolium*, 2-acid digestion, 3-sample solutions prepared for analysis

The prepared solutions were transferred into sterile plastic vials and stored at room temperature until analysis. The metal analysis was performed by using a flame atomic absorption spectrometer (FAAS), model AA240FS, Varian.

For the preparation of tea infusions, about 2.0000 g (± 0.0001 mg) of *Achillea millefolium* leaf sample was weighed on an analytical balance. Tea infusions were prepared according to the following procedure: 50 ml of ultrapure water was heated in laboratory beakers on a stove to the boiling point, then 2 g of the plant sample was added and allowed to boil for another 5 minutes with stands the plant sample was added and allowed to boil for another 5 minutes with occasional stirring. The cooled mixtures were filtered through qualitative filter paper into measuring vessels of 50 ml and diluted to the mark with ultrapure water.

Analytical quality control

All substances used were of analytical grade and supplied from Merck (Darmstadt, Germany). To ensure accurate heavy metal assessment, leaf and water infusion samples were spiked with the standard solution of each metal at three different levels of concentration to cover the measurement range. Recovery values are presented in Table 1.

Table 1. Recovery values of spiked leaf and tea water infusions of the *Achillea millefolium*

Metals	Recovery (%)	
	Leaves samples	Water infusion
Cu	96	96
Mn	98	99
Fe	96	95
Co	88	86
Ni	89	90
Cd	95	93
Pb	98	97
Zn	103	101

The recovery was calculated and ranged from 86% to 103% for all metals. The value of the detection limit (LOD) (calculated as three times the standard deviation of the blank signal) were: Cd (0.001 mg/L), Cr (0.006 mg/L), Cu (0.05 mg/L), Fe (6.15 mg/L), Mn (0.20 mg/L), Ni (0.015 mg/L), Pb (1.00 mg/L), and Zn (0.65 mg/L).

RESULTS AND DISCUSSION

Results of metal content (mean value ± standard deviation) in the leaf of *Achillea millefolium* sampled from the urban location are shown in Table 2, and in the Table 3 are shown results for metal content in the leaf sampled in the rural locations.

Results presented in Table 2. showed that the metal content in leaves of the *Achillea millefolium* sampled in all urban locations was arranged as the following diminishing series: Fe > Mn > Cu > Zn > Pb > Co > Ni, except from the urban location 5 (Turbe, Travnik), where diminishing serires were: Fe > Mn > Zn > Cu > Pb > Co > Ni. At the rural location, metal content in leaves of the *Achillea millefolium* varied from location to location. In rural locations 7 (Dogani, Jajce) and 8 (Vlašić-Galica), metal content was in the following diminishing series: Mn > Fe > Zn > Cu > Pb > Co > Ni. At location 6 (Kukavice, Kupres), the diminishing series: Mn > Pb > Fe > Cu > Zn > Co > Ni, while at location 9 (Smetovi, Zenica), it was: Fe > Mn > Zn > Cu > Pb > Co > Ni, and at location 10 (Nahorevska Brda, Sarajevo), it was: Fe > Mn > Cu > Zn > Pb > Co > Ni.

Table 2. Metal content in *Achillea millefolium* leaves sampled at urban location

Locations	**Metal content (mg/kg)**						
	Cu	**Mn**	**Fe**	**Co**	**Ni**	**Pb**	**Zn**
1	10.14 ± 0.36	68.47 ± 1.12	88.95 ± 8.61	0.132 ± 0.11	<LOD*	1.60 ± 0.45	6.30 ± 1.05
2	9.16 ± 0.05	73.63 ± 1.70	143 ± 19.54	<LOD*	<LOD*	0.684 ± 0.27	8.46 ± 0.68
3	7.42 ± 0.73	46.16 ± 1.13	82.14 ± 5	0.077 ± 0.06	<LOD*	2.59 ± 1.11	4.47 ± 2.53
4	9.70 ± 0.19	100 ± 1.76	138 ± 9.08	<LOD*	<LOD*	3.92 ± 1.46	5.98 ± 0.89
5	7.91 ± 0.01	52.88 ± 0.13	93.42 ± 4.64	<LOD*	<LOD*	1.22 ± 0.65	8.79 ± 0.96

<LOD* below the limit detection of the applied method

1-Pajić Polje, Gornji Vakuf; 2- Bugojno; 3-Rostovo; 4-Oborci, Donji Vakuf; 5-Turbe, Travnik

Table 3. Metal content in *Achillea millefolium* leaves sampled at rural location

Locations	**Metal content (mg/kg)**						
	Cu	**Mn**	**Fe**	**Co**	**Ni**	**Pb**	**Zn**
6	11.42 ± 1.04	78.50 ± 1.63	75.10 ± 4.73	<LOD*	<LOD*	77.72 ± 63.17	7.43 ± 1.12
7	7.76 ± 0.08	203 ± 7.35	52.14 ± 2.48	<LOD*	<LOD*	1.24 ± 0.23	24 ± 0.29
8	5.84 ± 0.11	82.72 ± 3.62	78.53 ± 8.17	<LOD*	<LOD*	4.12 ± 0.15	14.73 ± 1.52
9	11.16 ± 0.77	62.54 ± 0.15	230 ± 83.50	0.435 ± 0.01	<LOD*	2.46 ± 0.02	24.76 ± 15.86
10	11.54 ± 0.25	131.7 ± 0.95	245.7 ± 2.60	0.335 ± 0.27	<LOD*	3.32 ± 1.56	10.46 ± 2

<LOD* below the limit detection of the applied method

6- Kukavice, Kupres; 7- Dogani, Jajce; 8- Vlašić-Galica, Travnik; 9- Smetovi, Zenica; 10- Nahorevska Brda, Sarajevo

Based on the results presented in Table 2. and Table 3, the highest Cu content was recorded in three rural locations: Nahorevska Brda (Sarajevo), Kukavice (Kupres), and Smetovi (Zenica). Nahorevska Brda and Kukavice are rural areas where the population is mainly engaged in agriculture and animal husbandry, so the content of Cu can be explained by the use of fertilizers and pesticides, which represent an important anthropogenic source of Cu in the soil. Also, potential industrial and agricultural sources of Cu are batteries, pigments and dyes, alloys, fuels, and catalysts (Panagos et al., 2018; Rehman et al., 2019). On the other hand, Smetovi is a mountain located in the immediate vicinity of Zenica, a city known for its metal and metal processing industry. The ecosystems of the Zenica region have been exposed to the intense influence of heavy metals emitted from metallurgical plants for several decades. This affected the anthropogenic redistribution of heavy metals in the soil of the Zenica region. The soil and plants were heavily contaminated with heavy metals. However, the key metallurgical plants have not been operating since the beginning of 1992, which is why the soil load with heavy metals in that region has been greatly reduced (Goletić, 2003). Kabata-Pendias (2000) mentioned that the range of Cu content in plants from 5 mg/kg to 20 mg/kg is normal and necessary for proper growth and development of plants, while symptoms of Cu toxicity occur when the content exceeds the value of 30 mg/kg. The Cu content in this study at all sampling locations was within the range considered normal and sufficient for proper plant growth and development.

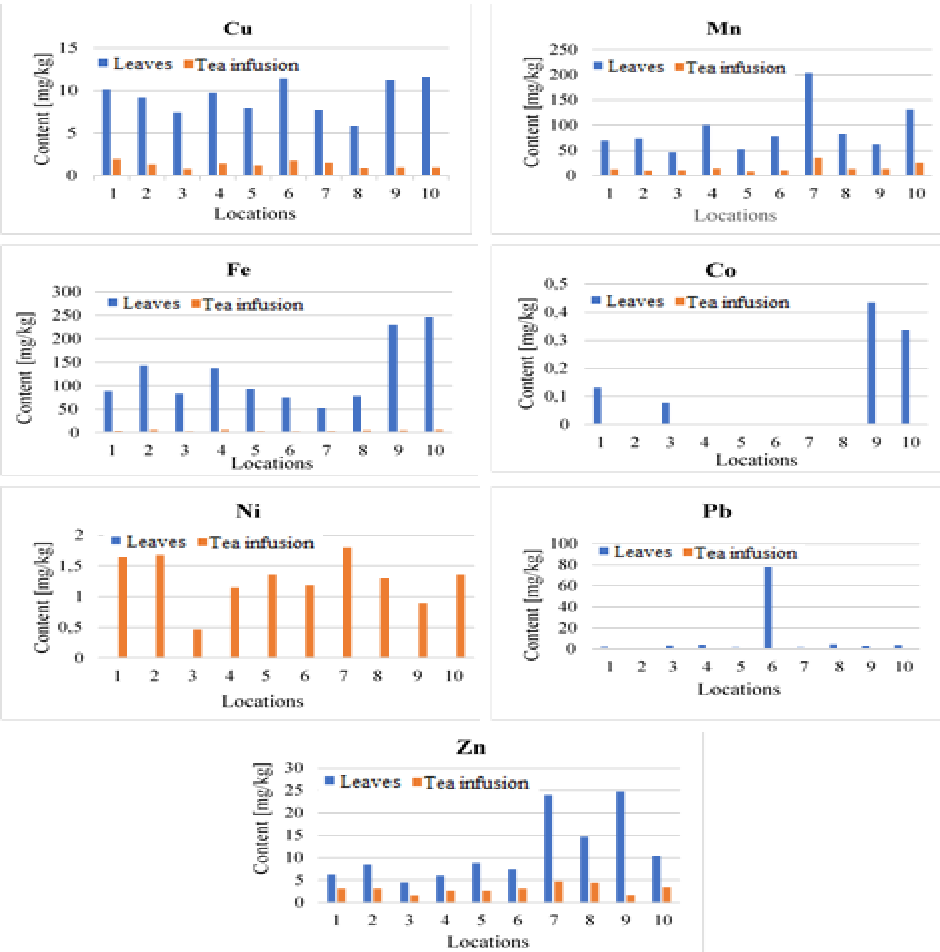
Kabata-Pendias (2000) mentioned that the normal range of Mn content in the leaves of medicinal plants is from 20 mg/kg to 300 mg/kg. Mn content at all sampling locations was within the specified normal range. However, a particularly high content compared to other locations was detected in the samples collected in Dogani, a village about 15 kilometers from the city Jajce. Namely, the factor that most determines the availability of Mn to plants is soil acidity; the more acidic the soil, the higher the availability of Mn. Soil analysis was not done in this experiment, but sampling at this location was done near wild blueberries (Vaccinium myrtillus), which are known to be adapted to acidic soil conditions and can only be found as wild species on such soil (Kabata-Pendias, 2000; Duralija and Konjević, 2022). Papludis et al., (2018) mentioned that Mn can enter the environment through the discharge of municipal waste water, waste sludge, mining and processing of mineral raw materials (smelting of metal ores), emissions during the production of steel and iron alloys, combustion of fossil fuels, and emissions during the combustion of fuel additives. Chizzola (2012) states that the critical value of Fe content below which deficiency symptoms can occur in plants is 50 mg/kg. On the other hand, the Fe content in the leaves is considered normal, even up to 2000 mg/kg. Due to the low toxicity of Fe, these high levels are not of concern to human health. Iron also has an important function in chlorophyll synthesis in the leaf (Živkov-Baloš et al., 2014).As can be seen in Table 2 and Table 3, the values of Fe content at all sampling locations are within the mentioned normal range. However, the highest Fe content was recorded on Nahorevska Brda and Smetovi. The high content of Fe in Nahorevska Brda can be explained by the fact that it is a rural area where fungicides and fertilizers are widely used, which are an important anthropogenic source of Fe in addition to mining and the metal industry. On the other hand, the high Fe content in Smetovi is explained by the fact that the mountain is located near Zenica, a city where steel production is still present, although to a lesser extent than before.

The content of Co in three urban locations (Bugojno, Oborci - Donji Vakuf, and Turbe - Travnik) and three rural locations (Kukavice - Kupres, Dogani – Jajce, and Vlašić - Travnik) was below the detection limit. Maiti (2003) states that the normal content of Co in plants is up to 1 mg/kg, while the range of critical content is between 15 mg/kg and 50 mg/kg. The Co content in this study was normal in all urban and rural locations where *Achillea millefolium* was sampled. According to the World Health Organization (WHO), the maximum permissible content of Pb in dried plant material is 10 mg/kg (WHO, 1998). The content of Pb in the leaf of *Achillea millefolium* was below the maximum permissible content according to WHO at nine out of ten sampling locations. An exception is the location of Kukavice, a village located about 5 kilometers from the center of Kupres, where a surprisingly high content of Pb was detected, almost eight times higher than the maximum allowed content. The reason for such a high concentration of Pb may be that there is a wood, textile, and metal industry in the sub-area of Kupres, as well as the fact that it is a rural location where Pb can originate from coal burning, car exhaust, mineral fertilizers, and municipal waste disposal sites.

The higher content of Zn in the leaves of *Achillea millefolium* was in the samples sampled from the locations Smetovi (Zenica) and in Dogani (Jajce). Kaur et al., (2014) state that one of the most important anthropogenic sources of zinc is steel production facilities. Given that the location of Smetovi is located near the city of Zenica, where there are steel production facilities, this could be one of the reasons for the increased zinc content in the samples of the leaves of *Achillea millefolium*. On the other hand, the higher content of Zn detected in Dogani is explained by the previously mentioned fact that it was caused by acidic soil. Kisić (2012) states that a higher Zn content can be expected on acidic soil. Maiti (2003) states that the critical content of Zn in plants at which symptoms of toxicity may occur ranges from 100 mg/kg to 400 mg/kg. Based on the results presented in Table 2 and Table 3, the Zn content in *Achillea millefolium* leaves was normal and far below the critical level at all sampling locations.

In Bosnia and Herzegovina, the Rulebook on the Maximum Permitted Amounts of Contaminants in Food does not prescribe the permitted amount of any tested metal in the tea infusion (Službeni glasnik BiH 37/09, 2009). On the other hand, in Croatia, the Ordinance on toxins, metals, metalloids, and other harmful substances that can be found in food prescribes a maximum permitted amount of Pb in domestic tea, which is 5 mg/kg (Narodne novine, 2005). The results of this study were compared with other studies (Diaconu et al., 2012; Gogoasa et al., 2013; Kočevar Glavač et al., 2017; Pehoiu et al., 2020; Popescu et al., 2021; Đelić et al., 2021), and the metal content was in the same or similar range.

One of the aims of the study was to determine the metal content in the tea infusion prepared from the leaves of *Achillea millefolium*. Figure 3 presents the results of the comparison of metal content in tea infusion and in the leaves of the *Achillea millefolium*.



* Note: 1 – Pajić Polje, Gornji Vakuf; 2 – Bugojno; 3 – Rostovo; 4 – Oborci, Donji Vakuf; 5 – Turbe, Travnik; 6 – Kukavice, Kupres; 7 – Dogani, Jajce; 8 – Vlašić-Galica, Travnik; 9 – Smetovi, Zenica; 10 – Nahorevska Brda, Sarajevo

Figure 3. Metal content in leaves and tea infusion of *Achillea millefolium*

By comparing the metal content in the leaves and the tea infusion of *Achillea millefolium*, it can be seen that the metal content at all sampling locations detected in the leaves was several times higher compared to the metal content in the tea infusion. The proportion of metals present in the leaf *of Achillea millefolium* that was soluble in water could be extracted by preparing tea infusions. The content of metals extracted in this way decreased in the following sequence: Mn>Fe>Zn>Cu>Ni>Pb.

As already mentioned, the content of Pb in the leaf of *Achillea millefolium* at the Kukavica location near Kupres was surprisingly high. By preparing a tea infusion from *Achillea millefolium* from the same location, an extremely low content of Pb (0.224 mg/kg) was detected, which did not significantly differ from the content of this metal in tea infusions from other locations. This tells us that Pb is present in this plant mostly in forms that are not soluble in water. The content of Co in all samples of tea infusion was below the detection limit of the applied method, while in the leaf of *Achillea millefolium* Co was detected in samples from four out of ten locations (Pajić Polje - Gornji Vakuf, Rostovo, Smetovi - Zenica, Nahorevska Brda, Sarajevo). On the other hand, Ni was detected in the tea infusion, but its content in the *Achillea millefolium* leaves itself was below the detection limit of the applied method. This phenomenon could have occurred for several potential reasons: (1) it is possible that Ni in the leaf of *Achillea millefolium* is present in a form that is more soluble in water than in nitric acid; (2) it is possible that Ni formed complex compounds with some of the components released by the decomposition of plant material during the acid digestion procedure, which made it impossible to detect it in the leaves of *Achillea millefolium* using FAAS. Results from the studies: Dobrinas et al., (2011); Diaconu et al., (2012); Altıntıg et al., (2014) and Popović et al., (2017).

CONCLUSIONS

The metal content in the leaves of *Achillea millefolium* depends on the place where the sampling was carried out and the potential natural or anthropogenic sources of heavy metals located nearby, as well as on the acidity of the soil. The metal content in tea infusions depends on the solubility in water of the particles found on the surface of the leaves or from the chemical form in which the metal is absorbed inside the leaves of *Achillea millefolium.*

Future research related to the preparation of the tea infusion and the amount of extracted metals could be related to the assessment of the influence of the shredding of the leaves, the amount of dry or raw sample, the length of the extraction time, as well as the use of different parts of the plant (root, stem, leaf, and flower).

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