Ripe and unripe seed of *Xanthium italicum* - elemental composition

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ABSTRACT

Elements content in plants varies considerably, which is a consequence of various factors, such as plant species, vegetative stage, pedological characteristics of the soil and environmental conditions. The subject of this work is an investigation of the elemental composition of *Xanthium italicum* ripe and unripe seed collected in Temska using ICP OES. Out of the analyzed macroelements, potassium is the one with the highest concentration, and its content significantly differs in the ripe and unripe seeds. Iron showed five times higher concentration in unripe (27.7 µg/g) compared to ripe (6.2 µg/g) seed. The content of Ba, Cr, Cu, Mn, Pb, and Zn also differ in the ripe and unripe stages of the analyzed seed, which indices that elemental composition is affected by the vegetative stage. Lead and arsenic content are higher than permissible limits, which might affect honey production in this region.

Keywords: Xanthium italicum, elemental composition, ICP OES, seed

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Introduction

Plant usage dates back to the first days of human history. Even today, in the era of great technological achievements, interest in plant usage in pharmacy, medicine, nutrition, as well as in other industries increases. The reason for that is the fact that plants are considered to be biochemical factories, with numerous and diverse products. For a plant to have the practical application it is necessary to know its chemical composition. However, the great natural products potential has not yet been sufficiently studied and used. Thus, chemical composition and product quality research represent an important field of scientific research because it helps to create conditions for successful plant usage, which can be of great importance for the economy and human health.

Xanthium species belong to important weed species, widely distributed, both worldwide and in Serbia. In many countries, they have the character of very important weed species, and in some, they are also classified as invasive species (Anastasiu et al., 2007; Galanos, 2015). They could be poisonous to animals. However, some of them have anticancerogenic effects and inhibit the development of lung and ovarian melanoma cells, as well as the central nervous system and colon (Ramirez-Erosa et al., 2007). It could also be used for the treatment of inflammatory diseases, such as rhinitis, empyema, and rheumatoid arthritis (Yoou et al., 2008).

X. italicum is an annual species, with large dispersion and propagation capacity, 30-100 cm tall, yellow-green, with an aromatic smell. As a weed, *Xanthium italicum* is present in wheat (Novák et al., 2009), sunflower (Manilov & Zhalnov, 2018), potato (Ilić & Nikolić, 2011), cotton (Economou et al., 2005), as well as in rural habitats (Fetvadjieva & Milanova, 1998). Its presence was also recorded in sports and recreational fields (Stevanović et al., 2009), indicating the great adaptability of *Xanthium* species and their potential to spread and persist in highly unstable plant communities. Its potential benefit could be reflected in the fact that it belongs to honey plants (Zima & Štefanić, 2018). *X. italicum* also possesses allelopathic activity, because xanthosine from its fruit reduces the germination of some weed seed species (Shao et al., 2012).

The chemical composition of plants is mostly affected by plant species, environmental composition and plant maturity. Previous studies on the biochemical composition of various plants fruits (Izonfuo & Omuaru, 1988; Baiyeri, 2000; Baiyeri and Unadike, 2001) reported that nutrient composition is significantly affected by plant maturity. Baiyeri (2000) found higher doses of N, P, K, Mg and Ca in ripe plantain pulp compared to unripe but lower Fe, Cu, Zn and Na concentrations in ripe fruits.

This study aimed to investigate the content of Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Si and Zn content in *Xanthium italicum* ripe and unripe seed using ICP OES. After the elemental composition is determined, differences in their content are studied to point out if the vegetative stage affects it. As far as we know, this is the first time ripe and unripe seed was analyzed.

Experimental

Plant and soil material

X. italicum plant was collected around the village of Temska. Samples were air-dried, homogenized and stored at 4°C until analysis.

Chemicals and instruments

Hydrochloric acid, nitric acid (65%), and hydrogen peroxide (30%), were purchased from Merck (Darmstadt, Germany). Multi - element standard solutions (20.00 ± 0.10 mg L^{-1}) used for ICP analysis was purchased from Ultra Scientific (North Kingstown, RI, U.S.A.).

The measurements were carried out with an ICP-OES iCAP 6000, Thermo Scientific. Table 1 shows the analytical parameters for ICP-OES.

Table 1. ICP-OES instrumental parameters

Flush Pump Rate	100 rpm
Analysis Pump Rate	50 rpm
Nebulizer gas	0.7 $Lmin^{-1}$
Coolant Gas Flow	12 $Lmin^{-1}$
Auxiliary Gas Flow	0.5 $Lmin^{-1}$
Plasma View	axial
Flush time	30 s

Table 2 shows the selected wavelengths of the investigated elements, limits of detection (LOD), limits of quantification (LOQ) and correlation coefficients of calibration curves (r).

Tabel 2. ICP OES method parameters

Element	**λ (nm)**	**LOD (ppm)**	**LOQ (ppm)**	**r**
Al	396.1	0.001234	0.004114	0.999625
As	189	0.003263	0.010877	0.999873
B	208.9	0.000982	0.003274	0.998998
Ba	455.4	0.000061	0.000202	1
Be	311.1	0.000435	0.001346	1
Ca	317.9	0.000333	0.001111	0.999927
Cd	226.5	0.000137	0.000456	0.999476
Co	228.6	0.000266	0.000888	0.999453
Cr	267.7	0.000561	0.001871	0.998638
Cu	324.7	0.000426	0.001418	0.999359
Fe	240.4	0.000547	0.001822	0.998834
K	766.4	0.001143	0.003811	0.994010
Mg	280.2	0.000127	0.000425	0.999967
Mn	259.3	0.000082	0.000273	0.998823
Na	589.5	0.000009	0.000031	1
Ni	231.6	0.000422	0.001408	0.999049
P	213.1	0.004049	0.013497	0.999962
Pb	220.3	0.001858	0.006195	0.999684
Si	251.1	0.001897	0.006323	0.999999
Zn	213.8	0.000097	0.000323	0.998704

Sample preparation

An acid digest of plant species was prepared by oxidizing 1 g of sample with conc. HNO_3 and left in the dark for 12 hours. After that, H_2O_2 (30%) and water were added. A digestion procedure was applied to obtained mixtures to reduce the volume and improve decomposition. Another portion of H_2O_2 was added and evaporation continued. After cooling concentrated HCl was added, and the mixture was left overnight. The resulting suspension was filtered and the rest is rinsed with hot HCl and then heated with deionized water. Filtrate was collected in a volumetric flask and diluted (US EPA, 1996). Each sample was analyzed twice, and the data were reported as a mean of the analyzed samples in µg/g.

Statistical analysis

The data were based on two replicates and subjected to statistical analysis in Statistica 8.0 software (StatSoft, Tulsa, Oklahoma, USA). A probability level of $p < 0.05$ was considered statistically significant.

Results and Discussion

All elements can be considered as biologically important elements, toxic and elements that have no biological role, but no toxic effect when reaching the body in small concentrations. However, it is not always easy to distinguish toxic elements from others, as they are all considered to be toxic in higher quantities. Metals can affect a long list of physiological and biochemical processes in plants and their toxicity varies with plant species. The inadequate supply of a nutrient, whether leading to deficiency or toxicity, affects plant growth and results in yield and quality losses in agricultural plants (Brdar-Jokanović, 2020).

Macroelements content

For normal plant growth and development, 17 elements are needed, of which 9 (C, O, H, N, S, Ca, K, Mg and P) are needed in higher amounts (>0.1 %) and these are called macro elements. Macro elements are structural components of tissues with certain functions in cells and metabolism, as well as in water and acid-base balance (Imelouane et al., 2011). The content of macroelements analyzed in *X. italicum* ripe and unripe seed are presented in Table 3.

Tabela 3. Macroelement content in unripe and ripe *X. italicum* seed (µg/g)

Element	Unripe	Ripe
Ca	1173 ± 94^{a}	1740 ± 106^{b}
Na	13.9 ± 0.9^{a}	16.6 ± 0.4^{a}
K	9918 ± 66^{a}	8813 ± 11^{b}
P	5044 ± 83^{a}	4347 ± 16^{b}
Mg	472 ± 5^{a}	483 ± 9^{a}

Values followed by the same letter are not significantly different at $p \leq 0.05$ significance.

The element with the highest concentration in the analyzed plant tissue is K with 9918±66 µg/g for unripe and 8813±11 µg/g for ripe seed. Potassium is important for ensuring optimal plant growth (White & Karley, 2010). Changes in potassium content might be explained by its great mobility in plants since it is an important component in protein synthesis and carbohydrate metabolism (Trankner et al., 2018). Phosphorus, an essential nutrient, is a component of the complex nucleic acid structure of plants, which regulates protein synthesis, and it is important in cell division and the development of new tissue. Phosphorus contributes to flower initiation and root, seed, and fruit development. This element is highly mobile in plants, and when deficient, it may be translocated from old plant tissue to young, actively growing areas. As a plant matures, phosphorus is translocated into the fruiting areas of the plant, where high-energy requirements are needed for the formation of seeds and fruit. Phosphorus content was also higher in unripe (5044±83 µg/g) compared to ripe *X. italicum* seed (4347±16 µg/g).

On the other hand, the content of three other analyzed macroelements (Ca, Na and Mg) is slightly higher in ripe compared to unripe seeds. Differences in Na and Mg content are not significant, whereas Ca content is higher in ripe seed for about 600 µg/g. Paul et al. (2012) concluded that relatively immobile element such as Ca migrates to the fruit at later stages of plant development, which agrees with our results. Calcium as an essential plant nutrient is required for various structural roles in the cell wall and membranes. This secondary nutrient, critical to crop development, is only xylem mobile, meaning it can only move up the plant, and once in place, it cannot be remobilized and moved to new developing tissues.

Among macroelements lowest concentration was recorded for Na (13.9±0.9 and 16.6±0.4 µg/g for unripe and ripe fruit, respectively). Higher plants require sodium to be able to grow to their full potential and increased growth rates. Compared to Chirigiu et al. (2003), macroelement content in analyzed *X. italcium* Mg and Na content was lower in the present study, but K and Ca content was a few times higher in our *X. italicum* seed samples. The reason for that could be environmental conditions and plant maturity, but also different instrumental techniques have been used for elemental analysis.

Microelements content

Microelements are essential elements that are required in relatively small amounts for plants' metabolic processes, and these include iron, manganese, zinc and copper (Wiedenhoeft, 2006). Microelements content in analyzed *X. italicum* seed are presented in Figure 1.

As can be seen from Figure 1, among microelements, an element with the highest concentration in analyzed samples is Fe, for unripe *X. italicum* seed (27.7 µg/g). In the case of ripe seed, Fe content is almost five times lower (6.3 µg/g). For iron adsorption to plant environmental conditions, a high pH value, but also phosphate and Ca^{2+} concentrations, are key factors, which reduce iron mobility from soil to plant. At the vegetative stage, most of the iron is translocated to aerial parts and used for photosynthesis, whereas at the reproductive stage, a large part of the Fe present in vegetative tissues is transferred to the seeds (Mari et al., 2020). Also, its important role is in pectin metabolism and influences tissue softening during ripening (Bai et al., 2021). However, in the case of *X. italicum* in this study, Fe content is much lower for ripe seed. The content of other analyzed microelements is lower in ripe seeds, compared to unripe ones.

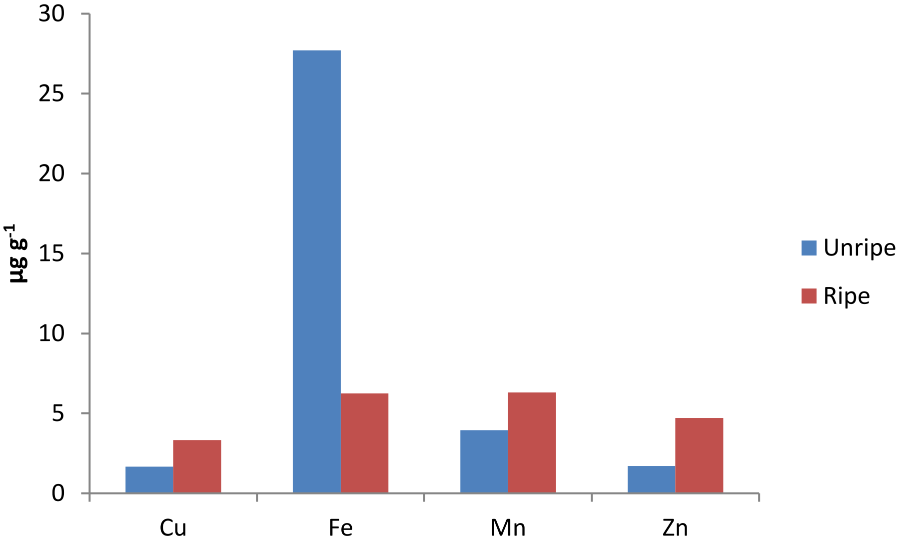

Figure 1. Content of Cu, Fe, Mn and Zn in analyzed *X. italicum* seed

Zinc is an essential element for all living organisms and plays an important role in the biosynthesis of enzymes, auxins, and other proteins in plants. The main signs of Zn toxicity in various plant species are indicated by a decrease in growth and development of the plant, alteration in metabolism processes and induction of oxidative damage (Versieren et al., 2017). Zn concentration is three times lower in ripe (1.7 µg/g) compared to unripe (4.7 µg/g) *X. italicum* seeds (Figure 1). Plants mainly absorb zinc in the form of Zn^{2+} cation, but in alkaline environment in the form of $ZnOH^+$ cation; Zn mobility in plants is moderate. Zinc increases the tolerance of plants to diseases. The range of 300-400 mg/kg d.w. is accepted as the toxicity limit for Zn in plants (Kabata-Pendias & Pendias, 1992). Compared to the Zn content of *X. strumarium* (average 0.129 µg/g) analyzed by Tadesse et al. (2018), *X. italicum* from this study had a higher Zn concentration (average 3.2 µg/g). However, Chirigiu et al. (2003) found 31.6 and 32.5 µg/g of Zn in *X. italicum* and *X. spinosum*, respectively, indicating that Zn content is not only affected by plant species but also the geographic origin and atmospheric conditions.

Copper is another essential element for plants and they mainly absorb copper in the form of Cu^{2+} ions and chelates. Excess Cu can cause a lack of other elements, especially manganese and iron. Studies have shown that plants that have high concentrations of oxygen and phosphorus are generally characterized by a low concentration of copper. Copper mobility in plants is medium and the first defects appear on the youngest organs. Twice a higher concentration of Cu is recorded in ripe (3.3 µg/g) compared to unripe seed (1.7 µg/g) (Figure 1).

Among microelements, there are ones with known biological function in plants, as well as ones with no function or toxic ones. For example, Zn, Ni, Cu, V, Co, W and Cr are considered to be toxic elements, but also essential in trace amounts, while As, Hg, Ag, Sb, Cd, Pb and U, have no known beneficial role and they are toxic to plants. Content of Al, As, B, Ba, Be, Cd, Co, Cr, Ni, Pb and Si content in analyzed *X. italicum* ripe and unripe seeds is presented in Figures 2 and 3.

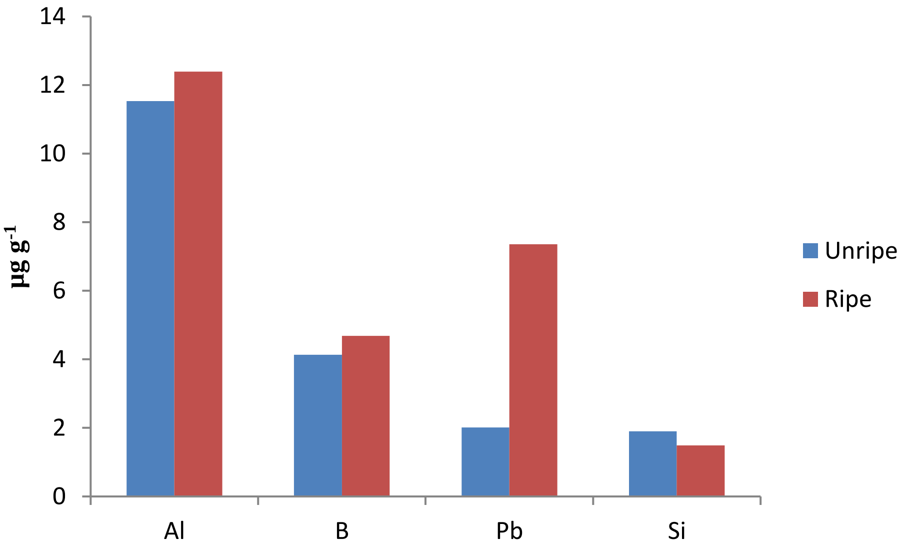

Figure 2. Content of Al, B, Pb and Si in analyzed *X. italicum* seed

Among elements presented in Figure 2, Al, B and Si have certain functions in plant tissues, in small amounts, whereas Pb is toxic to plant tissues.

Despite the abundance of aluminum (it is the third most abundant element after oxygen and silicon in the earth's crust), it is not considered as an essential element, and so far no experimental evidence has been put forward for a biological role. Its availability depends on soil pH. It is interesting that aluminum can have a beneficial or toxic effect, depending on different factors such as the chemical form of Al, metal concentration, plant species, physiological age, the duration of exposure to the metal and growth conditions. Aluminum content varied from 11.5 to 12.4 µg/g, which is not a significant difference for unripe and ripe *X. italicum* seeds. Si and B content was lower compared to Al. Boron is an essential or at least highly beneficial micronutrient for animal organisms, affecting the metabolism of macro minerals Ca, P, and Mg, proteins, triglyceride, amino acids, glucose, steroid hormones, and reactive oxygen species. Plants take up boron in the form of small uncharged boric acid molecules, as well as borate anions (Brdar-Jokanović, 2020). Same as in the case of aluminum, boron content was lower in unripe (4.1 µg/g), compared to ripe (4.7 µg/g) seed, but this difference was not statistically significant.

Lead is an extremely toxic element and plants have no channels for its uptake, so it is unknown how exactly it enters the root. It can remain attached to the carboxyl groups of uronic acids on the root surface. Lead is mostly absorbed by the root and remains in it, thus making the root the first barrier for further transport of Pb to the aerial parts of the plant, where its phytotoxicity could be fatal. Its adverse effects on mineral nutrition, water content, photosynthesis, morphology, seed germination, seedling growth, and enzymatic activities are confirmed for all plant species. In higher concentrations lead inhibits root and leaf growth, photosynthesis, and affects the morphological and anatomical structure of plants. Analyzed *X. italicum* seed showed high concentrations of Pb, 2.0 and 7.4 µg/g for unripe and ripe fruit, respectively (Figure 2).

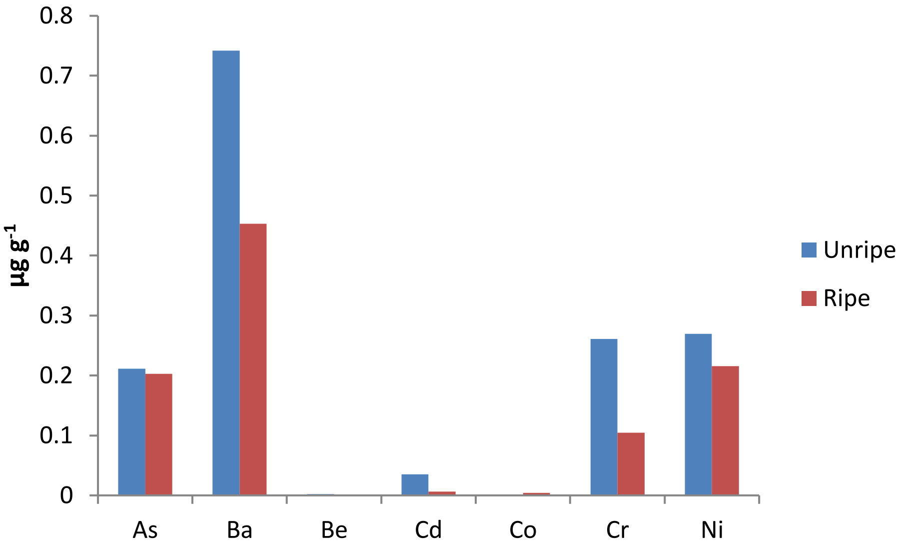

Figure 3. Content of As, Ba, Be, Cd, Co, Cr and Ni in analyzed *X. italicum* seed

According to WHO (1996) recommended permissible limit for Pb is 2 µg/g. The Pb level in the analyzed plant is higher than the permissible level, indicating that this plant could be used in Pb remediation from soil. The increased content of Pb can be explained by its presence in the soil, which originates from exhaust gases of motor vehicles, as well as from air transport and its deposition from air to soil and plant. Davies and Holmes (1972) found that if the content of Pb in gasoline is 0.45 g/L and if the flow rate of the vehicle is 24h, then on both sides road with a width of 15 m, with every 1000 vehicles, the concentration of lead in the air increases for 1 µg/m^3.

Arsenic is a non-essential and generally toxic element to plants, which inhibits root extension and proliferation, inhibits biomass production, interferes with metabolic processes and can severely inhibit plant growth by compromising plant reproductive capacity. As^{5+} is easily mobilized and taken up by plants through phosphate transport pathways. Due to their chemical similarity, As^{5+} competes with phosphates in the uptake process and it interferes with metabolic processes, such as the synthesis of ATP and oxidative phosphorylation. Arsenic can severely inhibit plant growth by compromising plant reproductive capacity. Arsenic content in unripe and ripe seeds did not vary significantly (0.21 µg/g and 0.20 µg/g, respectively) (Figure 3). According to FAO/WHO (1999) maximum permissible level for As in plants is 0.1 µg/g, which is twice lower than As content in *X. italicum* from this study. Since this plant is characterized as a honey plant, this could affect honey quality.

Chromium is an element classified as a carcinogen agent according to the International Agency for Research on Cancer. The toxic effects of Cr are correlated with the generation of reactive oxygen species (ROS), which cause oxidative stress in plants. The most common and stable states of chromium are hexavalent (Cr (VI)) and trivalent (Cr (III)) (IARC, 1987). Due to its high redox potential and intricate electronic and valence shell chemistry, chromium can easily convert from one oxidation state to another. Under physiological conditions, Cr (VI) enters the cells and may get reduced to Cr (V), Cr (IV), triradicals, hydroxyl radicals and finally Cr (III). All these oxidation states disrupt the cellular integrity of cells by attacking proteins, DNA and membrane lipids (Sharma et al., 2020). According to literature data, the concentration of chromium in plants is very low. The average chromium concentration in plant tissues is 0.2 to 4 mg/kg of dry plant matter. Cr excess in plants results in chlorosis and growth inhibition. Chromium concentration is twice higher in unripe (0.26 µg/g) compared to ripe seed (0.10 µg/g) in the presented study (Figure 3).

Compared to the maximum permissible level of Cr prescribed by FAO/WHO (1999) *X. italicum* from this study showed a 10 times lower concentration. Chirigiu et al. (2003) reported 5.525 µg g^{-1} of Cr in *X. italicum* and 0.562 µg g^{-1} in *X. spinosum*, so *X. italicum* from our study is more similar to *X. spinosum* by Cr content.

Same as Cr, nickel can also result in chlorosis in plant tissues in higher concentrations. Nickel helps plants to absorb Fe from the soil. It is very important for urease activity and has an impact on seed germination, too. Plants suffering from Ni deficiency show necrosis initiating from the tip of the leaf. Same as in the case of Cr, Ni concentration is higher in unripe (0.27 µg/g) compared to ripe (0.21 µg/g) seed (Figure 3).

Cadmium is an element with a very toxic effect on plants, animals, and humans. It is an extremely mobile element in the soil, easily transported through the plant and distributed to all plant organs subsequently. Cd and Zn are very similar, and in addition, Cd can imitate the behavior of some other essential elements in uptake from the soil and metabolism. The main cause of cadmium toxicity represents the high affinity of Cd for thiol groups in enzymes and proteins. Higher concentrations in plants inhibit iron metabolism and reduce the intensity of photosynthesis. The content of Cd (0.006 µg/g in ripe seed and 0.035 µg/g in unripe seed, Figure 3) is significantly lower compared to the WHO recommended value of 0.3 µg/g. It has been observed that high concentrations of Fe in the soil reduce Cd uptake by plants. Other metals, such as Zn, Ca, Mg and Cu, can also inhibit the uptake of Cd from the rhizosphere, among which the level of Ca has the strongest effect, as both these ions can pass through the membrane and via cation channels, their competition is very pronounced.

Conclusion

The content of 20 elements (Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Si, Zn) was determined in unripe and ripe *X. italicum* unripe and ripe seed. Out of the analyzed macroelements, K showed the highest concentrations in ripe and unripe seeds, while the lowest concentration was recorded for Na. Fe is the most abundant microelement, and it showed a five times higher concentration in unripe compared to a ripe seed. Surprisingly, Pb content was higher than WHO permissible limits, which might be because plant samples are collected near the road.

The content of Fe, Ba, Cr, Cu, Mn, Pb, and Zn differ in the ripe and unripe stages of the analyzed seed, which indices that elemental composition is affected by the vegetative stage.

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Conflict-of-Interest Statement

The authors did not declare any conflict of interest.

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