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MANGANESE POLLUTION IN AGRICULTURAL SOILS WITH IMPLICATIONS FOR FOOD SAFETY

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Summary

Introduction and objective: Manganese (Mn) is an essential element for the plant and it is necessary for maintain physiological processes, notably photosynthesis, but its higher content in the soil may negative affect the plant, and consequently human health. The objective of this study was to examine the Mn accumulation in edible parts of tested food crops growing on soils near two Mn ore deposits in Bužim municipality (active Mn mine Bužim and Mn ore deposits Radostovo). *Methods:* Atomic Absorption Spectroscopy (AAS) was used to determine Mn content in soils and edible parts of different food crops; onions, cabbage strawberry, garlic, potato, pepper, beans and raspberry. *Results:* The content of Mn available forms, and accumulation in edible parts of examined food crops was significantly higher in soils in the area around Mn mine Bužim although the content of the total Mn in the soils at the site Radostovo were much higher. Considering that soils in the area around Mn mine Bužim are much more acidic than soils at the site Radostovo, it is evident that soil pH is one of the key factors in the assessment of Mn availability in soil. The results of study also showed that the content of Mn in edible parts of all tested food crops did not exceed the toxic value for Mn in plants (400 mg/kg). *Conclusions:* From the point of view of soil pollution with Mn, both examined sites can be considered suitable for production of healthy food.

Keywords: ore deposits, fruits, vegetables, health

Introduction

Bužim is a town and municipality situated in the northwestern part of Bosnia and Herzegovina. Agriculture plays a strategic role in the process of economic development of this municipality, which is largely a result of the existing climatic and pedological features that provide favorable conditions for different types of agricultural production.
The wide geological diversity, diverse range of lithologies, and the peculiar hydrological conditions resulted in pronounced pedosphere heterogeneity in this area, with the accentuated representation of the following soil types: eutric cambisol, kalkocambisol, kalkomelanosol, rendzina, and in lowland areas hydromorphic soil (Čićić and Bašagić, 2001).
These soils, with their physical, chemical and biological properties, represent the appropriate medium for the cultivation of food crops, but the success of plant growth on them largely depends on the mineral composition of the lithological structure i.e. the parent material on which these soils are formed. The lithological structure is highly complex at the area of Bužim municipality (Čićić, 2002). In the complex formations of that parent material characterized by limestones, dolomites, and the basic igneous rocks there are also Mn ore deposits that greatly affect the chemical properties of the soil, and thus the possibility of cultivating food crops. At the area of Bužim municipality, there are few large deposits of Mn ore, and the largest among them is active Mn mine Bužim in the local community Vrhovska, located approximately 8 km northeast of the Bužim town.
Mn is essential for many plant functions, particularly for photosynthesis as part of the structure of photosynthetic proteins and enzymes playing an important role in water-splitting system of photosystem II (Mousavi et al., 2011), and for antioxidant defense system in plants as an enzyme antioxidant-cofactor (Millaleo et al., 2010). Contrarily, the excess of Mn in plants resulting in a reduction of biomass and photosynthesis, and biochemical disorders such as oxidative stress (Lei et al, 2007).
Given the above, the objective of this study was to examine the Mn accumulation in edible parts of tested food crops growing on soils near two Mn ore deposits in Bužim municipality.

Materials and methods

Study area

The experiment was carried out during 2018 at two sites in the Bužim municipality. The first study site was the area around the active Mn ore mine Bužim, located in the local community Vrhovska. At this site, the research involved three soil plots that were located at a very close distance from each other (up to 500 m).

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According to Soil Taxonomy, examined soils were classified as Cambisol (FAO, 1998), and characterized by the following physical properties: medium-texture without gravel and stones, fine crumb structures in the upper horizons that provide good soil aeration, permeability and water-holding capacity in root zone. The depth of the arable soils profile was 30 - 40 cm, and the food crops sampled on these soils were as follows: *Allium cepa* L. (onions), *Rubus idaeus* L. (raspberry), *Brassica oleracea* L. var. *capitata* (cabbage) and *Fragaria viridis* Weston (strawberry).
The second study site included three soil plots near the Mn ore deposits Radostovo, located approximately 1 km northeast of the Bužim town. According to FAO Soil Taxonomy, examined soils were classified as kalkocambisol, and characterized by the following physical and chemical properties: moderately fine texture, good water-holding capacity, neutral to slightly acidic with good availability of nutrients. The depth of the arable soils profile at this study site was 40 - 50 cm, and the food crops sampled on these soils were as follows: *Allium sativum* L. (garlic), *Solanum tuberosum* L. (potato), *Capsicum annuum* L. (pepper), *Phaseolus vulgaris* L. (young beans) and *Rubus idaeus* L. (raspberry).

Soil sampling and chemical analysis

The soil samples were taken from the tested soil plots before cultivation at a depth of 0 - 30 cm using a soil sampler probe. Average sample from each test soil was prepared by mixing of five individual samples. The chemical analyses of average soil samples were carried out at the laboratory of the Faculty of Agriculture and Food Sciences, University of Sarajevo, and the following parameters were analyzed: soil acidity, humus content, content of available forms of phosphorus (P_2O_5) and potassium (K_2O), as well as the most important parameter: the amount of total and available forms of Mn in the soil.
Soil acidity was determined by pH meter in accordance with ISO 10390 method (ISO, 2005), humus content by sulfochromic oxidation method (ISO, 1998), content of available forms of potassium and phosphorus by AL - method (Egner et al., 1960), and the total and available forms of Mn by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) according to the instructions specified in the ISO 11047 method (ISO, 1998).

Extraction of total Mn from soil

Extraction of total Mn from the soil sample was carried out using aqua regia solution in accordance with ISO 11466 method (ISO, 1995) as follows: 3 g of the air-dried soil was placed in 250 ml round bottom flask, 28 ml of aqua regia (21 ml 37% HCl and 7 ml 65% HNO_3) was added and then the flask was covered with a watch glass and allowed to stand 16 h (overnight) in the digester. After, the flask with mixture was heated on hotplate under reflux for 2 h, cooled to room temperature, and after the mixture was filtered through quantitative filter paper into 100 ml flask and diluted to the mark with deionized water.

Extraction of available forms of Mn from soil

The extraction of available forms of Mn was performed using the EDTA solution (Trierweler and Lindsay, 1969) as follows: 10 g of air-dried soils were placed into 100 ml plastic bottle then 20 ml EDTA solution (0.01 mol dm^{-3} ethylenediaminetetraacetic acid (EDTA) and 1 mol dm^{-3} $(NH_4)_2CO_3$, adjusted to pH 8.6) was added. The bottle was shaken 30 min in an orbital shaker at 180 rpm, then the mixture was filtered through quantitative filter paper into 25 ml flask and diluted to the mark with deionized water.

Plant sampling and analysis

Edible parts of plants from the examined soil plots were collected at the stage of commercial maturity in a quantity of approximately 300 g for each food crops. The content of Mn in the dry plant samples was also determined by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) according to method ISO 11047 (1998).
Previous extraction of Mn from the plant samples was performed using HNO_3-H_2SO_4 solution (Lisjak et al., 2009) as follows: 1 g of dry matter was placed into 100 ml round bottom flask, and 10 ml 65% HNO_3 and 4 ml 95-98% H_2SO_4 were added. The flask was covered with a watch glass, allowed to stand for few hours in the digester and then heated gently on a hot plate for 30 min. After cooling to room temperature, the mixture was filtered through quantitative filter paper into 50 ml flask and diluted to the mark with deionized water.

Statistical analysis

All measurements were done in triplicate. Statistical analysis was performed using Microsoft Excel 2016 and differences between means were tested using the least significance difference (LSD) test at $P < 0.05$.

Results and discussion

A summary of soil chemical properties near the active Mn mine Bužim and Mn ore deposits Radostovo is given in Table 1.

Table 1. Soil chemical properties at the examined sites

Parameter	Active Mn mine 'Bužim'			Radostovo			$LSD_{0.05}$
	Soil 1	Soil 2	Soil 3	Soil 1	Soil 2	Soil 3	
pH H_2O	5.8^{d}	5.9^{d}	6.0^{d}	7.1^{ab}	7.0^{abc}	7.2^{a}	0.41
pH KCl	5.0^{d}	5.2^{d}	5.1^{d}	6.4^{a}	6.2^{abc}	6.3^{ab}	0.43
Humus (%)	1.88^{abc}	1.92^{ab}	1.98^{a}	1.57^{e}	1.67^{de}	1.72^{d}	0.14
P_2O_5 (mg/100 g)	1.53^{d}	1.78^{d}	2.12^{d}	16.94^{a}	12.11^{b}	11.87^{bc}	2.08
K_2O (mg/100 g)	9.7^{d}	11.3^{d}	9.7^{d}	13.1^{bc}	16.11^{ab}	18.12^{a}	3.14
Total Mn (mg/kg)	1072.3^{d}	1011.5^{d}	1043.6^{d}	1914.9^{ab}	1998.1^{a}	1897.3^{abc}	254.2
Available Mn (mg/kg)	27.57^{a}	24.11^{b}	23.65^{bc}	19.78^{d}	18.97^{d}	19.11^{d}	2.67

Each value is a mean of three replicates. Different letters in each column represent significant difference ($P<0.05$)

The soils near the active Mn mine Bužim were acidic, with low content of available forms of phosphorus and potassium. Contrarily, the soils at the site Radostovo had higher pH pH (in H_2O 7 or slightly above), and moderate levels of available forms of phosphorus and potassium. The content of organic matter in all examined soils was moderate i.e. in the same categorization according to Egner et al. (1960). In accordance with results of soil chemical analysis, for all investigated soil plots were made appropriate nitrogen-phosphprus-potassium (NPK) fertilizers recommendation, which were carried out during food crops cultivation.

In the present study, the total Mn content of soils in both examined sites was above the average Mn content of 437 mg/kg dry mass reported by Kabata-Pendias and Pendias (2001). Kastori et al. (1997) noted that total Mn in the soil usually ranges from 200 to 2000 mg/kg, while some scientists mentioned lesser or higher values for the Mn content in the soil, from 134.79 to 247.62 mg/kg as reported by Shah et al. (2013) and 2500 mg/kg as reported by Pavilonis et al. (2015) respectively.

According to Škorić (1991), the content of Mn in soils differs considerably, primarily depending on the parent material from which soils have developed through the processes of pedogenesis. He also reported that Mn is more present as mineral in metamorphic and igneous rocks, and less in sedimentary rocks.

Although the high Mn presence in the soil may have a negative effect on the plants, and thus on humans (Gupta and Gupta, 1998), the legislature in Bosnia and Herzegovina does not establish the limit value of Mn which would indicate the pollution of soil by Mn, primarily because Mn is not considered as health hazard element. Regardless of the fact that this issue is not regulated, the value of 1000 mg/kg among scientists is taken as the permissible value for the Mn content in agricultural soils (Vukadinović i Lončarić, 1998).

As shown in Table 1, Mn total content of soils in both examined sites was higher than the previously mentioned limit value, and that was expected since the examined soil plots were located near the Mn ore deposits. These results indicate that all examined soils in this study have the potential to contaminate the agricultural crops with Mn.

If compared the results of total Mn in soils between studied sites it can be observed that the content of total Mn in soils at the site Radostovo was almost twice higher than the content of total Mn in soils around Mn mine Bužim, but the content of available forms of Mn in these soils were lower. These data suggest that Mn dynamics in soils is very complex, and that the higher content of the total Mn in the soil does not automatically mean the increased availability of Mn and thus toxicity to plants (Rengel, 2015).

Xiang and Banin (1996) reported that the Mn availability to plants is regulated by different factors, most notably the redox potential and soil pH. Namely, the release of most available Mn forms (Mn^{2+}) from Mn oxide minerals is much more pronounced in acid soils and under anaerobic conditions (Vukadinović and Vukadinović, 2011). As shown in Table 1, results related to pH value confirm the previously mentioned hypothesis that the soil pH greatly affects the availability of Mn in soil. Namely, the soils near the active Mn mine Bužim had significantly lower pH compared to soils at the site Radostovo, but also higher content of available forms of Mn, although the content of the total Mn in the soils at the site Radostovo were much higher, indicating that the Mn availability increases with the decrease of pH value (Marschner, 1995). The availability of Mn in neutral and alkaline soils is significantly reduced due to the formation of hard soluble Mn oxides, hydroxides and salts (Dučić and Polle, 2005; Khabaz-Saberi and Rengel, 2010). Vukadinović and Lončarić (1998) reported that the high presence of cations Ca^{2+} and Mg^{2+}, and higher Zn and Cu content in soils also negatively affect the Mn availability to plants, while Fageria at al. (2002) noted that the Mn phytoavailability increases in conditions when oxygen is depleted from the growing medium as result of waterlogging or very high organic matter content in soils.

The availability of Mn in soil also largely depends on the physical properties of the soil (Bradl, 2004). Millaleo et al. (2010) reported that the deficiency of Mn is particularly pronounced in sandy soil where the prevailing aerobic conditions, while in heavy soils Mn availability is generally higher, and such observations were also mentioned in numerous other studies (Kogelmann and Sharpe, 2006; Sharma et al., 2016). It is assumed that medium-texture of soils around the active Mn mine Bužim with dominance of silt and less air space contributed to higher Mn solubility and thus their availability for plants.

In addition, uptake of Mn and generally heavy metals by plants root, as well as their accumulation in the edible parts of food crops is also dependent on plant genetic potential (Sajwani et al., 1996).

The content of Mn in edible parts of food crops that have been grown in soils near the active Mn mine Bužim and Mn ore deposits Radostovo is given in Table 2 and 3.

Table 2. Mn content in edible parts of food crops grown on soils near the Mn mine Bužim

Plant species	Mn content (mg/kg dry mass)
strawberry	26.23 ± 2.02^{a}
raspberry	24.78 ± 2.11^{ab}
cabbage	24.04 ± 2.66^{abc}
onion	19.31 ± 2.34^{d}
$LSD_{0.05}$	2.289

Values expressed as main ± standard deviation. Different letters in each column represent significant difference (P<0.05)

Table 3. Mn content in edible parts of food crops grown on soils near the Mn ore deposits Radostovo

Plant species	Mn content (mg/kg dry mass)
garlic	5.34 ± 0.26^{c}
pepper	6.89 ± 3.45^{c}
young beans	10.42 ± 1.81^{ab}
potato	5.45 ± 1.02^{c}
raspberry	12.52 ± 3.00^{a}
$LSD_{0.05}$	2.228

Values expressed as main ± standard deviation. Different letters in each column represent significant difference (P<0.05)

As shown in Table 2 and 3, the food crops grown on soils near the Mn mine Bužim had higher content of Mn in the edible parts as compared to food crops from soils at the site Radostovo. Considering that soils around Mn mine Bužim had a higher content of Mn available forms, these results were expected. Moreover, Mn content in the fruits of raspberries from these soils was even twice higher in comparison with fruit of raspberry grown in soils at the site Radostovo. The results of the present study also showed that the Mn content in edible parts of strawberry, raspberry and cabbage grown on the soils around Mn mine Bužim did not differ significantly. The exception was only the onions, where the content of Mn was significantly lower compared to all other tested food crops. Interestingly, the lowest Mn content at the other studied site (Radostovo) was determined in edible parts of garlic. These results lead to the conclusion that some plants such as garlic or onions have evolved mechanism to translocate the high amont of Mn to upper part of plant or to reduce Mn entry into the plant roots which may also represent some adaptive mechanisms of plant to stress caused by high Mn content in soil.

Angelova et al. (2003) studied the absorption of heavy metals at several food crops (beans, lentils, chickpeas and soybeans) and they observed that the examined species, grown under the same agroecological conditions, differed considerably in their ability to absorb heavy metals from soil. Also, many scientists have found significant differences in the content of Mn and other heavy metals in different parts of the same plant (Cataldo et al., 1981; Guala et al., 2010; Skorbiłowicz et al., 2016). The data from the scientific literature indicate that Mn mostly accumulates in leaves, then in roots, and much less in the stem and fruits of plants (Goor and Wiersma, 1974; Page et al., 2006).

The results of this study also showed that the accumulation of Mn in edible parts of all tested food crops was not even close to the critical limit value for Mn in the plant, which according to Kastori (1993) is 400 mg/kg of dry mass.

Conclusions

The Mn availability, its absorption and accumulation in edible parts of food crops was considerably higher in acid soils, suggesting that soil pH is one of the key factors in determining the Mn dynamics in the 'soil - plant system'. Since the content of Mn in all tested food crops did not exceed the toxic value for Mn in plants, both examined sites from the point of view of contamination of the soil with Mn can be considered suitable for production of healthy food.

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