FACTA UNIVERSITATIS
Series: **Physics, Chemistry and Technology** Vol. 13, N^{o} 2, Special Issue, 2015, pp. 99 - 107
DOI: 10.2298/FUPCT1502099A

IRON CONTENT IN THE FRUITS OF THE GRAPEVINES AND PEACH TREES GROWING NEAR THE MINING AND SMELTING COMPLEX BOR, EAST SERBIA[†]

UDC 520.84 : 546.72

Slađana Č. Alagić[1*], Snežana B. Tošić[2], Mile D. Dimitrijević[1], Maja M. Nujkić[1]

[1]Technical Faculty Bor, University of Belgrade, Bor, Serbia
[2]Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Serbia

Abstract. *The samples of fruits of the grapevine (*Vitis vinifera*, cultivar Tamjanika) and the peach tree (*Prunus persica L. Batech*) from the Bor region were analyzed using an ICP-OES to determine the content of iron (Fe). This was done in order to assess possible health risks related to this essential element; the region of Bor's municipality is known as one of the most polluted areas in Serbia. The content of Fe in unwashed grapes seems not to be affected by the mining/metallurgical activities, as it was either in the normal concentration range or was at even lower than critical deficiency concentration in plants (21.8-98 mg/kg). The level of Fe in the samples of peaches ranged from 62.4 to 1418 mg/kg, which is much higher than that in grape samples and in one case, even higher than the phytotoxic threshold. The values of the enrichment factor (EF) were lower than 2 in the case of grape samples, while for peach samples, these values ranged from rather low (0.99) to extremely high (22.66). Based on the herein obtained results, in the region of Bor, it seems that the cultivation of grapevine should be favored over the cultivation of peach trees.*

Key words: *iron; grape; peach; phytotoxicity; deficiency; ICP-OES*

Received December 18th, 2014; accepted February 05th, 2016.
[†] Acknowledgement: Authors are grateful to the Ministry of Education, Science and Technological Development of Serbia for financial support (Project No. 46010).
Dedicated to Professor Radosav Palić on the happy occasion of his 70th birthday.
* Contacts of the corresponding author: E-mail: sladjaal@yahoo.com, salagic@tf.bor.ac.rs.

1. INTRODUCTION

Although many metals are essential for plants, all metals are toxic at higher concentrations (Alagić, 2014; Bhaduri and Fulekar, 2012; Lin and Aarts, 2012; Peralta-Videa, 2009; Rascio and Navari-Izzo, 2011). Iron (Fe) is not an exception in this regard. The role of Fe in higher plants is understood pretty well: Fe is an essential metal in energy transformations needed for syntheses and other vital processes of the cells due to the easy change of valence ($Fe^{2+}=Fe^{3+}+e^{-}$). It plays an important role in the formation of the chlorophyll molecule and, consequently, in the photosynthesis process (more than 75% of the Fe in plants is found in chloroplasts) (Marić et al., 2013; Palmer and Guerinot, 2009).

The proper content of Fe in plants is essential both for the health of plants and for the nutrient supply to animals and humans. Generally speaking, the sufficient amount of Fe in the leaf tissue, for most plants, is 50–100 mg/kg of dry weight, which is also the nutritional requirement of grazing animals (Marić et al., 2013). According to Nagajyoti et al. (2010), 140 mg/kg can be considered as normal concentration for plants. However, in soils, where Fe is easily soluble, plants may take up a very large quantity of Fe. This is the case with grasses grown in serpentine soils which contained Fe in the range of 2127–3580 mg/kg (Kabata-Pendias, 2011). The natural Fe concentration of fodder plants usually ranges from 18-1000 mg/kg, while the average Fe contents of different cereals amount: 31-98 mg/kg (Kabata-Pendias, 2011).

The so-called “lower” critical concentration, below which the plant shows the symptoms of deficiency, amounts to 50 mg/kg (Jones, 2005). The symptoms of iron deficiency are not always easy to distinguish from some other metal deficiency, so that they can be easily mistaken with S-, Mn-, or Zn-deficiency. One of the symptoms for the Fe deficiency is the absence of green plant color, especially in young leaves (chlorosis), which means the hammering of chlorophyll (Marić et al., 2013).

The difference among plants in their capacity to absorb Fe from the soil is not always consistent and is affected by varying conditions of the soil (pH, electrical conductivity (EC) and organic matter content (OM)) and climate, as well as by the type of plant. It is a well known fact that plants rich in nutrients, especially in Ca and SiO_2, can tolerate increased levels of Fe. Also, plants which are adapted to waterlogged environment are more tolerant to high Fe levels than plants grown in well-aerated soils. These plants can develop different strategies to avoid toxic effects, such as: oxidation, immobilization (deposition in cell walls), and exclusion of mobile Fe forms by roots, or cooperation with some soil microbes. In general, it can be said that although toxic in excessive concentrations, Fe is usually accumulated in most plants without any adverse effects. Also, the symptoms of Fe toxicity are not so specific in plants. However, injured leaves or necrotic spots on leaves are the first common indications of the Fe phytotoxicity for all higher plants (Kabata-Pendias, 2011; Nagajyoti et al., 2010; Palmer and Guerinot, 2009). According to Kabata-Pendias (2011), the Fe concentrations of above 1000 mg/kg can be considered as toxic (critical toxicity) for most plants. To date iron toxicity is not known to occur in vineyards.

In this study, fruit samples of the grapevine (*Vitis vinifera*, cultivar Tamjanika), and the peach tree (*Prunus persica L. Batech*) from vineyards and some other locations of Bor’s region, were analyzed to obtain the data on the concentration of Fe, because this element represents one of the key constituents of particulate matter originated from the copper smelter plant, which belongs to the Bor Mining and Smelting Complex (RTB Bor). RTB Bor is built in the zone of Bor town (East Serbia) which is known as one of the most polluted sites in Serbia, a hot spot of the entire Balkan Peninsula. The most important activities in Bor's region are mining and metallurgy. These activities cause large air, soil and water pollution in this region and most importantly, affect human health. Smelter plant is considered as a primary source of pollution due to the fact that the process of copper ore smelting produces massive emissions of sulphur dioxide (SO_2) and dust with elevated contents of heavy metals such as: Cu, Zn, Pb, As, and Cd. High concentrations of these toxic metals have been found in numerous air, water, soil and plant samples from the Bor region (Alagić et al., 2013; Alagić et al., 2014; Antonijevic et al., 2012; LEAP, 2003; Marić et al., 2013; Serbula et al., 2012). Also, some Fe enrichment was detected in soil samples which were collected across the Bor region (Dimitrijević et al., 2014), as well as in some fodder crops which were experimentally cultivated near old flotation tailing pond in Bor (Marić et al., 2013).

The described circumstances represent a hostile environment for crops and the vegetation on the whole. Even so, plants of grapevine (*Vitis vinifera*, cv Tamjanika) and peach tree (*Prunus persica* L. Batech) can be found in all types of soils which are present in the Bor region. They can be found even at the old, abandoned flotation tailing pond, in the close vicinity of copper smelter, where the pyrite tailing creates almost impossible situation for vegetation development. However, they are usually found in small vineyards in the rural zone in this region, as well as in numerous backyards on the territory of the town of Bor. Both plant species represent authentic sorts which are characteristic for the region of East Serbia and both give fruits full of flavor. Cultivar Tamjanika has a very tasty and aromatic fruit (berries) which is commonly used for production of small quantities of wine of superb quality; also, the delicious berries are very often consumed as fresh, or air-dried. Fruits of the peach tree are usually consumed in fresh condition, but they are also used for the production of tasty jam or juice.

The aim of this paper was to determine the concentrations of Fe in the fruits of grapevines and peach trees from Bor's region, thus providing important information about the possible excess and risks from this essential element for the investigated plant species, as well as for human population from the Bor region.

2. MATERIALS AND METHODS

2.1. Sampling area

The concentration of Fe was determined in grapes and peaches from the most endangered zones in Bor's municipality (Fig. 1; Table 1). The main factors which influenced the selection of the sites where the sampling of the bio-material was conducted were: the presence of investigated plant species (untreated with the pesticides), the position of the industrial facilities, the type of settlement, and the meteorological and topographic parameters (wind direction influences the distribution of pollutants from the industrial facilities to the town of Bor and its surrounding areas).

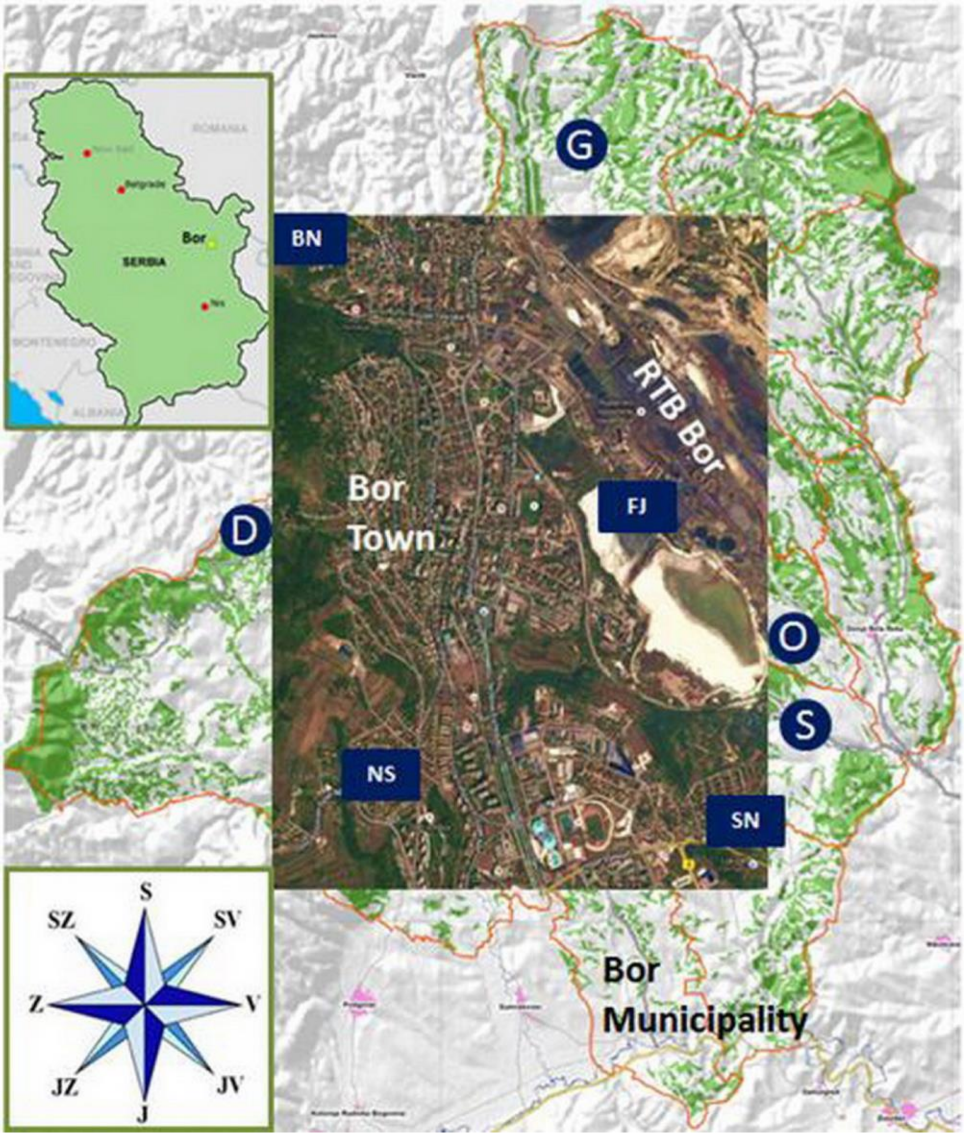

Fig. 1 Map of the study area

The urban-industrial (UI) zone included four sampling sites: Flotacijsko jalovište (FJ), an old abandoned flotation tailings pond, Bolničko naselje (BN), a part of the town which is situated near the city hospital, and two suburbs, Slatinsko naselje (SN) and Naselje Sunce (NS). All the sites from UI zone are localized very close to the copper smelter, a dominant source of pollution in the Bor region. The rural zone (R) included three rural settlements: Oštrelj (O), Slatina (S) and Dubašnica (D). The control zone (C) was in an unpolluted area of rural settlement Gornjane (G) which is located 19 km away from the Bor town (Table 1). This area is naturally protected from any pollution by the mountain Veliki Krš.

Table 1 Positions of the sampling sites in relation to the primary source of pollution

Sampling site	Zone	Distance (km)	Wind direction
Flotacijsko Jalovište (FJ)	UI	0.7	W
Bolničko naselje (BN)	UI	1.7	E-ESE
Slatinsko naselje (SN)	UI	2.3	WNW-NW
Naselje Sunce (NS)	UI	2.5	ENE-E
Oštrelj (O)	R	4	W-WNW
Slatina (S)	R	7	WNW-NW
Dubašnica (D)	R	17	E-ENE
Gornjane (G)	C	19	S

2.2. Experimental

The samples of fruits of grapevine (*Vitis vinifera*, cv Tamjanika) and peach tree (*Prunus persica L. Batech*) were taken from the selected sites during September/October 2012. At each site, samples of fruits were taken from three to five plants (from different quarters of each plant). These sub-samples were bulked to obtain a representative sample for each site. Fruit samples remained unwashed with the aim to detect a factual quantitative profile of Fe deposition as much as possible. All samples were air-dried to a constant weight in a well ventilated room, during a period of several months. Peach fruit was chopped into small pieces before the drying process, while grapevine fruit was dried according to the traditional manner, in one piece, hanging on a string, for the first three months of the drying process, after which, the berries were separated from rachises. The berries were left to dry for about a month and then subjected to further analysis, whereas rachises were discharged. All dried samples were homogenized in a laboratory mill. For complete mineralization of the samples, a representative 1 g (2 x 0.5 g) of each sample was treated with repeated additions of the nitric acid (65% HNO_3, Merck, Darmstadt), and hydrogen peroxide (10% H_2O_2, Merck, Darmstadt), according to a microwave assisted strong acid digestion method of complex matrices recommended by the United States Environmental Protection Agency (USEPA, method 3052) (Alagić et al., 2014). The digestion was performed in a microwave digestion system ETHOS 1 (Milestone, Bergamo, Italy) equipped with rotor SK 12. The conditions were as follows: 1000 W, 40 bar, with the temperature program: ramping time 10 min up to 180°C, and holding time 15 min at 180°C (constant). After cooling, the obtained solutions of each sample were merged, filtered and diluted to a volume of 50 mL with double distilled water. The obtained digests were stored in polyethylene bottles at $4^{o}C$ before the Fe analysis by ICP-OES.

An iCAP 6000 inductively coupled plasma optical emission spectrometer (Thermo Scientific, Cambridge, United Kingdom) with an Echelle optical design and a charge injection device (CID) solid state detector was used for determination of Fe content in fruit samples under the operate conditions as follows:

- Flush Pump Rate - 100 rpm
- Analysis Pump Rate - 50 rpm
- RF Power - 1150 W

- Nebulizer Gas Flow - 0.7 L/min
- Coolant Gas Flow - 12 L/min
- Auxiliary Gas Flow - 0.5 L/min
- Plasma View – Axial

The chosen wavelength for Fe, based upon the tables of known interferences, baseline shifts and the background correction (the highest signal-to-background ratio) which was manually selected for the quantitative measurements was 259.940 nm. The limit of detection (LOD), limit of quantification (LOQ) and correlation coefficient (r) were: 0.0005 mg/L, 0.0016 mg/L and 0.999883, respectively. Detection and quantification limits were expressed as: LOD = 3xSD/m and LOQ = 10xSD/m, where SD is the standard deviation of blank responses and m is the slope of the calibration graph. The multi-element standard solution of about 20.00±0.10 mg/L (Ultra scientific, USA) was used as a stock solution for calibration. Polyethylene bottles used for storing the samples were cleaned to avoid contamination of the samples. Containers were treated with 5% nitric acid, and washed with ultra-pure water 0.05 μS/cm (MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH). All results were calculated on a dry weight basis (mg/kg DW).

2.3. Data analysis

The enrichment factor (EF) is used for processing fruit samples from contaminated zones, i.e. to assess the degree of anthropogenic influence. The element enrichment factor is calculated as: EF=Cpolluted/Ccontrol, where Cpolluted and Ccontrol are the metal concentrations in fruit from the polluted sampling site and the control site, respectively. Element enrichment factors are evaluated by using local background values. Values of EF>2, point to the enriched samples (Mingorance et al., 2007). As the EF values increase, the contribution of the anthropogenic origin also increases.

3. Results and Discussion

The results of the ICP-OES analysis of grapes and peaches from the Bor region showed that the content of Fe varied significantly among samples: from 21.8 mg/kg in grapes from the site D to 1,418 mg/kg in peaches from the site FJ (Table 2). The results clearly showed that for locations, the contents of Fe were higher in peaches than in grapes. However, there was no correlation between Fe contents and the distance from the primary source of pollution (UI, R or C zone).

The samples of grapevine fruits had the content of Fe which was at the normal level for plants (50–100 mg/kg, i.e. 140 mg/kg) but in many samples, the concentrations were even lower than critical deficiency concentration (50 mg/kg) (Table 2). The content of Fe in nearly all peach samples was higher than normal levels for most plants and in one case (fruit from the site FJ) even higher than critical toxicity concentration (1,000 mg/kg). However, these concentrations were still in a range which is typical for some plants which naturally grow in serpentine soils, so it is not clear if the Fe level in this samples should be considered normal or elevated. Indeed, some serious symptoms of Fe toxicity were not visible in peach fruits or in other organs of this plant species: only a couple of dry fruits and several dead branches, as well as a few of chlorotic leaves found in plants from the sites FJ, BN, SN and S, during the process of sample collection. The same situation was found in the case of plant organs of grapevines from different locations, but when the fruits are the subject of interest, some of the mentioned incidences could be ascribed to the symptoms of deficiency rather than the symptoms of Fe toxicity, which is supported by the findings on Fe concentrations in these fruits. A similar situation of the lack of Fe-toxicity symptoms has already been observed in the experiment with fodder crops which were cultivated near old flotation tailing pond in the centre of the town of Bor. These plants contained Fe in a large quantity: from 3,589.6 mg/kg in *Lollium perenne*, up to 9,975.6 mg/kg in *Trifolium pratense* (Marić et al., 2013) but were without the symptoms of phytotoxicity. Finally, the described symptoms in the investigated grapevines and peach trees could be also ascribed to some other toxic elements which are constantly present in Bor's environment.

Table 2 Concentrations of Fe in the samples of grapes and peaches (mg/kg DW)*

Sampling site	Grape	Peach
FJ	98 ±2.0	1,418 ±30.0
BN	53.1±0.1	142 ± 3.0
SN	32.4±0.5	192 ± 3.0
NS	41.2±0.2	62.4 ± 0.2
O	44.8±0.4	94 ± 2.0
S	46.4±0.4	318 ± 4.0
D	21.8±0.4	106.9 ± 0.6
G	51.8±0.4	63 ± 3.0

* – Data are presented as the mean ± standard deviation (SD) for triplicate determinations

To resolve the ambiguities regarding Fe levels in the studied sampled (normal or elevated), and to assess the degree of anthropogenic influence, EFs were calculated. The values of EF varied from 0.63 for grapes from the site SN to a rather high value of 22.66 for peaches from the site FJ (Table 3). The values of EF higher than 2, were also calculated for peaches from the sites BN, SN and S, which reveals a serious enrichment in these cases and points to a significant influence of mining/metallurgical activities. However, this pollution which was so obviously present in the fruits of the peach tree was not reflected in the fruits of grapevine. Namely, all EF values which were calculated in this case, were lower than 2, wrongly pointing to the conclusion that mentioned locations were without any pollution by Fe. It is possible that smooth berries of the grapevine cultivar Tamjanika are less susceptible to the retention of airborne particulate matter (PM) which contains high concentrations of different metals. This may further suggest that the detected concentrations of Fe in unwashed grapevine fruits may be endorsed to a true bioaccumulation, mainly through root but in some extent also through leaves. Similar conclusions were achieved in the case of the contents of other metals: Cu, Zn, Pb, As, Cd, and Ni in the very same plant species from Bor's region (Alagić et al., 2014). This further allows for a conclusion that this grapevine cultivar utilizes some specific ways to protect its fruit from overloading of heavy metals which come from the highly contaminated environment. This is important not only for the plant, but also for human health. Unfortunately, the same arguments cannot be ascribed to the peach fruit. Obviously, its hairy surface was very suitable to retain PM originated from the main source of pollution, i.e. from the copper smelter.

Regarding the fact that PM may contain different concentrations of Fe as well as some other toxic metals, it can be said that the highest risks from consummation of this fruit exist in the case of the sites from the UI zone. The fact that fruits were subjected to analysis as unwashed cannot diminish these risks but obviously can favor the grapevine cv Tamjanika as a plant which can be recommended for cultivation in all areas which are exposed to severe pollution.

Table 3 Iron enrichment factor (EF)

Sampling site	Grape	Peach
FJ	1.89	22.66
BN	1.03	2.27
SN	0.63	3.07
NS	0.80	0.99
O	0.86	1.50
S	0.90	5.08
D	0.42	1.71

EF=Cpolluted/Ccontrol

4. Conclusion

The results of this work showed that the content of Fe seems not to be (highly) susceptible to mining/metallurgical activities: its levels were in the range of normal concentrations in plants, or in many cases even lower than critical deficiency concentration. However, the influence of anthropogenic activities was more pronounced in the case of peaches, as it can be seen from calculated enrichment factors. The concentration of Fe in peach samples was much higher than in grape samples, and in one case even higher than phytotoxic threshold. These facts may favor grapevine as a plant species which poses some more successful ways to protect its fruit from high metal concentrations, which further makes this fruit safe for consummation.

References

Alagić, S.Č., Šerbula, S.S., Tošić, S.B., Pavlović, A.N., Petrović, J.V., 2013. Arch. Environ. Con. Tox., 65 (4), 671-682. doi: 10.1007/s00244-013-9948-7

Alagić, S.Č., Tošić, S.B., Dimitrijević, M.D., Antonijević, M.M., Nujkić, M.M., 2014. Environ. Sci. Pollut. R., doi: 10.1007/s11356-014-3933-1.

Alagić, S.Č., 2014. Zaštita materijala, 55 (3), 313-322. doi:10.5937/ZasMat1403313A

Antonijevic, M.M., Dimitrijevic, M.D., Milic, S.M., Nujkic, M.M., 2012. J. Environ. Monitor., 14, 866-877. doi: 10.1039/C2EM10803H

Bhaduri, A.M., Fulekar, M.H., 2012. Rev. Environ. Sci. Bio., 11, 55–69. doi: 10.1007/s11157-011-9251-x

Dimitrijević, M., Alagić, S., Tošić, S., Nujkić, M., 2014. Heavy metal distribution in the topsoil from different locations near copper smelter in Bor (East Serbia). 46th International October Conference on Mining and Metallurgy, IOC 2014, Bor Serbia, October 1-4, Proceedings, pp. 273-276.

Jones, J.B., 2005. Hydroponics: a practical guide for the soilless grower, 2nd edn. CRC, Boca Raton.

Kabata-Pendias, A., 2011. Trace elements in soils and plants. Fourth edition, CRC Press, Taylor and Francis Group, LLC, Boca Raton, London, New York.

LEAP, 2003 (Local Environmental Action Plan of Bor, 2003). T. Marjanović, M. Trumić, Lj. Marković, Bor

Lin, Y.-F., Aarts, M.G.M., 2012. Cell. Mol. Life. Sci., 69, 3187–3206. doi: 10.1007/s00018-012-1089-z
Marić, M., Antonijević, M., Alagić, S., 2013. Environ. Sci. Pollut. Res., 20 (2), 1181-1188. doi: 10.1007/s11356-012-1007-9
Mingorance, M.D., Valdés, B., Oliva, R.S., 2007. Environ. Int., 33, 514–520. doi:10.1016/ j.envint.2007.01.005
Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M., 2010. Environ. Chem. Lett., 8, 199–216. doi: 10.1007/s10311-010-0297-8
Palmer, C.M., Guerinot, M.L., 2009. Nat. Chem. Biol., 5 (5), 333-340. doi: 10.1038/nchembio.166
Peralta-Videa, J.R., Lopez, M.L., Narayan, M., Saupe, G., Gardea-Torresdey, J., 2009. Int. J. Biochem. Cell. B., 41, 1665–1677. doi: 10.1016/j.biocel.2009.03.005
Rascio, N., Navari-Izzo, F., 2011. Plant. Sci., 180, 169-181. doi:10.1016/j.plantsci.2010.08.016
Serbula, S.M., Alagić, S.Č., Ilić, A.A., Kalinović, T.S., Strojić, J.V., 2012. Particulate Matter Originated From Mining-Metallurgical Processes, in Knudsen, H., Rasmunssen, N., (Eds.), Particulate Matter: Sources, Emission Rates and Health Effects. Nova Science Publishers US, New York, pp. 91-116.

SADRŽAJ GVOŽĐA U PLODOVIMA VINOVE LOZE I BRESKVE KOJE RASTU U BLIZINI RUDARSKO-TOPIONIČARSKOG KOMPLEKSA BOR, ISTOČNA SRBIJA

Uzorci plodova vinove loze (Vitis vinifera*, sorta Tamjanika*) *i vinogradarske breskve* (Prunus persica *L. Batech*) *iz regiona Bora su analizirani metodom ICP-OES, kako bi se odredio sadržaj gvožđa* (*Fe*)*. Analize su vršene da bi se procenio mogući zdravstveni rizik povezan sa ovim esencijalnim elementom; poznato je da je region Borske opštine jedan od najzagađenijih predela u Srbiji. Čini se da rudarsko-metalurške aktivnosti ne utiču na sadržaj Fe kod vinove loze, jer je isti bio ili u okviru koncentracija normalnih za većinu biljaka ili čak niži od kritične koncentracije deficijencije* (*21.8-98 mg/kg*)*. Koncentracija Fe u uzorcima breskve je bila u opsegu od 62.4-1418 mg/kg, što je mnogo više nego u uzorcima grožđa i u jednom slučaju čak iznad granice fitotoksičnosti. Vrednosti faktora obogaćenja bile su manje od 2 u slučaju uzoraka grožđa, dok su se kod uzoraka breskve ove vrednosti kretale od niskih (0,99) do ekstremno visokih (22,66). Na osnovu dobijenih rezultata, u borskom regionu, čini se da bi se trebalo forsirati uzgoj grožđa u odnosu na uzgoj breskve.*

Ključne reči: *gvožđe; grožđe; breskva; fitotoksičnost; deficijencija; ICP-OES*