Environmental pollution influence to soil–plant–air system in organic vineyard: bioavailability, environmental, and health risk assessment

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Abstract
This study was performed in organic vineyard to assess integrated pollution in soil–plant–air system by potentially toxic elements (PTE). Concentrations of 26 PTE were determined in soil, grapevine, and air biomonitor (moss bags) using ICP-OES and ICP-MS. Environmental implication assessment of soil did not show pollution by PTE, except for B in samples collected in the middle of grapevine season (July). Despite low total Cd concentrations in soil, it has the highest influence on increase of environmental risk. Based on biological accumulation concentration (BAC), grapevine is not hyperaccumulator of PTE from soil. Advanced classification algorithm, Kohonen self-organizing map (SOM), was applied to compare environmental implications in organic with conventional vineyards. PTE concentrations were significantly lower in organic than conventional grapevine. PTE concentrations were higher in the outer (leaf and petiole) than in the inner grapevine parts (skin, pulp, and seed). Some airborne elements have an influence on outer grapevine parts, especially on leaves (ratio factor—RF > 1). Moss bag technique testified about lower enrichment of airborne elements compared with the conventional vineyard and urban microenvironments. Environmental and health risk assessments confirmed that organic production is harmless for field workers and grape consumers.

Keywords Organic production · PTE · Environmental implications · Health risk assessment · Moss bag biomonitoring · Kohonen self-organizing maps

Abbreviations
PTE Potentially toxic elements
SOM Self-organizing maps
ICP-OES Inductively coupled plasma-optical emission spectrometry
ICP-MS Inductively coupled plasma-mass spectrometry
CRM Certified reference materials
RI Ecological risk
BGI Bio-geochemical
CF Contamination factor
PLI Pollution load index
Eri and RI environmental risk
BRAI Bioavailability risk assessment index
BAC Biological accumulation concentration
RF Ratio factor
LOQ Limit of quantification for moss bag method
RAF Relative accumulation factor
RAIS Risk assessment information system
DIR Daily intake rate
THQ Target hazard quotient
HI Hazard index
R Target cancer risk
OM Organic matter
Introduction

Organic viticulture started to spread at the end of the 20th century simultaneously with the development of contemporary technical methods for analyzing food quality, research on impact on human health and environmental awareness. Main arguments for encouraging organic production is its positive effect on environment and human health in comparison with conventional production (Häring et al. 2001). Advantages of organic production are safer environment with no frequent application of chemicals and safer products (vegetables and fruits), while disadvantages are more complex growth and higher costs.

Nowadays, organic wine production is present in almost all Europe, and the largest areas of organic vineyards are located in Italy, Spain, and France. Regarding land suitability for cultivation of organic vineyards, they should be grown far away from industrial plants, highways, and fields with conventional food production. In organic growing areas, trees and shrubs are usually planted as a barrier mitigating pollution from adjacent plots. If pesticides or other agrochemicals have been used on selected soil in past, it is necessary to pass 2–3 years to use the soil for organic production (National Gazette of Republic Serbia 2006). The standards for organic viticulture have been mostly set by the wine producers from Germany, Switzerland, and France. The first European regulation on organic food production was introduced in 1991 (EEC No 2092/91 n.d.). Following the years, the Commission in VI Research Framework Programme continuously revised initial regulations (Regulation EC No 834/2007), which finally lead to approval of new rules by Standing Committee on Organic Farming (SCOF) (Regulation EU–No.203/2012; European Commission 2012). In Serbia, law on organic production is implemented in National Gazette of Republic Serbia (2010b) and it is regulated by authorized institutions which are issuing permits and certificates (National Gazette of Republic of Serbia 2011a). The organic production differs from the conventional because the agrochemical use is strictly controlled and dosed by Regulative on Control and Certification in Organic Production and Methods of Organic Production (National Gazette of of Republic Serbia 2011b, 2012).

Elements in soil, plant, or air present in excess cause risk for plant growth, environment or human, and can exhibit toxicological characteristics and thus in this paper, are named as potentially toxic elements (PTE). Monitoring of PTE concentrations in agricultural soil represents the first measure of precaution related to food safety control, while mobility and bioavailability of PTEs in soil–plant–air system should be a further step in understanding PTE transportations and it could improve control production of fruits and vegetables. Investigation of element mobility and bioavailability in agricultural soils attracted attention worldwide in the last few decades (Pelfrêne et al. 2012). Single extraction procedures are one-step extraction procedures, recommended for studying the mobility (Santos et al. 2010) and bioavailability of elements in soils and for predicting their influence on plants (Vázquez Vázquez et al. 2016; Milicević et al. 2017a, 2018a, 2018b). They are cost-effective and simple-performing way to extract easy-available PTEs (Meers et al. 2007; Rao et al. 2008; Santos et al. 2010). Some of these procedures are included in the regulations, or they have been considered in framework of normalization bodies (CEN or ISO) (Quevauviller et al. 1996). Usually, in the literature, two or three single extraction procedures were compared for assessing mobility or bioavailability of elements from soil (Niesiołowski 2012), and these procedures have been used only in several studies conducted in conventional vineyards (Vázquez Vázquez et al. 2016; Milicević et al. 2017a, 2018a, 2018b).

Thus far, various equations for assessing environmental implications were developed. Soil pollution indices are based on normalization of PTE concentrations to dimensionless-unit “concentrations” which enable comparison between implications caused by PTE (Kim et al. 2015; Antoniadis et al. 2017a, 2017b, 2017c), but the use of these indices is also appropriate for distinguishing pollution of different sites (e.g., between conventional and organic vineyards). Kohonen self-organizing map (SOM) cluster data according to some similarity or distance measure surpasses traditional cluster methodology (Budayan et al. 2009) and it could be very effective when it is not possible to predict or define rules that lead to class formation. SOM has been successfully applied for clustering and visualization of element or POP concentrations in different studies (Mari et al. 2010; Deljanin et al. 2015).

Usually, air pollution assessment is performed by devices using the certified procedure (Directive 2004/107/EC n.d.; Directive 2008/50/EC n.d.). However, there are some limitations of instrumental monitoring (such as expensiveness of devices, electricity needs, technical maintenance, and the possibility of destroying the device), especially in rural and agricultural areas. Biomonitoring of air pollution using mosses has been developed as an easy operational and cost-effective method for air pollution assessment and it has been applied worldwide assessment of air pollutants in urban and industrial areas (Anićević Urošević and Milicević 2020 and references therein). Moss bag biomonitoring of PTEs has been rarely performed in agricultural regions (Capozzi et al. 2016a, 2016b; Milicević et al. 2017b). According to our knowledge, it has never been carried out in an ambient such as organic vineyard with presumably low air pollution level, which is of importance for defining the method application in potential pollution background areas in vineyards or agricultural ambient.
The main goal of this study was to estimate quality of organic grapevine production simultaneously assessing soil, grapevine, and air pollution (using a moss bag technique) by PTE. The aims were to (1) test in parallel which of nine investigated single extraction procedures are appropriate for assessing PTE mobility in the soil from organic vineyard; comparing with our previous investigations in conventional vineyards whether seven single extraction procedures were tested (deionized H₂O during 2 and 16 h, 0.01 mol L⁻¹ CaCl₂ during 3 h, 0.1 mol L⁻¹ NH₄NO₃ during 2 h, 0.05 mol L⁻¹ Na₂EDTA during 1 h and 0.11 mol L⁻¹ CH₃COOH during 16 h extracting), in this study for the first time nine different single extraction procedures were tested (two new than in previous study: 0.44 mol L⁻¹ CH₃COOH during 16 h and 1 mol L⁻¹ BaCl₂ during 3 h extracting) to compare them and suggest which of the procedures may be appropriate to assess mobility of which group of elements; (2) estimate environmental implications and human health risk caused by PTEs and to compare environmental implications in ambient with minor agrochemical treatments with those in the conventional vineyard using SOM algorithm; (3) assess bioavailability and origin of elements in grapevine parts, with special attention to those elements recognized as PTE; (4) assess air PTE pollution using moss bag biomonitoring technique, which is tested for the first time in organic vineyard ambient. This study, performed in the organic vineyard, represents a part of investigation of the environmental pollution influence on the different vineyard ambient, through several investigations conducted in the last 5 years (Milićević 2018). In our previous studies, we investigated separately air pollution using moss bags and bioavailability of PTEs from soil to grapevine (Milićević et al. 2017a, 2017b, 2018a), while in this study, we did an integrated investigation of soil–plant–air pollution. Finally, according to the best of our knowledge, this kind of experiment observing in one study soil–plant–air system accompanied by the environmental and human health risk assessment was for the first time performed in organic vineyard.

Materials and methods

Study area and experiment set up

Investigated organic vineyard is one of the three organic growth vineyards in the Republic of Serbia. It is located in settlement Grocka near the Danube River (parcels 4 and 5 are located 700 m from the Danube River) (Fig. 1) in Belgrade grapevine growing region (Ivanšević et al. 2015). Potential pollution sources in this region may be other agricultural fields, main road, or due to meteorological conditions some industrial activities from suburban region of the capital city Belgrade. In the past, this area was well-known as “Indigo hills”, because vineyards had growth in this region that were frequently treated by copper (II) sulfate. After 20 years of non-cultivating any plants on five agriculture parcels (Fig. 1), in 2008, the organic vineyard was established here. For preparing these parcels for organic production, they were in the conversion process (personal communication 2016).

Soil horizons investigated in this manuscript were named as reported by Food and Agricultural Organization of the United Nations (FAO 1998) and as in other studies (Hartemink et al. 2020). Organic soil, O horizon (0–5 cm), and topsoil A horizon (0–30 cm) samples (n = 30), leaf samples (n = 15), and grapevine samples (n = 30; seed n = 5, pulp n = 5, skin n = 5, whole berries n = 5, petioles n = 5, leaves n = 5) were collected from five vineyard parcels (1, 2, 3, 4, and 5) (Table S1; Fig. 1). Composite samples prepared form 10 subsamples were collected per each parcel (Table S1). The local background samples represent the subsoil samples from each investigated parcel (30–60 cm) (Table S1), the deepest soil layer we collected for this experiment (Ander et al. 2013).
Soil, leaf, and petiole samples were collected through the vegetation season (June–leaf set, July–veraison, and September–harvest), and two grapevine varieties were sampled (Table S1), *Pannonia* and *Regent*.

Active moss bag biomonitoring survey, using *Sphagnum girgensohni* moss species, was conducted from June to September covering the grapevine growing phases (leaf development and flowering–June, veraison–July, and harvest–September). More details about moss sampling and bag preparation are given in the previous study conducted in conventional vineyard (Milićević et al. 2017b). Moss bags were exposed for 2- and 4-month periods. Specifically, there were three different periods of the moss bag exposure: 2 × 2-month periods (June 1st to August 1st; August 1st to October 1st) and one 4-month period (June 1st to October 1st). The bags were exposed in the vineyard parcels on T-shaped wooden holders 1 m above grapevine rows (Milićević et al. 2017b). Due to the absence of pollution source spots near investigated parcels, composite samples made from 10 moss bag subsamples were prepared per each investigated parcel.

### Chemical analysis

**Samples’ preparation**

Air-dried soil samples were sieved (<2 mm) and crushed to a fine powder. Hydrosopic moisture of soil was determined by drying the samples at 105 °C until the dry weight. Soil acidity (pH) was determined (in a mixture (1:5) of soil–distilled H₂O, soil–1 mol L⁻¹ KCl, and soil–0.1 mol L⁻¹ CaCl₂). Organic matter (OM) was measured using the procedure described by Storer (1984). For obtaining which single extraction procedure is the most appropriate for PTE mobility and bioavailability assessment, nine different single extraction procedures were used in parallel: 0.1 mol L⁻¹ CH₃COOH during 16 h, 0.43 mol L⁻¹ CH₃COOH during 16 h, 0.05 mol L⁻¹ Na₂EDTA during 1 h, 0.01 mol L⁻¹ CaCl₂ during 3 h, 0.1 mol L⁻¹ BaCl₂, 0.1 mol L⁻¹ NH₄NO₃ during 2 h, 0.1 mol L⁻¹ NaNO₃, and deionized H₂O during 2 h and 16 h (Table S2). Also, pseudo-total digestion of soil was performed using aqua-regia solution and digestion was performed in microwave oven (ETHOS 1, Advanced Microwave Digestion System, Milestone, Italy) (US EPA Method Standard Solution 4, Speecpure (Alfa Aesar GmbH & Co KG, Germany). The concentrations of 15 elements (Al, B, Ba, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, S, Sr, V, and Zn) in soil samples were measured using inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo Scientific iCAP 6500 Duo, Thermo Scientific, UK). Calibration was done using a Multi-Element Plasma Standard (CRM). In the CRM M2, the recovery of the determined elements ranged between 76 and 128% (Table S3). For single extraction procedures, the recovery of the elements ranged between 72 to 133% (Table S4). The exceptions were recoveries for Cr and Cu extracted with CaCl₂ (135% and 138%, respectively). For validation, the analyses of the grapevine and moss samples, the moss *Pleurozium schreberi* (M2 and M3) was analyzed to check the single extraction protocol recovery. The pseudo-total soil analysis recovery for the determined elements ranged between 76 and 128% (Table S3). For single extraction procedures, the recovery of the elements ranged from 72 to 133% (Table S4). The exceptions were recoveries for Cr and Cu extracted with CaCl₂ (135% and 138%, respectively). For validation, the analyses of the grapevine and moss samples, the moss *Pleurozium schreberi* (M2 and M3) was used as the CRM. In the CRM M2, the recovery of the elements ranged from 75 to 106% (Table S5). The exception was the recovery of Cr, which was 65%. The element recoveries using the CRM M3 ranged from 76 to 117%; the exception was Cr recovery (60%) (Table S5). The recoveries are consistent with the recovery we observed in our previous researches which we conducted on the same equipment (Milićević et al. 2018a, 2018b).

**Quality control of the chemical analyses**

Control of the element chemical analyses was done by analyzing analytical blanks and certified reference materials (CRM). Different CRM: 2711a (Montana II Soil), BCR 143R (Sewage Sludge-Amended Soil), ERM CC 135a (Contaminated Brickworks Soil), and SARM 42 (Soil) were analyzed to check the recovery of the soil sample digestion. CRM BCR 483 (Sewage Sludge-Amended Soil) was analyzed to check the single extraction protocol recovery. The pseudo-total soil analysis recovery for the determined elements ranged between 76 and 128% (Table S3). For single extraction procedures, the recovery of the elements ranged from 72 to 133% (Table S4). The exceptions were recoveries for Cr and Cu extracted with CaCl₂ (135% and 138%, respectively). For validation, the analyses of the grapevine and moss samples, the moss *Pleurozium schreberi* (M2 and M3) was used as the CRM. In the CRM M2, the recovery of the elements ranged from 75 to 106% (Table S5). The exception was the recovery of Cr, which was 65%. The element recoveries using the CRM M3 ranged from 76 to 117%; the exception was Cr recovery (60%) (Table S5). The recoveries are consistent with the recovery we observed in our previous researches which we conducted on the same equipment (Milićević et al. 2018a, 2018b).

**Data processing**

Statistical analyses were performed using SPSS software version 21, Statistica 8 (StatSoft Inc., Tulsa, OK, USA) and R...
software environment for statistical computing (R Team 2012). Kolmogorov-Smirnov was applied for testing data normality ($p < 0.05$) and Wilcoxon signed-rank test was applied for determining differences ($p < 0.05$) in the element concentrations between studied grapevine varieties (*Pannonia* and *Regent*), different vineyard’s parcels, and soil layers. ANOVA (Friedman’s test) was used for testing differences between different grapevine parts (leaf, petiole, and berry) and berry parts (skin, pulp, and seed). Principal component (PCA) and correlation analysis were used to indicate associations between element concentrations in soil and to determine the origin of PTE in grapevine parts.

The detailed description of the theoretical background of SOM analysis has been given elsewhere (Kohonen 2013). Briefly, the SOM analysis, introduced by Kohonen (1982, 1991), represents a type of neural network method providing multidimensional data into regular projection nodes, usually two-dimensional grid. SOM analysis surpasses traditional cluster analysis (Budayan et al. 2009) and it has been commonly applied for investigation, clustering, and visualization of different pollutants (Mari et al. 2010; Deljani et al. 2015). It was applied using a software environment for statistical computing R (R Team 2012). In this study, SOM was applied for screening of relation between environmental implication indices in organic and conventional vineyards. More details about the SOM algorithm calculation are given in Supplementary material (Theoretical part, S2.3. Data processing).

### Environmental implications and health risk assessment

Element concentrations recognized as potentially toxic were considered in environmental implication and health risk assessment equations (US EPA 2007). One of the most used indices is potential ecological risk (RI), developed by Hakanson 1980, and it is a suitable way to comprehensively express PTE pollution (Hui-na et al. 2012; Ghasemi et al. 2018). For assessment of risk caused by easily available PTE, bioavailability risk (BRAI) was developed (Long et al. 1995; NOAA 1999; Jamshidi-Zanjani et al. 2015). Bio-Geochemical Index (BGI), contamination factor (CF), Pollution Load Index (PLI), Environmental Risk (Eri and RI), and Bioavailability Risk Assessment Index (BRAI) for soil samples were calculated (Table S6). For better assessing PTE bioaccumulation in soil–plant system, biological accumulation concentration (BAC) was calculated (Radulescu et al. 2013; Bravo et al. 2017) indicating if plant species is classified as metal excluder or hyper-accumulator. Comparing the PTE concentrations between plant parts which are directly exposed to atmospheric deposition with inner parts (ratio factor—RF) (Oliva and Mingorance 2006), air pollution influence to plant can be assessed. Limit of quantification for moss bag method (LOQT) and relative accumulation factor (RAF) was calculated to estimate enrichment of PTE in moss samples. In parallel, EF, CF, and PLI were calculated for moss samples, as well (Table S6).

To assess health risk for outdoor workers in the vineyard and grape consumers, the worst-case scenario was observed. To calculate non-carcinogenic and carcinogenic risk for outdoor workers with specific working conditions, we set a site-specific exposure scenario, including site-specific environmental and exposure parameters matching local lifestyle. Equations from RAIS (The Risk Assessment Information System) were adapted to local conditions which we introduced in the previous study (Milićević et al. 2018a). Estimated daily intake rate (DIR), target hazard quotient (THQ), hazard index (HI), and target cancer risk (R) values of PTE via consumption (mg kg$^{-1}$ day$^{-1}$) of organic grape were calculated applying equations (Table S7). For assessment of carcinogenic risk, the adjustable formula was applied (Table S7).

### Results and discussion

#### Soil

**Element concentrations in the soil samples**

The observed soil samples are neutral to mildly alkaline (pH-H$_2$O: 6.90–8.90; pH-KCl: 6.97–7.58) with low organic matter (OM) content (0.37–1.90%) and cation exchange capacity (CEC), ranged between 25 and 40 cmol kg$^{-1}$. Descriptive statistic of the element concentrations in O soil layer, A (topsoil) layer and subsoil (control) samples, is presented in Table S8. Concentrations of PTE were lower than maximum allowable concentrations (MAC) prescribed by the regulations (National Gazette of Republic Serbia 2010a; EU Council Directive 86/278/EEC n.d.). Although the concentrations of Cr and Ni in soil were around the MAC (Table S8), they were obtained in significantly lower concentrations than the concentrations observed in conventional vineyard (Milićević et al. 2018a, 2018b), but higher than those in experimental vineyard (Milićević et al. 2017a). In addition, the concentrations of these elements were significantly correlated with certain elements (Cr–Al, Cr–Fe, Cr–Mg, Cr–K, Ni–Al, Ni–Li) (Table S9) that represent the most important natural soil substrates (Kabata-Pendias and Mukherjee 2007). Moreover, there were no significant differences between the concentrations of these two elements between the studied soil layers. According to the obtained results for those elements, it seems that Cr and Ni have the geogenic origin, which is in accordance with some previous investigations of soils from the Balkan Peninsula (Lićina et al. 2017; Kanellopoulos et al. 2015; Dangić and Dangić 2007; Salminen et al. 2005).
Assessment of elements’ mobility in the soil

In this study, many single extractions were applied to assess which of the investigated procedures should be used in more comprehensive studies observing all organic vineyards in comparison with conventional ones and to make a selection which of the procedures may be appropriate to assess mobility of which group of elements. Among nine single extraction procedures, efficiency and selectivity imply that the most efficient extractants were 0.05 mol L$^{-1}$ Na$_2$EDTA and 0.44 mol L$^{-1}$ CH$_3$COOH (Table S10). Regarding the element concentrations isolated by extractants vs. pseudo-total element concentration, the highest percentage of Co, Cu, Fe, Pb, Sb, and Zn were extracted by complexing agent Na$_2$EDTA (Table S10), as it was observed also for the soil from the previous study in conventional vineyard (Miličević et al. 2018a). Chelating agent Na$_2$EDTA has proven to be the most effective and selective solution for extracting the elements which usually build very stable complexes (Inczeedy 1976). The procedure using 0.44 mol L$^{-1}$ CH$_3$COOH isolated the highest concentrations of some other elements (Al, B, Be, Cd, Cr, Mn, Ni, and P), probably because of its acidity and aggressive influence on carbonates which predominantly fix Cd and Mn in soil (Kabata-Pendias and Mukherjee 2007) but also it could make bonds with other elements.

Further, efficiency and selectivity were compared between extractants that have a similar chemical composition or molarities. The deionized H$_2$O extracted only the most water-soluble elements from the soil. Contrary to the previous study (Miličević et al. 2017a), the prolongation of extraction (from 2 to 16 h) with deionized H$_2$O, for this type of the soil, was not more effective. Even their molarities are noted equal, comparing the chloride salts (CaCl$_2$ and BaCl$_2$), more efficient for extraction of macroelements was 0.1 mol L$^{-1}$ BaCl$_2$, still this extraction procedure was not suitable for extracting some microelements (Table S10). Extraction by BaCl$_2$ is recommended for assessment soil CEC, and it is the most suitable for extraction of macroelements (e.g., Ca, Mg, Al, and K). The solution of 0.01 mol L$^{-1}$ CaCl$_2$ could not be the most effective for extracting all measured elements because the content of Ca makes an interference on determination of element concentrations (Hooda 2010), but it could be appropriate for extracting some metals from soil (e.g., Al, Fe, Ni, V, and Zn). Weak salt solution NH$_4$NO$_3$ extracted the highest Ba, Ca, K, Mg, and Sr content (Table S10). Comparing NO$_3^-$ salt solutions, 0.1 mol L$^{-1}$ NH$_4$NO$_3$ was more efficient for extracting most of the elements (Al, Ba, Ca, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, S, Si, Sr, and Zn), but NaNO$_3$ was more effective for extracting B, Be, Cd, Co, Pb, and V (Table S10). This is probably caused by NH$_4^+$, which binds metal in complexes and induces the additional release of these elements from the soil (Hooda 2010).

Environmental implications in the soil samples and health risk assessment for field workers

The environmental risk and environmental implication indices were used to compare normalized values of PTE concentrations, which enable distinguishing pollution of different sites, i.e., organic, conventional, and experimental vineyards. By creating these dimensionless-unit “concentrations”, investigated soils were more effectively classified according to the pollution.

To compare the concentrations in O soil layer with concentrations in A soil layer, BGI was calculated (Table S11). The median BGI values for most of the elements imply that there was no high PTE sorption in O layer (˂ 1 or ≈ 1; Table S11; Fig. S1). Thus, there was not frequent anthropogenic influence to O soil layer. Otherwise, it is worth mentioning that soil of the Balkan Peninsula is naturally enriched by As, Cr, Ni, and sometimes Pb (Ličina et al. 2017; Dangić and Dangić 2007; Salminen et al. 2005). Thus, according to obtained BGI values, most of the determined PTEs in the soil probably have a geogenic origin. High BGI values were observed only for several elements B, Na, S, and Si (Fig. S1) probably because of the application of natural fertilizers. Comparing with other studies conducted in agricultural areas, BGI values obtained for the elements in this study (Table S11) were lower than those BGI values obtained for the forest and the grassland soils (Mazurek et al. 2016).

To investigate whether any environmental implication caused by the element concentrations exists in O and A soil layers, CF values were calculated (Table S12; Fig. S2). CF values for both O and A layers were low (˂ 1 or ≈ 1; Table S12; Fig. S2), except for B in soil collected in the middle of the season (July) when CF values were very high in O soil layer (Fig. S2b). It seems, even the grapevine is not treated with commercial pesticides, in the organic vineyard B concentration in soil could originate from the neighbor parcels where B-based pesticides (Borax) had been used (Kabata-Pendias and Mukherjee 2007). PLI values for all the investigated parcels in the organic vineyard were low (PLI ≈ 1) (Table S12). These PLI values for A layer (0–30 cm) were similar or slightly higher than values obtained for the topsoil in experimental and conventional vineyards investigated in the previous studies (Miličević et al. 2017a, 2018a, 2018b). SOM algorithm, as a tool that exhibited advantages over commonly applied clustering techniques, was applied to propose whether any differences exist between PLI values depending on the vineyard ambient. According to applied SOM algorithm, there was identified only two clusters of particular samples as represented by SOM resulting map, neighbor distance plot, and dominant blue circles in contrast to light blue and yellow ones (Fig. 2a, b, c). The result implied that no distinction exists between the burden of soil samples by PTE in organic and conventional (Miličević et al. 2018b) vineyards (Fig. 2a, c). Exceptions
were PLI values for samples (25, 43, 48, 77, and 78) in light blue circles and samples (26 and 80) in a yellow circle (Fig. 2c). The samples with PLI which differ from others (26, 48, and 80) are the nearest samples from parcel located near the metal foundry in conventional vineyard (Miličević et al. 2018a), and other PLI values grouped in light blue circles were PLI of the samples located near the highway road (25, 77, 78) (Fig. 2c) in conventional vineyard, also investigated in one of the previous studies (Miličević et al. 2018b).

In this study, environmental risk assessment (Eri, RI) for PTE, such as As, Cd, Cr, Cu, Ni, Pb, and Zn, was < 40 (Table S13), and it was defined as low (Guo et al. 2010). The highest was environmental risk obtained for Cd (ErCd) (Fig. S3), which indicates that its concentration, even low in this soil, could have a prominent influence on environmental risk in the soil because of its high mobility. Moreover, according to the scale, obtained RI (31 < RI < 64; Table S13) in the organic vineyard was also low (Table S13). Environmental risk in organic vineyard assessed by RI values was significantly lower than RI obtained in the conventional vineyard (Fig. 3a). Complementary to the results of Wilcoxon signed-rank test, SOM analysis, as tool that exhibited advantages over commonly applied clustering techniques, distinguished patterns of RI dissimilarity in studied vineyards (Fig. 3b, c, d). A strong difference was observed regarding different vineyard ambient, as it is shown by dark blue circles which in counts plot represent samples from the conventional vineyard (Miličević et al. 2018b), and lighter ones illustrated samples from the organic vineyard (Fig. 3c). Thus, ecological risk (RI) in the organic vineyard was pointed out as significantly different (lower) than in the conventional vineyard (Miličević et al. 2018b).

According to BRAIprobable values, moderate to high bioavailability risk was observed, while according to BRAIapparent values, low to moderate bioavailability risk was observed (Table S14). Pseudo-total concentrations of PTE were lower than pseudo-total concentrations obtained in soil from the other conventional vineyards investigated in the previous studies (Miličević et al. 2017a; Miličević et al. 2018b). Still, it obtained higher BRAI for the soil in organic vineyard,
which is probably influenced by higher mobility of Cd, because it was extracted in higher concentrations from the organic than from the conventional vineyard’s soil using Na2EDTA (Miličević et al. 2018a). Thus, if the production is not organic, this soil type could be easily polluted by agrochemicals containing Cd due to its mobility investigated in this experiment, which could have an adverse influence to plant and further to human health. Between BRAIprobable and BRAIapparent, significant ($p < 0.01$) correlation ($R = 0.99$) was observed, which was also proved by regression analysis ($R^2 = 0.97$) between these values (Fig. S4). The obtained BRAIprobable in organic vineyard is higher than BRAIprobable observed in conventional vineyard in Serbia, while BRAIapparent is similar to BRAIapparent in the conventional vineyard (Miličević et al. 2018a) (Fig. 4). The median values of BRAIprobable in the organic vineyard (range 1 to 2.67; Table S14) were slightly lower than BRAIprobable of the urban soil (Madrid et al. 2008), while the values were significantly lower than BRAIprobable values for mining areas (Anju and Banerjee 2011), agricultural soils (Poggio et al. 2009), and residential sites (Poggio et al. 2009).

According to health risk assessment equations (Table S7), adjusted to simulate farmers exposed to toxic elements from the soil during grapevine season (from April to October), both non-carcinogenic ($\Sigma HI < 1$; Table S15) and carcinogenic ($\Sigma R \leq 10^{-5}$; Table S15) risks were low. These values were slightly lower than values calculated for workers in the conventional vineyard published in previous experiment (Miličević et al. 2018a), which indicates that during a long time of exposure, organic production environment could be healthier working place for the field workers. The oral intake had also the highest impact on non-carcinogenic risk in this study, as it was observed previously in the conventional vineyard, which leads to further ingestion and risk for human health. The total
carcinogenic risk in the organic vineyard was within the acceptable range proposed by EPA (US EPA 2007).

**Grapevine**

**Element concentrations in grapevine (leaf, petiole, whole berry, skin, pulp, and seed)**

The descriptive statistic of element concentrations in different grapevine parts (seed, pulp, skin, whole berry, petiole, and leaf) is presented in Table S16, and some elements’ concentration in grapevine parts is presented at Fig. 5. The concentrations in grapevine samples (leaves, petiole, and grape berries) did not vary significantly ($p<0.05$) between studied vineyard’s parcels and grapevine varieties. Observing the concentrations of elements in outer parts of grapevine (skin, petiole, and leaf), the highest element concentrations were observed in leaf samples for most of the investigated elements (Table S16; Fig. 5), which indicates that leaves more efficiently entrap air deposits than other grapevine parts, probably because of its plate and rough structure. Only Ba and Co were measured in higher concentrations in petioles than in leaf, while Na concentrations in petiole and leaf were similar (Table S16; Fig. 5). This indicates that these elements mostly originate from the soil, as it was observed in the previous study, where we assessed the bioavailability of the elements from soil to grapevine; it was concluded that Ba and Na in grapevine parts mostly originate from the soil (Miličević et al. 2018a, 2018b).

PTE concentrations in the grapevine berries were significantly lower than the concentrations in leaf and petiole (Table S16). Leaf in the organic vineyard had lower PTE concentrations than those measured in leaves from the conventional and experimental vineyards (Miličević et al. 2017a; Miličević et al. 2018a). National regulations of the Republic of Serbia prescribe maximum allowable concentrations (MAC) only for a few elements in fresh fruit (grape) (National Gazette of Republic of Serbia 2011c) and concentrations of PTEs (As, Cd, and Pb) in grape berries were lower than MAC (Table S16; Fig. 5). Among grape berry parts (skin,
pulp, and seed), the highest element concentrations were determined in grapevine seeds (Fig. 5), as it was also observed in the experiment conducted in conventional vineyard (Milčević et al. 2018a). Namely, the concentrations of the elements in grapevine parts were slightly lower than those observed in the previously studied varieties (Milčević et al. 2017a, 2018a).

Finally, observing the environmental implications obtained for soil that was previously described in “Soil”, B concentration influenced the soil contamination and Cd had an influence on the bioavailability risk. According to the distribution of concentrations obtained in different grapevine parts (seed, pulp, skin, petiole, and leaf), obtained by PCA analysis, it can be noticed that Cd from soil had a higher influence on inner parts of the grapevine (seed, and pulp) and B mostly influenced grapevine leaves (Fig. 6). Thus, these two elements probably originate from different mediums; Cd originates mostly from soil, while B originates from air deposition. This could be supported by the obtained higher concentrations of Cd in inner grapevine parts (seed and pulp) and B was accumulated in higher concentrations in outer grapevine parts (leaf and petiole) (Fig. 5; Table S16).

Environmental implications of the grapevine samples and health risk assessment for consumers

The grapevine varieties could not easily accumulate PTE from the soil since for the majority of the elements, BAC values were lower than 1. Although the investigated grapevine varieties are not metalofite, they probably slightly accumulated some quantities of PTE. According to observed BAC values, grapevine tends to accumulate B, K, Mg, and P mostly in leaves (Table S17) in case of neutral to low alkaline soil with low OM content. All these elements can be constituents of fertilizers applicable in some low quantities in organic production, but also could be deposited from the air and originate from the neighbor environment. In addition, B in higher concentrations could cause serious problems to the plant development and further could have slightly toxic effects on human health (Kabata-Pendias and Mukherjee 2007).

As defined by RF, some of the elements (Al, As, B, Ba, Be, Cu, Mn, Mo, Ni, P, Pb, Sr, V, and Zn), especially those in leaves, could originmate from air deposition (Table S18; Fig. S5), or some remote pollution sources. A similar result was also observed in the conventional vineyard (Milčević et al. 2018a). Due to less frequent applications of agrochemical and lower presence of PTE from the soil, leaves in the organic vineyard probably rather reflect the PTE deposition from the air, which could be a consequence of lower initial PTE levels in the organically grown leaves than those conventionally grown (Milčević et al. 2018a, 2018b).

Non-carcinogenic risk for human intake of organically grown grapes (adults and children) was not observed (ΣHI < 1; Table S19). According to the adjustable formula, total carcinogenic risk was not observed for grapevine consumers (ΣR ≤ 10⁻⁶). The values of the health risk indexes were lower than in the conventional vineyard published in the previous experiment (Milčević et al. 2018a), and for long-term conditions, grapevine varieties grown in organic vineyard could be safer for the consumption (Table S19).

Assessment of airborne element pollution by moss bag technique

The concentrations of elements in mosses were above LOQ in both exposure periods, except for K and Na which were also pronounced in previous studies (Adamo et al. 2003; Aničić et al. 2009; Milčević et al. 2017b). Thus, the moss bag technique could not be appropriate for biomonitoring of these two elements. Regarding the other element concentrations in the moss after 2-month exposure in organic vineyard ambient, reliable PTE “signal” was observed (Table S20). This result confirms that moss bag exposure for 2 months may be representative for reliable element pollution assessment in the organic vineyard. By this method, the organic vineyard ambient was recognized as not polluted agricultural ambient. Comparing with other studies where active moss biomonitoring was also performed during 2 months, the concentrations of most PTEs in this study were significantly lower than in the agricultural (conventional vineyard) and the urban (crossroad and urban background) areas (Fig. 7a) (Vuković et al. 2016; Milčević et al. 2017b). Further, in moss samples exposed for 4-month period, again lower PTE
concentrations (Table S20) were found in the mosses exposed in organic than those exposed in conventional vineyard (Milićević et al. 2017b) and suburban area (Aničić et al. 2009) (Fig. 7b). From the aspect of moss bag biomonitoring, organic vineyard ambient could be represented as a potential background agricultural ambient with presumable absence of frequent agrochemical additions. The reliable “signal” of PTE enrichment (>LOD) was detected in the biomonitor after 2-month exposure. It seems that previously selected exposure time of 2 months (Aničić et al. 2009) might be kept for future intercomparative studies with different land-use classes such as urban or industrial ambient.

For moss bag biomonitoring, it is of importance to exclude the influence of initial element concentrations specific for moss pristine area, from the concentrations in the moss after exposure by calculation of the relative accumulation. Previous claims of element accumulations in the moss have been confirmed by RAF values, which were also lower than in conventional vineyard (Milićević et al. 2017b) and urban area (Aničić et al. 2009; Vuković et al. 2016). Slightly higher RAF values were observed for Al, B, Cr, Cu, Sb, V, and Zn than for other PTE (Fig. S6). The prominent accumulation of elements in mosses could give information about potential adverse influence airborne PTEs. These results also judged on previous claims that most of the PTEs were observed in highest concentrations in outer grapevine parts (e.g., leaves), especially B (RAF = 6.30).

Higher accumulation of some elements (Al, As, Co, Cr, Fe, Sb and V) was observed during 4-month than 2-month exposure (Fig. S16), which confirms that in the agricultural area, a reliable cumulative pollution “signal” will be achieved if moss bags are exposed during whole vegetation season (Milićević et al. 2017b). Contrary, 2-month moss bag exposure gave a higher enrichment for B (RAF = 6.30) and Zn (RAF = 3.02) (Table S20), which were also significantly accumulated in the grapevine leaves (Fig. 5). It is worth mentioning that concentrations of B were also increased in mosses exposed in July, as it was the case for soil and leaf samples in this period. Two-month moss exposure (from June 1st to August 1st) represents the grapevine growing period and probably some manure and some quantities of pesticides were applied during this period. Also, these element enrichments in moss material and grapevine leaves could originate from the neighbor commercial parcels where some of the pesticides or fertilizers containing B and Zn could be used, and due to the influence of meteorological parameters (wind direction etc.), some pollution from the neighboring commercial parcels could affect the outer grapevine parts through the air deposition. RAF for B was significantly higher than RAF obtained for Cd (Table S20), which confirms the assumption that in the grapevine, B mostly originates from air and Cd mostly originates from soil (Figs. 5 and 6).

**Fig. 7** Median concentration (mg kg⁻¹) of the elements in *S. girgensohniia* exposed during 2 months (2M) in the organic vineyard (OV 2M) vs. the comparative values for conventional vineyard (2M CV) (Milićević et al. 2018b), the crossroads (CR 2M) and urban background (UB 2M) area of Belgrade (Vuković et al. 2016) and B exposed during 4 months (4M) in the organic vineyard (OV 4M) vs. conventional vineyard (CV 4M) and urban ambient (UA 4M) (Aničić et al. 2009)
Conclusion

To improve food quality in many developed countries, organic agricultural production has become a common practice. In this study, mobility of PTEs in agricultural ambient was investigated comparing nine single extraction procedures; Na$_2$EDTA and CH$_3$COOH were found as the most effective procedures. A total of 0.44 mol L$^{-1}$ CH$_3$COOH was more effective than 0.11 mol L$^{-1}$ CH$_3$COOH. Among Cl$^-$ salts, BaCl$_2$ was more effective for extracting macroelements than CaCl$_2$, which was more effective for microelements. However, Ca in CaCl$_2$ causes some interference in element determinations; thus, NO$_3^-$ salts could be more reliable for assessing mobility of microelements. Ammonium nitrate is more suitable than NaNO$_3$ for assessing the elements’ mobility, mainly because NH$_4^+$ could bind complexes with elements and induces the additional release of elements from soil. Prolongation of the extraction time for deionized H$_2$O did not prove as more effective for this soil type.

According to all environmental implications (BGI, CF, PLI, Eri, and RI), soil in organic vineyard was not polluted by PTE, except for B in topsoil samples, especially in July. Despite low pseudo-total Cd concentration in soil, it was highly mobile and it could have a high impact on environmental and bioavailability risk. SOM analysis distinguishes environmental risk (PLI and RI) between organic and conventional vineyards.

Lower PTE concentrations were obtained in grape in organic vineyard, than in conventional ones. Boron in grapevine parts mostly originates from air deposition and Cd mostly originates from soil. PTEs were measured in higher concentrations in outer grapevine parts than in inner indicating atmospheric deposition influence to outer plant parts, especially to leaves (RF > 1). However, deposition of airborne elements could not be controlled because meteorological parameters have an unpredicted influence on long-range element transport and deposition. Finally, the organic agricultural environments are safer for field workers and the quality of final products according to health risk assessment.

Indicated by moss bag biomonitoring, air pollution in organic vineyard is significantly less than in conventional vineyard and urban areas (crossroads, suburban areas, and urban background). According to the element concentrations and RAF values, partial influence of B is evident, especially in the period of grapevine growth. Organic vineyard could represent an adequate background ambient for application of moss bag technique in air pollution assessment in agricultural (vineyard) or urban ambient.

This study can be useful as a pilot study in further investigations in organically growth agricultural areas by giving useful information about the choices of specific single extraction procedure for elements’ mobility assessment. Also, this investigation can be an indication of environmental and health risk assessment in organic vineyard and it can serve for the comparison with other studies conducted in different vineyard ambient.

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