

TOTAL AND EXTRACTABLE FORMS OF Cu, Zn, Ni, Cr, Pb AND Fe IN VINEYARD SOIL (VALANDOVO VALLEY, MACEDONIA) DETERMINED BY A SEQUENTIAL EXTRACTION PROCEDURE

Elizabeta Karakaševa, Blažo Boev, Vesna Zajkova

*Faculty of Natural and Technical Sciences, “Goce Delčev” University,
Štip, Republic of Macedonia
elizabeta.karakaseva@ugd.edu.mk*

The total and extractable forms of Cu, Zn, Pb, Ni, Cr, and Fe in vineyard soils from the valley of Valandovo were analyzed in order to understand the existence of metals in soils and their potential availability to the flora and fauna. The content of metals was determined by ICP-EAS analysis, while metal binding was defined with a sequential extraction procedure. The total content of Cu, Zn, Pb, Ni, Fe and Cr, in almost all soil samples, did not exceed the maximum permissible content (MPC) of trace elements in soils proposed by the New Dutchlist. The only exception was the soil sample R(3), where a higher content was measured especially for Fe, Ni and Cr and to a lesser extent Cu and Zn. Sequential extraction showed that the distribution of metals increased in the order: oxidizable > residual fraction > reducible fraction > exchangeable, water and acid soluble in soil. From this study, it can be concluded that the increased content of heavy metals in the area around the village of Rabrovo can be explained by past mining activities and the partial transfer of heavy metals in the soil. However, the low value in the mobile exchangeable fraction and the physico-chemical characteristics of the studied area indicate a low potential risk to the surrounding environment.

Key words: Heavy metals; sequential extraction; accumulation; distribution

ВКУПНИ И ЕКСТРАХИРАНИ ФОРМИ НА Cu, Zn, Ni, Cr, Pb И Fe ВО ПОЧВИ ОД ЛОЗОВИ НАСАДИ (ВАЛАНДОВСКА КОТЛИНА, МАКЕДОНИЈА) ОПРЕДЕЛЕНИ СО ПОСТАПКА НА ПОСЛЕДОВАТЕЛНИ ЕКСТРАКЦИИ

Истражувањата во овој труд главно беа насочени кон определување на вкупните и формите на Cu, Zn, Pb, Ni, Cr и Fe кои можат да се екстрахираат од почви собрани од лозарски парцели од Валандовската Котлина, како чекор кон разбирање на однесувањето на овие метали во почвите и нивната потенцијална достапност до флората и фауната. Количеството на металите во почвата беше определено со ICP-AES, додека нивното фракционирање во почвата е дефинирано со помош на постапката на последователни екстракции. Вкупните количества на Cu, Zn, Pb, Ni, Cr и Fe во речиси сите почвени примероци не го надминаа максимално дозволеното количество (МДК), со исклучок на почвениот примерок R(3), каде што беше измерено повисоко количество на Ni, Cr, Fe, Cu и Zn. Фракциите екстрахирани од површинскиот слој на почвата го имаат следниот редослед на застапеност: фракција екстрахирана како резултат на оксидација > резидуална фракција > фракција екстрахирана како резултат на редукција > јоно-изменувачка фракција и фракција растворлива во слаби киселини. Доминацијата на металите во оксидационата фракција во почвата покажува дека оваа фракција има доминантна улога во контролата на однесувањето и транспортот на овие метали во почвата. Нискиот степен на застапеност на металите во првата (лесно мобилна) фракција укажува на ниска стапка на активност и мобилност на овие метали во почвениот раствор, предизвикана од моменталните физичко-хемиски услови во почвата. Високите вредности за Ni, Cr, Fe, Cu и Zn измерени во областа околу селото Раброво можат да се припишат на некогашната рударска активност и нивен делумен пренос во почвата, но ниските вредности добиени за лесно достапната фракција и физичко-хемиските карактеристики на почвата укажуваат на низок потенцијал на ризик за околината.

Клучни зборови: тешки метали; секвенцијална екстракција; акумулација; дистрибуција

1. INTRODUCTION

Metals are present in the soil in different chemical forms, which influence their reactivity and hence their mobility and bioavailability. The risks associated with the presence of heavy metals depend mainly on their chemical form (metals, oxides, salts and organometallics). The impact of these metals in soils is their possible transfer into water or plants, which is defined by the term "bioavailability". Cd and Pb are considered as the most important environmental pollutants in agricultural soils because of the potential harmful effects they commonly display on food quality and soil health [1, 2]. Copper, which is the active ingredient of fungicides, is reported as one of the most toxic metals to soil microorganisms and soil health [3]. Numerous studies have shown that the old practice of using copper salts (Bordeaux mixture, $\text{Ca}(\text{OH})_2 + \text{CuSO}_4$) and other fungicides for the protection of grapevines and other crops usually results in significant accumulation of heavy metals in soils. Despite its environmental and agricultural importance, the content, distribution and fractionation of both anthropogenic and naturally occurring copper in soil is poorly known. Although the total heavy metal content in soils is a useful indicator of soil deficiency and/or contamination, it does not provide enough information about its environmental impact.

Heavy metal availability to biota (as a nutrient or toxin) and its mobility are the most important factors to be considered when assessing effects on the soil environment. In order to understand the behavior of heavy metal in soils, it is quite important to not only measure the total content, but also to study the heavy metal distribution, using physical and chemical techniques [1]. Metal in soils can be divided into two fractions [4]: (i) the inert fraction, assumed to be the non-toxic fraction and (ii) the labile fraction, assumed to be potentially toxic. To assess the availability of heavy metals, only the soil labile fraction is taken into account, because this fraction is often called "the bioavailable fraction". However, the bioavailable fraction can differ

from one metal to another and from one receptor to another. The availability of metals for plants and microorganisms in soil depends on the composition of the different compartments of soil such as carbonates, (oxy)metal hydroxides, organic matter and silica.

Sequential extraction has been used to highlight the relationship between the compartments of soil and metals, and to assess the bioavailability and the potential threat of heavy metals to the environment. It involves conducting successive chemical attacks on soils; each attack is supposed to destroy the bond between the metal and the inorganic specific fraction of the soil. These sequential extractions commonly help to identify the portion of the solid phase which is the potential source for a metal released to the biota, where the solid sample is subjected to successive attacks with a series of progressively harsher reagents to dissolve increasingly refractory forms [5–6]. The BCR (the European Community Bureau of Reference) sequential extraction scheme [7–8] is the most commonly used in determining both actual and potential mobility of trace elements in soils. By using these four successive extractions, the procedure allows identification of three distinct parts (or compartments) and a residual.

The fraction bound to carbonates (F1) comprises metals adsorbed to the soil surface. It is the most accessible one and it represents the exchangeable fraction. Metals in this fraction are easily mobile and are assumed to be highly available. A decrease in the pH causes the migration of the metal ions from the solid phase to water and plants. The fraction bound to iron and manganese oxides (F2) is sensitive to redox potential changes and represents a fraction which can be solubilized under reducible conditions. The fraction bound to organic matter (F3) is temporarily inaccessible. It can be solubilized by chemical oxidation. The residual fraction (F4) includes mainly metals built into the crystal lattice of minerals. Under natural conditions, they are practically inaccessible to living organisms and can be considered as permanently immobile.

The main goals of the current study were (1) to determine the contents of Cr, Cu, Fe, Ni, Pb and Zn in agricultural soils samples from the vineyard area of the Valandovo valley, (2) to estimate the heavy metal distribution and mobility characteristics in soil samples using the sequential extraction procedure and (3) to establish relationships between heavy metals and soil properties.

2. MATERIALS AND METHODS

2.1. Study area

The Valandovo Valley is located in the south-eastern part of Macedonia, with an area of 331 km². Having an ideal Mediterranean climate featuring hot summers and cold winters, the Valandovo valley gives the impression of a large garden abundant with well-known and tasty early spring vegetables and fruits. The oldest formations follow the NW–SE direction and belong to the inner parts of the Vardar zone. This area presents the following geological units: Precambrian, old Paleozoic, altered gabbro diabase, serpentines, granite, quartzlatite and quaternary. The Paleozoic (Pz) metamorphic complex is present with two se-

ries: Paleozoic schist and serpentines that are present in the form of narrow belts along ruptures inside the Vardar zone. The north-eastern part of the study zone is covered with Paleozoic schist and metamorphosed limestone. In the southern part of the valley, Precambrian gneiss, Paleozoic serpentines and tertiary granite are present.

Pliocene lake sediments take up most of the study area, covered with quaternary layers, of which the majority are pro-alluvium sediments that developed along the Vardar, Kon-ska, Sermeninska and Kovanska rivers and the marginal part of the valley. This sediment contains rugged material from surrounding rocks, mixed with clay-sandy material.

2.2. Soil sampling and analysis

Seven surface soil samples (0–30 cm) from agricultural soils were collected in the study area (Figure 1). The soil samples were representative of major agricultural soils in the region. Each soil sample consisted of seven to nine subsamples. These were collected using a stainless steel sampling tube. The subsamples were mixed and homogenized. All soil samples were stored in plastic bags.

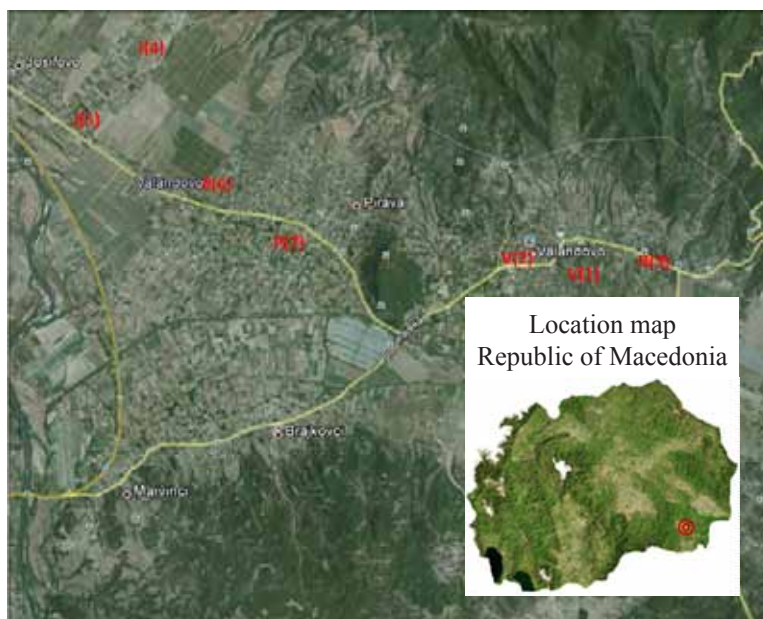


Fig. 1. Location of sampling

The soil samples were air-dried, ground afterwards and passed through a 2-mm sieve prior to physical analysis and then ground in a mechanical agate grinder to a fine powder ($< 63 \mu\text{m}$) for subsequent chemical analyses. Soil pH was measured in a 1:5 (w/v) ratio of soil to distilled water using a pH meter (HANNA HI 98150). Organic carbon was measured using the modified Walker-Black method [9] and calcium carbonate was measured by a volumetric method [10].

The CEC (cation exchange capacity) is the maximum amount of cations that 100 g of dry soil can absorb. It was determined using the $\text{CH}_3\text{COONH}_4$ method (1 M, pH 7) [10]. The particle size of soil was determined according to the standard reference method ISI 11277. The total content of metals in the soil was determined by acid attack using 5 ml of HNO_3 , 5 ml of HF and 1.5 ml HClO_4 [11]. After digestion, the sample solutions were analyzed by atomic emission spectrometry with inductively coupled plasma (ICP-AES).

Three different extraction procedures were used according to the BCR sequential scheme, as detailed by Quevauviller et al. [7]. The first step evaluates exchangeable metals and those bound to carbonates, which are respectively affected by adsorption-desorption processes or ion exchange, and by changes in pH. Approximately 1 g of sample was extracted with 40 ml of 0.11 mol/l of acetic acid, using a mechanical end-over-end shaker (30 rpm, 16 h, ambient temperature of 22°C). The second step evaluates acid reducible fractions, i.e. metals bound to Fe/Mn oxides, which are unstable under anoxic conditions. The residue from step 1 was treated with 40 ml of 0.1 mol/l hydroxylamine hydrochloride, at pH 2, and the extraction was conducted again using the end-over-end shaker. The third step extracts the oxidizable fraction, which entails metals bound to organic material, such as living organisms, detritus, organic coatings on mineral particles and sulfides. The step 2 residue was digested with 10 ml of 30% hydrogen peroxide (H_2O_2) for 1 h at room temperature, followed by the addition

of 10 ml H_2O_2 and digestion for 1 h at 85°C . After reducing the volume to almost dryness, the residue was extracted with 1 mol/l ammonium acetate (pH 2) using a mechanical end-over-end shaker. In the present study, a fourth step was included in order to evaluate the metal content bound to the silicate matrix or to crystalline Fe/Mn oxides, which are not released under natural conditions. The residual fraction was assessed with the analytical method SW-846 EPA3052 (US EPA, 1996) [11]. This extraction was performed with acid attack, i.e. 5 ml of ultrapurified water, 9 ml of nitric acid (HNO_3), 3 ml of hydrochloric acid (HCl) and 3 ml of hydrofluoric acid (HF) (Table 1).

After sequential extraction, the sample solutions were analyzed by atomic emission spectrometry with inductively coupled plasma (ICP-AES).

The sequential extraction procedures are summarized in Table 1.

Table 1

The BCR sequential scheme, as detailed by Quevauviller et al. [7], and residual fraction

Tests fractions		
Step 1	40 ml of 0.11M CH_3COOH , shaking time: 16 h, room temperature	Exchangeable and acid extractable fraction (F1)
Step 2	40 ml of 0.1 M $\text{NH}_2\text{OH}\cdot\text{HCl}$, pH 2, shaking time: 16 h, room temperature	Reducible fraction (F2)
Step 3	10 ml of 8.8 M H_2O_2 (pH 2), 1 h, heated for 1 h + 50 ml of $\text{CH}_3\text{COONH}_4$ 1 M, shaking time: 16 h, room temperature	Oxidizable fraction (F3)
Step 4	5 ml of HNO_3 , 5 ml of HF and 1.5 ml of HClO_4 were successively added to the residue	Residual fraction (F4)

2.3. Quality parameters

The validation of the applied sequential extraction procedures in this study on the distribution of heavy metals in soil was carried out according to the statistical parameters of accuracy, precision and limit of detection. For this purpose, a certified standard material – bright alluviums meadow soil (PS-3, COOMET № 0001-1999 BG, SOD № 310a 98) [12] was used. Accuracy was evaluated by calculating the recovery test of each metal, determined by ICP-AES in the extraction solution after sequential extraction according to the BCR method (Table 2).

The precision was calculated as the standard deviation of all measurements performed on the content of metal by ICP-AES in the solutions after sequential extraction according to the BCR method. Detection limits were calculated as $3 \times$ the value of the standard deviation

of 10 measurements of the blank solutions after sequential extraction.

The results obtained from the application of the sequential extraction procedure show that the variances of the tested procedure gave comparable and reproducible results in the determination of heavy metals by the ICP-AES method in extraction solution. The sum of metal contents obtained from each fraction of the sequential extraction procedure was compared with the total contents of metals obtained by direct digestion of soil samples using HF and HClO₄. Analyses were performed in triplicate for the four soil samples (numbered 1, 2, 6 and std) randomly chosen by using the dissolving procedure, as explained above. The results obtained are given in Table 3.

The relative recovery between total extraction and the sum of four sequential phases was generally satisfactory.

Table 2

Statistical parameters obtained by analyzing certified standard soil [12]

Mean content \pm SD (mg/kg), $n = 3$					
Metals:	Cu	Pb	Ni	Zn	Cr
Fraction I	9.98 \pm 0.4 DL (0.03)	6.27 \pm 0.8 DL (0.08)	0.86 \pm 0.6 DL (0.04)	7.61 \pm 0.8 DL (0.1)	2.32 \pm 0.5 DL (0.01)
Fraction II	11.71 \pm 0.9 DL (0.05)	17.90 \pm 3.7 DL (0.02)	10.87 \pm 1.5 DL (0.02)	13.81 \pm 2.9 DL (0.15)	2.39 \pm 0.6 DL (0.01)
Fraction III	25.01 \pm 3.6 DL (0.1)	42.27 \pm 3.5 DL (0.04)	34.03 \pm 2.7 DL (0.03)	70.68 \pm 4.8 DL (0.3)	49.07 \pm 3.8 DL (0.01)
Fraction IV	7.17 \pm 1.9 DL (0.03)	6.25 \pm 1.6 DL (0.02)	13.22 \pm 1.3 DL (0.01)	33.46 \pm 3.1 DL (0.2)	26.23 \pm 2.7 DL (0.01)
Sum of the fractions	56.21 \pm 2.7	72.69 \pm 5.7	48.98 \pm 4.3	128.56 \pm 12.2	80.01 \pm 9.2
Certified value [12]	53.8 \pm 2	85.35 \pm 5.2	51.75 \pm 5.76	125.56 \pm 12.95	88.15 \pm 9.2
Recovery (%)	104.4	85.2	94.6	102.3	90.7

DL – detection limit, SD – standard deviation, R – recovery

Table 3

Comparative results of the metal levels of the sequential extraction procedure and trace metal content digested with a 5:1 mixture of hydrofluoric and perchloric acids for four soil samples (in mg/ kg)

	Num. of sample	Sum of fractions	Total content	R %
Cu	1	27.80±3.5	26.22±2.7	106
	2	34.87±1.2	32.58±2.1	107
	6	20.27±1.9	18.76±2.1	108
	Std	56.21±2.7	54.21±3.2	99
Pb	1	28.54±3.1	30.11±4.2	94
	2	33.14±3.6	35.21±2.7	94
	6	11.75±0.8	13.01±0.4	91
	Std	72.69±4.7	74.98±5.3	96
Ni	1	22.80±2.1	20.1±1.9	113
	2	29.74±1.5	31.2±4.6	95
	6	20.27±2.1	20.68±1.5	98
	Std	48.98±3.3	51.75±3.76	94
Zn	1	92.4±8.9	95.22±5.2	96
	2	85.59±5.6	85.22±5.2	101
	6	67.82±4.2	69.9±4.8	97
	Std	128.56±12.2	131.02±10.1	97
Cr	1	103.81±9.4	108.4±9.3	103
	2	92.20±8.4	98.3±7.1	95
	6	75.87±6.8	78.2±5.6	93
	Std	80.01±12.2	82.11±5.5	96

STD – certified standard soil

3. RESULTS AND DISCUSSION

3.1. Soil properties

The characteristics of the studied soil samples are presented in Table 4. Soil textural class varied from sandy loam (R3) and sandy clay loam (J4, J5, Pi6 and Pi7) to clay loam (Vi1 and Vi2). The organic matter (OM) content was low due to intensive use of the soil and rapid mineralization of OM influenced by climatic conditions. For example, the soil from J4 had 2.0%, while Vi2 and Pi7 soil had only 0.5% OM.

Cation exchange capacity (CEC) refers to the preservation and supply of soil fertilizer and buffering capacity. The CEC varied from 9.8 to 21.3 cmol/kg (Table 4), implying that soil in the Valandovo Valley had a moderate capac-

ity to retain and supply fertility. The content of carbonate varied depending on the place. Soils Va(4), Va(5) and R(3) contained higher amount of carbonates, unlike the Pi(6), Pi(7), J(1) and J(2) which were poor in terms of carbonates.

The pH of the soil is a parameter that influences the adsorption, retention and movement of heavy metals in the ground. As organic acids are degraded, the pH increases to an almost neutral level, buffered by the bicarbonate system [13]. This may explain the observed alkalinity in most soils (Table 4).

The pH depends significantly on the content of carbonates. Therefore, soil with a significant content of CaCO_3 and MgCO_3 has a higher pH. Alkaline pH increases the adsorption capacity of soil particles which become good and strong natural adsorbents [13]. Metals are thus adsorbed or co-precipitated.

Table 4

Locations of sampling and some physical properties of the soils

ID	Location	pH (H ₂ O)	OM (%)	CEC (cmol/kg)	CaCO ₃ (%)	Clay (%)
Va(1)	Valandovo (between two rivers)	8.15	0.6	19.8	11.4	42
Va(2)	Valandovo (unknown)	8.03	0.5	21.3	10.8	45
Ra(3)	Rabrovo (Nerezini)	7.52	0.8	9.8	2.1	15
J(4)	Josifovo (Kalkovo)	6.73	0.5	16.3	1.3	24
J(5)	Josifovo (under the highway)	7.76	1.2	17.6	5.2	26
Pi(6)	Pirava (Cardakli bunar)	6.78	1	10.9	1.5	18
Pi(7)	Pirava (Rakavec)	7.12	2	12.4	1.8	21

OM – organic matter; CEC – cation exchange capacity

3.2. Total heavy metals content

Table 5 shows total metal content of studied surface soils in the Valandovo valley and, for comparison, the maximum permissible content in soils according to the New Dutchlist [14].

The examined soils can be considered unpolluted, since their contents were under the maximum permissible content (MPC) of trace elements in agricultural soils proposed by the New Dutchlist, with the exception of R(3), were

the data clearly show that the soil samples from this location contain a slightly increased heavy metal content.

The values for the total heavy metal content in this soil sample exceeded the limit emission values reported by the New Dutchlist [14]. (Table 5).

The highest deviations were observed for chromium, nickel and copper, which in this wine parcel exceed the MPC proposed by the New Dutchlist [14].

Table 5

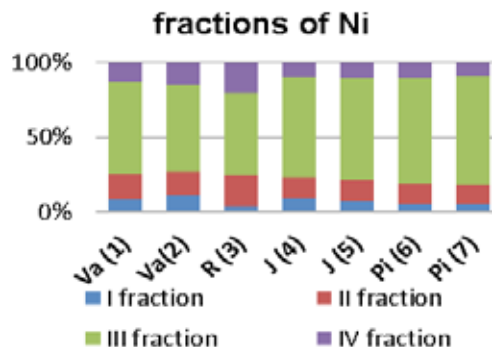
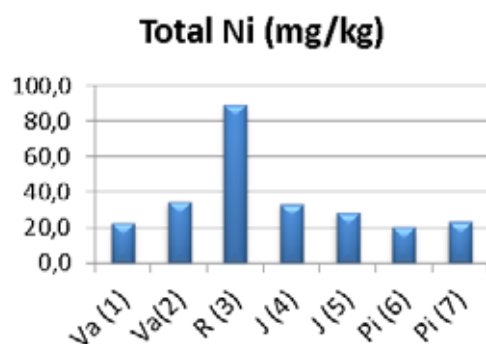
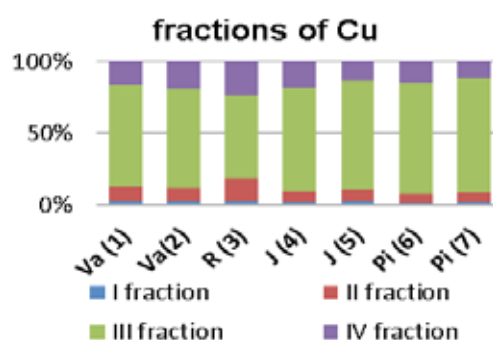
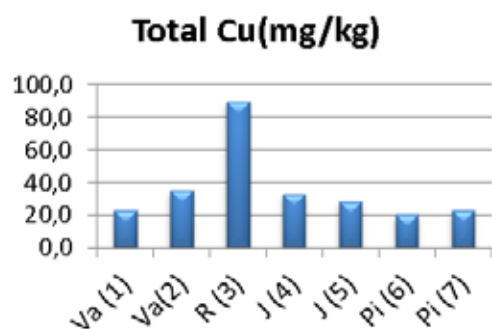
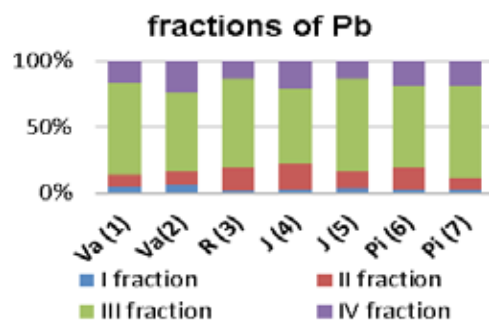
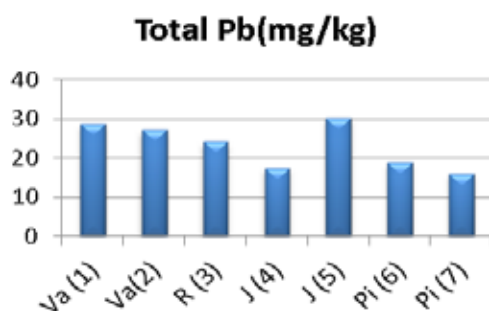
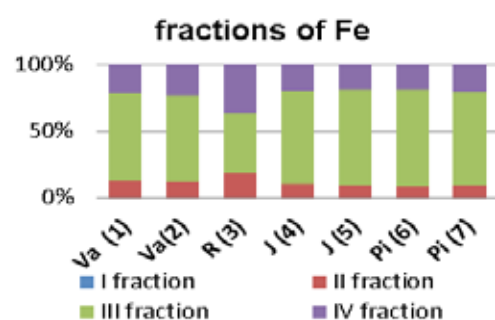
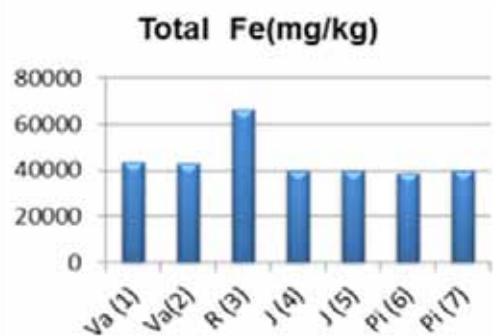
Total content of metals and maximum permissible agricultural soil content in some European countries (the New Dutchlist [14].)

ID	mg/kg dry weight					
	Cu	Ni	Zn	Cr	Pb	Fe
Va(1)	27.80	22.80	92.4	103.81	28.54	43511
Va(2)	34.87	29.74	85.59	92.20	33.14	42992
R(3)	89.24	153.61	108.91	286.83	24.15	66051
J(4)	33.23	25.06	79.6	79.98	17.5	40075
J(5)	28.32	37.70	81.2	91.82	30.22	40063
Pi(6)	20.27	20.27	67.82	75.87	11.75	38874
Pi(7)	23.02	23.02	71.6	98.52	11.91	39612
New Dutchlist [14].	36	35	140	100	85	

3.3. Sequential extraction

The distribution of heavy metals in a sample allows us to predict their mobility and bioavailability. The percentages of heavy metal contents in

various fractions of the studied soils are presented in Figure 2. In general, most of the studied metals were found to be mostly present in the oxidizable fraction, followed by the residual, reduction, easily leachable and ion exchange fractions.



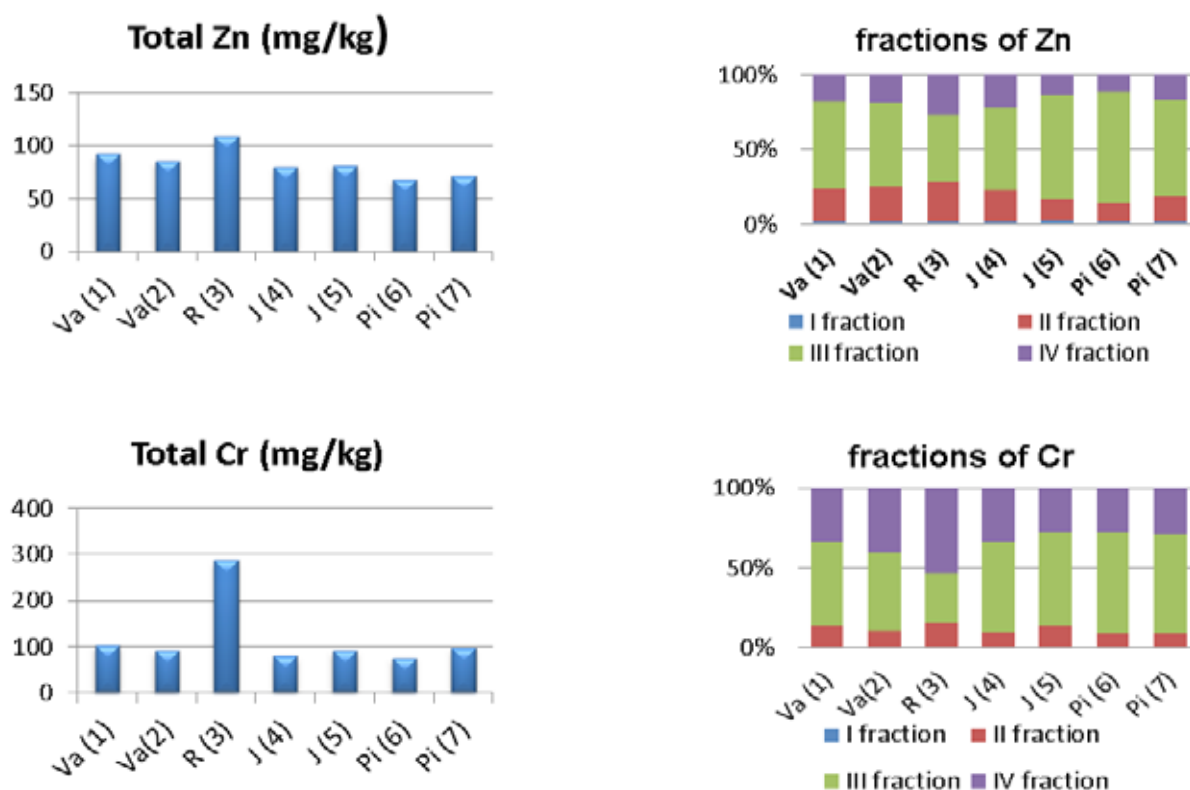


Fig. 2. Contents of Fe, Cu, Pb, Zn, Cr, and Ni in each of the fractions obtained by sequential extraction.

Chromium. In the studied soils, Cr was found to have a higher content in the organic and residual fractions, with average content of total Cr content ranging between 51.4 and 33.5%, respectively. In natural soil environments, Cr is present as a structural impurity in a variety of aluminosilicate minerals, or as chromite, a resistant Cr oxide mineral that accounts for most of the residual Cr fraction [3]. Chromium also showed the highest affinity for the organic fraction (Figure 2), which is related to its strong chelating capacity, and the role played by organic matter as an electron donor in the reduction of Cr(VI) to Cr(III) [15]. The Fe-Mn-oxide fraction was the third largest contributor to Cr retention, averaging 11% of the total Cr. Chromate ions can be easily absorbed to hydrous oxide surfaces at pH levels > 4, while Cr(III) tends to co-precipitate with Fe or Al hydroxides. That is because its ionic size and geochemical properties are very similar to those of Fe(III) and Al(III) [15]. Very small amounts of Cr were found in the exchangeable and the “carbonate-bound” frac-

tion. This is not surprising because at the soil pH levels encountered (6.8–8.12), Cr(III) precipitates readily [10], while carbonate minerals were absent in most studied soils. Large contributions of Cr in the organic fraction may raise concerns about its bioavailability and toxicity. Particularly under conditions of accelerated OM decomposition, chromium has a high mobility and toxicity potential, considering that nearly 55% of the total content was present in the non-residual fraction. However, the oxidation state of Cr must be defined before reliable Cr toxicity assessments can be made.

Copper. Copper exists mainly as Cu^{2+} in natural environments. In spite of the general perception that Cu is usually associated with organic and inorganic particle surfaces [16], nearly 71.8% of Cu in this study was found in the oxidizable fraction (Figure 2). The residual fraction (18%) and Fe-Mn oxide (11%) were the other contributors, but with significantly lower Cu contents compared to the oxidizable fraction. The association of Cu with Fe-Mn oxides

is attributed to Cu(II)-Fe(II) competitive sorption or co-precipitation processes [15]. The participation of Cu in the exchangeable and “carbonate” fractions was very small (2.6%). Since Cu was mainly associated with the oxidizable, residual and the Fe-Mn oxide fractions, which are relatively insoluble under natural conditions in soil (high pH), its status in the soils studied is considered stable. Copper bound to the Fe-Mn oxide fraction could be mobilized if soil drainage conditions deteriorate causing Fe and Mn reduction, but Cu toxicity problems are unlikely to occur. Cu bound to the organic matter and sulfide in soil can be mobilized if the soil is under low pH and oxidation conditions.

Nickel. Figure 2 shows that Ni was strongly associated with the oxidizable fraction (64.6%). High Ni contents were found in the oxidizable fraction, possibly due to an association with illuviated organic colloidal particles or an association with sulfide under reduced conditions. The Fe-Mn oxide fraction was the next most important Ni retention contributor, with an average soil profile contribution of 15.4% of the total Ni content. The sorption of Ni on Fe-Mn oxide surfaces usually occurs through Ni(II) substitutions of Mn, which could be enhanced by elevated pH in the soil [13, 15]. The highest proportion of Ni in the residual fraction was found in the soil sample from location R(3). According to Tessier et al. [5], the origin of Ni in natural soil environments is mainly detrital, where it is usually found as an occlusion in the structure of weathering silicate minerals. Although very small amounts of Ni were found in the exchangeable and “carbonate fraction,” they were greater compared to other metals. Clay content and the CEC, as the main contributors to total soil exchangeable charge, were good predictors of Ni content in the exchangeable fraction (Table 5). The mobility and bioavailability potential of this metal is a matter of concern in the studied soils. Particularly its bioavailability is affected if the soil pH drops significantly or if complexes with organic substances are created.

Zinc. Like most other metals, the majority of Zn was present in the oxidizable fraction, accounting for nearly 60.7% of the total content in the soil (Figure 2). The Fe-Mn oxide fraction (19.2%) and residual fraction (18.2%) were the next most important contributors in Zn retention, in terms of the total Zn content in the soil. This significant content Zn content in the oxidizable and Fe-Mn oxide fractions suggests strong associations with organic and clay particle surfaces [3, 15]. The retention of Zn in the Fe-Mn oxide fraction is explained by the high stability constants of Zn oxides and the ability of Zn to replace Mn in the oxide surface [2]. The highest amount of Zn in the residual and reducible fractions was found in the soil sample from the R(3) sampling site, as with the other metals. Relatively low levels of Zn were associated with the “carbonate fraction” (most likely phosphate-bound) and exchangeable fraction, contributing to 2.2% of the total Zn content. Increases in the exchangeable Zn content were generally affected by CEC increases in the soil.

Lead. Lead exists predominantly as Pb^{2+} in the environment, but when oxidized to Pb^{4+} , it can be incorporated into clay mineral and Fe-Mn oxide structures [3]. The results of the extractable fractions of Pb in soils are shown in Figure 2 Pb and exhibited similar trends in the chemical fraction distributions in all soils. The largest portion was found in the oxidizable fraction, accounting for 62.6% of total Pb, followed by the residual fraction (17.6%) and the reducible fraction (12.9%). The exchangeable fraction of Pb was below 3.5% of the total Pb content in soils. The relatively low content of Pb found in the relatively labile fractions (exchangeable, “carbonate,” organic) of these soils suggest a low risk for potential toxicity problems as a result of anthropogenic deposition processes.

Iron. Iron, like the other metals considered, followed the sequence: oxidizable fraction > residual fraction > reducible fraction > fraction soluble in weak acids. The highest percentage of total iron was measured in the oxidizable fraction. The reason is that pedogenic

processes, caused by extensive processing of agricultural soils (plowing, fertilization, etc.) leads to redistribution of heavy metals in the humus-accumulative part of the soil.

The proportion of iron extracted from the residual fraction contained silicate mineral crystals and some forms of iron unavailable to plants.

3.4. Correlation between the parameters determined in the soil

The iron content of soil does not change because of its high levels in the Earth's crust, for example, 0.5 to 10%, unless a source point exists in a particular environment, because it is used as a reference element for determining the origin of metals. To determine the correlation between iron and the other metals, statistical processing of all parameters was performed using the Pearson

correlation matrix. The correlations between soil properties were also determined (Tables 6 and 7).

The results show that most elements had a high linear dependence with iron (for nickel $R^2 = 0.94$; for Cr $R^2 = 0.98$; copper $R^2 = 0.94$; zinc $R^2 = 0.78$). A significant positive correlation indicates a strong geological or pedogenic influence.

Among the soil parameters considered, a rather high positive correlation was determined between the content of carbonates and the pH of the soil ($R^2 = 0.891$) as well as between the clay content of the soil and CEC ($R^2 = 0.932$). This indicates that the pH value of the soil depends on the content of carbonates and CEC increases with an increasing content of clay in the soil. The alkaline pH and high clay content in soil increases the capacity of adsorption of the soil, which become a good and strong natural adsorbent [13]. Metals are thus adsorbed or co-precipitated.

Table 6

Pearson correlation matrix for total metal contents

	Pb (total)	Cu (total)	Ni (total)	Zn (total)	Cr (total)	Fe (total)
Pb (total)	1.000	0.334	0.334	0.440	0.192	0.241
Cu (total)	0.334	1.000	1.000	0.835	0.962	0.972
Ni (total)	0.334	1.000	1.000	0.835	0.962	0.972
Zn (total)	0.440	0.835	0.835	1.000	0.842	0.885
Cr (total)	0.192	0.962	0.962	0.842	1.000	0.994
Fe (total)	0.241	0.972	0.972	0.885	0.994	1.000

Table 7

Pearson correlation matrix between soil parameters

	pH	OM (%)	CEC (cmol/kg)	CaCO ₃ (%)	Clay (%)
pH	1.000	0.267	0.657	0.891	0.740
OM (%)	0.262	1.000	0.458	0.436	0.458
CEC (cmol/kg)	0.654	0.458	1.000	0.844	0.932
CaCO ₃ (%)	0.891	0.436	0.844	1.000	0.951
Clay (%)	0.740	0.458	0.932	0.951	1.000

4. CONCLUSIONS

The present study focused on studying the accumulation and distribution of Fe, Pb, Cu, Ni, Cr and Zn in vineyard soil from the Valandovo valley, Macedonia. The content of metals was determined by ICP-EAS analysis, while metal binding was defined using a sequential extraction procedure. The results show that the measured values for the total content of trace metals do not exceed the maximum allowable limits. The only except (section R3) was a soil sample collected from the vicinity of the village of Rabrovo (east of Valandovo) that exhibits an elevated content of Cr and Ni over the limits proposed by the maximum permissible content in soil according to the New Dutchlist [14]. The high correlation between total iron and the part of iron extracted from the residual fraction and the high correlation between total iron and total Cu, Zn, Ni, and Cr suggest a geological or pedogenic influence, probably caused by the presence of serpentines in this area. On the other hand, the data obtained with the sequential extraction procedure shows that the highest percentage of heavy metal is found in the oxidizable fraction in the soil. This means that this fraction has a dominant role in controlling the behavior and transport of these metals in the soil. The relatively low values obtained with the easily accessible content of metal in all soil samples suggest a low rate of mobilization of metals, indicating that most metals are found in an inactive form in soil. This finding implicates that, in the considered areas, heavy usage of various types of pesticides and fertilizers has not appeared to increase the toxic metal content in soil.

The main mechanism of metal retention in soil is co-precipitation due to the pH of the soil solution. The measured high pH and other physico-chemical characteristics of the studied vineyard soil make metal mobility almost impossible.

From this study, we can conclude that the increased content of heavy metals in the area around the village of Rabrovo could be

explained by past mining activities and partial transfer of heavy metal in soil. However, the low values in the mobile exchangeable fraction and physico-chemical characteristics of the studied vineyard soil indicate a low potential risk to the surrounding environment.

REFERENCES

- [1] M. A. Kashem, B. R. Singh, T. Kondo, S. Kawai, Comparison of extractability of Cd, Cu, Pb and Zn with sequential extraction in contaminated and non-contaminated soils, *Int. J. Environ. Sci. Tech.*, **4** (2), 169–176 (2007).
- [2] M. J. McLaughlin, R. E. Hamon, R. G. McLaren, T. W. Speir, S. L. Rogers, A bioavailability-based rationale for controlling metal and metalloid contamination of agricultural land in Australia and New Zealand, *Aust. J. Soil Res.*, **38**, 1037–1086 (2000).
- [3] A. Kabata-Pendias, and H. Pendias, *Trace Elements in Soils and Plant*, 3rd edition, CRS Press, Boca Raton, Florida, 2001, p. 413.
- [4] J. Rachou, S. Sauvé, Evaluation of affinity constants of Cu, Cd, Ca and H for active soil surfaces for a solid phase-controlled soil ligand model, *Environ. Chem.*, **5**, 150–160 (2008).
- [5] A. Tessier, P. G. C. Campbell, M. Bisson, Sequential extraction procedure for the speciation of particulate trace metals, *Analy. Chem.*, **51**, 844–850 (1979).
- [6] M. Anju, D. K. Banerjee, Comparison of two sequential extraction procedures for heavy metal partitioning in mine tailings, *Chemosphere*, **78**, 1393–1402 (2010).
- [7] P. Quevauviller, G. Rauret, J. F. Lopez-Sanchez, R. Rubio, A. Ure, H. Muntau, Certification of trace metal extractable contents in a sediment reference material (CRM 601) following a three-step sequential extraction procedure, *Science of the Total Environment*, **205**, 223–234 (1997).
- [8] G. Rauret, J. F. López-Sánchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, Ph. Quevauviller, Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials, *J. Environ. Monitor*, **1**, 57–61 (1999).
- [9] D. W. Nelson, L. E. Sommers, *Total carbon, organic carbon and organic matter*. In: *Methods of soil analysis*, Soil Science Society of America, 1982.

- [10] A. L. Page, *Methods of Soil Analysis*, Part 2, *Chemical and Microbiological Properties*, 2nd ed, American Society of Agronomics, ASA, SSSA, Madison, 1982.
- [11] US EPA:, Method EPA 3051: Microwave assisted acid digestion of sediments, sludges, soils and oils, in *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods – SW-846* US, EPA, Washington, DC, USA, 1995.
- [12] P. Zapranova, V. Angelova, L. Dospatliev, K. Ivanov, Comparative study of methods for the preparation of soil samples for analysis AAS of Cd, Pb, Cr and Ni, *Scientific papers*, 34, 5, 2006 (in Bulgarian).
- [13] S. Bozkurt, L. Monero, I. Neretnieks, Long-term processes in waste deposits, *Elsevier, the Science of the Total Environment*, **250**, 101–121 (2000).
- [14] New Dutchlist, <http://www.contaminated-land.co.uk/std-guid/dutch-1.htm>
- [15] A. Kabata-Pendias, Behaviour properties of trace metals in soils. *Applied Geochemistry*, **2**, 3–9 (1993).
- [16] M. Dussault, V. Bécaert, M. François, S. Sauvé, L. Deschênes, Effect of copper on soil functional stability measured by relative soil stability index (RSSI) based on two enzyme activities, *Chemosphere*, **72**, 755–762 (2008).