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Scientific paper

COPPER AND ZINC FRACTIONATION IN APPLE ORCHARD SOIL IN THE VILLAGE OF BUKEVJE (CROATIA) USING THE REVISED FOUR-STEP BCR EXTRACTION PROCEDURE

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The aim of this study was to establish the fractionation of copper and zinc in a small apple orchard using the revised (four-step) Bureau Communautaire de Référence (BCR) sequential extraction procedure and assess their potential mobility in soil. Soil samples were collected at the depth of 10 cm to 25 cm, sixteen from the orchard and five control samples from a meadow located some 200 m away from the orchard. As the distribution of trace-element concentrations in the control samples was normal, they were used for comparison as background levels. We also determined soil mineralogical composition, carbonate content, soil pH, cation exchange capacity, and soil organic matter. The extraction yields of Cu and Zn from the control soil were lower than from the orchard soil (25 % *vs*. 34 % and 47 % *vs*. 52 %, respectively), which pointed to natural processes behind metal bonding in the control soil and greater influence of man-made activities in the orchard soil. Compared to control, the orchard soil had significantly higher concentrations of total Cu (P=0.0009), possibly due to the application of Cu-based fungicides. This assumption was further supported by greater speciation variability of Cu than of zinc, which points to different origins of the two, Cu from pesticides and Zn from the parent bedrock. Copper levels significantly better (P=0.01) correlated with the oxidisable fraction of the orchard soil than of control soil. Residual and organically bound copper and zinc constituted the most important fractions in the studied soils. However, the use of Cu-based fungicides in the apple orchard did not impose environmental and health risk from Cu exposure.

KEY WORDS: *BCR extraction, bedrock lithology, floodplain, fungicides, Sava River, trace elements*

Agricultural soil is a well-studied environmental compartment, since intensive land use has been associated with inputs of soil amendments and agrochemicals (1-3) and the risk of element accumulation at phytotoxic levels. Even though copper is one of the seven micronutrients (Zn, Cu, Mn, Fe, B, Mo, Cl) essential for normal plant growth (4), its excessive use in chemicals such as fungicidal sprays to control weeds and pests may adversely affect beneficial soil organisms and ultimately the entire agroecosystem. Therefore, much attention has been given to Cu behaviour in vineyard and orchard soils in terms of long-term or intensive use of agricultural chemicals containing this element (5-8). It is now widely recognised that micronutrient uptake by crops correlates with extractable fractions of the element in soils (4). Oreščanin et al. (9) found a twofold increase in Cu content in vineyard soil compared to the background level, most of it in exchangeable form which highly correlated with Cu content in grapes, most likely due to absorption from soil. More and more authors claim to be able to predict copper phytotoxicity from extractable Cu concentrations in soils (10-14). A number of single and multi-step extraction protocols have been developed to estimate the fraction of a micronutrient or contaminant that is bioavailable in the short term (15, 16). In order to harmonise methodology throughout the European Union so that the analytical results could be comparable, the Community Bureau of Référence (BCR) invented a simple, three-stage sequential extraction protocol for speciation or fractionation of trace elements in sediment and soil samples (17). The protocol was later revised to include one more stage (18, 19).

The aim of this study was to apply this revised BCR protocol in order to assess the potential mobility of pollutants in soil. This procedure is designed so that reagents release metals associated with specific soil phases: acid soluble, reducible, oxidisable, and residual. The first, acid soluble phase is presumed to be the most mobile, and therefor phytoavailable fraction.

Our second objective was to assemble a new set of data on the physicochemical forms of elements in an apple orchard classified between organic and conventional in terms of agricultural practices to see how it fits into the general belief - prevailing over the past few decades - that organically grown fruit is healthier and safer for consumption.

MATERIALS AND METHODS

Study area

For this study we selected a typical, small orchard (with an area of about 3750 m^2) with dense apple tree arrangement, situated in the village of Bukevje, which is being intensively urbanised due to the near vicinity of the town of Velika Gorica and the city of Zagreb (Croatia). The orchard owner is a small apple producer, who has been applying pesticides for years, but at rates substantially lower than those used for commercial fruit production, i.e. up to five to six sprays a year. These include copper oxychloride- and dithiocarbamate-based fungicides, which contain Cu and Zn, respectively. The apples are sprayed lightly using hand lances from hand-operated pumps. Otherwise, there are no major sources of pollution in the close vicinity of the study area.

Geological description

The study area is part of the Western Pannonian plain, situated in the north-west of Croatia and delimited by the hilly area of Vukomeričke Gorice to the southwest and the Sava River and its tributaries to the north, west and east. Its lithology is determined by the Sava River deposits, composed of the Quaternary sand and gravel complex in the form of three floodplain terraces. The last time the Sava flooded this area was in September 2010. The terrace consists of clastic sediments, varying from gravels to sands, and of sandy and silty clays. The gravel pebbles are mostly carbonate, then sandstone, chert, igneous rocks, metamorphic rocks, and quartz (20). According to Pavlović et al. (21), the Sava sediments are composed of low-lying carbonates and overlying silicates as a result of the intense erosive action of melting glaciers exerted on limestones and dolomites in the alpine region, followed by local inputs mainly of silicate composition. This heterogeneity of the parent material has resulted in a wide variety of soil types, but the prevailing soils are hydromorphic, including Molic and Calcaric Fluvisols, Eutric Cambisols, and Eutric and Calcic Greysols (2).

Sampling and analytical methods

Sixteen soil samples were collected from the apple orchard with a shovel as follows: a) nine samples from nine apple rows (one per row, across the orchard) at a distance of 10 cm to 15 cm from the apple tree trunk at a depth of 10 cm to 25 cm (after removing the layer of organic debris; this depth is common in geochemical mapping); b) seven samples were collected from between adjacent rows, also at the depth of 10 cm to 25 cm. We did that with the assumption that the apple row samples would be more affected by agrochemicals than samples from in between rows. Five control samples were taken in the same way from an adjacent meadow, some 200 m away. To our knowledge, the meadow had not received any artificial inputs of Cu, thus representing the local background metal levels.

Mineral composition was determined using a Philips PW 3040/60 X'Pert PRO powder X-ray diffractometer (PANalytical; Almelo, The Netherlands) with a CuKα line (λ=1.54055 Å) at 40 kV and 40 mA.

To identify phyllosilicates, the samples were additionally treated in ethyleneglycol vapour for 24 h and heated at 400 °C and 550 °C for 30 min.

Cation exchange capacity (CEC) was determined by mixing the samples with the 0.01 mol L^{-1} solution of copper ethylenediamine complex $[Cu(en)_2]^{2+}$ (22). The subsequent change in Cu^{2+} concentration due to sample adsorption was determined with a ultraviolet-visible spectrophotometer Hach DR/4000 U (Hach Company, Loveland, CO, USA) at 548 nm. The pH of the $[Cu(en)_2]^{2+}$ solution upon mixing with each sample was around 7. Soil pH was measured in H_2O.

Carbonate content was determined gravimetrically by weight loss after leaching the samples with 1 mol L^{-1} HCl.

Loss on ignition (LOI), as an indicator of organic matter content, was also determined by gravimetry after dry ashing at 375 °C over 24 h; determinations were performed in duplicate per sample.

Sample preparation and the methods used to determine Cu and Zn [energy dispersive X-ray fluorescence (EDXRF) and the revised BCR sequential extraction method] have been described earlier (23). The level of extractable copper in soil tells us how much of it is available to plants (4). As phytoavailability of metals depends on various abiotic and biotic processes, including adsorption onto and desorption from mineral surfaces, precipitation, dissolution of minerals, and interactions with soil, numerous sequential extraction schemes have been devised to assess trace metal content under different environmental conditions (e.g. pH, Eh), with various degrees of precision (12, 17). We opted for the revised BCR (1999) method because it has been widely applied to soil and sediment samples by a number of authors (15-19). The analytical verification of the method has been reported elsewhere (23, 24). The accuracy of the BCR method, expressed by the recovery of measured elements, ranged from 90 % to 112 %, which is considered satisfactory. Its limits of detection are also considered acceptable for environmental analyses. The overall uncertainty of the measurement was 9 % for all elements. The precision of the EDXRF method was better than 0.5 % for K, Mn, Fe, and Ti, and for other elements better than 5 %.

Statistical analysis

Data were processed with the STATISTICA software (Version 7, StatSoft. Inc.). Normality of distribution was tested with the Shapiro-Wilk test. We used Kendall's tau correlation coefficient to evaluate the relationships between variables for every group of samples (apple row, between rows, combined orchard, and control). Differences between the groups were tested with non-parametric Kruskal Wallis and Mann-Whitney U tests.

RESULTS AND DISCUSSION

Physicochemical characteristics of the soils

Selected physico-chemical characteristics of the studied soils are listed in Table 1. Generally, these carbonate-rich soils have pH values within the neutral range. The average cation exchange capacity (CEC) was 114.3 μEq g^{-1}, which corresponds to loam and silty loam soil textures. In terms of mineral composition, the soil samples consisted of quartz (semi-quantitative range: 20 % to 39 %), dolomite (3 % to 26 %), muscovite/illite (29 % to 52 %), plagioclase (7 % to 13 %), chlorite (7 % to 10 %), and kaolinite (5 % to 8 %). Minor chlorite-smectite occurrences were observed only in one orchard sample and in the control soil while kaolinite was found in the orchard samples but not in control. This mineral composition largely supports low CEC, whose range between 50 μEq g^{-1} and 250 μEq g^{-1} strongly correlates with these phyllosilicate minerals (22). Considered alone, this physico-chemical profile suggests that this soil has a low potential for phytoavailable Cu or Zn.

Total trace element levels

It has been widely recognised that elevated Cu levels in soil decrease microbial activity (6), and that Cu levels as low as 15 mg kg^{-1} can adversely affect earthworms (25). Wang et al. (7) have shown that fungicide-derived Cu in an apple orchard accounts for a large part of the total variance of soil microbial and enzyme properties and that it slows down the processes mediated by microbes. Roussos and Gasparatos (26) found lower Cu levels in the peel of conventionally grown than in organically grown apples, which was attributed to delayed application of Cu-based fungicides in the organic orchard to prevent apple scab infection at the later stages of the fruit development. These studies illustrate the beneficial and detrimental effects of Cu, but also the complexity of its behaviour following the application of pest-control chemicals.

Table 1 *Mineral and chemical properties of randomly selected control and apple orchard soil samples*

Sample	Mineral composition	Carbonate content / %	pH	LOI / %	CEC / µEq g^{-1}
Control 1	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, chlorite-smectite	22.28	6.72	7.0	165.3
Control 5	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, chlorite-smectite	20.16	6.80	9.5	161.1
Between apple rows 2	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, kaolinite, chlorite-smectite	20.47	7.38	11.7	-120.8
Between apple rows 10	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, kaolinite	22.09	7.16	6.8	-67.4
Between apple rows 15	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, kaolinite	24.02	7.23	7.3	-75.4
Apple row 3	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, kaolinite	22.97	7.60	9.3	-132.3
Apple row 7	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, kaolinite	20.78	7.45	11.6	-116.6
Apple row 13	Quartz, dolomite, plagioclase, muscovite/ illite, chlorite, kaolinite, chlorite-smectite	23.15	7.23	11.3	-75.5

LOI - Loss on ignition; CEC - Cation exchange capacity

This raises the question about finding an optimal dose in terms of environmental protection.

To understand Cu and Zn findings (presented as range, mean and SD values in Table 2) in our apple orchard we first need to discuss the characteristics of control soil. The Shapiro-Wilk test showed moderate to perfect normality for all variables in control samples (P=0.61 for Cu and P=0.55 for Zn). Normality presumes natural element composition that may serve as background data for trace elements in the study area.

In addition, we calculated Kendall's tau correlation matrix (P<0.05) for Al, Fe, Co, Ni, Rb, Sr, LOI, Cu, Zn, As, and Pb (Table 2) to get a broader view of geochemical relations. The first seven variables represent conservative components, whose values are commonly unaffected by contaminant input, whereas the last four are heavy metals whose levels reflect human activity (28). The correlations are mainly positive but not statistically significant, and could have resulted from geogenic processes in soils formed by the weathering of the local bedrock lithology. In other words, these correlations in control soil could be valid for the entire study area.

With the exception of Cu and Zn, total mean levels of all other variables in the orchard were nearly identical to control. In contrast, Cu and Zn in orchard samples (combined) were significantly higher than control (P=0.0009 and P=0.0132, respectively, Mann-Whitney U test), but did not exceed respective Croatian reference limits in agricultural soils of (60 to 90) mg kg^{-1} and (60 to 150) mg kg^{-1} (29). Furthermore, the differences between control and orchard sites using Cu and Zn values normalised for the LOI content were not statistically significant, which suggests that both metals have strong affinity for organic matter, as reported elsewhere (4).

We also found a deviation from normal distribution for Cu (P=0.10) and Zn (P=0.14), which is consistent with copper contamination of the orchard soil.

Similarly, Kendall's tau correlations between Cu/ Zn and other variables (Table 3) were positive in control samples, suggesting a common geochemical origin (30). In orchard samples, several correlations were even negative, which points to different sources and/or different chemical behaviour of these elements in soil, as suggested by Abollino et al. (31) or to common geogenic processes (30).

Copper and zinc fractionation

Table 3 shows the four extraction steps that correspond to exchangeable, reducible, oxidisable, and residual fractions of Cu and Zn. The ratio of total Cu, determined with EDXRF, to extractable Cu (sum of steps 1 to 3) (EDXRF:EF ratio), in control samples was distinctly higher than in the apple rows and between-row samples. These ratios have a roughly

Table 2 *The Kendall's tau correlation matrix calculated for the studied soil groups*

	Cu				Zn			
	C	**O**	**A**	**M**	**C**	**O**	**A**	**M**
Al	0.50	-0.03	0.00	0.21	0.12	0.11	0.30	0.10
Fe	0.22	-0.11	-0.06	0.05	0.11	0.28	0.22	0.43
Co	0.47	-0.06	0.37	-0.41	0.22	0.13	0.43	-0.10
Ni	0.32	0.00	0.33	-0.52	0.20	-0.10	0.06	-0.33
Cu	1.00	1.00	1.00	1.00	0.74	0.35	0.39	0.24
Zn	0.74	0.35	0.39	0.24	1.00	1.00	1.00	1.00
As	0.11	0.03	0.11	-0.05	0.00	0.23	0.34	0.14
Rb	0.73	0.11	0.28	0.05	0.60	**0.41**	**0.56**	0.43
Sr	0.31	0.14	0.20	-0.05	0.60	0.02	0.03	-0.24
Pb	0.53	-0.04	0.08	-0.05	**0.80**	0.18	-0.20	**0.71**
LOI	0.32	0.28	0.39	0.33	0.20	0.34	0.00	**0.71**

Bolded figures denote significant correlations at $P<0.05$*; LOI - loss on ignition; C - control samples,* n=5*; O - orchard soil samples combined (M+A),* n=16*; A - apple row samples,* n=9*; M - samples from between the apple rows,* n=7*). Al, Fe, and LOI (loss on ignition) are expressed in %, other values in mg kg*$^{-1}$.

Table 3 *Basic statistical parameters of the total (EXDRF) and the BCR-extracted Cu and Zn values in orchard and control soil*

Decriptive parameters	**Mass fraction / mg kg^{-1}**					
	Control, *n*=5		**Orchard, n=16**			
			Apple row, *n*=9		**Middle row, *n*=7**	
	Mean (SD)	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**
			Copper			
Step 1	0.00 (0.00)	0.00 to 0.00	0.00 (0.00)	0.00 to 0.00	0.00 (0.00)	0.00 to 0.00
Step 2	0.00 (0.00)	0.00 to 0.00	0.12 (0.24)	0.00 to 0.69	0.52 (1.37)	0.00 to 3.63
Step 3	0.06 (0.13)	0.00 to 0.31	3.59 (1.69)	0.00 to 5.72	4.35 (6.08)	0.00 to 17.53
Step 4	8.92 (0.69)	8.23 to 10.01	12.63 (4.99)	9.08 to 25.30	10.06 (1.60)	7.69 to 12.62
EDXRF	34.96 (1.97)	32.80 to 37.90	46.70 (4.31)	39.00 to 53.20	45.80 (1.66)	43.30 to 48.10
EDXRF:EF	582		12		9	
			Zinc			
Step 1	1.24 (0.94)	0.48 to 2.80	0.86 (0.52)	0.25 to 1.67	0.42 (0.30)	0.00 to 0.84
Step 2	10.33 (2.30)	8.23 to 14.11	10.42 (1.59)	7.65 to 13.26	12.60 (1.30)	11.03 to 14.15
Step 3	16.51 (3.54)	12.83 to 20.46	20.80 (4.07)	15.34 to 26.95	22.54 (2.78)	19.05 to 25.05
Step 4	35.67 (5.07)	31.77 to 44.51	40.09 (5.08)	34.61 to 48.94	37.91 (5.27)	33.75 to 46.48
EDXRF	133.64 (1.30)	132.30 to 135.70	139.73 (4.32)	130.40 to 146.30	139.68 (5.10)	136.40 to 143.20
EDXRF:EF	5		4		4	

Step 1: exchangeable fraction; Step 2: reducible fraction; Step 3: oxidisable fraction; Step 4: residual fraction; Steps 1+2+3: extractable fraction (EF)

similar trend as reported by Pietrzak and McPhail (10).

Figure 1 shows Cu and Zn fractionation in all soils, where the relative fractions are in the following descending order: residual > oxidisable > reducible > exchangeable. In contrast to orchard soil, control soil Cu was almost exclusively (>99 %) bound to the residual fraction (non-silicate bound metal, Step 4) and mineral lattice (labelled as EDXRF on Figure 1), and therefore not available to the plant under environmental conditions. This agrees with reports that metals of anthropogenic origin generally exhibit greater mobility in soil compared to metals of natural origin, which are strongly bound to soil components (4, 8).

The ratios of total to extractable Zn were rather comparable in all soils, indicating mainly geogenic origin (32).

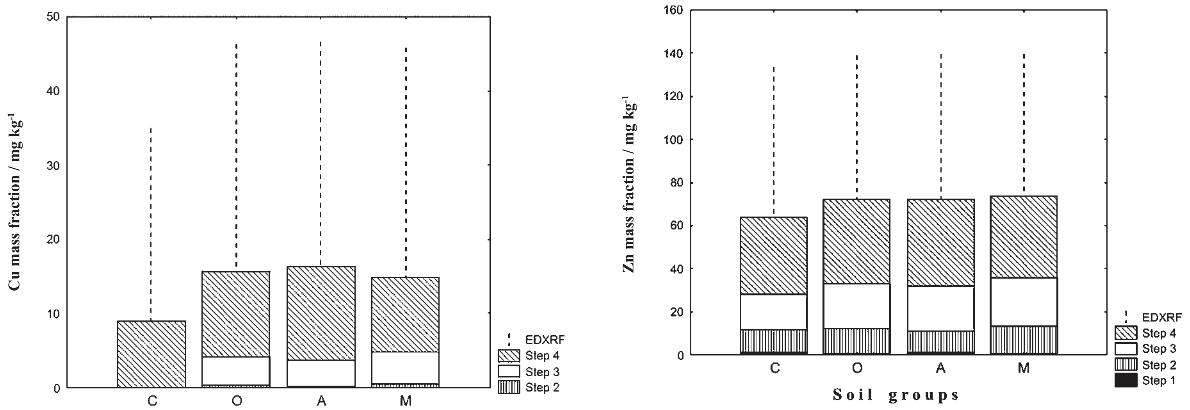

Figure 1 *Chemical fractionation of Cu and Zn in the studied soils*
Data represent mean values. Soil groups: C - control samples, n=*5; O - orchard soil samples combined (M+A),* n=*16; A - apple row samples,* n=*9; M - samples from between the apple rows,* n=*7; Step 1: exchangeable fraction; Step 2: reducible fraction; Step 3: oxidisable fraction; Step 4: residual fraction.*

Generally, our results are comparable with similar studies (11, 13, 14, 32). Quite expectedly, the extractable amounts were rather low due to low total metal content, neutral to alkaline properties of the soils (4, 17), and low CEC (Table 1), but important because they are phytoavailable.

The Mann-Whitney U and Kruskal-Wallis tests showed statistically higher Cu levels in the oxidisable fraction of the combined orchard and apple row soil compared to control (P=0.01 and P=0.02, respectively), which is consistent with other studies (11-14), confirming that Cu is one of the least mobile metals in soils due to its strong sorption on and complexation with organic matter (4). With the exception of Zn in control samples, the correlations between oxidisable fractions of both metals and the respective recoverable fractions (sums of steps 1-3) were highly significant (P=0.77 to 0.99). This points to sorption on organic matter as the most important retention mechanism for Cu in the studied apple orchard soil.

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Sažetak

KEMIJSKI OBLICI BAKRA I CINKA U TLU NASADA JABUKA U SELU BUKEVJE (HRVATSKA) ISPITIVANI REVIDIRANOM BCR EKSTRAKCIJSKOM SHEMOM U ČETIRI KORAKA

Analizirani su uzorci tla iz obiteljskog voćnjaka u selu Bukevju kako bi se utvrdilo ponašanje metala u tragovima u okolišu u kontekstu poljoprivrednih postupaka proteklih nekoliko desetljeća. Unos kemijskih sredstava za zaštitu bilja navodno je nizak. Nakon provedene tzv. BCR sekvencijske ekstrakcijske analize, kemijski oblici bakra i cinka razmotreni su iz geokemijskoga/geološkoga gledišta. Uzeto je šesnaest uzoraka tla iz voćnjaka te pet uzoraka kontrolnog tla s obližnje livade. Analize su obuhvaćale određivanje mineralnog sastava tla, kationsko izmjenjivačkog kompleksa (KIK), pH tla i gubitka žarenjem. Koncentracije mjerenih varijabli u kontrolnim uzorcima, određene XRF metodom, upućuju na njihove prirodne razine na temelju normalnosti raspodjela. Tlo iz voćnjaka neznatno je onečišćeno bakrom, moguće od primjene fungicida na bazi bakra, što je utvrđeno Mann-Whitneyevim U testom, koji je pokazao statistički značajno više koncentracije ukupnog Cu ($P = 0.0009$) u tlu voćnjaka u usporedbi s uzorcima kontrolnog tla. Učinkovitost BCR ekstrakcije bakra i cinka izračunata za kontrolno tlo (25 % odnosno 47 %) bila je niža nego ona izračunata za tlo iz voćnjaka (34 % odnosno 52 %). Bakar, potječući djelomice od fungicida, pokazao je veću specijacijsku varijabilnost u istraživanim tlima u usporedbi s cinkom, za koji se pretpostavlja da potječe uglavnom od trošenja stijenske podloge. Mann-Whitneyev U test pokazao je statistički značajno ($P = 0,01$) više vrijednosti Cu povezane s frakcijom tla iz voćnjaka podložnoj oksidaciji u odnosu na istu frakciju kontrolnih uzoraka. Bakar i cink u istraživanim tlima čvrsto su vezani za rezidualnu i organsku frakciju, a ukupne im vrijednosti ne premašuju maksimalno dopuštenu količinu onečišćujućih tvari u poljoprivrednom zemljištu, pa ne bi trebali predstavljati toksikološku opasnost za ljudsko zdravlje.

KLJUČNE RIJEČI: *BCR ekstrakcijska analiza, fungicidi, litologija podine, poplavna ravnica, rijeka Sava*

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