Heavy Metals Accumulation in Topsoils from the Wine-growing Regions

Part 2. Relationships between soil properties and extractable copper contents

Marija ROMIĆ*
Davor ROMIĆ
Gabrijel ONDRAŠEK

SUMMARY

The present study analyses the effects of high copper concentrations on its mobility in the soils from the wine-growing regions of the Northwestern Croatia. The aims of this study were to determine the copper distribution in vineyard soils by single extraction procedures, and to relate the total, mobile and mobilisable copper content to several chemical soil characteristics using multiple regression analysis. The total Cu concentrations were determined by an aqua regia procedure. Two other methods were used for evaluating soil available copper: DTPA extraction and calcium chloride extraction. The physical and chemical characteristics (texture, organic C, pH, $CaCO_3$, total P, CEC) were determined as well. Both DTPA- and $CaCl_2$-extractable copper were largely explained by the total copper contents, and they were also highly correlated to each other. It was found that the DTPA-extractable copper decreased with increasing cation exchange capacity, but concentrations of $CaCl_2$-extractable copper mainly depend on pH.

KEY WORDS

extractable copper, multiple regression analysis, plant availability, single extraction, soil characteristics

University of Zagreb, Faculty of Agriculture, Department of Amelioration
Svetošimunska 25, HR-10000 Zagreb, Croatia
*Corresponding author: M. Romic
E-mail: mromic@agr.hr

INTRODUCTION

Numerous investigations have proven that the century-old practice of using Cu-sulphates and other copper containing fungicides to protect grapevine, but also other agricultural crops, in temperate and tropic climatic regions, resulted in significant Cu accumulation in soils. Most of the copper accumulated in leaves and soil by spraying will be retained in topsoil through the biological cycle and tillage (WALSH ET AL., 1972; FLORES-VELEZ ET AL., 1996; DELUISA ET AL., 1996). Comparison of copper contents of 110 mg/kg to 1500 mg kg^{-1} with its usual content in agricultural soils (20 – 30 mg kg^{-1}) points to their connection with such practice (BAKER, 1990). Copper can be either a micro nutrient or a toxic element which depends on the copper concentration.

Determination of the total content of metals in soils is an important step in estimating the hazards to the vital roles of soil in the ecosystem, and also in comparison with the quality standards in terms of the effects of pollution and sustainability of the system. From the ecotoxicological aspect, however, it is equally important to determine the bioavailability of Cu accumulated in vineyards, i.e., the fraction of the total metal content in soil that can be utilized by biota (LEPP, 2003).

It depends on the soils properties, temperature, water content and aeration, and also on plant species. The toxicity of copper is essentially observed in acid soils, but not in calcareous soils and for copper contents as high as those reported in vineyard soils. A number of authors have found positive correlation between copper retention and pH (TYLER AND MCBRIDE, 1982; GUPTA AND ATEN, 1993) and sum of bases or exchangeable calcium (HARTER, 1979). The bioavailability of copper has also been reported to decrease when the cation exchange capacity or the level of organic matter increases (MCBRIDE ET AL., 1981; GUPTA AND ATEN, 1993). Various electrolytes such as water, buffered or unbuffered salt solutions, chelating agents, diluted acids or a mix of these reagents can be used to estimate the biavailability of copper in soils (reviews given by LAKE ET AL., 1984; ROSS, 1994).

Hence, the objective of the investigations was to establish the actual risk of high concentrations of copper and its mobility in vineyard soils of northwestern Croatia. The aims of this study were: (1) to determine the copper distribution in soils of vineyards by single extraction procedures, (2) to relate the total, mobile and mobilisable copper content to several chemical soil characteristics using multiple regression analysis.

MATERIAL AND METHODS

Study area

Research was carried out on a total area of 242 km[2] in two wine-producing subregions in northwestern Croatia, in the wider Zagreb region: the vine-growing subregion of Zelina (approximate coordinates: latitude, 45º 55' and longitude, 16º 10') and the wine-growing subregion of Plesivica (approximate coordinates: latitude, 45º 42' and longitude, 15º 37'). Anthropogenic vineyard soils were classified as Aric Anthrosols (FAO, 1990). More details on sampling and the geological and pedological characteristics of the region are provided by Romic et al. (submitted).

Chemical analysis

Total soil copper was evaluated after digestion with aqua regia in accordance with the ISO 11466 procedure (ISO, 1995) as well as available copper. Two other methods were used for evaluating soil available copper: DTPA extraction (ISO/DIS 14870, 1997) and calcium chloride extraction (HOUBA ET AL., 1999).

Extraction by means of the aqua regia microwave technique: 0.5 g soil (weighed on an analytical scale Mettler Toledo PM 480 Delta range, precision 0.001 g) with addition of 6 ml concentrated HCl + 2 ml concentrated HNO_3 was subjected to microwave digestion. The obtained soil extract was filtered through slow-filtering paper S&S 583 and made up with 1% v/v HNO_3 to a 50 ml volume. The extraction process was done by the microwave technique on a Perkin Elmer Multiwave 6MF 100 (1000 W) apparatus in closed TFM vessels and with automatic pressure and temperature regulation.

DTPA extraction: 10 g of soil were extracted with 20 ml DTPA 0.005 M + TEA 0.1 M + $CaCl_2$ 0.01 M for 2 h at 20ºC under stirring (Heidolph PROMAX 2200 used), prior to being filtered.

Calcium chloride extraction: 0.5 g of soil were extracted with 50 ml of $CaCl_2$ 0.01 M for 2 h at 20ºC under stirring, prior to being filtered.

Copper concentrations in soil extracts were determined by flame atomic absorption spectrometry (AAS Perkin Elmer 3110).

All chemicals were declared pro analysi, and all solutions were prepared with double-distilled water. Standard working solutions were prepared from original certified stock solutions (MERCK), concentration 1000 mg l^{-1} in 1% (v/v) HNO_3. All samples were analyzed at the Laboratory of Soil and Water Analysis of the Faculty of Agriculture, University of Zagreb. The quality control procedure consisted of reagent blanks, duplicate samples and several referenced soil and sediment samples of a similar matrix from the interlaboratory calibration

programme in which the laboratory participates (HOUBA ET AL., 1996).

Statistical analysis and data management

Linear regression: Relations between extractible copper in soils and soil properties were analyzed by simple and multiple linear regression (SAS INSTITUTE, 1999).

General conditions for model application were verified after parameter determination. Thus, residuals should follow normal distribution, the assumption of homoscedasticity of variable variances should be proven, and they should be independent of one another. Distribution normality was tested using the Shapiro-Wilk test, while the other hypotheses were checked visually after the graphs were drawn. Details given by Romić (2002).

All soil data were incorporated into the GIS database.

RESULTS

Total copper contents

To compare copper concentrations in adjacent soils that had never been treated with copper fungicides and those in vineyard soils, eight samples of forest and field soils were taken. The determined copper contents ranged from 14 to 71 mg kg^{-1}. This number of samples does not allow estimation of the so-called geochemical or natural concentration, nor characterization of original geogenic concentrations, nor determination of anthropogenic contamination; however, these plots can be treated as check in the given agroecological environment. Total copper concentrations in anthropogenic vineyard soils ranged from 30 to 700 mg kg^{-1}, while total copper in 88% samples exceeded the maximum tolerant concentration under the Croatian regulation (100 mg kg^{-1}).

The amount of copper contained in the topsoil of vineyard soil was estimated on a per ha basis taking into account the total copper concentration (mg kg^{-1}) and the weight of 10 cm thick topsoil, assuming a bulk density of 1.5. Approximate plantation age (with within-decade precision) was estimated for most plots, and a detailed history for some plots: time of the first and possibly second deep ploughing, time of vineyard restoration or supplementary planting, common growing practices in the past, and thereby also approximate frequency of copper fungicide applications. More than 64% vineyards are more than 40 years old and it is assumed that the same percent of all plots received similar annual amounts of copper, ranging from 2 to 5 kg ha^{-1}. This, naturally, does not allow an exact estimate of the overall copper

input into soil throughout the vineyard history. The upper layer of plots planted with about a century old vines exibited high copper contents.

Correlation between total, extractable Cu and soil properties

A detailed description of the soil properties as well as the statistical summary was presented by ROMIC ET AL. (submitted). Table 1 provides the data on copper extracted in different ways per locations. In the total copper content extracted with aqua regia, DTPA-extractable copper amounted to 12-81% and CaCl$_2$-extractable to 0.2-3.1%.

Both DTPA- and CaCl$_2$-extractable copper were largely explained by the total copper contents, as attested by their high correlation coefficient (R = 0.899 and R = 0.896). They were also highly correlated to each other (R = 0.763) (Table 2).

Table 1. Total Cu, extractable Cu and total amount of Cu per ha in the upper layer (10 cm) of vineyard soils (n=67)

Plot[1]	Cu$_{TOT}$ (mg kg^{-1})	Cu$_{DTPA}$ (mg kg^{-1})	Cu$_{CaCl2}$ (mg kg^{-1})	Cu$_{TOT}$/ha (kg ha^{-1})[2]
351	363	136	1.15	491
352	154	48	0.63	208
353	380	142	1.17	514
354	389	121	0.74	525
355	181	56	0.73	244
356	166	54	0.55	224
357	655	368	1.45	884
358	586	296	1.96	790
359	369	135	0.94	498
360	565	296	1.35	763
361	633	340	2.08	855
362	641	228	1.68	866
363	336	196	1.24	454
364	385	152	1.15	519
365	263	101	0.82	355
366	667	316	1.36	901
367	615	332	1.56	830
368	419	143	1.19	565
369	494	166	1.47	667
370	244	95	0.93	330
371	519	282	1.15	701
372	134	47	0.42	181
373	199	71	0.52	268
374	623	348	1.84	841
375	35	4.32	0.10	47
376	389	140	1.26	525
377	442	138	0.96	597
379	252	104	0.92	340
380	30	808	0.93	40
381	531	198	1.17	717
382	305	117	1.04	412
383	453	199	1.35	612
384	360	131	1.2	486
385	79	21.2	0.21	107
386	398	146	1.36	537
387	324	120	0.94	437

continue (Table 1)

390	51	8.28	0.21	69
391	74	11.8	0.11	100
392	228	89	0.83	307
395	533	170	1.46	720
396	627	254	1.66	847
397	32	12.4	0.10	43
398	43	10.6	0.21	58
399	610	180	1.98	823
401	691	278	1.93	933
402	427	154	1.35	576
404	539	191.	1.58	728
405	393	135	1.66	530
406	590	298	1.64	797
407	345	118	1.03	465
408	597	274	1.57	806
410	295	100	1.24	398
411	157	46.6	0.73	212
412	159	44	0.32	215
413	332	108	0.96	448
414	700	336	2.07	945
415	559	286	1.56	755
416	614	498	1.16	829
417	493	376	1.03	666
418	524	159	1.06	707
419	251	65.8	0.74	338
420	461	138	1.06	623
421	304	74.6	0.88	411
422	115	35.8	0.31	156
423	303	95.2	0.73	409
425	381	116	1.50	515
426	83	20	0.32	112

[1] Plot: All the data available from the GIS database
[2] Cu/ha (kg ha-1) Calculated from the data on total Cu (mg kg-1) in the top 10 cm assuming a bulk density of 1.5

Highly significant positive correlation was also determined between total copper and organic matter in soil, and a weaker correlation, but still significant, between total copper and cation exchange capacity and carbonate content (Table 2). In the case of extractable copper, a significant correlation was determined between Cu_{DTPA} and organic carbon content and pH, but not between cation exchange capacity and carbonate content. Significant correlation was recorded between all the analyzed soil properties and Cu_{CaCl2}.

Linear regression of total and extractable copper and selected soil properties

To establish the relation between copper fractions after particular extractions (aqua regia, DTPA and $CaCl_2$) and soil properties that may affect their behavior in soil and availability to plants, the multiple linear regression analysis was done. The model included those variables for which correlation probability $p<0.05$ was determined (SAS INSTITUTE, 1999). For the regression model of aqua regia extracted copper, this condition was met by the following properties: Cu_{DTPA}, Cu_{CaCl2} and cation exchange capacity (CEC). For total copper, the regression model explains 92% of total variance (Table 3). The largest contribution to the variance in regression was that of Cu_{DTPA}. Concentration of total copper in vineyard topsoil went up with an increase in the cation exchange capacity.

Square root transformation (SQRT) was applied to the regression model of DTPA-extractible copper, whereby the model conditions were satisfied, and the transformed variables $SQRT(Cu_{DTPA})$ were predominantly dependent on Cu_{UK}. As this relation was already determined, it was omitted from the model. Two other properties met the condition of correlation probability $p<0.05$: organic matter content (Org-C) and $CaCl_2$-extractible copper (Cu_{CaCl2}), and they were included into the model. For Cu_{DTPA}, the regression model explains 85% of total variance (Table 4).

Two parameters were included into the regression model of $CaCl_2$-extractible copper: organic matter content (Org-C) and pH, and the model explains 62% of total variance (Table 5).

Concentrations of CaCl2- extractable copper mainly depend on pH, which relation was also confirmed by this investigation. However, since these are predominantly alkaline soils, this relation is not as strong as in the case of soils with a more varying pH (Brun et al., 1998).

DISCUSSION

Vineyard age parameter was not included in the multiple linear regression model because the data were

Table 2. Correlation matrix, upper triangle

	Cu_{total}	Cu_{DTPA}	Cu_{CaCl2}	pH_{H2O}	Org. C	CEC	$CaCO_3$
Cu_{total}	1	0.899^{***}	0.896^{***}	0.388^{**}	0.601^{***}	0.277^{*}	0.314^{**}
Cu_{DTPA}		1	0.763^{***}	0.321^{**}	0.395^{***}	0.084^{ns}	0.236^{ns}
Cu_{CaCl2}			1	0.392^{***}	0.734^{***}	0.257^{*}	0.341^{**}
pH_{H2O}				1	0.121^{ns}	0.315^{**}	0.587^{***}
Org. C					1	0.339^{**}	0.173^{ns}
CEC						1	0.130^{ns}
$CaCO_3$							1

ns - not significant; * Correlation significant at $p < 0.05$; ** Correlation significant at $p < 0.01$: *** Correlation significant at $p < 0.001$

Table 3. Linear regression of total copper (Cu_{TOT}) as a function of DTPA-extractable (Cu_{DTPA}), $CaCl_2$-extractable (Cu_{CaCl2}) and cation exchange capacity (CEC)

Source of variation	Degree of freedom	Sum of squares	F	Pr > F
Cu_{DTPA}	1	301395	101.8	0.0000
Cu_{CaCl2}	1	192403	65.0	0.0000
CEC	1	30384	10.3	0.0021
Total	63	2319596		

Variable explained: Cu_{TOT}
Regression equation:
Cu_{TOT} = -63.42 + 0.956* Cu_{DTPA} + 170.9*Cu_{CaCl2} + 3.761*CEC
R^2 = 0.92

Table 4. Linear regression of DTPA-extractable (Cu_{DTPA}), as a function of $CaCl_2$-extractable (Cu_{CaCl2}) and soil organic matter (Org. C)

Source of variation	Degree of freedom	Sum of squares	F	Pr > F
Cu_{CaCl2}	1	685.7	240.0	0.0000
Org-C	1	44.54	15.59	0.0002
Total	63	1224		

Variable explained: SQRT (Cu_{DTPA})
Regression equation:
SQRT(Cu_{DTPA}) = 5.534 + 9.400*Cu_{CaCl2} − 1.326* Org-C
R^2 = 0.853

Table 5. Linear regression of $CaCl_2$-extractable copper as a function of soil organic matter (Org. C) and pH

Source of variation	Degree of freedom	Sum of squares	F	Pr > F
Org-C	1	8.30	83.37	0.0000
pH	1	1.62	16.23	0.0002
Total	64	17.34		

Variable explained: Cu_{CaCl2}
Regression equation:
Cu_{CaCl2} = -2.673 + 0.379* Org-C + 0.337* pH
R^2 = 0.621

Figure 1. Vine age as a function of total Cu

y = 16.101 + 0.069*TOT Cu
R^2 = 0.485

wine boom period at the end of the 19th century. Recognizing the benefits, wine-growers often did not observe the recommended concentrations and application times, and a large number of treatments, as many as 8 to 14, were often applied at positions exposed to disease attacks (MIROŠEVIĆ, 2001, written personal communication; ROMIĆ ET AL., 2000b). However, numerous other factors, such as scattering during applications, washing off the leaves by rain, input of treated plant residues into soil, tillage and erosion, make it difficult to establish the relation between vineyard age and accumulated copper.

Diethylenetriaminepentaacetic acid (DTPA) is a potent synthetic chelating agent, and the method of extraction with DTPA was developed for the purpose of determining zinc, iron, manganese or copper deficiency in neutral and carbonate soils (LINDSAY AND NORVELL, 1978). HAQ AND MILLER (1972) reported negative results of the DTPA test, which they explained by their failure to determine sufficiently significant relations between concentrations of metals (copper and manganese) extracted from soil and those found in the tested plants. Mention should be also made of the research done by O'CONNOR (1988), who gave a number of comments on the DTPA test, based also on non-significant correlation between DTPA-extractable metals in soil and their concentrations in plants. Regardless of the above considerations, DTPA is the most widely used agent for extraction of "available" cadmium, copper, nickel and zinc, and thereby also the most standardized one (SINGH AND MYHR, 1998; ISO/DIS 14870, 1997; AMACHER, 1996). Starting from the fact that the data on total copper content reveals very little about its bio-availability, such strong correlation between copper extracted with aqua regia and DTPA actually indicates that neither the latter extraction method is suitable for assessing copper availability to plants. MERRY ET AL. (1983) recorded in vineyard soils 25-35 times higher contents of copper, lead and arsenic, originating from plant protection agents, than their

not obtained by measurement but were estimated. There is, however, strong statistical evidence that an increase in vineyard age is related to the increase of expected total copper content (Fig. 1).

It was estimated that more than 64% vineyards were over 40 years old and it is assumed that the same percent of parcels received a similar annual amount of copper, ranging from 2 to 5 kg ha^{-1}. This, naturally, does not allow an exact estimate of the overall copper input into soil throughout the vineyard history. According to historical documents, the vine downy mildew infection started spreading in the vineyards of northwestern Croatian in 1882, and the Bordeaux mixture application became indispensable during the
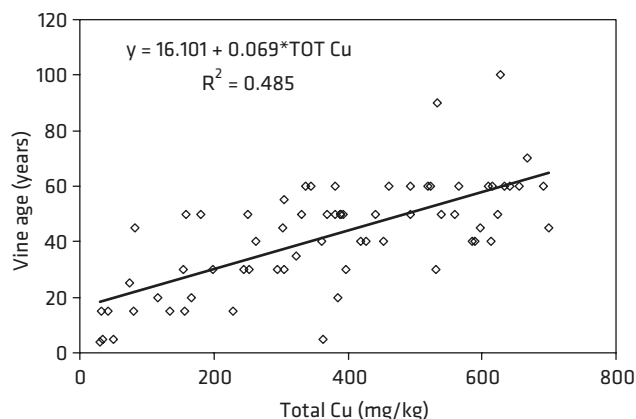
*CS

Agric. conspec. sci. Vol. 69 (2004) No. 2–3

common values in uncontaminated soils; they also determined a strong correlation between total and DTPA-extractable copper ($0.93 < r < 0.96$). Also BRUN ET AL. (1998) found that the regression model Cu$_{DTPA}$ explained 90% of total variance in vineyard soils, its largest part referring to total copper content. When cation exchange capacity was included into the model, it was found that the DTPA-extractable copper decreased with increasing cation exchange capacity.

Soil extraction 0.01 M CaCl$_2$ is the method that was increasingly used in the last decade for soil testing to determine soil fertility and the behavior of nutrients and contaminants in the soil. The capabilities of instrumental chemical analysis have improved to such an extent, even in the last few years, that it become possible to determine very low concentrations of nutrients and pollutants in soil extracts (HOUBA et al., 1999). The advantage of this method for determining metal concentrations in soil is that the concentration of electrolytes stays practically constant and metal concentrations reflect the difference in binding strength or solubility between soils. The extractant is an unbuffered solution and therefore the measured metals reflect their availability at the pH of the soil. The best criterion of the efficiency of the method for determining the soil bioavailable fraction is the high correlation between the Cu content observed in plants grown in situ, at least for neutral to acid soils (BRUN ET AL., 1998). It is expected that the continuation of investigations, which will also include copper uptake by plants, will enable evaluation of the application of unbuffered salt solutions for estimating the transfer of soil copper into plants.

REFERENCES

Amacher, M.C. (1996): Nickel, Cadmuim and Lead. U Methods of Soil Analysis. Part 3-Chemical Methods. SSSA-ASA Publ., Madison, USA. Str. 739-768.

Baker, D.E. (1990): Copper. U Heavy Metals in Soils. Ed. B.J. Alloway, Blackie and Son Ltd. Glasgow. Str.151-175.

Brun, L.A., Maillet, J., Richarte, J., Herrmann, P., Remy, J.C., 1998. Relationships between extractable copper, soil properties and copper uptake by wild plants in vineyard soils. Environmental Pollution, 102 (2-3): 151-161.

Deluisa, A., Giandon, P., Aichner, M., Bortolami, P., Bruna, L., Lupetti, A., Nardelli, F., Stringari, G., 1996. Copper pollution in Italian vineyard soils. Communications in Soil Science and Plant Analysis, 27 (5-8): 1537-1548.

FAO (1990): Soil Map of the World. Revised Legend. World Resources Reports 60, FAO/UNESCO/ISRIC. Rome. Italy.

Flores-Velez, L.M., Ducaroir, J., Jaunet, A.M., Robert, M. (1996): Study of the distribution of copper in an acid sandy vineyard soil by three different methods. European Journal of Soil Science, 47 (4): 523-532.

Golden Software, 1999. Surfer for Windows. Version 7. Grid-based graphics program. Colorado, USA.

Gupta, S.K., Aten, C. (1993): Comparison and evaluation of extraction media and their suitability in a simple-model to predict the biological relevance of heavy-metal concentrations in contaminated soils. International Journal Of Environmental Analytical Chemistry, 51 (1-4): 25-46.

Haq, A.U., Miller, M.H. 1972. Prediction of available soil Zn, Cu, and Mn using chemical extractans. Agronomy Journal, 64:779-782.

Harter, R.D. (1979): Adsorption of copper and lead by Ap and B2 horizons of several northeastern United-States Soils. Soil Science Society of America Journal, 43 (4): 679-683.

Houba, V.J.G., Uittenbogaard. J., Pellen, P., 1996. Wageningen evaluating programmes for analytical laboratories (WEPAL), organization and purpose. Communications in Soil Science and Plant Analysis 27 (3-4): 421-431.

Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., van Vark, W. (1999): Soil analysis procedures extraction with 0.01 M CaCl$_2$. Department of Environmental Science, Sub-department of Soil Science and Plant Nutrition. Wageningen Agricultural University. Nizozemska.

ISO/DIS 14870 (1997): Soil quality: Extraction of trace elements by buffered DTPA solution. International Organisation for Standardisation.

Lake, D. L., Kirk, P. W. W., Lester, J. N. 1984. Fractionation, Characterization, and Speciation of Heavy Metals in Sewage Sludge and Sludge-Amended Soils: A Review. Journal of Environmental Quality, 13:175-183.

Lepp, N. 2003. Bioavailability – A concept driven by science or legisation? Conference proceedings of the 7th International Conference on the Biogeochemistry of Trace Elements. Uppsala, Sweden, June 15-19, 2003, pp. 12-12.

Lindsay, W.L., Norvell, W.A. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal, 42: 421-428.

Merry, R.H., Tiller, K.G., Alston, A.M. (1983): Accumulation of Copper, Lead and Arsenic in some Australian Orchard Soils. Australian Journal of Soil Research, 21 (4): 549-561.

McBride, M.B., 1981. Forms and distribution of copper in solid and solution phases in soil. U Copper in soils and plants. Ed. J.F. Lonergan, A.D. Robson, R.D. Graham. Academic Press Australia. Str. 25-45.

Mirošević, N. (2001): Kroz povijest vinogradarstva Plešivice i Prigorja i Kratki osvrt na povijest peronospore. Pisana osobna komunikacija. Rujan, 2002.

O'Connor, G.A. (1988): Use and misuse of the DTPA soil test. Journal of Environmental Quality, 17: 715-718.

SAS, 1999. SAS Procedures Guide for Personal Computers, Version 8th Edition. SAS Institute Inc., Cary, NC.

Singh, B.R., Myhr, K. (1998): Cadmium uptake by barley as affected by Cd sources and pH levels. Geoderma, 84 (1-3): 185-194.

Romić, M., 2002. Heavy metal contents, forms and redistribution in agricultural soils of the Zagreb region. Unpublished PhD Thesis. University of Zagreb, Faculty of Agriculture.

Romić, M., Romić, D., Dolanjski, D. Heavy metals accumulation in topsoils from the wine-growing regions: Part 1. Factors which control retention. (submitted)

Ross, S. M. (1994): Toxic Metals in Soil-Plant System. Ed. S.M. Ross. John Wiley & Sons Ltd.

Tyler, L.D., McBride, M.B. 1982. Mobility and extractability of cadmium, copper, nickel, and zinc in organic and mineral soil columns. Soil Science, 134, 198-205.

Walsh, L.M., Erhardt, W.H., Seibel, H.D. (1972): Copper toxicity in snap beans (Phaseolus vulgaris L.). Journal of Environmental Quality, 1: 197-200.

acs69_06