

Elevated Soil Copper Content in a Bohemian Vineyard as a Result of Fungicide Application

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Abstract: A set of fifty samples were taken from soil surface layers of an anonymous vineyard in the Bohemia region of the Czech Republic. Samples were analysed for basic soil parameters including pH and humus content and quality and for potentially toxic elements Cu, Pb, Zn, Cd and Mn. When compared to soil guideline values, mean Cu exceeded the limit by 280%. Although other elements did not exceed the guideline value, Zn and Cd surpassed background concentrations by 108 and 187%, respectively. Mn did not occur in concentrations that are considered excessive with respect to common natural soil levels. A statistically significant correlation was observed for Cu and C_{ox} , but no significant correlation could be made between Cu and humus quality, suggesting that in this case, Cu retention in surface soil is more dependent on quantity of humic substances rather than humus quality. The unnaturally high accumulation of Cu in the surface horizon is certainly of anthropogenic origin; most likely due to long term application of copper-based fungicides. High observed values of Cd are probably a result of the soils parent material and application of fertilizers which may have contained cadmium compounds. Distribution maps illustrated a common trend of potentially toxic element accumulation in the western section of the study area plot. However, many variables which were unmeasured in this study could account for the distribution and therefore more detailed study is required before conclusions can be made regarding this occurrence. An analysis of wine produced at the vineyard showed that although the end product beverage contained insignificant amounts of Cu in both red and white wines, the white wine contained 0.026 mg/l of Cd; a value which could contribute considerably to exceeding the dietary allowable intake of cadmium. The investigation should be extended to analyse a wider range of wines produced from the vineyard with a focus on soil-plant-fruit-wine transfer of cadmium and/or production processes.

Keywords: Bordeaux mixture; copper toxicity; fungicide; potentially toxic elements; vineyard soil

The application of liquid copper based treatments is a well established practice for combating a range of fungal plant diseases, and their use in vineyards are extensive. The Bordeaux mixture ($CuSO_4$ and $Ca(OH)_2$) is a common and long-used solution for such treatments worldwide. A well known property of Cu is its potential to accumulate in soil surface horizons, and due to the fact that many European vineyards are often centuries old, repeated use of copper based fungicides has resulted in the

intensification of soil Cu concentrations beyond regulation toxicity limits. KOMÁREK *et al.* (2008) proved that this is evident in various vineyards of the Czech Republic whereby in most of their studied vineyard soils, soil Cu levels did not meet the regulatory limits for agricultural soils set by the Czech Ministry of the Environment, and total Cd exceeded the limits in all cases. In the same study, from 5 vineyards of differing regions, the average Cu concentration in the upper 10 cm was

shown to be 1.8 times greater than the underlying 10 cm layer, highlighting the persistence of copper complexes which form with soil organic matter (SOM) at the surface. In addition to its affinity for organic matter, Cu forms and mobility are strongly controlled by pH, with most bioavailable Cu being bound to Fe and Mn hydroxides or converted to residual form at pH greater than ~ 6 (KOMÁREK *et al.* 2010; FAN *et al.* 2011). However, mobility can increase at pH values above ~ 7.5 due to the solubilization of SOM and formation of Cu-SOM complexes (KOMÁREK *et al.* 2010). Ion activity measurements on soil samples by KARLSSON *et al.* (2006) showed that less than 0.2% of total Cu was in the free form Cu^{2+} at pH 4.8–6.3.

When present in toxic concentrations, Cu is known to inhibit plant growth and to interfere with important cellular processes such as photosynthesis and respiration, therefore plants grown in the presence of excessive levels of Cu normally show reduced biomass and symptoms of chlorosis (YRUELA 2005). Besides the direct toxicity toward plants from potentially toxic elements (PTE), fungicides are thought to reduce the biochemical functions in soil making them more susceptible to contamination. WANG *et al.* (2009) made a comparison of soil microbial properties between apple orchards of differing ages and a reference soil to determine the relationship between Cu accumulation and microbial activity. A significant correlation was observed between CaCl_2 -extractable Cu and soil microbial biomass carbon, suggesting that elevated soil Cu concentrations were responsible for significant reductions in soil microbial health and activity. Enzyme activity assays also showed a similar trend, and declined with the increasing levels of Cu in orchard soils. Conversely, in a study by NORGROVE (2007), little to no negative consequences were observed within 3 years of high application rates of copper fungicide with respect to earthworm activity and there was no evidence of any negative effect on litter decomposition or soil bulk density.

MATERIAL AND METHODS

This study focuses on an anonymous small wine producer of the Bohemia region in the Czech Republic. The studied vineyard is situated between 220–265 m a.s.l. and covers an area of 3.5 ha. The area is part of the Czech Cretaceous Basin, consist-

ing largely of Proterozoic rocks (shales, greywackes, lydites, spilite) covered with Quaternary sediments of variable thickness, made up of mostly sandy gravels and sands, occasioning the presence of eolian sediments – loess or calcareous drift sands. Samples were taken using an irregular sampling network from fifty points within the vineyard (Figure. 1). Surface layer of soil was collected to a depth of 20 cm. Samples were air dried, sieved to < 2 mm and the following basic soil characteristics were assessed: C_{ox} , quality of humic substances, $\text{pH}_{\text{H}_2\text{O}}$ and pH_{KCl} . 2M HNO_3 extractable concentrations of selected PTE (Cu, Pb, Zn, Cd) were identified and mapped using ArcGIS software. PTE levels were compared to Czech soil guideline values for agricultural soils (SGV) and background concentrations in order to establish the extent of the contamination.

C_{ox} was measured by a modified Tyurin's oxidimetric titration; potassium dichromate in sulphuric acid titrated with Mohr's salt (POSPÍŠIL 1964). Quality of humic substances was calculated from spectrophotometric analysis, taken as the ratio of a pyrophosphate soil extract absorbance at wavelengths 400 and 600 nm ($A_{400}/600$) according to PODLEŠÁKOVÁ *et al.* (1992). For PTE analysis, 5 g of soil sample (< 2 mm) was shaken with 50 ml of 2M HNO_3 for 6 h. The sample was then centrifuged and the supernatant analysed for risk elements by FAAS spectrometry; Varian 280 FS with SIPS 20 using Analytika standards (Analytika Ltd., Prague, Czech Republic). $\text{pH}_{\text{H}_2\text{O}}$ and pH_{KCl} were measured with a WTW inolab pH level 1 pH probe. Wine which had been produced from grapes grown at the study area was purchased; one white (Riesling Kabinett 2011) and one red (Pinot Noir 2010), and analysed for PTEs. The method was reproduced from ŠPERKOVÁ and SUCHÁNEK (2005). 50 ml of

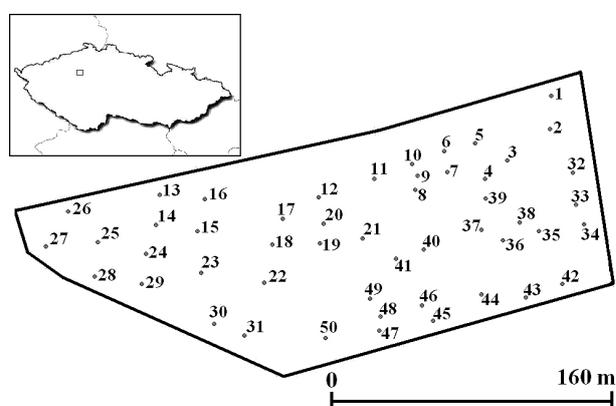


Figure 1. Vineyard outline map and sampling points

Table 1. Mean and standard deviation of potentially toxic elements (PTE) from fifty samples (mg/kg)

	Cu	Pb	Zn	Cd	Mn
Mean	139.564	40.81	65.298	0.75	657.128
SD	66.84	22.37	60.32	82.07	246.29

SD – standard deviation

wine was heated at 190°C for approximately 4 h to volatilize the ethanol and the residual wine dissolved in 25 ml of 0.1M HNO₃. Samples were prepared in triplicate and the solution was analysed for elements by FAAS. Detection limits for elements were as follows: Cu 0.035, Mn 0.020, Cd 0.018, Pb 0.09, Zn 0.013 mg/l.

Correlation statistics were prepared in MS Excel and Chi-square analysis calculated according to a method written by TAYLOR (2007). Distribution maps were created using ESRI ArcGIS 10.0 (Esri, Redlands, CA, USA).

RESULTS AND DISCUSSION

Potentially toxic elements. To assess the degree of enrichment of PTE in the upper 20 cm of vineyard soil, concentrations were related to SGV and background concentrations for the Czech Republic according to BENEŠ (1993). Average soil PTE contents are expressed as a percentage of the reference levels (Table 2.).

Copper was the only element to occur higher on average than the Czech SGV (2.8 times higher), although in some soil samples Zn and Cd also exceed the SGV. Reasons for an excess of soil Cd may be attributable to the nature of the parent material of the vineyard, which was derived primarily from sandstones, known for its high natural content of Cd (ADRIANO 2001), and is also likely to have arisen as a direct result of traditional fungicide applications which may have contained Cd compounds or from phosphorus-fertilizers, manures

Table 2. Mean soil potentially toxic elements (PTE) values as a percentage of soil guideline values (SGV) and background concentration

	Cu	Pb	Zn	Cd
SGV (%)	280	57	65	75
Background (%)	400	100	108	187

or to a lesser extent liming (FMAF 2000). Cu is by far the most significant pollutant as almost every sample is contaminated as indicated by the standard deviation in Figure 2. In this study the mean soil Cu concentration is one of the highest seen in a Czech vineyard. However, in a study by PIETRZAK and MCPHAIL (2004) at a vineyard in Victoria, Australia, total mean Cu reached as high as 228.9 mg/kg.

Cu, Zn and Cd all exceed the Czech background concentration on average, whereas Pb is on the upper limit (Table 2). Pb appears to be the least problematic PTE at the study area as even the upper error bar does not surpass the SGV (Figure 2). These findings are consistent with the data obtained by KOMÁREK *et al.* (2008) for the same study area whereby total contents of soil Cu, Pb and Cd as a percentage of SGV were 228, 77 and 76%, respectively, closely matching the results of our study. Other vineyards across the Czech Republic showed slightly different trends. Pb was far lower in all cases and Cd was greater than the SGV at one Moravian vineyard (KOMÁREK *et al.* 2008). However, each individual vineyard should be treated on a case by case basis as there are many variables to consider. Mn was measured in all samples but as there is no SGV or background level published for this element in the Czech Republic it has been omitted from the tables. The average Mn concentration from samples was 657.1 mg/kg, a level at which Mn is not likely to exhibit toxic effects given that normal soils contain an average Mn concentration of 850 mg/kg (range of 100 to

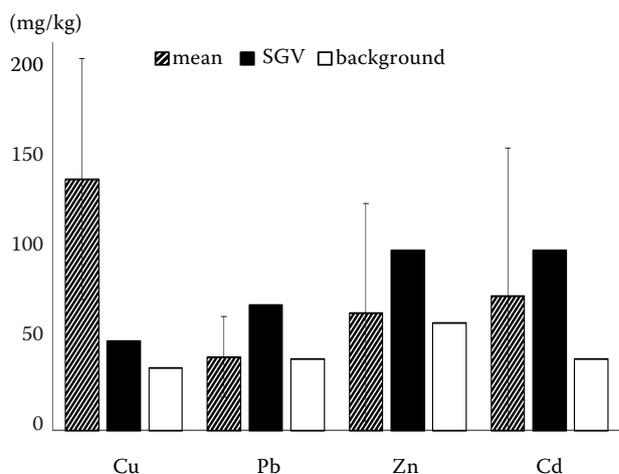


Figure 2. Mean soil potentially toxic elements (PTE) compared to soil guideline values (SGV) and background concentrations of the Czech Republic

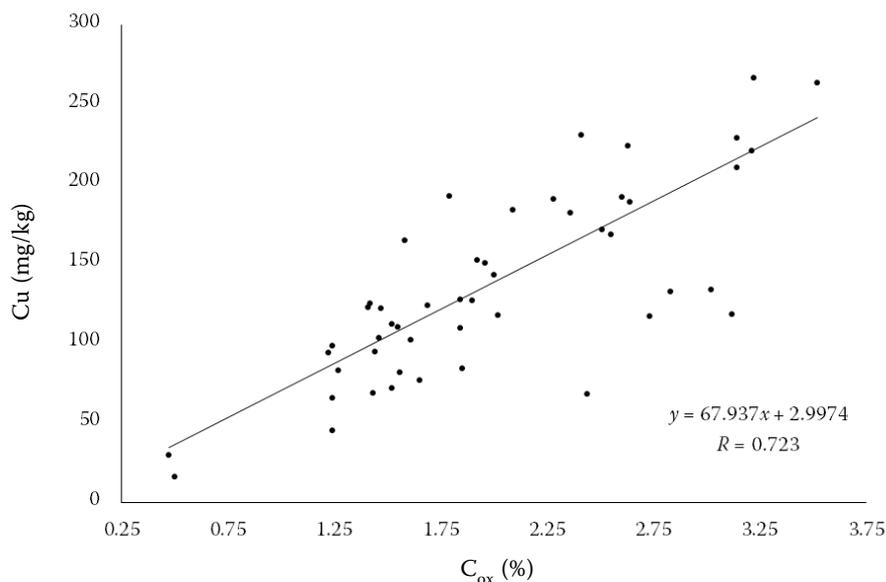


Figure 3. Dependence of Cu retained in the upper 20 cm of soil on oxidizable carbon percentage

4000 mg/kg) (ADRIANO 2001). Mn toxicity is dependent on soil pH and redox and is most dangerous for plants in the exchangeable Mn^{2+} form occurring mainly under acidic conditions, whereas other species of manganese which predominate at higher pH (Mn^{3+} , Mn^{4+}) are not available for plant uptake (MILLALEO *et al.* 2010).

Organic matter. An assessment was made to relate the distribution of Cu to known parameters which affect its mobility. Figure 3 confirms the dependence between surface horizon Cu retention and organic matter, in this case nitric acid

extractable Cu correlated with percentage of oxidizable carbon. The *R* statistic indicates a strong positive correlation which is highly significant due to exceeding the critical value of 0.304 for Pearson's correlation. The relationship was further confirmed by Chi-square analysis, whereby a statistically strong dependence was achieved between variables C_{ox} and Cu concentration (test statistic 34.6 > critical $P = 9.488$). However, Figure 4 indicates that there is no statistical significance for the correlation of humus quality with the occurrence of Cu in the upper 20 cm of soil (critical $P = 0.304$

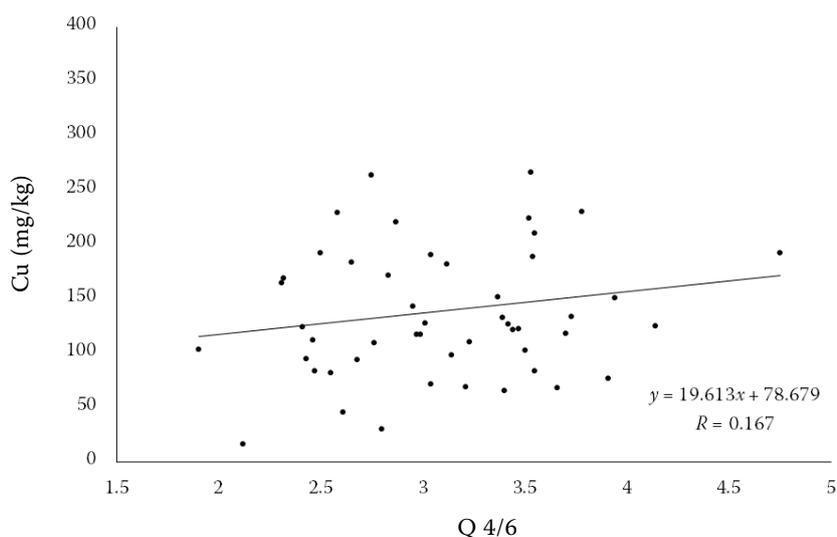


Figure 4. Dependence of Cu retained in the upper 20 cm of soil on humus quality

Table 3. pH_{H₂O} and pH_{KCl}

	pH	
	H ₂ O	KCl
Mean	6.17	5.68
SD	0.97	1.17
Variance	0.95	1.36

SD – standard deviation

> $R = 0.167$) suggesting that Cu retention in the vineyard surface layers is more strongly determined by humus quantity rather than humus quality. For further study, multiple horizon sampling to allow for a leaching depth analysis of PTEs would serve as an expedient tool in better understanding Cu mobility. In other studies it was determined that Cu concentrations are always greatest at the surface due to retention on the soil sorption complex and their concentrations decrease with increasing depth, confirming the anthropogenic origin of Cu in vineyard soils (PIETRZAK & MCPHAIL 2004; KOMÁREK *et al.* 2008).

When C_{ox} is mapped with Cu concentration among samples, a pattern becomes clear that the areas with the greatest accumulation of copper correspond to those with the higher contents of oxidizable carbon at the north western corner of the vineyard. Further investigation is required to determine reasons for the higher portion of or-

ganic matter in this part of the vineyard, possibly considering factors such as wind direction, slope, plant species and mass of plants grown at differing sample points etc. Also, the spraying regime by employees is not known and may well have a significant impact on the distribution of fungicide throughout the vineyard. Additionally, knowing hydrological and soil water parameters such as soil permeability, infiltration and runoff rates, water retention etc. and K_{ow} value of fungicides would assist in the assessment of fungicide movement and hence PTE transport within the vineyard.

Soil pH. No apparent relationship between soil pH and distribution of PTE was observed in this case.

Distribution maps. Figure 6 illustrates the distribution of elements throughout the studied vineyard. There appears to be a common trend of accumulation in the western part of the site for all elements. There are some apparent hot-spots of Cd contamination where values are considerably higher than the surrounding values, the reason for which is undetermined. Pb shows a particularly strong trend of surface soil accumulation in the western corner of the vineyard which is very similar to the pattern for Zn. The map of Cu distribution illustrates a trend of overall contamination although the highest values also occur in the far western corner of the study area.

Wine analysis. Two bottles of wine produced at the studied vineyard were purchased; one bottle of white (Riesling Kabinett 2011) and one red

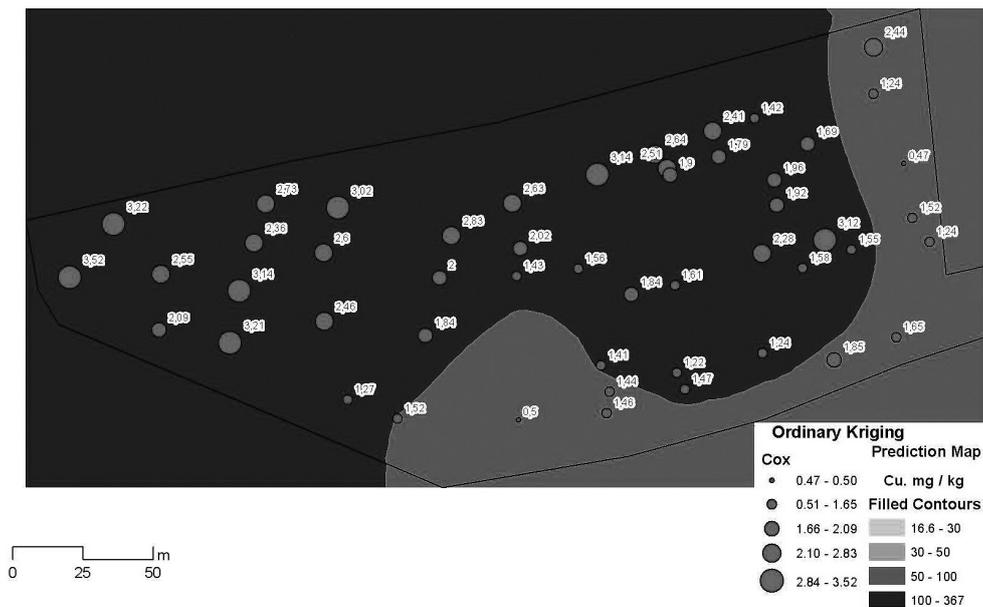


Figure 5. Overlay of oxidizable carbon and Cu occurrence in samples

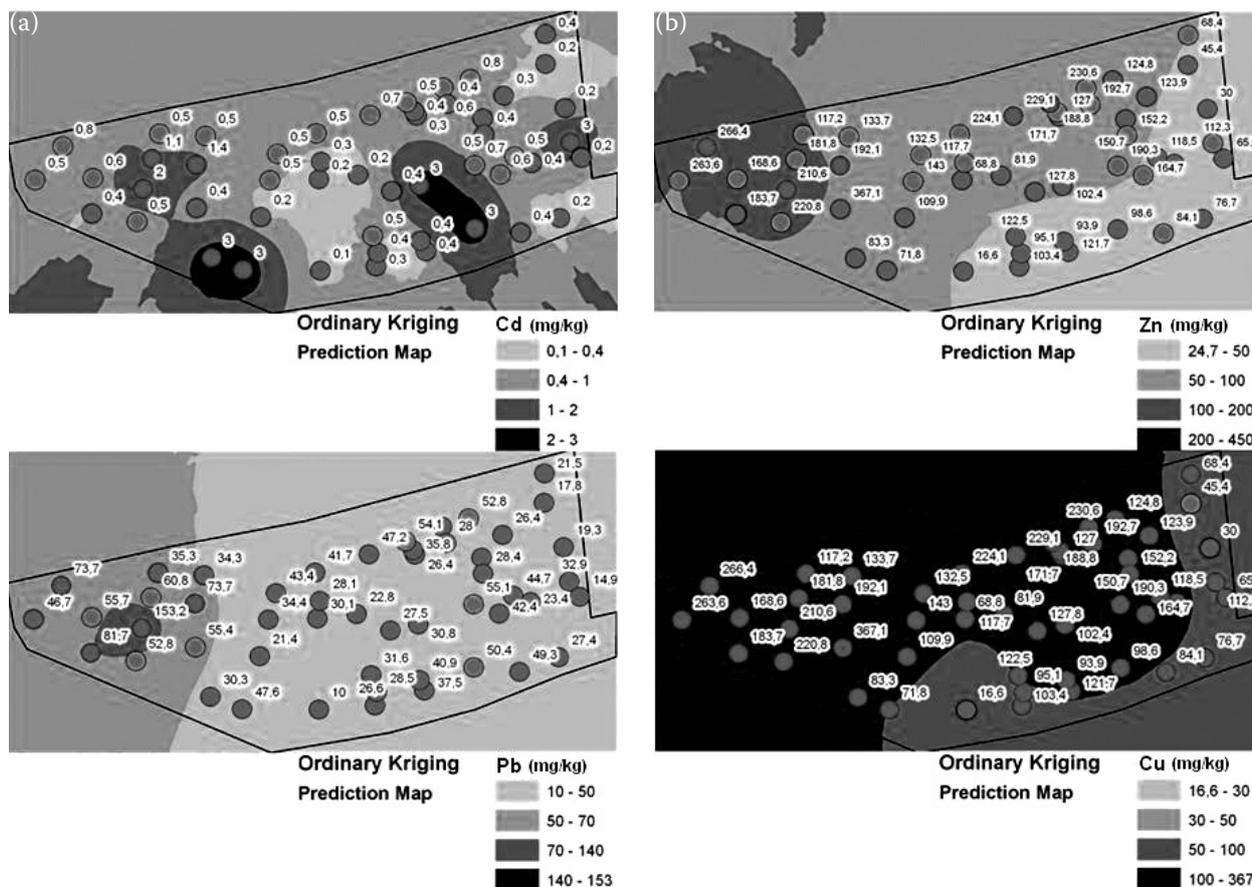


Figure 6. Distribution maps: (a) potentially toxic elements (PTE) Cd and Pb, (b) potentially toxic elements (PTE) Zn and Cu

(Pinot Noir 2010). The wine was analysed for its PTE contents to determine whether or not there is a significant threat of soil contaminants reaching the end product beverage. Mean results from 3 replicates are given in Table 4 with two supermarket wines for reference.

A guideline was set by IPCS (1998) delineating a rough estimate of no more than approximately 3 mg/day oral intake of Cu, and EPA (2004) publish guidelines for human exposure to Cu which includes a drinking water standard of 1.3 mg/l.

With regards to copper, which is the main element of concern, both of our study vineyard wines show insignificant amounts which would have a minimal contribution to reaching the daily allowable intake of Cu. The white wine values were all below detection limits suggesting that red wine production results in greater Cu accumulation, although this should be the subject of further and more thorough investigation. The supermarket wines had a greater content of Cu than for the studied vineyard wine, but the value still represents a fraction of the guideline al-

Table 4. Mean values (standard deviation) of analysed wine samples (in mg/l)

	Cu	Mn	Cd	Pb	Zn
Study area red	0.032 (0.012)	2.393 (0.208)	b.d.l.	b.d.l.	0.296 (0.039)
Study area white	b.d.l.	1.206 (0.053)	0.026 (0.001)	b.d.l.	0.163 (0.008)
Modry portugal	0.086 (0.021)	1.072 (0.040)	b.d.l.	b.d.l.	0.368 (0.011)
Poezie cervena	0.083 (0.023)	1.100 (0.055)	b.d.l.	b.d.l.	0.920 (0.036)

b.d.l. – below the determination limit

lowances. Manganese in the studied vineyard red wine was almost twice the value for the white and more than twice the supermarket wines. The only cadmium which was in a large enough concentration to be detected by the analysis equipment was in the studied vineyard white wine. Unpolluted water usually contains below 0.001 mg/l (FRIBERG *et al.* 1986) and the dietary daily intake of Cd for most areas is estimated to be in the range of 0.01–0.040 mg (IPCS 1992). Therefore, the white wine produced from grapes at the studied vineyard contains an amount of cadmium which in conjunction with normal dietary consumption, could increase Cd intake to well above average. All Pb analysis gave results below the determination limit, and concentrations of zinc were highest in the two supermarket wines.

CONCLUSIONS

Excessive concentrations of Cu (respective of background concentrations and guideline values for agricultural soils) were consistently evident in the upper 20 cm of the vineyard soil. It is possible to say with confidence that the copper is of anthropogenic origin, most likely as a result of repeated fungicide application. It is apparent from statistical analysis that in this case the retention of copper in surface soil is more dependent on humus quantity rather than the quality of humic substances (although the range of obtained values for Q 4/6 is small). On average, Cd and Zn were found to exceed background concentrations only. Grape vines are very deep rooting plants, therefore suggestions for further study include sampling at depth from all horizons to gain more data concerning PTE leaching, retention and potential uptake.

By studying the distribution maps, a common trend of PTE accumulation was observed in the western corner of the map. Further analysis and more specific data are needed to determine the reason for the distribution.

The analysis of wine samples showed that Cu, although highly concentrated in the surface soil layer, is not being transferred to the end product beverage at a level that is considered dangerous. However, the study area white wine contained an unacceptable level of cadmium, which may have occurred as soil-plant-fruit-wine transfer or as a result of production processes. It is therefore recommended that the study be extended to sample a wide range of wines produced at the studied

vineyard, with an analytical focus on all potential transfer stages within the grape vine plant.

Although the observed high soil copper content does not result in any apparent detrimental effect with respect to the consumers beverage, it is not practicable to claim that the elevated levels of soil Cu do not effect the health of the plant and its grape production and/or the health of soil micro and macro fauna which maintain soil quality.

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