Bull. Environ. Contam. Toxicol. (2005) 75:968–974

DOI: 10.1007/s00128-005-0844-3

Bulletin of Environmental Contamination and Toxicology

Copper-Based Fungicide Contamination and Metal Distribution in Brazilian Grape Products

N. Mirlean,[1] A. Roisenberg,[2] J. O. Chies[2]

[1] Fundação Universidade do Rio Grande, Department of Geosciences, Av. Italia–km 08, Campus Carreiros, CEP 96201-900, Rio Grande-RS, Brazil
[2] Universidade Federal do Rio Grande do Sul, Institute of Geosciences, Av. Bento Gonçalves, 9500–Porto Alegre-RS, Brazil

Received: 29 July 2005/Accepted: 19 September 2005

After almost 30-years of decrease, a new growth of global wine consumption is denoted. It is known that a wine may contains increased quantities of some metals such as copper and among them other known toxic elements such as lead and cadmium (Scheinberg, 1991). Alcohol strengthens toxic effect of metals and also their accumulation in an organism which has been shown especially for lead (Roses et al., 1997, Brivet et al., 1990, Nation et al., 1990). A case of severe lead poisoning through wine consumption has been described (Mangas et al., 2001). In a background of traditionally high consumption of wine, as in viniculture areas, the elevated copper content is, probably, the reason for the greater occurrence of liver diseases among the population (Mirlean and Burgelia, 1986). The trace element composition of grape wines and other alcoholic products is influenced by the type of soil, wine processing equipment, vinification methods and also composition of used fungicides, insecticides and fertilizers (Eschnauer and Neeb, 1988). The detection of the factor having the main influence on the high content of metals in wine and other grape products should help to improve their quality and reduce possible health hazards.

Viniculture in Brazil appeared more than one hundred of years ago together with mass Italian immigration which brought extensive cultivation of grapes and vinification techniques. Actually, the vinification branch in Brazil is booming, vine areas have increased and output of wine and other grape products are growing. Practically all Brazilian wine production is concentrated in mountainous areas of the Rio Grande do Sul State, southern Brazil, where rather cold winters facilitate the successful cultivation of grapes. However, because of high intensity of atmospheric precipitation (1900 MM/yr) this area is, probably, the dampest viniculture area in the world. High humidity and high periodicity of rains demand more frequent processing by fungicides which consequently should affect metal content in grape products.

Till now there were no publications about metals in Brazilian grape products such as wine, vinegar, grape juice and grape jam. The present study involves the analysis of Brazilian market and home-made grape products, grape bagasse, vineyard soils and copper-based fungicide. Analyses were performed for

Correspondence to: N. Mirlean

cadmium, copper, lead, and zinc, which could be pollutants of the grapes and normally are controlled in wines. Chromium and nickel, which are characteristic for local ferrous soils developed in basaltic rocks, were also analyzed.

MATERIALS AND METHODS

Twenty five wine samples from 14 wine types were investigated. A part of the wine samples were obtained directly from the wineries and the others were obtained from the local market. Four wine vinegar and four grape juice samples were taken from the local market and two home-made samples of these products were obtained from the home owners. Four samples of grape jam, including one home-made, were purchased in the local market. Two samples of fresh grape bagasse were taken from a dump close to a winery. Twelve surface soil (0-5 cm) samples were collected in 6 vineyards belonging to different wineries and histories of copper applications. A sample of copper sulphate was obtained from the local distributor of agro-chemicals. All analysed samples were from the same region of Bento Gonçalves in Rio Grande do Sul State, southern Brazil. The names of the producers and specific locations cannot be disclosed because of confidentiality agreements with the owners.

Water extraction from grape pomace was prepared by 1:5 pomace:deionized water ratio shaking during 2 hours and consecutive filtering through membrane 0.45 μm. A copper sulphate sample was prepared by dilution of 1g of $Cu\ S0_4\ 2H_2O$ in 1L of deionised water acidified (1% v/v) by s.p. nitric acid. Soil samples were air-dried, lightly ground and sieved through a 2 mm stainless steel sieve. Trace-metal clean procedures detailed in Mart (1982) were employed to minimize a contamination risk when sampling and sample treating.

The digestion of samples of wine, vinegar, juice, bagasse water extract and jam was performed by treating 10 ml of liquid sample or 1 g of jam with 3 ml conc. HNO_3 and 5 ml H_2O_2 according to Pietrzak and McPhail (2004). After complete digestion, the samples were made up by deionized Milli-Q water to 50 ml. For soil samples the digestion method $HF/HClO_4/HNO_3$ was employed.

Zn, Cu, Cr and Ni in soil samples were analyzed by flame atomic absorption spectrophotometry (GBS 932). For Pb and Cd analysis in soil samples and Cu, Zn, Cr, Ni, Pb and Cd analysis in wine and other grape products the graphite furnace atomic absorption spectrophotometry was used. Maximum value of relative standard deviation for 3 replicate analyses was less than 4%.

Since qualified international standard organizations do not support certified reference materials of wine and vinery products, the method accuracy and digestion recovery was tested by preparing three quality control samples (Galani-Nikolakaki et al., 2002). The absolute ethanol was added (12% v/v) to Milli-Q achieving a final alcohol concentration close to that in wines. Copper and zinc were added to 200 $\mu g\ L^{-1}$; lead, chromium and nickel to 20 $\mu g\ L^{-1}$ and cadmium to 2 $\mu g\ L^{-1}$. These samples were digested under the same conditions used for wines and vinery products. Quite good recoveries for metals were found falling within the 95% confidence limits for prepared samples. The accuracy and precision of soil material analyses were ensured by sequential digestion and analysis NRCC-PACS-2 Certificate Reference Material (CRM). For majority of metals good recovery was found within the 95% confidence limits for the CRM. For cadmium those fall outside the limits but recovery was still within 10% of the certified value. Therefore, we consider the reported data of sufficient accuracy and acceptable precision.

RESULTS AND DISCUSSION

The vineyard top soil contains very high copper concentration which on its maximal and the average values considerably surpasses copper concentration in vineyard soils of other countries (Pietrzak and McPhail, 2004). Such a high copper concentration in Brazilian vineyard soils is caused by local climatic conditions, favorable for more frequent flashes of mildew, and, as a consequence, to more frequent application of copper-based fungicide.

Table 1. Metal concentrations in grape products and bagasse (μg L^{-1}, μg kg^{-1}), soil and fungicide (mg kg^{-1}), in brackets-mean concentration.

Sample (n)*	Cu	Zn	Pb	Cd	Ni	Cr
Red wine (25)	69-1011 (197)	80-358 (194)	5-121 (54)	0.2-0.9 (0.5)	4-14 (9)	8-34 (16)
White wine (4)	81-264 (181)	54-552 (326)	5-79 (29)	0.3-1.3 (0.6)	5-27 (12)	14-35 (22)
Grape juice (4)	240-1023 (526)	96-528 (307)	40-212 (117)	1.0-1.9 (1.3)	6-35 (19)	28-54 (38)
Grape jam (4)	87-934 (404)	67-587 (271)	15-187 (93)	0.4-2.3 (1.4)	12-34 (22)	18-44 (28)
Vinegar (4)	65-158 (112)	110-211 (165)	13-47 (25)	0.2-1.0 (0.5)	7-24 (15)	13-28 (19)
Home-made juice (1)	1839	522	290	1.9	8	46
Home-made vinegar (1)	87	106	35	4.3	10	12
Bagasse, white-red (2)	297-6624	456-2843	198-365	1.4-4.9	44-110	70-32
Vineyard topsoil (14)	196-2497 (1175)	91-355 (193)	8-47 (16)	0.3-3.6 (1.6)	5-67 (35)	9-113 (55)
Copper sulphate (1)	326000	1309	96	1.4	13	20

* number of samples

The copper sulphate used for fungicide preparation contains considerable quantities of other metals; zinc and lead concentrations in copper sulphate is several times higher than in vineyard soils (Table 1).

Water extracts from fresh grape bagasse demonstrate very high metal concentrations. Besides the copper, zinc and lead concentrations are particularly increased in bagasse extracts which correspond to distribution of these metals in copper sulphate used in vineyards. Water extract from red grape bagasse in comparison to white grape bagasse is several times richer in lead, zinc and cadmium, and 20 times richer in copper.

Metal concentrations in Brazilian wines varied in a wide range (Table 1). Red wines on the average contained more copper and lead than white ones, which coincided with the published data for other countries (Lara et al., 2005; Karadjova et al., 2000). The copper concentration above the permitted level established in Brazil (1000 $\mu g\ L^{-1}$) was met only in Merlot.

Though the lead concentration in analyzed wines does not exceed the Brazilian limit of 300 $\mu g\ L^{-1}$, it was higher than lead concentration in wines of other countries. The concentration of the other metals in wine was comparable to the values generally found in literature (Gomes et al., 2004; Salvo et al., 2003; Conde et al., 2002; Galani-Nikolakaki et al., 2002).

In grape vinegars the concentration of Cu, Zn, Pb and Cd was on the average lower than in wines. That is, probably, related to the additional process of fermentation and also to dilution of the end-product in order to achieve commodity concentrations of acetic acid. The higher concentration of cadmium in domestically prepared vinegar may be explained by contamination from the metal container.

Grape juices contain noticeably more metals than wine. In one of the four analyzed market juices the copper concentration exceeded the Brazilian norms (1 $mg\ L^{-1}$), lead concentration exceeded recommendations of the Office International de la Vigne et du Vin-OIV (200 $\mu g\ L^{-1}$) and zinc concentration exceeded European Community norms (500 $\mu g\ L^{-1}$). In domestically prepared grape juice (sold by manufacturers during grape harvesting) the copper concentration exceeded the Brazilian norms and the majority norms of other countries. The lead concentration was very close to Brazilian norms but surpassed the OIV norms, and zinc concentration was higher than European Community norms. The cadmium concentration is generally higher in juices in comparison to wines (Table 1).

Grape jams are characterized by noticeably increased metal concentrations compared to wines, but lesser than juices. The highest metal concentrations were found in home-made jam. Local market jams have lower metal concentrations, probably, due to addition of components (pectin, soy-milk) not related to the fruit.

As a whole, distribution of metals in the analyzed grape products depends on the degree of its processing; the more technological stages the product passes through, lesser the metal concentrations. These products form the following line in decreasing order of metal concentrations: juice – jam – wine - wine vinegar.

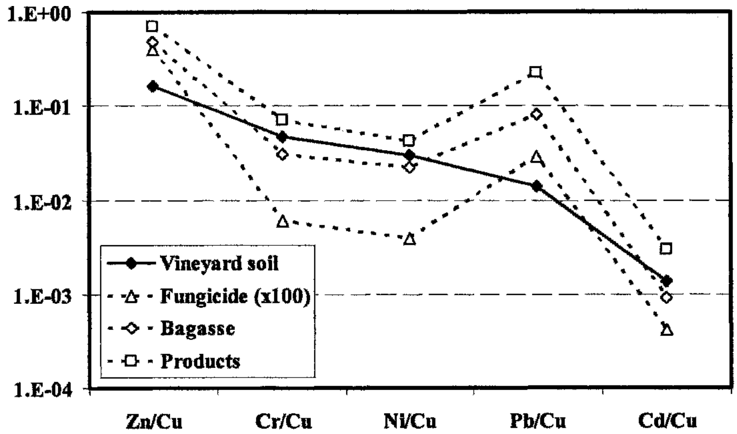

Figure 1. Average metal content normalized by copper concentration in studied sample sets.

Herein, the metal contents in home-made products are higher than in the industrial ones. Presumably, that is the result of an infraction of technological norms, pollution from the container or smaller dilution.

In spite of apparent evidence in the significant contribution of fungicide in the metal concentrations in grape products, there is no common opinion concerning this subject in literature. Even the elevated copper concentrations in wines are not always attributed to the influence of the use of fungicide (Lara et al. 2005). Zinc in wine, according to Lara et al. (2005), increases when zincware containers during the processing and ageing stages are used and also when special zinc-based pesticides are applied. Elevated lead concentrations in wine are frequently attributed to atmospheric pollution (Lara et al., 2005; Medina et al., 2000).

Figure 1 presents the distribution of metal concentrations normalized to copper concentrations in grape products, grape bagasse, soil and fungicide (metals are located on x-axis in decreasing order of their normalized values in soil). It can be seen that lines of metal distribution in the studied sample sets show two different configurations. Configuration for fungicide, bagasse and grape products differs from that for soil by higher values of normalized concentrations of lead and zinc. Such concurrence of metal distribution lines in fungicide and grape products testifies that product pollution most likely occurs as a result of direct contamination by residual quantities of fungicide adhered to the fruit surface instead of contamination by soil material or selective metal accumulation by plants through root system.

The increased lead concentration in grape products due to atmospheric pollution is

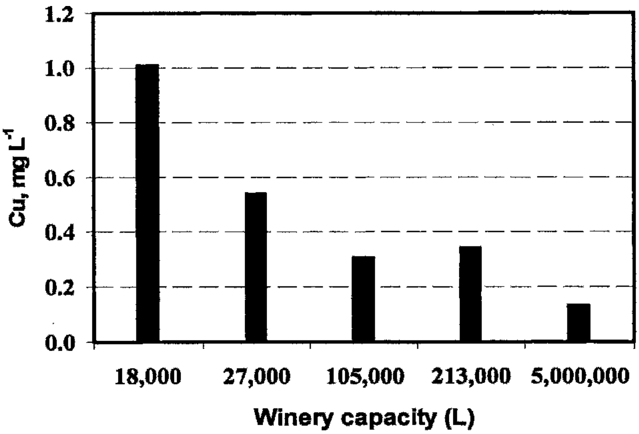

Figure 2. Copper concentrations in wine Merlot versus winery's capacity.

unlikely, at least in the investigated area. There is no large industrial activity or intensive traffic in studied region, apart from that the lead additive to gasoline has not been applied in Brazil since 1980. The significant correlation between copper and lead in grape products ($r=0,79$, $p<0.05$, $n=39$) confirms the direct contribution of fungicide to the lead contamination.

Observed variations of metal concentrations in the same type of product from different manufacturers, probably, are related to distinctions in technology (or violations of standard technological schemes), mixture of different kinds of grapes or products being manufactured in different years when distinct quantities of fungicide were applied. Purposely, to check the influence of manufacturer on metal concentrations in wine, we carried out additional analysis of the 2002 crop Merlot wine (this wine is made of only one kind of grape), from 5 different manufacturers in the same area. Results presented in figure 2 demonstrate a tendency of inverse correlation between copper concentration in wine and manufacturer output capacity. Such correlation could be atributed to deviations in the technological process of small-scale manufacturers by reducing certain technological operations.

REFERENCES

Brivet I, Chomard PH, Dumas P, Lallemant AM, Theven M (1990) Ethanol-lead in the Sprague Dawley rat. Food Add Contam 7:150–151

Conde JE, Estevez D, Rodriguez-Bencomo JJ, Montelongo FJG, Perez-Trujillo JP (2002) Characterization of bottled wines from the Tenerife Island (Spain) by their metal ion concentration Italian J Food Sci 14: 375-387

Eschnauer H, Neeb R (1988) Micro-element analysis in wine and grapes in: modern methods of plant analysis. In: Linskens HI, Jackson JF (ed) Wine Analysis 6, Springer-Verlag, Berlin, p 67

Galani-Nikolakaki S, Kallithrakas-Kontos N, Katsanos AA (2002) Trace element analysis of Cretan wines and wine products. Sci Tot Env 285: 155-163

Gomes MMG, Feldman I, Jakubowski N, Andersson JT (2004) Classification of German white wines with sertified brand of origin by multielement quantitation and pattern recognition techniques. J Agric Food Chem 52: 2962-2974

Mangas S, Visvanathan R, Alphen M (2001) Lead poisoning from homemade wine: A case study. Env Health Persp 109:433-435

Lara R, Cerutti S, Salonia JA, Olsina RA, Martinez LD (2005)Trace element determination of Argentine wines using ETAAS and USN-ICP-OES. Food Chem Tox 43: 293-297

Karadjova I, Izgi B, Gucer S (2000) Fractionation and speciation of Cu, Zn and Fe in wine samples by atomic absorption spectrometry. Spectr Acta Part B: Atom Spectr 57: 581-590

Mart L (1982) Minimization of accuracy risks in voltammetric ultratrace determination of heavy metals in natural waters. Talanta 29: 1035-1042

Medina B, Augagneur S, Barbaste M, Grouset FE, Buat-Meard P (2000) Influence of atmospheric pollution on the lead content of wines. Food Add Contam 17: 435-445

Mirlean N, Burgelia N (1986) After-effects of contamination by heavy metals. In: ANMSSR. (ed) Anthropogenic impact in Moldova. Kishinev, p 66

Nation JR, Dugger LM, Dwyer KK, Braton GR, Grorer CA , (1990) The effects of dietary lead on ethanol-reinforced responding. Alc-Alcoholism 26: 473–480

Pietrzak U, McPhail DC (2004) Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma 122: 151-166

Roses OE, Gonzalez DE, Lopez CM, Pineiro AE , Villaamil EC (1997) Lead levels in argentine market wines. Bull Environ Contam Toxicol 59: 210–215

Salvo F, Pera L, Bella Giuseppa, Nicotina M, Dugo G (2003) Influence of different mineral and organic pesticide treatments on Cd,Cu,Pb,and Zn contents determined by derivative potetiometric stripping analysis in Italian white and red wines. J.Agric. Food Chem. 51: 1090-1094

Scheinberg IH, (1991) Copper. In: Merian E (ed) Metals and their compounds in the environment, VCH, Weinheim p 45