# HEAVY METAL (Pb, Cu, Zn and Cd) CONTENT IN WINE PRODUCED FROM GRAPE CULTIVAR MAVRUD, GROWN IN AN INDUSTRIALLY POLLUTED REGION

## TAUX DE MÉTAUX LOURDS (Pb, Cu, Zn and Cd) DANS LES VINS DU CÉPAGE MAVRUD PROVENANT D'UNE RÉGION POLLUÉE PAR LES INDUSTRIES

Violina ANGELOVA*, A. IVANOV**, D. BRAIKOV** et K. IVANOV*

University of Agriculture, *Departement of Chemistry,**Departement of Viticulture
Mendeleev str.12, 400 Plovdiv, Bulgaria

**Abstract** : The investigation was carried out in the period 1991-1993 with cv. Mavrud, grown in the region with a major industrial pollutant the Non-Ferrous-Metal Works (NFMW) and a region with no industrial pollutants (as a control). The heavy metal content in soil, grapes and wine was determined. Most of the heavy metals in the grapes precipitate during fermentation into the sediments, which is the reason for their significantly lower content in the wine. Water washing of grape before processing leads to about 2 time decrease in the Pb, Cu, Zn and Cd contents of wine. The pre-washing of grapes does not lead to any quality deterioration in the wine produced. The amounts of Cu, Zn and Cd in the wine from cv. Mavrud, grown in the region of the NFMW-Plovdiv, are lower than the maximum admissible levels, while the Pb content exceeds them about two times.

**Résumé** :L'étude a été menée de 1991 à 1993, sur le cépage Mavrud, cultivé dans deux régions, l'une, dont le polluant principal est le Combinat de Metallurgie non-ferrese et l'autre sans aucun polluant industriel (contrôle). Les taux de métaux lourds présents dans le sol, le raisin et le vin ont été déterminés. La plupart des métaux que l'on trouve dans le vin précipitent au cours de la fermentation, ce qui explique la baisse considérable de leur concentration dans le vin. Le taux de métaux lourds est divisé par deux, lorsque le raisin est lavé avec de l'eau. Le lavage préalable du raisin n'altère pas la qualité du vin produit. Les taux de Cu, Zn et Cd dans le vin du cépage Mavrud sont beaucop moins élevés que les limites de concentration admissibles, trandis que le taux de Pb est environ deux fois au-dessus de la norme.

**Key words** : heavy metals, grape, wine
**Mots clés** : métaux lourds, raisins, vin

## INTRODUCTION

The heavy metal amounts in wine vary depending on the specific cultivar, the grapevine-growing soil, the climatic conditions, the methods of pest and disease control, the grape processing method and vinification. The heavy metal deposition on grapevine plants through air aerosols in result of the production activities of man could lead to increase in their content in wine (BANER *et al.*, 1994). The use of copper containing fungicides for the disease control of plants could also lead to increase in the Cu amounts of grapes, as well as of the products obtained from their processing (MAGALHAES *et al.*, 1985; RENAN, 1994).

According to HENICK-KLING and STOEWSAND (1993), Pb is the most toxic element of the heavy metals. It is established that the consumption of Pb containing wine (150 mg/l) and beer leads to increase of its amount in blood (SHERLOCK *et al.*, 1986). FAO/WHO (1972) recommends for the Pb content in the daily meals and drinks of people not to exceed 0.43 mg. In Europe, the maximum allowable rates for its content in wine vary from 0.2 to 0.35 mg/l. According to O.I.V. (1990) recommendations, wine must contain not more than 0.3 mg/l Pb, 0.01mg/l Cd, 1 mg/l Cu and 5 mg/l Zn. The investigations made from GARTEL (1987), BRENDEL *et al.* (1989) and BANER *et al.* (1994) show that the Pb, Cu and Cd amount in wine are less than legal limit, as a great part of them are precipitated as sulphides during the fermentation processes.

In the vicinity of the Non-Ferrous-Metal Works (NFMW) near Plovdiv, more than 2 100 ha have been polluted by heavy metals. It is estimated that around 460 tons of dust containing mainly Pb, less Zn and Cd,

1 : correspondance : aviolina@hotmail.com

are annually emitted to the atmosphere (BULLETIN FOR THE ENVIRONMENTAL POLLUTION IN BULGARIA, 1989 ; 1990). The data analysis from the Non-Ferrous-Metal Works emissions (ECOLOGICAL PROBLEMS AND OUTLOOKS OF LEAD - ZINC PRODUCTION IN BULGARIA, 1990) showed that the main source of environmental pollution with lead, zinc, cadmium and sulfur dioxide are the technological and ventilation gases released from lead production. The second important pollution sources are the gases from Roller workshops and drying furnaces in zinc plants. Another environment pollution threat is posed by the shaft furnace in the periods of its starting and stopping, as well as in regimes of break-down operations, when the purification installations are turned off and the dust-gas mixture is directly released into the atmosphere. The heavy metal distribution in the separate parts of the wine cultivar grapes exerts, no doubt, an effect on the heavy metal amounts in the wines produced therefrom. For the heavy metal pollution of this region, it is necessary to establish what amounts of them are accumulated in grapes and what part of these amounts has passed into the wine.

The objective of the present study was to determinate the heavy metal amounts in red wine produced from grapes grown in an industrially polluted region, as well as holding out opportunities for reducing their content in it.

## MATERIAL AND METHODS

The investigation was carried out during the period 1991-1993 in the experimental vineyard of the Department of Viticulture (in Brestnik), located at a distance of about 3 500 m from the Non-Ferrous-Metal Works (NFMW) - Plovdiv. Samples from the Experimental Estate of the Institute of Viticulture and Enology - Pleven (a region with no industrial pollutant) were used as a control. Position and climatic characteristics of the investigation regions are shown in figures 1 and 2. The distance between investigation regions was 200 km.

Grapevines from the cultivar Mavrud (*Proles pentica* Neg.), intended for the production of dry red wines, was included in the study. The spacing of the plants was : 3.2 m (row)/ 1.2 m (intrarow) and were grafted to the rootstock Kober 5BB. A total of 120 plants (60 plants from industrially polluted and 60 plants from non-polluted region) were included in the trial.

### I - SOILS

Soil samples were taken in 10 cm layers up to 100 cm in the Plovdiv region and up to 40 cm in the Pleven estate farm. The testing scheme was according to MAGALHAES *et al.* (1985). It allows the observation of concentration changes in the xy plane and along z axis (depth), accounting for the eventual effect of the fertilization and the aerosol pollution. Soil of plantations located near the vineyards were also tested (background samples). Two hundred and eighty samples were collected, dried, ground and passed through a 1 mm sieve. 3 g air-dry soil was weighed in a 250 ml conical flask and moistened by bidistilled water. Then 21 ml of a mixture of $HNO_3$ and HCl (1:3) was carefully added to avoid any losses in the course of the reaction with the organic matter of soil. The sample was heated for 3 h on a sand bath. After cooling, it was transferred into a 100 ml flask and brought to volume with bidistilled water.

Soil pH ($H_2O$) was determined using a soil - water ratio of 1:2.5. The total content of carbonates was determined by titration. Total amount of humus according the method of TURIN (1965). Soil chemical properties of the samples are listed in table I.

### II - GRAPE

The grape analyses were conducted during the technological ripeness (in the beginning of October) with a must sugar content of 22 - 23 p. cent and titrable acids - 6 - 6.5 g/dm$^3$. Grape berries (approximately 3 000 g per sample) were cut using grape shears and placed in freezer bags by samplers wearing rubber gloves. The grape samples were investigated in two variants - unwashed and water - washed grapes. In the washed variant, the grape was washed for 3 minutes in running water for removal of dust. Skins, pulp and seeds of the grapes from the different varieties were separated using stainless steel forceps. Skins, pulp, seeds and stalks from unwashed and washed berries were then ashed separatly. All samples were treated by the method of dry mineralization. A 10 g sample of skin (pulp, seeds and


**Fig.1 - Bulgarian map showing the investigation regions (Plovdiv and Pleven, Bulgaria)**

**Fig. 1 - Carte des régions étudiées (Plovdiv et Pleven, Bulgarie)**

stalks, respectively) was weighed in a quarz crucible and put into a furnace (T = 400°C) until ashing has occured. After cooling to a room temperature, 1 ml $NHO_3$ (1:1) was added, evaporated in a sand bath and put again into the furnace (T = 400°C). The procedures were repeated until the ash was white. It was finally dissolved in 2 ml 20 p.cent. v/v HCl, transferred in a graduated 10 ml cylinder and brought to volume with bidistilled water.

III - WINE

Part of the grape was initially washed with water and the rest - processed without washing. For this purpose, a day before vintage, we defoliated the grapevines marked to be washed in the region of their bunches. Then the grape was washed through a Perla sprayer at a water consumption rate of 100 l/dka. The next day, the grapes were hand-harvested into plastic buckets and transported to the experimental estate of the Department of Viticulture for vinification. The following wine-production operations were conducted: separating the berries from stalks; crushing the berries; sulfurating the mash; inoculation with cultured yeast; active fermentation for 8-10 days; separating the young wine and the marc (solid grape particles - berry skins and seeds) when a relative density is reached 1 030-1 050 and a bottom fermentation. The wines produced from unwashed and water-washed grapes were analyzed for the contents of Pb, Cu, Zn and Cd. After separating, the young wine samples were taken from the yeast sediment, too. The latter was also investigated for heavy metal amounts.

Wine samples were treated by the method of wet mineralization. A sample of 50 ml red wine was put into a glass 5 ml c. $HNO_3$ was added and heated on a sand bath. If the sample was not discoloration for 1 hour, it was cooled and then 2 ml c. $HNO_3$ was added. Heating was continued till the solution discoloration, after which the latter was transferred into a graduated 10 ml cylinder and brought to volume.

Yeast sediment samples were treated by the method of dry mineralization, described above.

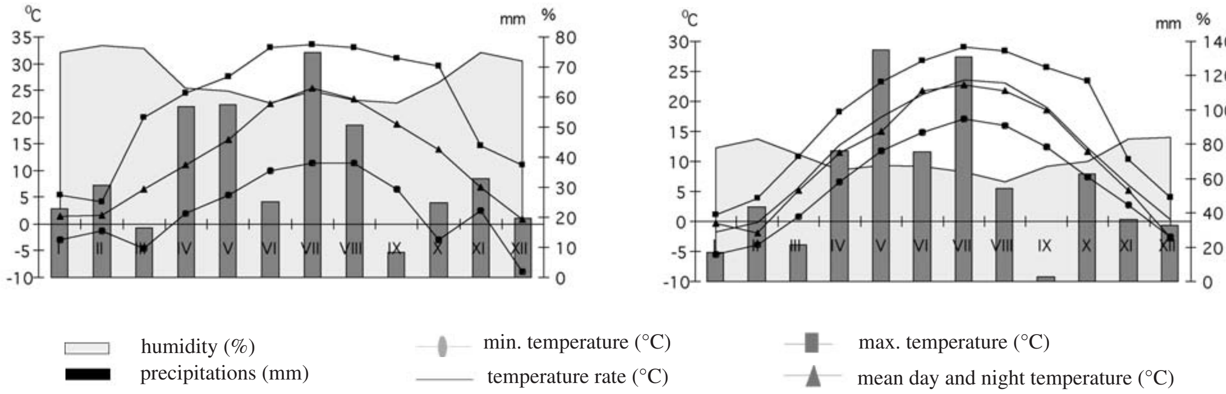


**Fig. 2 - Climatic characteristics (a regimen of temperature, precipitations and air humidity) of the investigated regions**
**A: industrially polluted region ; B: non-industrially polluted region**

**Fig. 2 - Caractéristiques climatiques des régions étudiées (température, précipitation et humidité de l'air). A : région industrielle polluée ; B : région industrielle non polluée**

**TABLE I**
**Some chemical characteristics of the investigated soils**

**Tableau I - Caractéristiques chimiques des sols étudiés**

| Soil | Depth (cm) | pH | $CaCO_3$ (%) | Humus (%) | Soil classification |
|---|---|---|---|---|---|
| industrially polluted region | 0 - 25 | 7.0 | 13.26 | 1.52 | alluvial meadow soil |
| | 25 - 50 | 7.3 | 21.02 | 1.10 | |
| | 50 - 80 | 7.5 | 23.0 | 0.97 | |
| | 80 - 105 | 7.7 | 25.2 | 0.68 | |
| non-industrially polluted region | 0 - 25 | 6.6 | - | 1.83 | cinnamonic meadow soil |
| | 25 - 50 | 6.3 | - | 1.24 | |
| | 50 - 80 | 7.5 | 24.18 | 0.79 | |
| | 80 - 105 | 7.8 | 26.18 | 0.76 | |

**TABLE II**
**Contents of Pb, Cu, Zn and Cd (mg/kg) in vineyards near the NFMW - Plovdiv**
(x : average value from 5 repetitions ; sd : mean standard deviation)

**Tableau II - Teneurs en Pb, Cu, Zn et Cd (mg/kg) dans des sols viticoles situés à proximité des aciéries de Plovdiv (NFMW)**
(x : moyenne des 5 répétitions ; sd : écart-type)

| | Depth (cm) | Pb (x ± sd) | Cu (x ± sd) | Zn (x ± sd) | Cd (x ± sd) |
|---|---|---|---|---|---|
| Left intra row | 0 - 10 | 68.9 ±9.1 | 66.4 ±1.4 | 249 ±20 | 1.8 ±0.6 |
| | 10 - 20 | 50.8 ±9.0 | 58.9 ±1.4 | 223 ±18 | 1.7 ±0.6 |
| | 20 - 30 | 43.3 ±6.5 | 52.6 ±1.2 | 211 ±18 | 1.3 ±0.4 |
| | 30 - 40 | 42.0 ±6.2 | 48.7 ±1.1 | 185 ±16 | 0.8 ±0.2 |
| | 40 - 50 | 31.6 ±5.5 | 47.1 ±1.0 | 139 ±14 | 0.4 ±0.1 |
| | 50 - 60 | 26.3 ±3.2 | 45.6 ±1.0 | 117 ±12 | 0.2 ±0.1 |
| | 60 - 70 | 24.1 ±2.8 | 42.9 ±1.1 | 111 ±12 | < 0.2 |
| | 70 - 80 | 23.5 ±2.3 | 40.1 ±1.0 | 98 ±10 | < 0.2 |
| | 80 - 90 | 19.7 ±1.9 | 39.7 ±1.0 | 89 ±8 | < 0.2 |
| | 90 - 100 | 18.2 ±1.5 | 40.3 ±1.1 | 67 ±8 | < 0.2 |
| Row | 0 - 10 | 74.8 ±9.1 | 72.3 ±1.6 | 247 ±20 | 2.5 ±0.7 |
| | 10 - 20 | 52.3 ±9.0 | 63.0 ±1.6 | 215 ±19 | 1.4 ±0.5 |
| | 20 - 30 | 42.8 ±6.3 | 58.8 ±1.3 | 189 ±18 | 1.0 ±0.2 |
| | 30 - 40 | 41.0 ±6.2 | 47.2 ±1.2 | 170 ±16 | 0.8 ±0.1 |
| | 40 - 50 | 33.3 ±5.3 | 56.3 ±1.0 | 129 ±14 | 0.3 ±0.1 |
| | 50 - 60 | 27.2 ±3.3 | 46.7 ±1.0 | 98 ±12 | 0.2 ±0.1 |
| | 60 - 70 | 23.7 ±2.3 | 45.5 ±1.0 | 94 ±12 | < 0.2 |
| | 70 - 80 | 23.5 ±2.3 | 40.8 ±1.1 | 91 ±10 | < 0.2 |
| | 80 - 90 | 18.8 ±1.9 | 39.5 ±1.0 | 71 ±8 | < 0.2 |
| | 90 - 100 | 17.3 ±1.6 | 38.8 ±1.1 | 58 ±8 | < 0.2 |
| Right intra row | 0 - 10 | 72.6 ±9.0 | 69.7 ±1.5 | 243 ±20 | 1.9 ±0.6 |
| | 10 - 20 | 61.4 ±8.5 | 64.2 ±1.4 | 235 ±19 | 1.6 ±0.5 |
| | 20 - 30 | 46.3 ±6.5 | 58.1 ±1.2 | 214 ±18 | 1.4 ±0.4 |
| | 30 - 40 | 42.1 ±5.5 | 48.3 ±1.1 | 187 ±16 | 1.1 ±0.2 |
| | 40 - 50 | 35.6 ±5.3 | 43.9 ±1.0 | 138 ±14 | 0.3 ±0.1 |
| | 50 - 60 | 31.2 ±2.8 | 45.1 ±1.1 | 116 ±12 | 0.2 ±0.1 |
| | 60 - 70 | 25.7 ±2.3 | 43.7 ±1.1 | 114 ±12 | < 0.2 |
| | 70 - 80 | 24.1 ±2.0 | 42.6 ±1.0 | 100 ±10 | < 0.2 |
| | 80 - 90 | 20.3 ±1.5 | 40.3 ±1.0 | 79 ±8 | < 0.2 |
| | 90 - 100 | 18.4 ±1.5 | 41.8 ±1.0 | 56 ±6 | < 0.2 |
| Background sample | 0 - 10 | 177 ±15 | 63.4 ±1.6 | 238 ±19 | 5.6 ±0.9 |
| | 10 - 20 | 174 ±15 | 44.2 ±1.4 | 146 ±15 | 2.4 ±0.7 |
| | 20 - 30 | 61.4 ±8.2 | 47.5 ±1.1 | 124 ±13 | 1.7 ±0.5 |
| | 30 - 40 | 42.3 ±6.5 | 43.6 ±1.0 | 109 ±10 | 1.2 ±0.4 |
| | 40 - 50 | 33.7 ±5.3 | 41.4 ±1.0 | 104 ±10 | 0.3 ±0.1 |
| | 50 - 60 | 27.4 ±2.3 | 48.1 ±1.1 | 98 ±8 | 0.2 ±0.1 |
| | 60 - 70 | 23.9 ±2.1 | 39.3 ±1.0 | 96 ±8 | < 0.2 |
| | 70 - 80 | 22.7 ±1.9 | 41.2 ±1.0 | 89 ±6 | < 0.2 |
| | 80 - 90 | 20.1 ±1.9 | 38.9 ±1.0 | 73 ±6 | < 0.2 |
| | 90 - 100 | 19.4 ±1.5 | 39.7 ±0.9 | 52 ±4 | < 0.2 |

Wine chemical analysis are listed in table VIII. Alcohol content was determined by OIV's chemical method, anthocianins - by method of P. RIBÉREAU-GAYON et STONESTREET. Colour intensity and hue were determined by OIV's method (used for assessing the chromatic characteristics of red wines). These techniques were described in PRACTICAL HANDBOOK OF ENOLOGY by IVANOV *et al.* (1979).

To determine the heavy metal content in the samples, atomic absorption spectrophotometry (Perkin-Elmer 3030 B) was used. The working wave lengths were as follows: Cu - 324.8 nm; Zn - 213.9 nm; Pb - 217 nm; Cd - 228.8 nm. The calibration was performed using five aqueous standard solutions in 2 p. cent v/v $HNO_3$. A commercial multielement standard solution (Merck) with concentration 100 mg/l was used as a stock solution. The calibration standard solutions have the following concentrations: 0; 0.2; 0.5; 2.0 and 5.0 mg/l. The acidity of the standard and sample solutions was the same (2 p. cent v/v $HNO_3$).

## RESULTS AND DISCUSSION

I - SOILS

In the vineyards located at about 3.5 km from the NFMW, the heavy metal amounts in soil were considerably higher than those in the vineyards in non-polluted region. In the region of the NFMW - Plovdiv, the Pb amounts in the 0-10 cm layer varied from 68.9 to 74.8 mg/kg, Zn - from 243 to 249 mg/kg, Cd - from 1.8 to 2.5 mg/kg (table II), while in the surface layer of the vineyards of the non-polluted region the Pb content ranges from 18.2 to 21.3 mg/kg, Cu - from 67.5 to 70.3 mg/kg, Zn -from 72 to 75 mg/kg and Cd - from 0.8 to 1.3 mg/kg (table III). With increasing depth heavy metal contents decreased in both investigated regions. In the region of the NFMW - Plovdiv, in the deepest layer (90-100 cm) the Pb content reached no more than 18.4 mg/kg, Cu - 41.8 mg/kg, Zn - 67 mg/kg, Cd - 0.2 mg/kg. The amounts established in the region with industrial pollutants were most equal to those registered in the non-polluted region, but at a depth of 30-40 cm. Therefore it can be assumed that the lead-aerosol soil pollution in the region of the NFMW occurred to about 80 cm in depth. Obviously, the subsoiling practices at the preparation of soil for grapevine planting, as well as the later tillages contributed to the deeper penetration of this metal into the soil.

Pb tended to be somewhat higher in the interrows as compared to the samples taken from the rows. This is due to the fact that that zone is open, as well as small amounts of Pb may come from the equipment used for soil tillage, disease and pest control.

In the samples from the NFMW region, the Cu content of soil was a little bit higher than in the region with no industrial pollutants. No differences could be find out between samples from the rows and interrows. In the background samples in both investigated regions,

**TABLE III**
**Content of Pb, Cu, Zn and Cd (mg/kg) in vineyards of non-polluted region (Pleven)**
(x: average value from 5 repetitions; sd : mean standard deviation)

**Tableau III - Teneurs en Pb, Cu, Zn et Cd (mg/kg) des sols viticoles dans la zone non polluée de Pleven**
(x : moyenne des 5 répétitions ; sd : écart-type)

| Sample | Depth (cm) | Pb (x ± sd) | Cu (x ± sd) | Zn (x ± sd) | Cd (x ± sd) |
|---|---|---|---|---|---|
| Left intra row | 0 - 10 | 21.3 ± 4.0 | 68.6 ±1.4 | 73 ± 8 | 1.0 ±0.2 |
| | 10 - 20 | 17.3 ±2.0 | 56.4 ±1.4 | 73 ±8 | 0.7 ±0.2 |
| | 20 - 30 | 17.6 ±2.0 | 43.2 ±1.2 | 61 ±6 | 0.7 ±0.2 |
| | 30 - 40 | 16.3 ±1.8 | 27.1 ±0.8 | 60 ± 6 | 0.3 ±0.1 |
| Row | 0 - 10 | 18.2 ±2.1 | 67.5 ±1.4 | 72 ±7 | 0.8 ±0.2 |
| | 10 - 20 | 16.7 ±1.8 | 53.5 ±1.2 | 72 ±7 | 0.7 ±0.1 |
| | 20 - 30 | 16.7 ±1.8 | 46.7 ±1.0 | 72 ±7 | 0.6 ±0.2 |
| | 30 - 40 | 15.8 ±1.7 | 29.2 ±0.8 | 55 ±5 | 0.3 ±0.1 |
| Right intra row | 0 - 10 | 20.2 ±3.2 | 70.3 ±1.4 | 75 ±8 | 1.3 ±0.3 |
| | 10 - 20 | 16.2 ±1.8 | 57.2 ±1.2 | 73 ±8 | 0.7 ±0.2 |
| | 20 - 30 | 17.3 ±2.0 | 46.6 ±1.0 | 69 ±7 | 0.5 ±0.2 |
| | 30 - 40 | 14.3 ±1.6 | 30.2 ±0.9 | 54 ±5 | 0.3 ±0.1 |
| Background | 0 - 10 | 16.7 ±2.8 | 61.5 ±1.4 | 67 ±9 | 1.0 ±0.2 |
| | 10 - 20 | 16.9 ±2.9 | 51.7 ±1.4 | 65 ±6 | 0.7 ±0.2 |
| | 20 - 30 | 16.2 ±2.2 | 43.2 ±1.2 | 62 ±6 | 0.6 ±0.1 |
| | 30 - 40 | 15.2 ±1.9 | 27.3 ±0.8 | 57 ± 5 | 0.4 ±0.1 |

**TABLE IV**
**Content of Pb, Cu, Zn Cd (mg/kg) in grapes from cv. Mavrud, grown in an industrially polluted region**
**Tableau IV - Taux de Pb, Cu, Zn et Cd (mg/kg) déterminés dans les baies du cépage Mavrud provenant d'une région polluée**

| Year | Unwashed grape | | | | | | | | Washed grape | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Skins | | Pulp | | Seeds | | Stalks | | Skins | | Pulp | | Seeds | | Stalks | |
| | x± sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) |
| Pb (ALC - 0.4 mg/kg) | | | | | | | | | | | | | | | | |
| 1991 | 2.3± 0.3 | 13.0 | 1.1±0.2 | 18.2 | 2.6±0.3 | 11.5 | 31.7±4.2 | 13.2 | 1.5±0.2 | 13.3 | 1.0±0.2 | 20.0 | 2.5±0.3 | 32.0 | 20.4±2.7 | 13.2 |
| 1992 | 1.4±0.3 | 21.4 | 1.1±0.2 | 18.2 | 1.4±0.3 | 21.4 | 39.3±5.6 | 14.2 | 1.1±0.2 | 18.2 | 0.4±0.2 | 50.0 | 1.3±0.3 | 23.1 | 25.4±3.7 | 14.6 |
| 1993 | 0.9±0.1 | 11.1 | 0.6±0.1 | 16.7 | 2.6±0.3 | 11.5 | 21.7±2.4 | 11.1 | 0.5±0.1 | 20.0 | 0.5±0.1 | 20.0 | 2.6±0.3 | 11.5 | 16.8±1.2 | 14.6 |
| Average | 1.5±0.2 | 13.3 | 0.9±0.2 | 22.2 | 2.2±0.3 | 13.6 | 30.9±4.1 | 13.3 | 1.0±0.2 | 20.0 | 0.6±0.2 | 33.3 | 2.1±0.3 | 14.3 | 20.9±2.5 | 12.0 |
| Cu (ALC - 5.0 mg/kg) | | | | | | | | | | | | | | | | |
| 1991 | 4.8±0.2 | 4.2 | 2.1±0.2 | 9.5 | 16.6±0.3 | 1.8 | 60.9±1.4 | 2.3 | 3.1±0.2 | 6.5 | 1.4±0.2 | 14.3 | 16.4±0.3 | 1.8 | 41.2±1.0 | 2.4 |
| 1992 | 3.1±0.2 | 6.5 | 2.1±0.2 | 9.5 | 13.2±0.3 | 2.3 | 40.5±0.8 | 2.0 | 2.3±0.2 | 8.7 | 1.4±0.2 | 14.3 | 12.3±0.3 | 2.4 | 35.4±0.9 | 2.5 |
| 1993 | 3.7±0.2 | 5.4 | 2.1±0.2 | 9.5 | 16.6±0.3 | 1.8 | 46.8±0.8 | 1.7 | 2.5±0.2 | 8.0 | 1.4±0.2 | 14.3 | 16.1±0.3 | 1.9 | 28.4±0.4 | 1.4 |
| Average | 3.9±0.2 | 5.1 | 2.1±0.2 | 9.5 | 15.5±0.3 | 1.9 | 31.4±1.0 | 3.2 | 2.6±0.2 | 7.7 | 1.4±0.2 | 14.3 | 14.9±0.3 | 2.0 | 35.0±0.8 | 2.3 |
| Zn (ALC - 10 mg /kg) | | | | | | | | | | | | | | | | |
| 1991 | 4.2±0.4 | 9.5 | 2.2±0.3 | 13.6 | 14.7±1.3 | 8.8 | 54.7±4.7 | 8.6 | 3.3±0.3 | 9.1 | 2.2±0.3 | 13.6 | 14.2±1.3 | 9.2 | 35.8±3.1 | 8.7 |
| 1992 | 3.2±0.4 | 12.5 | 2.3±0.3 | 12.5 | 23.8±2.1 | 8.8 | 62.4±5.2 | 8.3 | 2.1±0.2 | 9.5 | 2.0±0.2 | 10.0 | 24.1±2.1 | 8.7 | 42.3±4.3 | 10.1 |
| 1993 | 1.9±0.1 | 5.3 | 1.1±0.1 | 9.1 | 21.6±2.2 | 10.2 | 55.2±4.2 | 7.6 | 1.4±0.1 | 7.1 | 1.0±0.1 | 10.0 | 23.1±2.2 | 9.5 | 45.2±3.5 | 7.7 |
| Average | 3.1±0.3 | 9.7 | 1.9±0.3 | 15.8 | 20.0±1.9 | 9.5 | 57.4±4.7 | 8.6 | 2.3±0.2 | 8.7 | 1.7±0.2 | 11.8 | 20.5±1.9 | 9.3 | 41.1±3.6 | 8.8 |
| Cd (ALC - 0.2 mg/kg) | | | | | | | | | | | | | | | | |
| 1991 | 0.2±0.1 | 50.0 | <0.2 | - | 0.2±0.1 | 50.0 | 0.5±0.1 | 20.0 | 0.2±0.1 | 50.0 | <0.2 | - | 0.2±0.1 | 50.0 | 0.3±0.1 | 33.3 |
| 1992 | 0.4±0.1 | 25.0 | <0.2 | - | 0.3±0.1 | 33.3 | 1.0±0.2 | 20.0 | 0.3±0.1 | 33.3 | <0.2 | - | 0.3±0.1 | 33.3 | 0.3±0.1 | 33.3 |
| 1993 | 0.2±0.1 | 50.0 | <0.2 | - | 0.3±0.1 | 33.3 | 0.4±0.1 | 25.0 | <0.2 | - | <0.2 | - | 0.3±0.1 | 33.3 | 0.3±01 | 33.3 |
| Average | 0.3±0.1 | 33.3 | <0.2 | - | 0.3±0.1 | 33.3 | 0.6±0.1 | 16.7 | 0.2±0.1 | 50.0 | <0.2 | - | 0.3±0.1 | 33.3 | 0.3±0.1 | 33.3 |

x : average value (mg/kg) from 5 repetitions; sd: mean standard deviation; n.d. - non detected; ALC: admissible limit of concentration (approved for Bulgaria)
x: moyenne de 5 répétitions ; sd: écart type ; n.d.:non determiné; ALC: concentration autorisée en Bulgarie

**TABLE V**

**Content of Pb, Cu, Zn Cd (mg/kg) in grapes from cv. Mavrud, grown in an non-industrially polluted region**

**Tableau V - Taux de Pb, Cu, Zn et Cd (mg/kg) déterminés dans les baies du cépage Mavrud provenant d'une région non polluée**

| Year | Unwashed grape | | | | | | | | Washed grape | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Skins | | Pulp | | Seeds | | Stalks | | Skins | | Pulp | | Seeds | | Stalks | |
| | x± sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) | x±sd (mg/kg) | c.v. (%) |
| Pb (ALC - 0.4 mg/kg) | | | | | | | | | | | | | | | | |
| 1992 | 0.6±0.1 | 16.7 | n.d. | - | 1.8±0.2 | 11.1 | 6.6±0.4 | 6.1 | 0.5±0.1 | 20.0 | n.d. | - | 1.8±0.2 | 11.1 | 5.8±0.3 | 5.2 |
| 1993 | 0.6±0.1 | 16.7 | n.d. | - | 1.4±0.3 | 21.4 | 5.7±0.4 | 7.0 | 0.5±0.1 | 20.0 | n.d. | - | 1.4±0.2 | 21.4 | 4.9±0.4 | 8.2 |
| Average | 0.6±0.1 | 16.7 | n.d. | - | 1.6±0.3 | 18.8 | 6.2±0.4 | 6.5 | 0.5±0.1 | 20.0 | n.d. | - | 1.6±0.3 | 18.8 | 5.4±0.4 | 7.4 |
| Cu (ALC - 5.0 mg/kg) | | | | | | | | | | | | | | | | |
| 1992 | 2.3±0.2 | 8.7 | 0,8±0.1 | 12.5 | 4.5±0.3 | 6.7 | 34.8±0.8 | 2.3 | 1.9±0.2 | 10.5 | 0.8±0.1 | 12.5 | 4.5±0.3 | 7.3 | 26.7±0.4 | 1.4 |
| 1993 | 2.4±0.2 | 8.3 | 0,3±0.1 | 33.3 | 4.2±0.3 | 7.1 | 34.8±0.8 | 2.3 | 1.9±0.2 | 10.5 | 0.3±0.1 | 33.3 | 4.1±0.3 | 7.3 | 28.8±0.4 | 1.4 |
| Average | 2.4±0.2 | 8.3 | 0,6±0.1 | 16.7 | 4.4±0.3 | 6.8 | 34.8±0.8 | 2.3 | 1.9±0.2 | 10.5 | 0.6±0.1 | 16.7 | 4.3±0.3 | 7.0 | 27.8±0.8 | 1.4 |
| Zn (ALC - 10 mg /kg) | | | | | | | | | | | | | | | | |
| 1992 | 3.7±0.1 | 2.7 | 0.6±0. | 16.7 | 4.2±0,2 | 4.8 | 28.2±0,5 | 1.8 | 3.0±0.1 | 3.3 | 0.6±0.1 | 16.7 | 4.2±0.2 | 4.8 | 23.4±0.5 | 2.1 |
| 1993 | 3.4±0.3 | 8.8 | 0.5±0. | 20.0 | 4.0±0,2 | 5.0 | 27.5±0.5 | 1.8 | 3.0±0.1 | 3.3 | 0.5±0.1 | 20.0 | 3.9±0.2 | 5.1 | 23.5±0.5 | 2.1 |
| Average | 3.6±0.2 | 5.6 | 0.6±0. | 16.7 | 4.1±0,2 | 4.9 | 27.9±0.5 | 1.8 | 3.0±0.1 | 3.3 | 0.6±0.1 | 16.7 | 4.1±0.2 | 4.9 | 23.5±0.5 | 2.1 |
| Cd (ALC - 0.2 mg/kg) | | | | | | | | | | | | | | | | |
| 1992 | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - |
| 1993 | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - |
| Average | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - | n.d. | - |

x : average value (mg/kg) from 5 repetitions; sd: mean standard deviation; n.d. - non detected; ALC: admissible limit of concentration (approved for Bulgaria)
x: moyenne de 5 répétitions; sd: écart type; n.d.:non determine; ALC: concentration autorisée en Bulgarie



*J. Int. Sci. Vigne Vin*, 1999, **33**, n°3, 119-131

lesser amount of copper were found. This may be due to the fact that no copper-containing preparations for disease control were used. With increase of soil depth the Cu content decreased.

In the background samples from the NFMW region from the surface 0-10 cm layer, significantly higher Pb and Cd values were registered - up to 177 and 5.6 mg/kg soil, respectively (table II). This was obviously due to the fact that this terrain was open, not cultivated and considerable amounts of these metals were accumulated via aerosols in the 0-10 cm horizon. With the increase of depth their content sharply decreased. In the 20-30 cm layer Pb and Cd reached 61.4 and 1.7 mg/kg, respectively, comparable to the surface layers of the vineyards. No significant differences in the Cd and Zn content between the vineyard and background samples were established.

II - GRAPE

The data obtained demonstrated that most heavy metals were accumulated in the grape stalks. In cv. Mavrud Pb varied from 21.7 to 39.3 mg/kg, Cu - from 40.5 to 60.9 mg/kg, Zn - from 54.7 to 62.4 mg/kg, Cd - from 0.4 to 1.0 mg/kg (table IV). Heavy metal amounts in the berry seeds ranked in the second place. Pb varied from 1.4 to 2.6 mg/kg, Cu from 13.2 to 16.6 mg/kg, Zn from 14.7 to 23.8 mg/kg and Cd from 0.2 to 0.3 mg/kg. The different heavy metal amounts occurring in the region via aerosols did not exert any significant effect on the Pb, Cu, Zn and Cd accumulation in the seeds, which remained almost the same in the separate years. In the berry skins, changes in the Pb content during the three years of investigation ranged from 0.9 to 2.3 mg/kg, Cu - from 3.1 to 4.8 mg/kg, Zn - from 1.9 to 4.2 mg/kg, Cd - from 0.2 to 0.4 mg/kg. Differences in the overall weather conditions, as well as in the course and intensity of the production processes of the NFMW - Plovdiv contributed to the changes in the Pb, Zn and Cd amounts in the berry skins of the tested cultivar.

The lowest heavy metal contents was established in the berry pulp - from 0.6 to 1.1 mg/kg Pb, from 1.1 to 2.4 mg/kg Zn, 2.1 mg/kg Cu and <0.2 mg/kg Cd. This proves that even under conditions of high soil Pb, Zn and Cd (table II), the grapevines of the tested cultivars did not show any tendency to accumulate them in the berry pulp. This is in agreement with the finding of LAMAND (1981) and TEISSEDRE *et al.* (1994) who state mineral elements are stored in the storage tissues, like seeds. The variance of data matches well with those from FAVRETTO *et al.* (1986), who found increasing contents of Pb in the order: skins, pulp and seeds.

Grape from the same experimental vines were rinsed with water for 3 minutes. After drying, they were analyzed for heavy metal content. During all years the rinsing of the grapes with water considerably decreased the heavy metal contents. In the skins, Pb in an average decreased by 50 p. cent, with distinct variation in separate years. Obviously, some of the aerosol pollutants adhered mechanically to the berries while others bound chemically with the bloom and could not be washed with water. After this cleaning process, the Pb content in the berry skins varied during the years from 0.5 to 1.5 mg/kg (table IV). Despite this treatment the Pb content in the berry skins of cv. Mavrud was still over the maximum admissible level of 0.4 mg/kg. A similar change in the skin was observed for Cu, Zn and Cd. After rinsing they were reduced for about 30 p. cent.

After washing with water, in spite of the considerable decrease, the highest heavy metal amounts were registered again in the stalks. Probably, part of the heavy metals may be taken by plants and accumulated in the stalks and the washing procedure can only decontaminate those which are deposited on the surface, hence the content of the stalks stay high. The decrease of Pb ranged from 25 to 35 p. cent, Cu - from 10 to 35 p. cent, Zn - from 20 to 40 p. cent. Cd decreased for 66 p. cent.

No changes of the heavy metal contents in the berry pulp and seeds could be detected after rinsing the grapes with water. This is easily to explain because water cannot penetrate through skins and stalkses.

The results obtained for the heavy metal contents in the grape of cv. Mavrud, grown in the regions with and without industrial pollutants (Plovdiv and Pleven), showed significant differences. Their content was considerably higher in the grapes from vines grown in the vicinity of the NFMW - Plovdiv. Significant differences were detected mainly for those grape parts that were subjected to aerosol pollution (skins and stalks). In the berry pulp and seeds, these differences were insignificant.

The heavy metal content in the samples from the non-polluted region varied in comparatively smaller ranges. Although no direct pollutant was available in the region of Pleven some heavy metals were detected on the berry skins. In the berry skins from cv. Mavrud, Pb was 0.6 mg/kg, that of Cu - 2.4 mg/kg, and of Zn - 3.4 - 4.3 mg/kg (table V). After rinsing with water, the heavy metal contents decreased. Their content in the pulp was significantly lower than in the skins. The amount of Zn varied from 0.5 to 0.6 mg/kg and that of Cu - from 0.3 to 0.8 mg/kg. Neither Pb nor Cd was detected. Significantly larger amounts of heavy metals were proved in the seeds in comparison to skins and pulp. Probably the grapevine plants were prone to the accumulation of definite heavy metal amounts in the berry seeds. It was found out that the heavy metal contents in the seeds ranged from 1.4 to 1.8 mg/kg for Pb, from 4.2 mg/kg to 4.5 mg/kg for Cu and from 4.0 mg/kg to 4.2 mg/kg for Zn in cv. Mavrud. The highest heavy metal amounts in the grapes from the non - polluted region were again found in their stalks, these amounts exceeding several times the contents found in the other grape parts. The Cu content was 34.8 mg/kg. Similar results were obtained for Pb - 5.7 - 6.6 mg/kg. More significant differences were detected with respect to Zn - 28.2 - 27.5 mg/kg. Water treatment of grapes led also to a significant decrease in the heavy metal amounts of stalks.

## III - WINE

At the separating the berries from stalks and crushing the berries, some of the heavy metals together with grape juice pass into the mash. The pre-washing of grapes led to an average decrease of about 40 p. cent in the heavy metal amounts in berry skins in result of which a large part of them is prevented from passing into the mash. During the alcoholic fermentation and the following vinification steps, most of the lead contained in the must is removed. This process is affected by chemical (alchohol content, must acidity) and physical parameters (temperature). This agrees with recent work by TEISSEDRE *et al.* (1993a) who reported that 40 to 80 p. cent was removed during fermentation. MOHR (1979) attributes the removal the lead to the reaction of lead with $H_2S$ which is performed during the yeast fermentation. Lead sulphid* precipitates is adsorbed to the yeast, and removed with the yeast lees.

The data in table VI show that the water washing of grapes leads to a significant decrease in the heavy metal amounts in wine and sediments. The Pb content in the wine produced from non-washed grapes was from 0.60 to 0.80 mg/l, while in the wine from washed grapes, it decreased significantly - 0.4 mg/l. The same tendency was also observed in the sediments. After washing of grapes, the Pb content in the sediment was in the range of 1.1 to 1.9 mg/kg, while in the variant with no washing it was from 1.7 to 2.6 mg/kg. When comparing the Pb amounts in the berry pulp (table IV), the wine and the sediment (table VI), it was established that its value in the wine was always less than that in the pulp, but the content in the sediment was significantly higher. These differences are due to the sedimentation of part of Pb during the fermentation, in result of which less amounts of Pb remain in the wine. The significant increase of Pb in the sediment, as compared to that in the berry pulp, is obviously due to the

**TABLE VI**
**Content of Pb, Cu, Zn Cd in wine (mg/l) and sediment(mg/kg) from cv. Mavrud, grown in an industrially polluted region**

**Tableau VI - Taux de Pb, Cu, Zn et Cd (mg/kg) déterminé dans le vin du cépage Mavrud provenant d'une région polluée**

| Element | Year | Variants | | | | | | | | ALC |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Unwashed grape | | | | Washed grape | | | | |
| | | wine | | sediment | | wine | | sediment | | |
| | | x±sd | c.v. | | c.v. | x±sd | c.v. | x±sd | c.v. | |
| Pb | 1991 | 0.80±0.03 | 3.8 | 2.5±0.3 | 12.0 | 0.40±0.03 | 7.5 | 1.4±0.2 | 14.3 | 0.3 |
| | 1992 | 0.60±0.03 | 5.0 | 1.7±0.2 | 11.8 | 0.40±0.03 | 7.5 | 1.1±0.2 | 18.2 | |
| | 1993 | 0.60±0.03 | 5.0 | 2.6±0.3 | 11.5 | 0.40±0.03 | 7.5 | 1.9±0.2 | 10.5 | |
| | average | 0.67±0.03 | 4.5 | 2.3±0.3 | 13.0 | 0.40±0.03 | 7.5 | 1.5±0.2 | 13.3 | |
| Cu | 1991 | 0.60±0.04 | 6.7 | 14.2±0.4 | 2.8 | 0.40±0.04 | 10.0 | 8.2±0.3 | 3.7 | 5.0 |
| | 1992 | 0.31±0.04 | 12.9 | 13.3±0.4 | 3.0 | 0.18±0.04 | 22.2 | 8.5±0.3 | 3.5 | |
| | 1993 | 0.44±0.04 | 9.1 | 13.0±0.4 | 3.1 | 0.22±0.04 | 18.2 | 7.5±0.3 | 4.0 | |
| | Average | 0.45±0.04 | 8.9 | 13.5±0.4 | 3.1 | 0.27±0.04 | 14.8 | 8.1±0.3 | 3.7 | |
| Zn | 1991 | 0.90±0.03 | 3.3 | 12.3±0.3 | 2.4 | 0.80±0.03 | 3.8 | 8.9±0.3 | 3.4 | 10.0 |
| | 1992 | 1.20±0.03 | 2.5 | 17.3±0.3 | 1.7 | 0.70±0.03 | 4.3 | 10.3±0.4 | 3.9 | |
| | 1993 | 1.00±0.03 | 3.0 | 17.2±0.2 | 1.2 | 0.93±0.03 | 3.2 | 6.3±0.3 | 4.2 | |
| | Average | 1.0±0.03 | 3.0 | 15.6±0.3 | 1.9 | 0..81±0.03 | 3.7 | 8.5±0.3 | 3.5 | |
| Cd | 1991 | 0.02±0.002 | 10.0 | 0.08±0.002 | 2.5 | 0.003±0.001 | 33.3 | 0.04±0.002 | 5.0 | |
| | 1992 | 0.01±0.002 | 20.0 | 0.30±0.002 | 0.7 | 0.006±0.002 | 33.3 | 0.20±0.001 | 0.5 | |
| | 1993 | 0.01±0.002 | 20.0 | 0.09±0.002 | 2.2 | n.d | - | 0.07±0.001 | 1.4 | |
| | Average | 0.01±0.002 | 20.0 | 0.16±0.002 | 1.3 | 0.005±0.001 | 20.0 | 0.10±0.001 | 1.0 | |

x: average value (mg/kg) from 5 repetitions; sd: mean standard deviation; n.d.: non detected; ALC: admissible limit of concentration (approved for Bulgaria)
x : moyenne de 5 répétitions ; sd : écart type ; n.d. : non determiné ; ALC : concentration autorisée en Bulgarie

sedimentation of part of it through the berry skins, too. Though the Pb content in the wine was less in comparison with that in the pulp, its amount was higher than the ultimate concentrations allowed even if after initial water washing of grapes (ALC - 0.3 mg/l).

The levels of Pb detected in the wine samples from industrially polluted region are generally much higher than those reported by several authors in other countries (TEISSEDRE et CABANIS, 1993b ; BOLLETTI *et al.*, 1994 ; BULINSKI *et al.,* 1995 ; MENA *et al.*, 1996, ROSES *et al.,* 1997.

The amount of Cu in the wine produced from grapes without washing was about 2 times higher than that in the « washed grape » variant. The same tendency was also observed in the sediments. If compared to the lead, however, here the difference between the Cu amounts in the wine and the sediments was significantly bigger.

It is seen from the results obtained in table IV and table VI, that the Cu content in the wine is less than that in the berry pulp. Its significant concentration in the wine sediments, however, allows our assertion that the main part of the copper, present in the berry pulp and skins, precipitates also during fermentation. For these reasons, in both variants the content of that metal in the wine produced is under the ultimate concentrations allowed (ALC - 5.0 mg/l), i.e. from 0.33 to 0.60 mg/l.

The levels of Cu in wine are similar to those detected by LAY et LIEB (1988), DUCANOVIC *et al.* (1992), BOLLETI *et al.* (1994) and BULINSKI *et al.* (1995).

The water washing of grapes led to some reduction in the Zn amounts of wine, but the differences between the two variants were not so great as those in Pb and Cu. In the unwashed grapes, the content of Zn in the wine was from 0.90 to 1.20 mg/l, while that in the washed grapes - from 0.70 to 0.93 mg/l. Considerably more Zn was found in the sediment: from 7.2 to 17.3 mg/l in the unwashed grapes and from 6.3 to 10.3 mg/kg in the washed grapes. The difference between the wine and the sediments with respect to Zn was from 6 to 14 times, while that with the berry pulp - from 2 to 3 times. Obviously, a significant part of that metal, contained in the berry pulp and skins, was sedimented, too. In result of that in all three years of investigation, the Zn content in the wine in both variants was significantly lower than the ultimate concentrations allowed (ALC - 5.0 mg/l), i.e. from 0.70 to 1.20 mg/l.

The levels of Zn in wine are similar to those detected by LAY et LIEB (1988), BOLLETI *et al.* (1994) and BULINSKI *et al.* (1995).

**TABLE VII**
**Content of Pb, Cu, Zn Cd in wine (mg/l) and sediment(mg/kg) from cv. Mavrud, grown in an non-industrially polluted region**

**Tableau VII - Taux de Pb, Cu, Zn et Cd (mg/kg) déterminé dans le vin du cépage Mavrud provenant d'une région non-polluée**

| Element | Year | Variants | | | | | | | | ALC |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Unwashed grape | | | | Washed grape | | | | |
| | | wine | | sediment | | wine | | sediment | | |
| | | x±sd | c.v. | x±sd | c.v. | x±sd | c.v. | x±sd | c.v. | |
| Pb | 1991 | 0.17±0.03 | 17.6 | 1.00±0.02 | 2.0 | 0.09±0.002 | 2.2 | 0.70±0.03 | 4.3 | 0.3 |
| | 1992 | 0.12±0.03 | 25.0 | 0.80±0.02 | 2.5 | 0.06±0.002 | 3.3 | 0.55±0.03 | 5.5 | |
| | average | 0.15±0.03 | 20.0 | 0.90±0.02 | 2.2 | 0.075±0.002 | 2.7 | 0.60±0.03 | 5.0 | |
| Cu | 1991 | 0.29±0.04 | 13.8 | 5.7±0.2 | 3.5 | 0.17±0.04 | 23.3 | 3.5±0.2 | 5.7 | 5.0 |
| | 1992 | 0.32±0.04 | 12.5 | 5.9±0.2 | 3.4 | 0.20±0.04 | 20.0 | 3.9±0.2 | 5.1 | |
| | Average | 0.31±0.04 | 12.9 | 5.8±0.2 | 3.4 | 0.19±0.04 | 21.1 | 3.7±0.2 | 5.4 | |
| Zn | 1991 | 0.82±0.03 | 3.7 | 6.3±0.3 | 4.8 | 0.56±0.03 | 5.4 | 4.0±0.3 | 7.5 | 10.0 |
| | 1992 | 1.00±0.03 | 3.0 | 6.6±0.3 | 4.5 | 0.55±0.03 | 5.5 | 4.4±0.3 | 6.8 | |
| | Average | 0.91±0.03 | 3.3 | 6.5±0.3 | 4.6 | 0.56±0.03 | 5.4 | 4.2±0.3 | 7.1 | |
| Cd | 1991 | n.d | - | 0.02±0.001 | 5.0 | n.d | - | n.d | - | |
| | 1992 | n.d | - | 0.02±0.001 | 5.0 | n.d | - | n.d | - | |
| | Average | n.d | - | 0.02±0.001 | 5.0 | n.d | - | n.d | - | |

x: average value (mg/kg) from 5 repetitions; sd: mean standard deviation; n.d.: non detected; ALC: admissible limit of concentration (approved for Bulgaria)
x : moyenne de 5 répetitions ; sd : écart type ; n.d. :non déterminé; ALC : concentration autorisée en Bulgarie

In Cd, the same tendency as that in the rest of the elements was observed. Water washing of grapes led to decrease in its amount in the wine and sediments. Its content in the wine produced from the variant without washing was from 0.01 to 0.02 mg/l, while in the variant with washing the maximum recorded amount was 0.006 mg/l. In both cases, it was lower than that in the berry pulp for the reasons given so far. In the sediments, significantly higher Cd amounts (from 0.04 to 0.20 mg/kg) were established.

**TABLE VIII**
**Wine analysis (1992 and 1993)**

**Tableau VIII - Analyses des vins (1992 et 1993)**

| Indexes | Year | Wine, produced from unwashed grape | Wine, produced from washed grape |
|---|---|---|---|
| Relative density | 1992 | 0.9939 | 0.9943 |
| | 1993 | 0.9933 | 0.9933 |
| | Average | 0.9936 | 0.9938 |
| Alcohol (vol. %) | 1992 | 12.5 | 12.4 |
| | 1993 | 13.1 | 13.2 |
| | Average | 12.8 | 12.8 |
| Total extract ($g/dm^3$) | 1992 | 26.73 | 27.23 |
| | 1993 | 26.73 | 27.23 |
| | Average | 26.73 | 27.23 |
| Sugar content ($g/dm^3$) | 1992 | 1.81 | 2.12 |
| | 1993 | 3.79 | 3.44 |
| | Average | 2.80 | 2.78 |
| Without sugar extract ($g/dm^3$) | 1992 | 24.92 | 25.11 |
| | 1993 | 22.94 | 23.79 |
| | Average | 23.93 | 24.45 |
| Titrable acids content ($g/dm^3$) (g tartaric acids/$dm^3$) | 1992 | 5.7 | 5.4 |
| | 1993 | 6.2 | 6.2 |
| | Average | 5.95 | 5.8 |
| Volatile acids content ($g/dm^3$) | 1992 | 0.44 | 0.34 |
| | 1993 | 0.51 | 0.74 |
| | Average | 0.48 | 0.54 |
| Free $SO_2$ ($mg/dm^3$) | 1992 | 5.76 | 5.76 |
| | 1993 | 8.64 | 8.64 |
| | Average | 7.20 | 7.20 |
| Total $SO_2$ ($mg/dm^3$) | 1992 | 57.60 | 79.49 |
| | 1993 | 129.00 | 163.58 |
| | Average | 93.30 | 121.54 |
| Anthocyanins, ($mg/dm^3$) | 1992 | 138.75 | 146.25 |
| | 1993 | 120.00 | 135.00 |
| | Average | 129.38 | 140.63 |
| Colour intensity | 1992 | 6.0 | 6.7 |
| | 1993 | 3.6 | 3.7 |
| | Average | 4.8 | 5.2 |
| Hue | 1992 | 0.875 | 0.811 |
| | 1993 | 0.741 | 0.698 |
| | Average | 0.808 | 0.755 |
| pH | 1992 | 3.5 | 3.5 |
| | 1993 | 3.7 | 3.7 |
| | Average | 3.6 | 3.6 |

The levels of Cd detected in the wine samples from industrially polluted region are generally much higher than those reported by several authors in other countries (DANILATOS et SALACHA-MONTSOPOULOU (1983), BULINSKI *et al.* (1995), MENA *et al.* (1996).

In the wine from the non-polluted region, produced without grape washing, the Pb content varied from 0.17 mg/l for 1992 to 0.12 mg/l for 1993. After water washing of grapes, the Pb content decreased to 0.06 mg/l. A similar tendency was observed in Cu and Zn. The copper amount in the wine, produced without washing of grapes, was from 0.29 to 0.32 mg/l, or about two times higher than that in the washed grape variant (from 0.17 to 0.20 mg/l). The content of Zn in the unwashed grape variant was from 0.81 to 1.0 mg/l, as against 0.55 mg/l in the washed one. No Cd was found in the investigated wines from the non-polluted region (table VII).

The pre-washing of grapes does not lead to any quality deterioration in the wine produced. On the basis of the results obtained from the wine chemical analysis it was established that no significant differences existed in the wines produced (table VIII).

## CONCLUSIONS

On the basis of the results obtained for the heavy metal amounts in soils, grapes and wine from cv. Mavrud, grown in the regions with and without pollutants (Plovdiv and Pleven), the following conclusions can be made:

1. In the vineyards located in a distance of about 3.5 km from the Non - Ferrous Metal Factory (NFMW) the heavy metal contamination of soils is considerably higher than in comparable vineyards near Pleven (non-polluted area). In the surface soil layer the respective differences in terms of Pb are 10 times, Zn - 4 times and Cd - 2 times higher. The copper values are almost equal.

Differences between the contents of Pb and Cd in the surface soil layers of the vineyard and the background samples were established. The Pb and Cd contents in the background samples were about 3 times higher than those taken from the vineyards, due to the fact that the background soil was completely open and allowed the continuous accumulation of new Pb and Cd immissions from the NFMW. Bellow the 70-80 cm soil horizon, the differences between background samples and vineyards disappear. The values for Pb, Cu and Cd at depths of 70-80 cm, 80-90 cm and 90-100 cm remaining almost the same and correspond to the respective values of the 30-40 cm horizon in the non-polluted region of Pleven. Probably, deep ploughing as preplanting operation as well as the permanent tilling operation during the growing cycle may contribute to the deep penetration of heavy metals, spread as aerosols in the NFMW region.

2. A selective heavy metal accumulation was found for the different grape parts. Most of them are localized in the stalks, less in the berry skins and seeds and least in the pulp. Significant differences in the heavy metal amounts of grapes were established between the two regions in terms of berry skins and stalks. As for the pulp and seeds, the differences were small despite great differences in the heavy metal amounts of soil.

3. Rinsing the grapes with water led to an average decrease of about 40 p. cent in the heavy metal amounts of stalks and berry skins. This shows that part of them have not been assimilated by soil and have fallen upon the berry skin by aerosols.

4. Most of the heavy metals in the berry pulp and skins precipitate during fermentation into the sediments, which is the reason for their significantly lower content in the wine.

5. Water washing of grape before processing leads to about 2 time decrease in the Pb, Cu, Zn and Cd contents of wine. The pre-washing of grapes does not lead to any quality deterioration in the wine produced.

6. The amounts of Cu, Zn and Cd in the wine from cv. Mavrud, grown in the region of the NFMW (village of Brestnik), are lower than the maximum admissible levels, while the Pb content exceeds them about two times.

## REFERENCES


ANONIMOUS, 1989. *Bulletin for the Environmental Pollution in Bulgaria*. Centre for Ecological monitoring at Ministry of Environment, Sofia.

ANONIMOUS, 1990. *Bulletin for the Environmental Pollution in Bulgaria*. Centre for Ecological monitoring at Ministry of Environment, Sofia.

ANONIMOUS, 1990. *Ecological problems and outlooks of lead-zinc production in Bulgaria*, Sofia.

BANER K.H., HINKEL S., NEEB R., EICHLER P. and ESCHNANER H.R., 1994. Gehalte von Fe, Cu, Zn, Mn und Al in deutschen weinen. Simultanbestimmung mit ICP - OES. *Wein - wissenschaft.*, **49**, 209-214.

BOLLETTI P., MENCI L. and TELLINI L., 1994. Lead, copper and zinc in wines. Study of local production in the Arezzo Province. *Industrie delle Bervande*, **23**, 327-328.

BRENDEL G., HOFER M. and ROMER T., 1989. Investigations on the influence of copper-containing vine protection products on biological effects, aging and copper residues in musts and wines of the vintage 1986. *Deutsch Weinbau-Jahrbuch*, **40**, 211-222.

BULINSKI R., WYSZOGRODZKA-KOMA L. and MARZEC Z., 1995. Determination of metal levels in cocktails, grape and fruit wine. *Bromatol. Chem. Toksykol.*, **28**, 273-257.

DANILATOS N. and SALACHA-MONTSOPOULOU M., 1983. Amount of trace elements in greek wines. 1.Lead-cadmium. *Hellenica-Oenologica Chronica*, **3**, 85-99.

DICANOVIC - LUCAY Z., PALIC A., VAHCIC N. and GACIC M., 1992. Copper and iron content in wines. *Riv. Vitic. Enol.*, **45**, 29-33.

FAVRETTO L., MARASPIN M. and VOJNOVIC D., 1986. Indagine sullinquinamento da carmio e piombo dei chicchi d,uva del vino. *Riv. Soc. Ital. Sci. Aliment*, **15**, 63-68.

GARTEL W., 1987. Heavy metals in pesticides as impurties - their importance as contaminants of wine. *Nachrictenblatt des Deutschen Pflanzenschutzdienstes,* **36**, 154-157.

HENICK-KLING T. and STOEWSAND G.S., 1993. Lead in wine. *Am. J. Enol. Vitic.*, **44**, 459-463.

IVANOV T., GEROV S., JANKOV, A., BAMBALOV G., TONCHEV T., NACHKOV D. and MARINOV M., 1979. *Practical handbook of Enology*, Plovdiv, 925 p.

LAMAND M., 1981. *Le rapport sol - plante - pathologie animale. Milieu, pathologie et prévention chez les ruminants*, 111-121, INRA Pub.

LAY H. and LIEB W., 1988. The contents of zinc, cadmium, lead and copper in the must, wine and by-products of wine making. *Wein-Wissenshaft*, **43**, 107-115.

MAGALHAES M.J., SEQUEIRA E.M. and LUCAS M.D., 1985. Copper and zinc in vineyards of Central Portugal. *Water, Air and Soil pollution*, **26**, 1-17.

MENA C.M., CABRERA C., LORENZO M.L. and LOPEZ M.C., 1996. Cadmium levels in wine, beer and other alcoholic beverages: possible sources of contamination. *Sci. Total Environment*, **181**, 201-208.

MENA C.M., CABRERA C., LORENZO M.L. and LOPEZ M.C., 1997. Determination of lead contamination in Spanish alcohol beverages by flow injection atomic absorption spectrometry. *J. Agric. Food Chemistry*, **45**, 1812 -1815.

MOHR H.D., 1979. Untersuchungen zum Verbleib von Schwermetallen, die Traubenmost zugesetzt wurden, nach Ablauf der Garung. *Weinberg. Keller*, **26**, 277 - 288.

O.I.V., 1990. *Recueil des méthodes internationales d'analyse des vins et des moûts*. O.I.V, Paris

RENAN L.,1994. Effect of long term aplications of copper on soil and grape copper (*Vitis vinifera*). *Can. J. Soil Sci*., **74**, 345-347.

ROSES O.E., GONZALEZ D.E., LOPEZ C.M., PINEIRO A.E and VILLAAMIL E.C., 1997. Lead levels in Argentin market wines. *Bull. Environmental Contam. Toxicology*, **59**, 210-215.

SHERLOCK J.C., PICKFORD C.J. and WHILE G.F.1986. Lead in alcoholic beverages. *Food Addit. Contam*., **3**, 347-354.

TEISSEDRE P.L., CABANIS M.T., DANMAS F. and CABANIS G.S., 1993a. Evolution of lead content during winemaking of cotes and phone and phone valley wines. *Rev. Fr. Œnol*., **33**, 6-18.

TEISSEDRE P.L. and CABANIS M.T., 1993b. Origin and reduction of lead content of wines. *Rev. Fr. Œnol*., **33**, 41-44.

TEISSEDRE P.L., CABANIS M.T., CHAMPAGNOL F. and CABANIS G.S., 1994. Lead distribution in grape berries. *Am. J. Enol. Vitic*., **45**, 220 -226.

TURIN I. V., 1965. To the method of analysis for comparative study of the soil humus composition. *Annual Book of Soil Science Institute V. V. Dokuchaev*, **38**, Moscow.

WHO., 1972. Joint FAO/WHO expert committee on food additives. *WHO technical report series 50*5, WHO, Geneva 13.

Reçu le 30 mars 1998 ; révisé le 24 juin 1999
accepté pour publication le 19 août 1999