



HAL
open science

Statistical study of the influence of fungicides treatments (mancozeb, zoxamide and copper oxychloride) on heavy metals concentration in Sicilian red wines.

Lara La Pera, Giacomo Dugo, Rossana Rando, Giuseppa Di Bella, Roberta Maisano, Francesco Salvo

► To cite this version:

Lara La Pera, Giacomo Dugo, Rossana Rando, Giuseppa Di Bella, Roberta Maisano, et al.. Statistical study of the influence of fungicides treatments (mancozeb, zoxamide and copper oxychloride) on heavy metals concentration in Sicilian red wines.. Food Additives and Contaminants, 2008, 25 (03), pp.302-313. 10.1080/02652030701329603 . hal-00577453

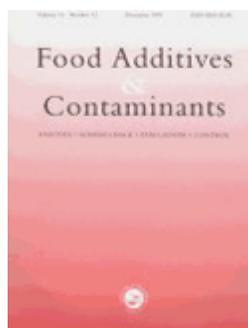
HAL Id: hal-00577453

<https://hal.science/hal-00577453>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Statistical study of the influence of fungicides treatments (mancozeb, zoxamide and copper oxychloride) on heavy metals concentration in Sicilian red wines.

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2006-269.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	06-Mar-2007
Complete List of Authors:	La Pera, Lara; University of Messina, Food and Environmental Science Dugo, Giacomo; University of Messina, Food and Environmental Science Rando, Rossana; University of Messina, Food and Environmental Science Di Bella, Giuseppa; University of Messina, Dept. of Veterinary Public Health Maisano, Roberta; University of Messina, Theoric Physic Salvo, Francesco; University of Messina, Food and Environmental Science
Methods/Techniques:	Stripping chronopotentiometry, Statistical analysis
Additives/Contaminants:	fungicides, Heavy metals
Food Types:	Grape, Wine

SCHOLARONE™
Manuscripts

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1 **Statistical study of the influence of fungicide treatments (mancozeb, zoxamide**
2 **and copper oxychloride) on heavy metals concentrations in Sicilian red wine**

3
4
5
6
7
8
9
10
11 5 Lara La Pera ¹, Giacomo Dugo ^{§1}, Rossana Rando ¹, Giuseppa Di Bella ², Roberta Maisano ³ and
12 6 Francesco Salvo ¹

13
14
15
16
17 8
18
19 9 ¹ *Dept. of Food and Environmental Science, University of Messina, Salita Sperone 31, 98166*
20
21 10 *Messina, Italy.*

22
23
24 11 ² *Dept. of Veterinary Public Health, University of Messina, V.le SS. Annunziata, 98165 Messina,*
25
26 12 *Italy*

27
28 13 ³ *Dept. of Theoretical Physics, University of Messina, Salita Sperone 31, 98166 Messina, Italy.*
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

The purpose of this work was to assess the influence of mancozeb, zoxamide and copper oxychloride fungicide treatments on Mn, Zn, Cu, Cd and Pb concentrations in Sicilian red wines, grapes, marcs and grape stalks; the experimentation was carried out over two crop years, 2003 and 2004. Trace metals analysis was performed by derivative stripping chronopotentiometry which allowed detection of concentrations lower than 1 ng g^{-1} . The data which was obtained gave evidence that the levels of Mn and Zn in wines from plots treated with zoxamide-mancozeb were about 3-fold higher than those observed in the control. Wines treated with Cu oxychloride had a significant increase in Cu (II) concentrations with respect to the control; in particular samples from 2004 showed a 50% increase in Cu levels. Furthermore, as shown in a previous paper, the fungicides treatments which were studied led to a moderate increase in Pb (II) and Cd (II) levels in treated samples with respect to the control. Wines from 2004 had higher Cu and Pb amounts than wines from 2003; but the concentrations of all the other metals were similar. The statistical analysis of the data Linear Discriminant Analysis (LDA) and Kruskal-Wallis test confirmed that both zoxamide-mancozeb treatments and copper oxychloride treatments exerted a significant influence on Mn (II), Zn (II) Cu (II), Pb (II) and Cd (II) concentrations in wines, grapes, marcs and grape stalks samples from both the studied vintages.

Running head: Fungicide treatments influence on metals content of wine

Keywords: fungicide treatment, grape, grape-stalk, heavy metals, marc, statistical analysis, wine.

[§] Corresponding Author

Tel: +39 (0)90 6765180

Fax: +39 (0)90 6765436

E mail: dugogia@isengard.unime.it; laralapera20@libero.it

1
2
3 41 **INTRODUCTION**

4
5 42
6
7 43 In recent years, great importance has been directed towards the monitoring of food products for
8
9 44 heavy metals contamination, and the assessment of the source of food contamination. The presence
10
11 45 of trace elements in wines, depends on many factors such as the type of soil, wine processing
12
13 46 equipment, vinification methods (Hsia et al., 1975; Golimowsky et al, 1979 b; Karadjova et al.,
14
15 47 2002). Nowadays, a number of different metallic compounds are used in agricultural chemicals and
16
17 48 can, as a result, contaminate crops. Fungicides are considered to be indispensable aids in agriculture
18
19 49 and are extensively used in vine-growing; many studies report the toxic effect of these chemicals on
20
21 50 human health but there are few data about the influence of the use of pesticides on wine
22
23 51 composition and particularly on metal concentrations. Trace metals in wine, including Cu, Mn and
24
25 52 Zn are essential for human, animals, and plants and particularly Zn at low concentrations plays an
26
27 53 important role in wine fermentation. On the other hand, Cu and Mn at high concentrations
28
29 54 participate significantly in the destabilization of wines and in their oxidative evolution (Benitez et
30
31 55 al., 2002). At the same time the analysis for certain elements, such as Pb and Cd, in wines is of
32
33 56 special interest due to their toxicity in case of excessive intake (Ribéreau-Gayon and Peynaud, 1976;
34
35 57 Gennaro et al, 1986; Mc Kinnon and Scollary, 1997; Fernandez Pereira, 1998). A recent study has
36
37 58 provided evidence that the use of some organic and inorganic fungicides - quinoxifen, dinocap,
38
39 59 penconazole, azoxystrobin and powder of sulphur-in wine-growing, may moderately influence the
40
41 60 concentration of trace elements (Cd, Cu, Pb and Zn) in red and white wines (Salvo et al., 2003).

42
43
44
45
46
47
48
49 61
50
51 62 The present study has been focussed on the possible correlation between the presence of Mn (II), Zn
52
53 63 (II), Cu (II), Cd, (II), and Pb (II), in samples of Sicilian red wines and mancozeb-zoxamide and
54
55 64 copper oxychloride treatments that occurred during viticulture; in order to better investigate the
56
57 65 effects of these fungicide treatments, heavy metals analysis were also performed on grapes, marcs,
58
59 66 and grape stalks; furthermore data concerning heavy metal levels in soil near the plots were

1
2
3 67 available as background data. The experimentation was carried out over two crop years, 2003 and
4
5 68 2004. Ethylenebisdithiocarbamate of manganese and zinc (mancozeb)- $[C_4H_6N_2S_4Mn]_x(Zn)_y$ -
6
7
8 69 belongs to a wider group of ethylenebisdithiocarbamate fungicides and is widely used in
9
10 70 agriculture, mainly on fruits, ornamental plants and vegetables (Maroni et al., 2000). It is
11
12 71 characterized by low acute toxicity and short environmental persistence, for this reason the total
13
14 72 amount of mancozeb used is increasing worldwide. Zoxamide [3, 5-dichloro-N-(3-chloro-1-ethyl-1-
15
16 73 methyl-2-oxopropyl)-4-methylbenzamide] is a new fungicide for foliar use on potatoes, grapevines
17
18 74 and vegetables to control Oomycete fungi (Egan et al., 1998). Despite the production of a wide
19
20 75 variety of synthetic organic fungicides, copper oxychloride- $CuCl_2 \cdot 3Cu(OH)_2$ -still predominates
21
22 76 the field of fungicidal plants disease control and its wide use is also due to its general low toxicity
23
24 77 for humans (Helling et al., 2000).
25
26
27
28
29
30

31 78
32 79 The determination of Mn, Zn, Cu, Cd and Pb in grapes, grape stalks, marcs and wines was carried
33
34 80 out by using derivative stripping chronopotentiometry (dSCP), an electro-analytical technique
35
36 81 widely employed for trace and ultra-trace metals analysis in food and environmental matrices
37
38 82 (Salvo et al, 2003; La Pera et al., 2003; Dugo et al., 2004; Dugo et al., 2005a; Dugo et al., 2005b;
39
40 83 Dugo et al., 2005c; Matarese Palmieri et al., 2005). The analytical techniques conventionally used
41
42 84 for metal determinations are both emission and absorption spectrophotometry, as well as
43
44 85 voltammetry (Balduini et al., 1999). These methods require sample pre-treatment which enable the
45
46 86 complete destruction of the organic matrix such as wet digestion, dry ashing and microwave oven
47
48 87 dissolution (Reid et al., 1995). Derivative stripping chronopotentiometry allows the direct
49
50 88 determination of heavy metals in wines without the need of a laborious sample preparation
51
52 89 procedure but acid adjustment, as the glassy (GCMFE) is inert towards organic molecules
53
54 90 interferences during the signal collection phase (Town and van Leewen, 2001 and 2002).
55
56
57
58
59
60

92 **Materials and methods**

93 *Apparatus*

94 Metals analysis were carried out by using a PSA ION 3 potentiometric stripping analyzer
95 (Steroglass, S. Martino in Campo, Perugia, Italy). The analyzer was equipped with a conventional
96 three-electrodes cell. The plating process with mercury of the glassy carbon working electrode, the
97 reference and the auxiliary electrodes, have been described in previous papers (Salvo et al., 2003;
98 La Pera, et al. 2003; Dugo et al, 2005 a, b, c; Matarese Palmieri et. al 2005; Dugo et al., 2004 b)

99 *Reagents*

100 All glassware was rinsed with 10% (v/v) nitric acid. Ultra-pure water ($18.2 \text{ Mohm cm}^{-1}$) was
101 obtained from a Pure Lab RO and a Pure Lab UV system (USF, Ransbach-Baumbach, Germany).
102 Ultra pure hydrochloric acid (34-37%), Hg (II) (1000 mgL^{-1} , 1M in hydrochloric acid) and Cd (II),
103 Cu (II), Pb (II), Zn (II), and Mn (II) (1000 mgL^{-1} , 0.5 N in nitric acid) standard solutions were
104 purchased from Panreac Quimica (Barcelona, Spain). Glucose, fructose, gallic acid, tartaric acid
105 and potassium tartrate used to prepare the model wine solution (14%v/v aqueous ethanol, 2 gL^{-1}
106 fructose, 2 gL^{-1} glucose, 1 gL^{-1} gallic acid, saturated with potassium hydrogen tartrate and adjusted
107 to pH 3.5 with tartaric acid), were purchased from Sigma Aldrich (Milwaukee, WI, USA). Supel-
108 ENVI carbon columns were obtained from Supelco (Bellefonte, PA, USA)

109 *Wines characteristics and pesticides treatments*

110 Trials were conducted in five plots located in the Sicilian wine-producing zone of Etna; grapevines
111 were from the Nerello Mascalese variety. Plots were investigated over two crop years 2003 and
112 2004. Plots B-C were characterized by the use of mancozeb and zoxamide and they differed for the
113 time elapsed from the last fungicide treatment and the grape harvesting (Δt) ; plots D-E by the use
114 of copper oxychloride and they also differed for the Δt , and plot A was characterized by the use of
115 water and represented the control plot (Table 1). All the studied plots measured between 0.25-0.5

1
2
3 116 ha. Pesticides were used at the doses recommended by the manufacturer and the treatments were
4
5
6 117 applied by the wine farmers with a manual sprayer. In all the considered crop years (2003 and
7
8 118 2004), the grapes were harvested in the period from Aug. 25 to Sept.10. Newly cropped *Vitis*
9
10 119 *vinifera* were crushed and de-stemmed, then spiked with SO₂ (5 g/hL), selected yeasts (30 g/hL)
11
12
13 120 and let to ferment for 10 days at 28°C, making three fullings a day. The wine was drawn from the
14
15 121 vat and the marcs were crushed by a hydraulic press. After 4 weeks the lees were removed and the
16
17 122 wine was spiked with SO₂ (2 g/hL). Subsequently the wine was spiked again with SO₂ (2 g/hL) and
18
19
20 123 bottled in dark bottles at 4°C for the duration of the experimentation. Each sample was separately
21
22 124 fermented and bottled, following the protocol mentioned above. From each investigated plot, both
23
24
25 125 in 2003 and 2004, representative samples of grapes (about 10 kg), grape-stalks (about 1.4 kg),
26
27 126 marcs (about 0.5 Kg), and wines (7.5 L) were delivered to the laboratory and stored at -10°C.
28

29 127 *Pedoclimatic conditions*

30
31
32 128 The vineyards lay on calcareous soil (300 m asl) that contains on average 37% of sand, 36 % of
33
34 129 silt, 27% of clay and 4.5 % of organic matter; water contents at 10–20 cm depths were 30.1% by
35
36
37 130 volume. Heavy metal concentrations of soil and groundwater samples collected in different points
38
39 131 of each plot before the first pesticides application both in 2003 and 2004, determined in an external
40
41
42 132 laboratory by ICP-AES, were available as background data (Table 2). In the studied area the climate
43
44 133 is arid and the low rainfall results in a lack of water except in the winter months. The environmental
45
46 134 conditions were recorded by an AD-2 automatic weather station from April to September; during
47
48
49 135 the experiments, the minimum and maximum average temperatures were 12-35 °C in 2003 and
50
51 136 12.6-35.5 °C in 2004. The average precipitation rate over the studied area was 665 mm/year in 2003
52
53
54 137 and 600 mm/year in 2004; in particular the rainfall was 0.7 mm in spring and 0.1 mm in summer on
55
56 138 1 rainy day in 2003 and 0.55 mm in spring and 0.0 mm in summer on 1 rainy day in 2004.
57

58 139 *Sample preparation*

1
2
3 140 Wine samples were directly analyzed after acidification to pH 2 with 5 M HCl and filtration through
4
5
6 141 a Carbon column as described in previous studies (Salvo et al, 2003; Dugo et al, 2005 b). Grapes
7
8 142 from each plot were beaten by a blender equipped with titanium blades to avoid metal
9
10 143 contamination, until a homogeneous juice was obtained. An aliquot of 2 mL of the juice was placed
11
12
13 144 into a teflon beaker with 4.0 mL of 37% HCl and 6.0 mL of ultrapure water and extracted as
14
15 145 described in a previous paper (Dugo et al., 2005 a; Dugo et al., 2004 b). Marcs and grape-stalks
16
17 146 from each plot were separately dried at 110 C° to constant weight in order to measure their moisture
18
19
20 147 contents. An aliquot of 1 g of dried samples was dissolved in 10.0 mL of 35% HCl and extracted at
21
22 148 80 °C as discussed in previous studies concerning metal analysis in vegetable matrices (Dugo et al,
23
24
25 149 2005 c, Matarese Palmieri et. al 2005).

27 150 *Derivative chronopotentiometric stripping analysis*

30 151 Cd (II), Cu (II), Pb (II) and Zn (II) were determined simultaneously in wine, grapes, grape-stalks
31
32
33 152 and marcs as described in a previous papers (Salvo et al, 2003, Dugo et al, 2005 b, c; Matarese
34
35 153 Palmieri et. al 2005); Mn (II) was determined separately by placing 500 µL of acidified wine (or
36
37
38 154 acid extract of grapes, marc, grape-stalks) in the electrochemical cell together with 1 mL of 0.5 M
39
40 155 ammonia-ammonium chloride buffer (pH 9.5), 10.0 mL of ultrapure water and 1.0 mL of 1000 µg
41
42 156 mL⁻¹ Hg (II) standard solution as oxidant agent, and de-oxygenating for 5 min by N₂ purging. The
43
44
45 157 best electrochemical conditions were optimized by fixing the electrolysis potential at -1700 mV, the
46
47 158 final acquisition potential and the cleaning potential respectively at -500 mV and -1500 mV, and
48
49
50 159 varying the electrolysis time as afterwards described; the solution was stirred at 1000 rpm; the
51
52 160 manganese peak was registered around -1460 mV (Dugo et al., 2005 c). The quantitative analysis
53
54 161 of metals was done by the multiple-point standard additions method. Optimum precision and
55
56
57 162 accuracy were obtained with two additions of appropriate aliquots of standard solution of Mn (II) in
58
59 163 order to double the sample peak area, and performing the measurements five times for each addition
60
164 (La Pera, et al. 2003; Dugo et al, 2005 a, b, c; Matarese Palmieri et. al 2005; Renman and Jagner,

1
2
3 165 1997). Calibration curve with correlation coefficients $> 99.5\%$ were obtained. The linear
4
5
6 166 concentration range was $0-10000 \mu\text{gL}^{-1}$. Under optimized electrochemical conditions, the analysis
7
8 167 lasted 25 minutes.
9

11 168 ***Method performance: sensitivity, accuracy and repeatability***

12
13 169 Method performances for Cu, Cd, Mn, Pb and Zn chronopotentiometric analysis in wines and
14
15
16 170 vegetables have been reported in previous papers (Salvo et al, 2003; La Pera, et al. 2003; Dugo et
17
18 171 al, 2005 a, b, c; Matarese Palmieri et. al 2005; Dugo, et al.2004).
19

20 172 ***Statistical methods***

21
22
23 173 The statistical elaboration of the data was performed by two softwares: SPSS for Windows (version
24
25 174 12.0; 2003) and STATISTICA package for Windows (version 6.0, 2000). The non parametric test
26
27
28 175 of Kruskal-Wallis and Linear Discriminant Analysis (LDA) were performed grouping wines,
29
30 176 grapes, marcs and grape stalks according to the pesticide treatments applied; data expressing
31
32 177 concentrations of Mn, Zn, Cu, Pb and Cd in wines, marcs, grapes and grape-stalks samples from
33
34
35 178 2003 and 2004 vintages were processed. In order to define statistically the difference between
36
37 179 samples submitted to a specific fungicide treatment (A, B, C, D, E), the data were elaborated using
38
39 180 a multivariate non-parametric Analysis of Variance and subsequently a Linear Discriminant
40
41
42 181 Analysis in order to distinguish samples coming from one of the five treatment groups according to
43
44 182 a simple statistical model (Dugo et al., 2005 b). The choice to perform a non-parametric analysis
45
46
47 183 was related to the fact that the distribution of Mn, Zn, Pb, Cd and Cu levels did not seem to meet
48
49 184 the necessary conditions required to apply a simple Analysis of Variance (ANOVA), because there
50
51 185 was not a homogeneity of variance between samples coming from different groups. Moreover
52
53
54 186 samples belonging to different treatments did not show a normal distribution over the same group.
55
56 187 Therefore the five variables (Mn, Zn, Pb, Cd and Cu concentrations expressed in mg/L), were
57
58 188 submitted to the non-parametric test of Kruskal-Wallis to verify the significances of differences
59
60 189 between the control and treated samples. This procedure tested the hypothesis that n independent
190 samples came from populations having the same distribution.

1
2
3 191
4
5
6 192
7
8 193
9
10 194
11
12 195
13
14
15 196
16
17 197
18
19 198
20
21 199
22
23 200
24
25 201
26
27 202
28
29 203
30
31 204
32
33 205
34
35 206
36
37 207
38
39 208
40
41 209
42
43 210
44
45 211
46
47 212
48
49 213
50
51 214
52
53 215
54
55 216
56
57
58
59
60

RESULTS AND DISCUSSION

Derivative stripping chronopotentiometry for the analysis of metals in enological samples

Growing concern about heavy metals food contamination has led to increasing needs to develop sensitive analytical techniques capable to detect trace and ultra-trace concentrations of metals in a variety of alimentary matrices. Derivative stripping chronopotentiometry has been recognised as a powerful tool for measuring trace metals in food samples. Its remarkable sensitivity is attributed to the concentration step during which the metals are accumulated onto the working electrode. Under optimized analytical conditions, detection limits lower than 1 ng g^{-1} have been obtained for the studied metals in different food matrices; the analytical performances of SCP tested on certified reference materials are reported in the cited literature (La Pera, et al. 2003; Dugo et al, 2005 a, b, c; Matarese Palmieri et. al 2005; Renman and Jagner, 1997). Sample preparation played an important role in ensuring the accuracy of determination, because metals were present in very low concentrations in complex matrices such as wine, grape, marcs and stalks. Interferences from organic molecules are minimized in SCP, hence a simple and rapid sample pre-treatment based on concentrated hydrochloric acid extraction at 80°C was required which enabled the extraction of the metals in less than 1 h. Wine may be analysed after acidification to pH 2, followed by filtration through a carbon column, without the need for sample pre-treatment. A speciation study performed on a certified reference wine has shown that at $\text{pH} < 3$ heavy metals are present in the soluble form (Azenha and Vasconcelos, 2000; Karadjova et al., 2002). A recovery test carried out on a model wine spiked at different levels with Cu (II), Mn (II), Zn (II), Cd (II) and Pb (II) standard solutions, acidified to pH 2 with HCl and filtered trough a carbon column. The obtained recovery factors (95-99%) provided evidence that metal loss did not occur during sample pre-treatment. Comparative analysis between filtered and unfiltered red wine discussed in a previous paper, demonstrated that metal quantification was not affected by the filtration procedure and a higher analytical response was achieved by passing the wine sample trough the carbon column prior to the

1
2
3 217 chronopotentiometric measures. (Dugo et al, 2005b); the repeatability of the chronopotentiometric
4
5
6 218 method, expressed as RSD%, was $1.1\pm 0.1\%$ for Cd, $2.0\pm 0.3\%$ for Cu, $1.5\pm 0.2\%$ for Pb, $3.0\pm 0.1\%$
7
8 219 for Mn and $1.0\pm 0.1\%$ for Zn (La Pera and Dugo, 2005). A previous study reports parallel analysis
9
10 220 of red and white wine samples by graphite furnace atomic absorption spectroscopic and the
11
12
13 221 obtained results agreed within 5% (Salvo et al., 2003). SCP coupled high sensitivity and resolution,
14
15 222 rapid sample preparation with low costs of the equipment; moreover the small dimensions of the
16
17
18 223 analyzer allowed its transport for in situ analysis. For all these features, SCP is well suited for
19
20 224 routine analysis.

22 23 225 *Influence of fungicide treatments on Cu, Cd, Mn, Pb and Zn levels*

24 25 226 *Results from untreated plots*

26
27 227 In control wines samples from 2003 (plot A), metal levels decreased in the order
28
29
30 228 Zn>Mn>Cu>Pb>Cd, whereas in samples from the following crop year in the order Zn \cong Mn
31
32 229 \cong Cu>Pb>Cd (Table 3). Mn, Cu and Zn were the most abundant metals in grape-stalks from 2003
33
34
35 230 and 2004, followed by Cd and Pb (Table 4). As it was observed for wine samples, in grapes from
36
37 231 2003 metals concentrations decreased in the order Zn>Cu>Mn>Pb>Cd, whereas in samples from
38
39
40 232 the following crop year in the order Cu>Zn>Mn > Cd > Pb (Table 5). Furthermore, at **vintaging**
41
42 233 both in 2003 and 2004 grapes from untreated plots did not show any fungal infection since weather
43
44 234 conditions, characterized by high temperatures and reduced rainfall were unfavourable for fungal
45
46
47 235 development. In marcs samples from both vintages, metal concentrations decreased in the order
48
49 236 Cu>Mn>Zn, Cd and Pb levels in the controls were lower than the instrumental detection limits
50
51 237 (Table 6). Cadmium levels in soil from plot A sampled in 2003 were higher than those found in the
52
53
54 238 other plots; this might have resulted in an increased cadmium concentration in grape-stalks and
55
56 239 grapes grown in the control vineyard.

57 58 240 59 60 241 *Results from plots treated with mancozeb-zoxamide*

1
2
3 242 As Table 3 reports, Mn and Zn levels in 2003 wines from zoxamide-mancozeb treatments (plot B
4
5
6 243 and C) were about 3 fold and 2 fold higher than those observed in the control samples, respectively;
7
8 244 the Δt parameter did not greatly influence the concentration of these elements. Mn levels in 2004
9
10
11 245 wines from zoxamide-mancozeb treatments (plot B and C) were about 3-fold higher than those
12
13 246 observed in the control samples; the Δt parameter seemed to exert a significant influence on Zn
14
15
16 247 level as the metal concentration was 3.5 fold higher than that observed in the control in samples
17
18 248 harvested 14 days after the last treatment (plot B) and 6.5 fold higher in samples harvested 28 days
19
20 249 after the last treatment (plot C). Moreover the studied treatments lead to a significant increase of Pb
21
22
23 250 and Cd concentrations (an average of 150% over the control) in wines from 2003, meanwhile only a
24
25 251 slight enhancement in Pb levels in samples from 2004 was observed. As given in Table 4, Mn levels
26
27 252 in 2003 grape-stalks from plots B and C were from 2.4 to 6.5-fold higher than those observed in the
28
29
30 253 control and from 5.6 to 6.8-fold higher in 2004 grape-stalks; in particular, samples harvested 28
31
32 254 days after the last treatment showed the highest values. Zn levels were from 1.5 to 2.6-fold higher
33
34 255 with respect to the control in grape-stalks from 2003 and from 4 to 6 higher in 2004 samples; in
35
36
37 256 particular, samples harvested 14 days after the last treatment showed the highest values. Pb and Cd
38
39 257 concentrations in 2003 grape-stalks from zoxamide-mancozeb treatments were from 5 to 7 fold
40
41
42 258 higher than those observed in the blanc, whereas samples from 2004 showed a slight increase of Pb
43
44 259 concentration as compared to the control and a great enhancement of Cd levels (7 to 11 fold higher
45
46 260 with respect to the blanc). As Table 5 reports, the Δt parameter seemed to exert a significant
47
48
49 261 influence on Mn level in grapes from both the crop years: samples from 2003 harvested 14 days
50
51 262 after the last treatment (plot B) presented Mn concentrations 11 fold higher with respect to the
52
53
54 263 control, whereas samples harvested 28 days after the last treatment (plot C) 4-fold higher than the
55
56 264 control; on the contrary samples from 2004 harvested in the plot B showed Mn levels 2-fold higher
57
58 265 with respect to the blanc, whereas samples harvested in the plot C presented Mn levels 4-fold higher
59
60 266 than the blanc. A similar profile was observed for Zn levels, particularly for grapes from 2004.

267 Furthermore a remarkable influence of the treatments over Pb and Cd levels, particularly in grapes

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

268 from 2003 was noticed. Mn and Zn levels in 2003 and 2004 marcs from plots B and C were from 2
269 to 4-fold higher than those observed in the controls; treated samples from 2004 showed an increase
270 of Pb levels with respect to the control, whereas no significant influence of the treatments on Cd
271 levels in marcs was noticed (Table 6).

Results from plots treated with copper-oxychloride

274 Cu levels in wines from 2003 were very much lower with respect to those observed in the following
275 crop year; moreover Cu concentrations in 2003 wines from all the plots were very similar, whereas
276 a significant influence of copper-oxychloride treatments was noticed in wines from 2004 (plots D
277 and E) that showed a Cu amount increased by 50% with respect to the control; moreover copper-
278 oxychloride applications lead to a moderate increase of Pb and Cd concentrations in wines from
279 both the crop years (Table 3). Copper-oxychloride treatments greatly influenced Cu levels in grape-
280 stalks from 2003 and 2004 that presented concentrations from 4 to 50-fold higher than those
281 detected in the control; samples harvested 28 days (Δt) after the last treatment (plot E) showed the
282 highest Cu amounts. In particular, copper-oxychloride applications seemed to exert a remarkable
283 effect on Pb levels in grape-stalks which are 24-fold higher than that of the control in samples from
284 2003, nevertheless this effect was not observed in samples from the following crop year (Table 4).
285 As was already observed for wines, grapes samples from 2003 presented lower Cu concentrations
286 than those detected in samples from 2004; the influence of copper-oxychloride treatments over Cu
287 concentrations in grapes from both the vintages was particularly evident since treated samples
288 presented values 3-4 fold higher than those of the controls (Table 5). A significant influence of Cu
289 oxychloride treatments over Cu levels in marcs from both the crop years was noticed since samples
290 from plots D and E had concentrations 3-5 fold higher than the control; moreover treated samples
291 from 2004 showed an increase of Pb levels with respect to the control, whereas no significant
292 influence of the treatments on Cd levels in marcs was noticed (Table 6).

293

1
2
3 294 *Statistical analysis*

4
5
6 295 As shown in Tables 3-6, all the four types of enological matrices (wines, marcs, grapes and grape-
7 stalks) presented a significant difference through the five treatments. This is explained by the
8 296 calculated significance levels (p) from Kruskal-Wallis test, that resulted $\leq .001$ for all of the five
9
10 297 variables considered independently over the two years. This result suggests that the studied
11
12 298 fungicide treatments greatly influenced the concentrations of the Mn, Zn, Pb, Cd and Cu in the
13
14 299 wines, marcs, grapes and grape-stalks samples from both the vintages. The concentrations of Mn,
15
16 300 Zn, Pb, Cd and Cu have been further studied using *Linear Discriminant Analysis (LDA)* in order to
17
18 301 create a model that, through some classification functions, could separate samples in relation to the
19
20 302 particular treatment used. LDA was performed separately on wines, marcs, grapes and grape-stalks
21
22 303 samples from the two crop years. The results have shown a very good degree of classification
23
24 304 (100%) of the samples according to the functions created in every analysis. Scatterplots (Figures 1-
25
26 305 4) of discriminant functions, that explained on average the 93% of the total variance over the
27
28 306 samples, have shown a good separation of the five groups representing various fungicide treatments
29
30 307 applied to grapevines. Generally it can be observed (overall for the wine samples) a little proximity
31
32 308 between samples belonging to treatments “B” with those from the treatment “C”; and between
33
34 309 samples from treatments “D” and “E”. This means that these groups of treatments influence in a
35
36 310 common way the concentrations of metals in the samples. Moreover any classification function can
37
38 311 be represented as a linear combination of the five variables, providing a good statistical model to
39
40 312 characterize the treatments. For example, the two classification functions regarding wines from
41
42 313 2003 can be described by the following equations:
43
44 314
45
46 315
47
48 316
49
50
51 317
52
53 318

$$\text{Root1} = -2,420\text{Mn} - 5,477\text{Zn} + 53,807\text{Cu} - 76,533\text{Pb} - 230,871\text{Cd} + 10,013;$$

$$\text{Root2} = 2,995\text{Mn} - 0,379\text{Zn} + 472,674\text{Cu} - 117,649\text{Pb} + 190,004\text{Cd} - 22,525;$$

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

319 where Root1 is strongly negatively correlated with the Zn level and Root2 is positively correlated
320 with the Cu level.

321
322 On the whole the statistical analysis of the data has evidenced that wines, grapes, grape-stalks and
323 marcs from both the crop years treated with zoxamide-mancozeb and with copper oxychloride
324 showed a significant increase of Mn (II), Zn (II) and Cu (II) concentrations with respect to the
325 control; the effect of time elapsed between the last application of fungicides and the harvesting (Δt)
326 on the concentration of Mn, Zn, Pb, Cd and Cu in wines, grapes, grape-stalks and marcs has not
327 been yet elucidated as heavy metal levels in treated samples irregularly increased in a Δt -dependent
328 manner. Furthermore Mancozeb-zoxamide and copper-oxychloride treatments lead to a moderate
329 increase of Pb (II) levels in treated wines, grape-stalks, grapes and marcs with respect to the control.
330 As shown in a previous paper (Salvo et al, 2003), the increase of Cd and Pb levels in treated
331 samples could be the result of the presence of trace concentrations of these contaminants in the
332 chemicals used for the treatments.

334 *Inter-seasonal variations*

335 Pesticide treatments were the variables that mostly influenced metal levels in the enological
336 matrices from 2003 and 2004 vintages . Therefore, in order to estimate inter-seasonal variations due
337 to the environmental conditions, only the control plots have been considered. Tables 3-6 give
338 evidence that Cu and Pb levels in 2004 wines, grapes, and marcs were much higher than those
339 found in 2003, whereas Mn and particularly Zn levels in 2003 wines, grapes, grape-stalks and marcs
340 were higher than those found in 2004. As Table 2 reports, these results are related to metal levels in
341 the soil/groundwater sampled in the control plots in both the crop years. The relationship between
342 metal levels in the enological matrices and in soil/groundwater system may explain the observed
343 inter-seasonal variations. It must be added that it is very difficult to asses all the factors including,

1
2
3 344 pollution, volcanic emissions, harvesting and storage that that may influence metal levels in food
4
5
6 345 plants grown in the same zone in two different crop years.
7

8 346

9
10 347 ***Nutritional evaluation***
11

12
13 348 Zinc, copper and manganese are essential micronutrients since they are co-factors of many
14
15 349 enzymes, a low dietary intake of these microelements may cause severe deficiency syndromes; on
16
17 350 the other hand at high concentrations these trace elements may have toxic effects. For this reason
18
19
20 351 Recommended Daily Allowances (RDAs) have been established by the Food and Drug
21
22 352 Administration; for a 70 kg adult they were 18 mg Zn/day, 5 mg Mn/day, 3 mg Cu/day (La Pera and
23
24
25 353 Dugo, 2005). Considering the concentration ranges of Zn ($0.4\text{-}3\text{ mgL}^{-1}$), Mn ($0.5\text{-}1.6\text{ mgL}^{-1}$) and Cu
26
27 354 ($0.05\text{-}1\text{ mgL}^{-1}$) found in this study and a 0.25 L daily wine consumption, the RDA % were
28
29 355 respectively of 0.5-4 %, 2-8 % and 0.3-8 %. Moreover Cu and Zn levels were always below the
30
31
32 356 legal limits established by the Italian Republic, 1.0 and 5.0 mgL^{-1} respectively (Italian Republic,
33
34 357 1986). Toxic elements such as Cd and Pb, present in foodstuff mainly as a result of a contamination
35
36 358 process, were found in the studied treated and untreated wines in concentrations well below the
37
38
39 359 legal limits established by the European Community (0.2 mg Pb L^{-1} and 0.1 mg Cd L^{-1}) (European
40
41 360 Community, 2001). The nutritional evaluation of the obtained data has shown that a moderate daily
42
43
44 361 consumption of treated wines from both the crop years, provides a Cu, Mn and Zn intake below the
45
46 362 RDA and that Pb and Cd levels are within the legal limits; therefore a correct use of these
47
48 363 fungicides in wine-growing does not represent a risk for human health.
49

50
51 364

52 365 **References**
53

54
55 366 Azenha M A G O., Vasconcelos M T S D. 2000. Assessment of the Pb and Cu in vitro availability
56
57 367 in wines by means of speciation procedures Food and Chemical Toxicology 38: 899-912
58
59 368 Balduini P L, Cavalli S, Lal Sharma J. 1999. Determination of Transition Metals in Wine by IC,
60
369 DPASV-DPCSV and ZGFAAS Coupled with UV Photolysis. J. Agric. Food Chem. 47:1993-1998.

- 1
2
3 370 Benitez P, Castro R., Barroso C G. 2002. Removal of iron, copper and manganese from white
4
5
6 371 wines through ion exchange techniques: effects on their organoleptic characteristics and
7
8 372 susceptibility to browning. *Analytica Chimica Acta* 458:197–202
9
10 373 Dugo G.mo, La Pera L, La Torre G L, Giuffrida D 2004a. Determination of Cd (II), Cu (II), Pb (II),
11
12 374 and Zn (II) content in commercial vegetable oils using derivative potentiometric stripping analysis
13
14
15 375 (dPSA) *Food Chem.* 87:639-645.
16
17 376 Dugo G.mo, La Pera L, Lo Turco V, Giuffrida D, Restuccia S 2004b. Determination of Cu, Zn, Se,
18
19
20 377 Pb and Cd using potentiometric stripping methods in potatoes (*Solanum tuberosum L.*) *Food*
21
22 378 *Additives and Contaminants* 21:649-657
23
24 379 Dugo G.mo, La Pera L, Lo Turco V, Di Bella G. 2005a. Speciation of inorganic arsenic in
25
26
27 380 alimentary and environmental aqueous samples by using derivative anodic stripping
28
29 381 chronopotentiometry (dASCP) *Chemosphere* 61: 1093-1101
30
31 382 Dugo G.mo, La Pera L, Pellicanò T M, Di Bella G, D’Imperio M 2005b. Determination of some
32
33
34 383 inorganic anions and heavy metals in D.O.C. Golden and Amber Marsala wines: statistical study of
35
36 384 the influence of the ageing period, color and sugar content. *Food Chem* 91: 355-363.
37
38 385 Dugo G.mo, La Pera, L, Lo Turco V, Matarese Palmieri R, Giuffrida D. 2005c. Effect of boiling
39
40
41 386 and peeling on manganese content of some vegetables determined by derivative anodic stripping
42
43 387 chronopotentiometry (dASCP). *Food Chem.* 93:703-711.
44
45 388 Egan A R, Michelotti E L, Young D H, Wilson W J, Mattioda H. 1998. RH 7281: a novel fungicide
46
47
48 389 for control of downy mildew and late blight. *Proc. Br. Crop. Prot. Pest. Dis.* 2:335-340.
49
50 390 European Community, CE Rule 466/2001, *Europ. Off. Gaz.* March 2001
51
52
53 391 Fernandez Pereira C. 1998. The Importance of Metallic Elements in Wine. A Literature Survey. *Z.*
54
55 392 *Lebensm. Unters. Forsh.* 186:295-300.
56
57 393 Gennaro M C, Mentasti E, Sarzanini C, Pesticcio A. 1986. Undesiderable and harmful metals in
58
59
60 394 wines – determination and removal. *Food Chem.* 19:93-104.

- 1
2
3 395 Golimowsky J, Valenta P, Wolfgang-Nurnberg H. 1979. Toxic Trace Metals in Food II. A
4
5
6 396 Comparative Study of The Levels of Toxic Trace Metals in Wine by Differential Pulse Anodic
7
8 397 Stripping Voltammetry and Electrothermal Atomic Absorption Spectrometry Z.Lebensm. Unters.
9
10 398 Forsh. 168:439-443.
11
12
13 399 Helling B, Reinecke S A, Reinecke A J 2000. Effect of fungicide oxychloride on the growth
14
15 400 and reproduction of *Eisenia fetida* (Oligochaeta). *Ecotox. And Env. Safety* 46:108-116.
16
17 401 Hsia, C., Planck, R.W., Nagel, C. W. 1975. Influence of Must Processing on Iron and Copper
18
19
20 402 contents of Experimental Wines. *Am. J. Enol. Vitic.* 26:57-61.
21
22 403 Italian Republic. Caratteristiche e Limiti di alcune Sostanze nel Vino. 1986, december D.M. 29.
23
24
25 404 Karadjova I, Izgi B, Gucer S. 2002 Fractionation and speciation of Cu, Zn and Fe in wine samples
26
27 405 by atomic absorption spectrometry_ *Spectrochimica Acta Part B* 57:581-590
28
29 406 La Pera L, Saitta M, Di Bella G, Dugo Gmo. 2003. Simultaneous determination of Cd (II), Cu (II)
30
31
32 407 Pb (II) and Zn (II) in citrus essential oils by derivative potentiometric stripping analysis. *J. Agric.*
33
34 408 *Food Chem.*, 51:1125-1129.
35
36 409 La Pera L, Dugo Gmo. Metalli pesanti negli alimenti, aspetti nutrizionali e tossicologici. In: Alberto
37
38
39 410 Chiriotti eds. *Metalli pesanti negli alimenti* pp 16-56, Turin, Italy, 2005.
40
41 411 Maroni M, Colosso C, Ferioli A, Fait A. 2000. Biological monitoring of pesticides exposure: a
42
43
44 412 review. *Toxicology*, 143:1-23
45
46 413 Matarese Palmieri R, La Pera L, Di Bella G, Dugo G.mo. 2005. Simultaneous determination of Cd
47
48 414 (II), Cu (II), Pb (II) and Zn (II) by stripping chronopotentiometry in *Pittosporum tobira* leaves: a
49
50
51 415 measurement of local atmospheric pollution in Messina (Sicily, Italy) *Chemosphere* 59:1161-1168.
52
53 416 Mc Kinnon A J, Scollary G R. 1997. Size Fractionation of Metals in Wine Using Ultrafiltration.
54
55 417 *Talanta* 44:1649-1658.
56
57
58 418 Reid H J, Greenfield S, Edmonds T E. 1995. Investigation of Decomposition Products of
59
60 419 Microwave Digestion of Food Sample. *Analyst (London)* 120: 1543-1548.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 420 Renman R, Jagner D. 1997. Asymmetric distribution of results in calibration curve and standard
421 addition evaluation *Analytica Chimica Acta*, 357:157-166.
- 422 Ribéreau-Gayon J, Peynaud E, Ribéreau-Gayon P. *Precipitation dans le Vins*. In : *Sciences et*
423 *Techniques du vin 2nd ed.* Bordas: Paris, 1976, vol 3, pp 567-616.
- 424 Salvo F, La Pera L, Di Bella G, Nicotina M, Dugo Gmo. 2003. Influence of mineral and organic
425 pesticides treatments on Cd (II), Cu (II) Pb (II) and Zn (II) content determined by Derivative
426 Potentiometric Stripping Analysis in Italian red and white wines. *J. Agric. Food Chem*, 51:1090-
427 1094
- 428 Town R M, van Leewen, H P 2001. Fundamental features of metal ion determination by stripping
429 chronopotentiometry. *J of Electroanal Chem* 509: 58-65.
- 430 Town R M, van Leewen, H. P. 2002. Effects of adsorption in stripping chronopotentiometric metal
431 speciation analysis *J of Electroanal Chem* 523:1-15.

1
2
3 **Table 1.** Fungicide treatments applied for each plot.
4

PLOT	Fungicide	N. applications	Frequency of applications (days)	Δt (days)*
A	H ₂ O	4	7	28
B	200g/hL of Zoxamide 8.3% and Mancozeb 66.7%	4	7	14
C	200g/hL of Zoxamide 8.3% and Mancozeb 66.7%	4	7	28
D	350g/hL of Cu oxychloride 50%WP**	4	7	14
E	350g/hL of Cu oxychloride 50%WP	4	7	28

2 *Δt (days): time elapsed from the last fungicides treatment and the grape harvesting

3 **WP= wet powder

4
5 **Table 2.** Heavy metal concentrations (as well as pH) in soil (mgkg⁻¹ d.w.) and groundwater (μgL⁻¹)
6 samples collected in different points of the studied plots. Each result is the mean of three replicates.

Plot	pH	Mn	Zn	Cu	Pb	Cd
2003						
A s*	7.9	100.2±11.3	85.4±7.2	11.1±1.3	1.0±0.2	1.5±0.1
A g*	7.3	198.2±15.6	67.1±8.8	5.9±0.5	0.1±0.0	0.2±0.0
B s	8.0	95.5±19.1	73.2±8.4	10.5±1.1	1.5±0.5	1.0±0.3
B g	7.4	177.5±25.9	58.3±5.5	5.0±0.5	0.2±0.0	<0.1
C s	7.9	103.4±16.3	78.8±5.1	8.9±0.9	1.6±0.4	1.3±0.3
C g	7.2	212.2±25.3	66.3±7.1	3.8±0.3	0.2±0.0	0.1±0.0
D s	7.9	89.0±9.6	81.4±9.4	9.1±0.9	1.3±0.7	1.6±0.2
D g	7.3	161.1±14.3	70.0±5.9	4.1±0.3	0.1±0.0	0.1±0.0
E s	7.8	106.7±13.5	75.6±8.3	11.7±1.9	1.3±0.6	1.0±0.2
E g	7.5	224.0±21.5	62.5±5.8	6.0±0.6	0.1±0.0	<0.1
2004						
A s	8.0	80.2±10.0	74.3±7.2	13.2±1.0	1.7±0.3	1.1±0.1
A g	7.5	68.5±6.1	49.9±5.1	7.3±0.6	0.2±0.0	<0.1
B s	8.1	85.5±9.4	83.0±8.5	15.2±2.0	1.9±0.7	1.0±0.3
B g	7.5	62.3±5.5	54.2±5.9	8.5±0.6	0.2±0.0	<0.1
C s	7.8	103.0±11.2	94.2±7.3	10.5±1.5	2.0±0.2	1.9±0.3
C g	7.3	70.0±6.9	58.6±4.8	7.0±0.5	0.2±0.0	0.1±0.0
D s	7.9	101.7±8.5	82.8±8.8	8.5±0.9	2.1±0.5	1.4±0.2
D g	7.4	69.8±6.3	55.6±6.1	7.0±0.6	0.2±0.0	<0.1
E s	7.9	78.9±7.5	67.4±6.7	8.2±1.1	1.8±0.6	1.0±0.2
E g	7.3	75.2±7.0	41.5±3.9	6.3±0.4	0.1±0.0	0.1

7 *s=soil, g=groundwater

1
2
3 **Table 3** Metal concentrations (mean±st.dev) in wines subjected to different pesticide treatments, as
4 indicated in Table 1; five samples from each plot were analysed in triplicate (mean±st.dev).

5
6 Significances of differences between blank and treated samples are also reported as chi-square (χ^2)
7 and p values from Kruskal- Wallis test*
8

9
10 *the treatments number was considered as the grouping variable; the freedom degrees were 4
11
12
13

year	2003				
PLOT	Mn (mgL ⁻¹)	Zn (mgL ⁻¹)	Cu (µgL ⁻¹)	Pb (µgL ⁻¹)	Cd (µgL ⁻¹)
A	0.5±0.1	1.4±0.2	43.5±2.0	10.4±10.1	5.5±1.0
B	1.7±0.1	3.0±0.1	50.2±1.3	31.3±5.2	9.3±4.2
C	1.2±0.2	2.6±0.2	47.3±3.4	18.4±1.5	13.5±3.9
D	0.5±0.1	0.5±0.1	57.2±2.7	13.0±1.5	2.2±1.0
E	0.6±0.1	0.5±0.0	44.0±2.1	19.3±1.4	6.3±1.0
χ^2	19.579	21.840	19.818	20.427	18.298
p <	0.000	0.000	0.000	0.000	0.000
	2004				
A	0.5±0.0	0.4±0.0	552.2±92.1	117.1±25.5	<0.5
B	1.5±0.1	1.4±0.1	335.4±16.4	190.4±21.9	<0.5
C	1.7±0.2	2.6±0.3	760.4±14.4	<0.1	<0.5
D	0.3±0.1	0.7±0.0	1112.1±84.5	32.6±7.3	<0.5
E	0.6±0.0	1.1±0.1	1100.0±73.3	152.2±6.3	<0.5
χ^2	21.948	22.995	21.940	22.564	0.0
p <	0.000	0.000	0.000	0.000	1

1
2
3
4
5
6
7
8
9
10
11
1214
15
16
17
18
19

Table 4. Metal concentrations in grape-stalks subjected to different pesticide treatments; five samples for each plot were analysed in triplicate (mean±st.dev). Significances of differences between blank and treated samples are also reported as chi-square (χ^2) and p values from Kruskal-Wallis test

year	2003					20
PLOT	Mn (mgL ⁻¹)	Zn (mgL ⁻¹)	Cu (mgL ⁻¹)	Pb (µgL ⁻¹)	Cd (µgL ⁻¹)	21
A	7.4±1.2	17.3±1.4	6.44±0.75	8.3±1.2	17.2±1.2	22
B	18.8±1.0	47.2±2.0	8.84±1.47	55.1±1.4	86.1±2.2	23
C	48.4±1.6	26.2±1.0	11.2±1.2	58.4±2.9	73.9±2.5	24
D	10.4±1.5	15.6±1.4	196±12	191.1±11.0	62.1±1.3	25
E	4.5±0.6	16.2±1.2	314±12	192.8±2.5	17.4±2.1	26
χ^2	22.900	19.483	22.390	21.986	23.529	27
p <	0.000	0.000	0.000	0.000	0.000	28
	2004					30
A	7.0±0.2	5.0±0.4	28.08±0.92	87.0±9.1	11.1±1.7	31
B	39.8±5.9	29.0±4.2	24.5±2.3	92.3±8.6	82.2±11.0	32
C	47.7±3.2	19.2±1.3	54.7±4.5	103.0±12.1	126.8±80.3	33
D	7.6±0.8	4.4±0.4	124.0±13.4	164.8±13.4	67.2±7.4	34
E	15.6±1.7	6.4±0.7	177.0±15.1	<0.1	<0.1	35
χ^2	21.629	22.250	22.900	19.672	21.718	36
p <	0.000	0.000	0.000	0.000	0.000	37

25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

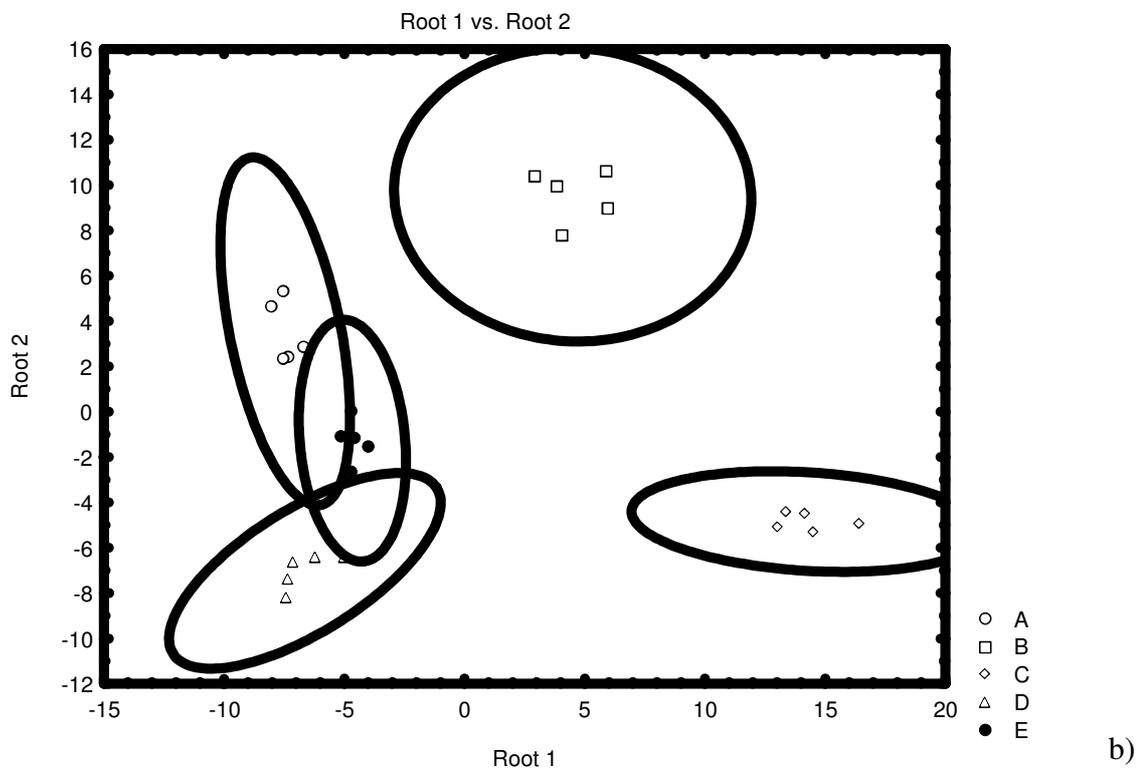
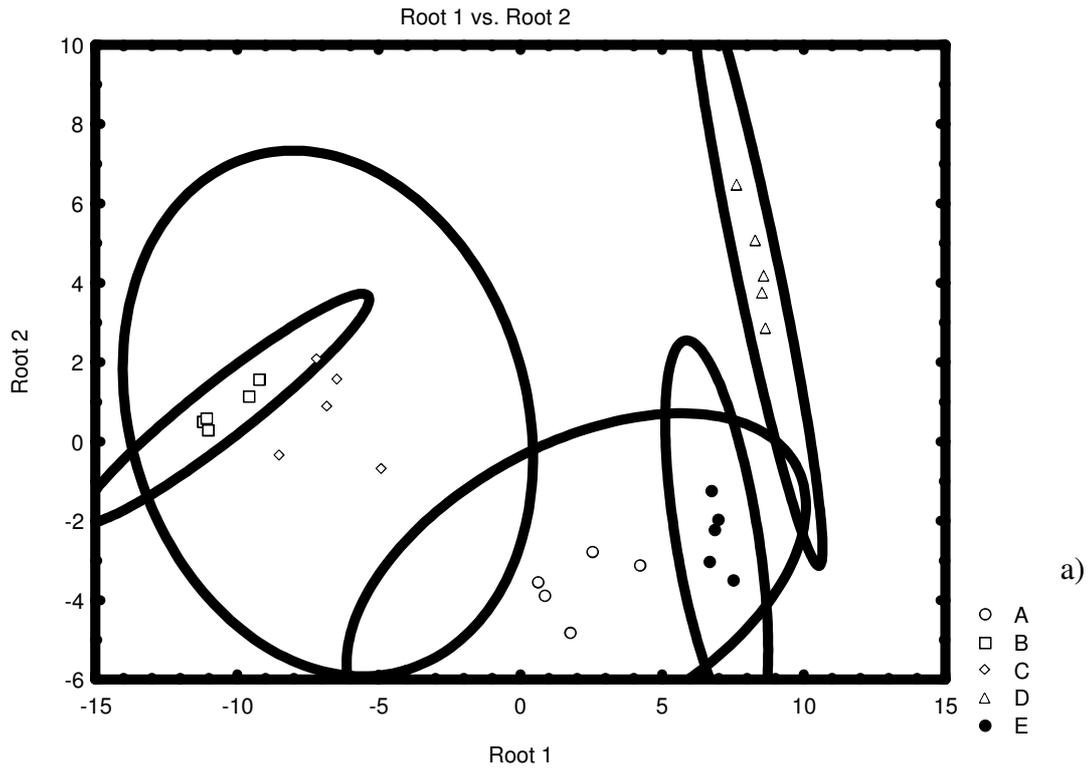
Table 5. Metal concentrations in grapes subjected to different pesticide treatments; five samples for each plot were analysed in triplicate (mean±st.dev). Significances of differences between blank and treated samples are also reported as chi-square (χ^2) and p values from Kruskal- Wallis test

year		2003				
PLOT	Mn (mgL ⁻¹)	Zn (mgL ⁻¹)	Cu (mgL ⁻¹)	Pb (µgL ⁻¹)	Cd (µgL ⁻¹)	
A	2.2±0.7	24.2±3.3	3.60±0.77	<0.1	<0.1	
B	23.3±4.1	61.8±10.0	5.40±1.29	196.1±46.4	143.2±20	
C	8.0±1.8	27.0±6.0	12.9±1.8	22.3±9.5	76.1±18	
D	4.3±1.4	24.2±6.7	12.9±2.1	265.4±28.1	40.2±9.9	
E	3.3±0.5	15.1±1.7	9.78±1.92	288.0±22.3	52.9±10.4	
χ^2	21.494	19.080	19.947	21.492	22.825	
p <	0.000	0.001	0.001	0.000	0.000	
		2004				
A	5.0±0.7	11.5±0.7	20.0±0.9	<0.1	54.2±3.2	
B	8.9±0.9	19.8±0.9	18.6±1.0	41.1±1.3	66.1±4.5	
C	21.5±2.3	54.9±2.6	10.6±1.4	<0.1	156.3±10.2	
D	4.7±0.7	14.0±0.8	83.2±2.7	<0.1	161.2±7.8	
E	2.0±0.4	3.9±0.50	120±4	<0.1	183.4±8.2	
χ^2	21.998	23.077	22.530	23.641	22.030	
p <	0.000	0.000	0.000	0.000	0.000	

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

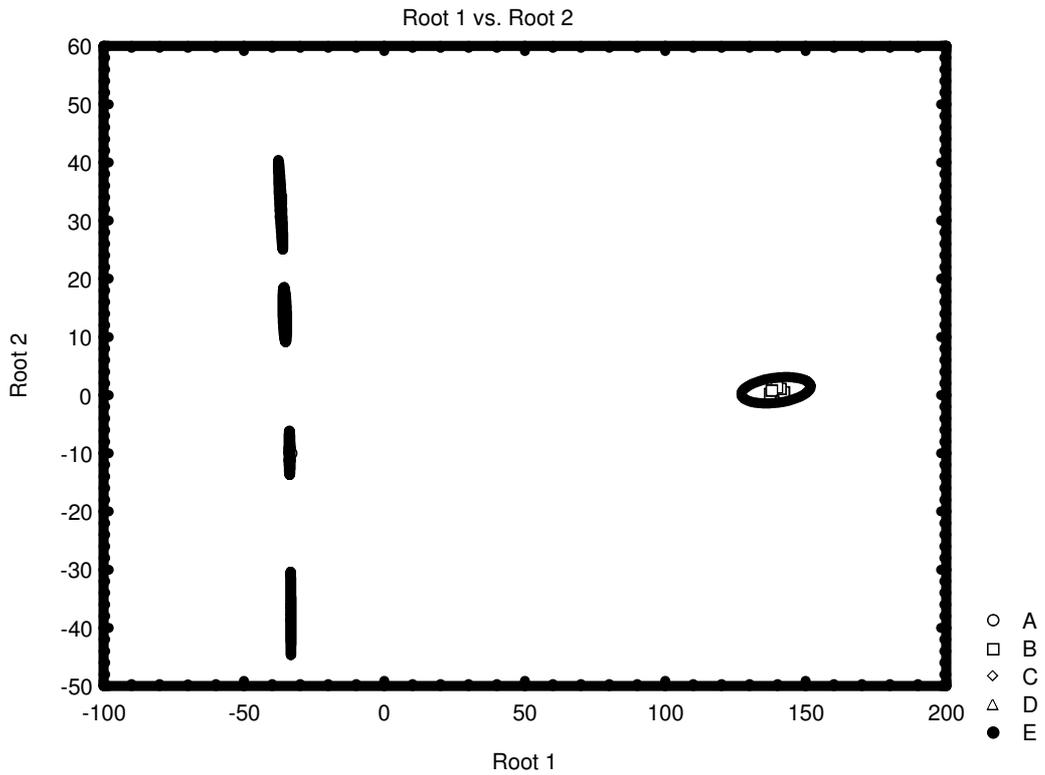
Table 6. Metal concentrations in marcs subjected to different pesticide treatments; five samples for each plot were analysed in triplicate (mean±st.dev). Significances of differences between blank and treated samples are also reported as chi-square (χ^2) and p values from Kruskal- Wallis test

year		2003				
PLOT	Mn (mgL ⁻¹)	Zn (mgL ⁻¹)	Cu (mgL ⁻¹)	Pb (µgL ⁻¹)	Cd (µgL ⁻¹)	
A	7.4±1.6	2.5±0.3	31.2±3.7	<0.1	<0.5	
B	19.3±2.8	7.2±0.7	56.1±6.9	<0.1	31.3±7.2	
C	33.2±4.0	9.1±1.5	51.4±2.9	<0.1	80.0±6.1	
D	3.9±0.7	8.7±0.5	161.7±10.1	<0.1	<0.5	
E	12.2±1.7	4.6±0.4	150.3±12.6	<0.1	<0.5	
χ^2	23.077	21.150	21.267	23.622	23.529	
p <	0.000	0.000	0.000	0.000	0.000	
		2004				
A	5.6±0.92	3.0±0.5	32.2±4.8	<0.1	<0.5	
B	10.7±2.0	4.7±0.7	33.2±3.6	71.1±2.2	<0.5	
C	12.4±1.2	10.9±1.4	31.7±2.9	42.3±6.4	63.4±8.3	
D	5.5±0.9	0.6±0.1	86.1±7.4	46.8±7.1	<0.5	
E	2.8±0.8	9.2±0.9	93.5±3.7	<0.1	<0.5	
χ^2	21.186	22.457	17.935	22.401	23.622	
p <	0.000	0.000	0.001	0.000	0.000	

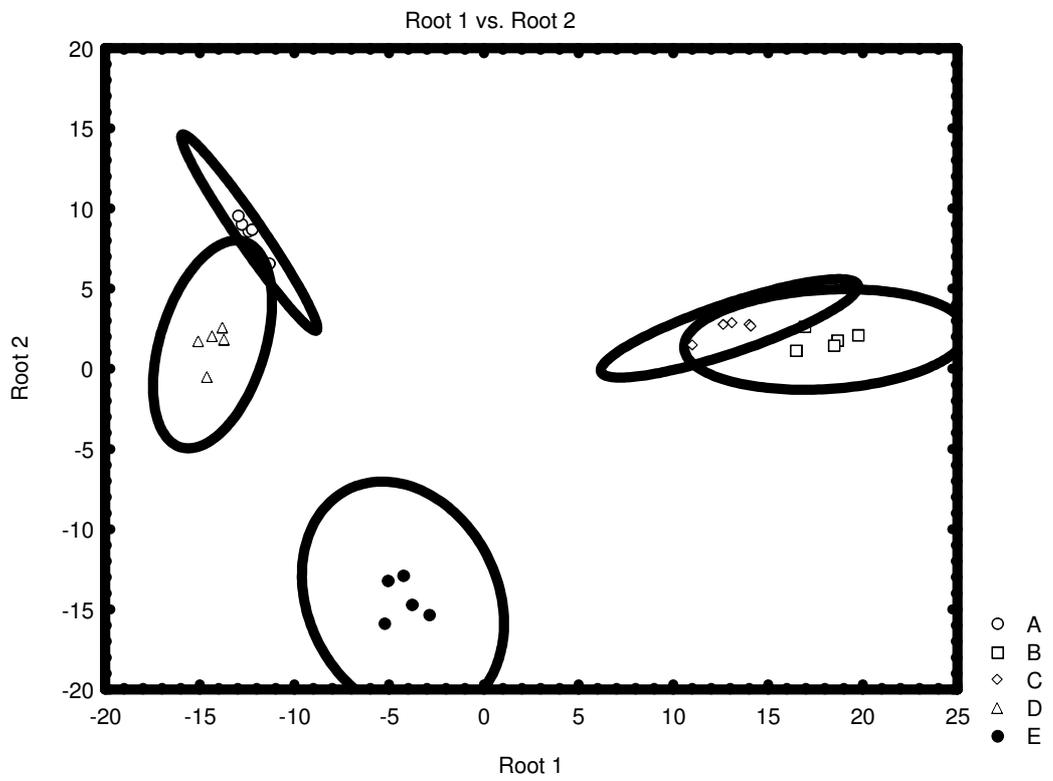


57
58
59
60
8 **Figure 1:** Linear Discriminant Analysis for wines from 2003 (a) and 2004 (b)

1
2
3 9
4 10
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30 11
31 12
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57 13
58 14
59 15
60



a)



b)

Figure 2: Linear Discriminant Analysis for grape stalks from 2003 (a) and 2004 (b)

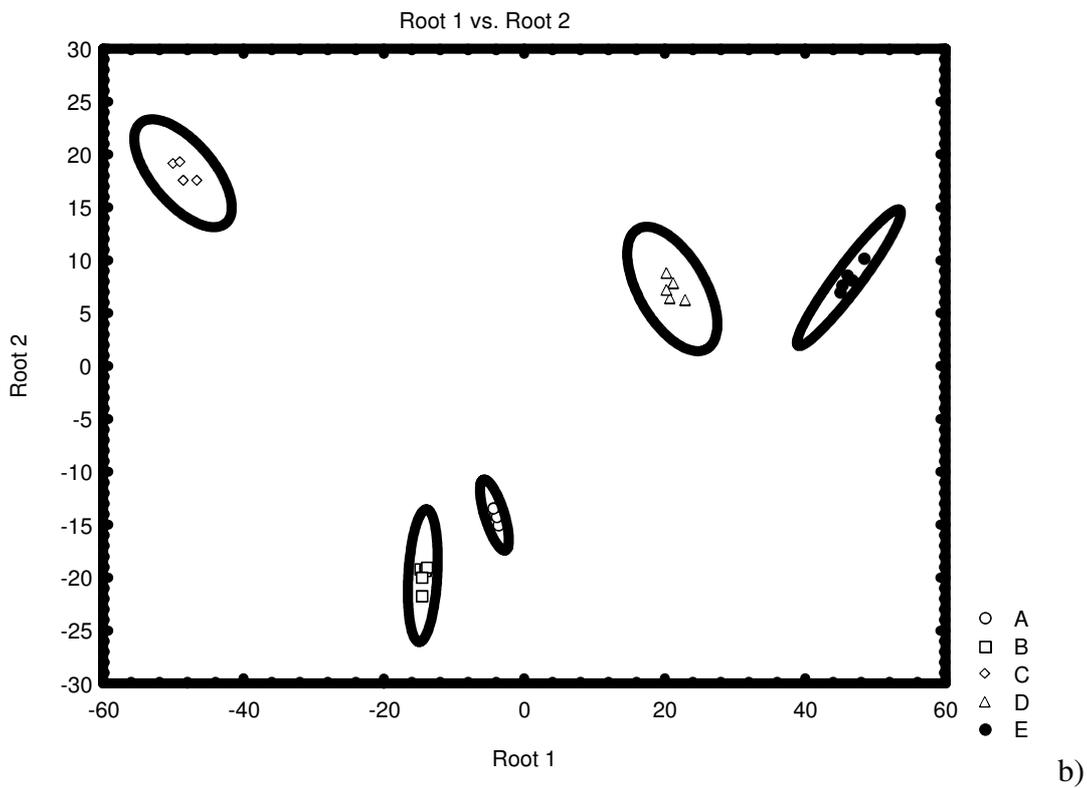
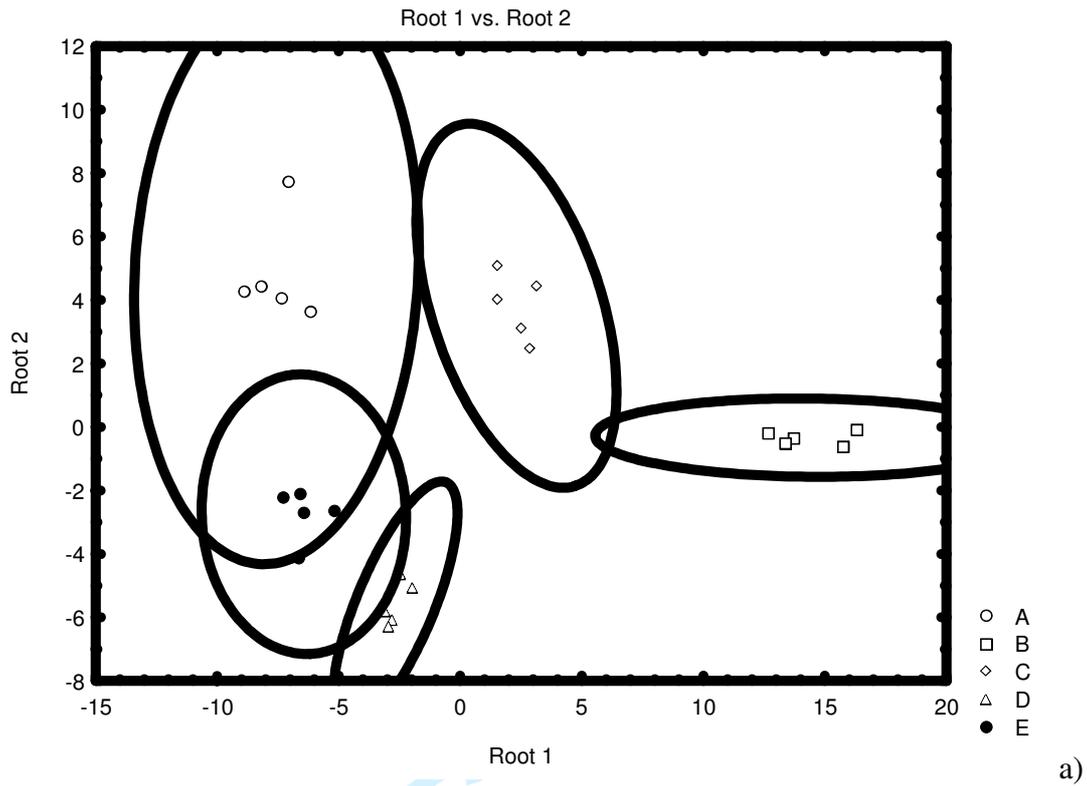


Figure 3: Linear Discriminant Analysis for grapes from 2003 (a) and 2004 (b)

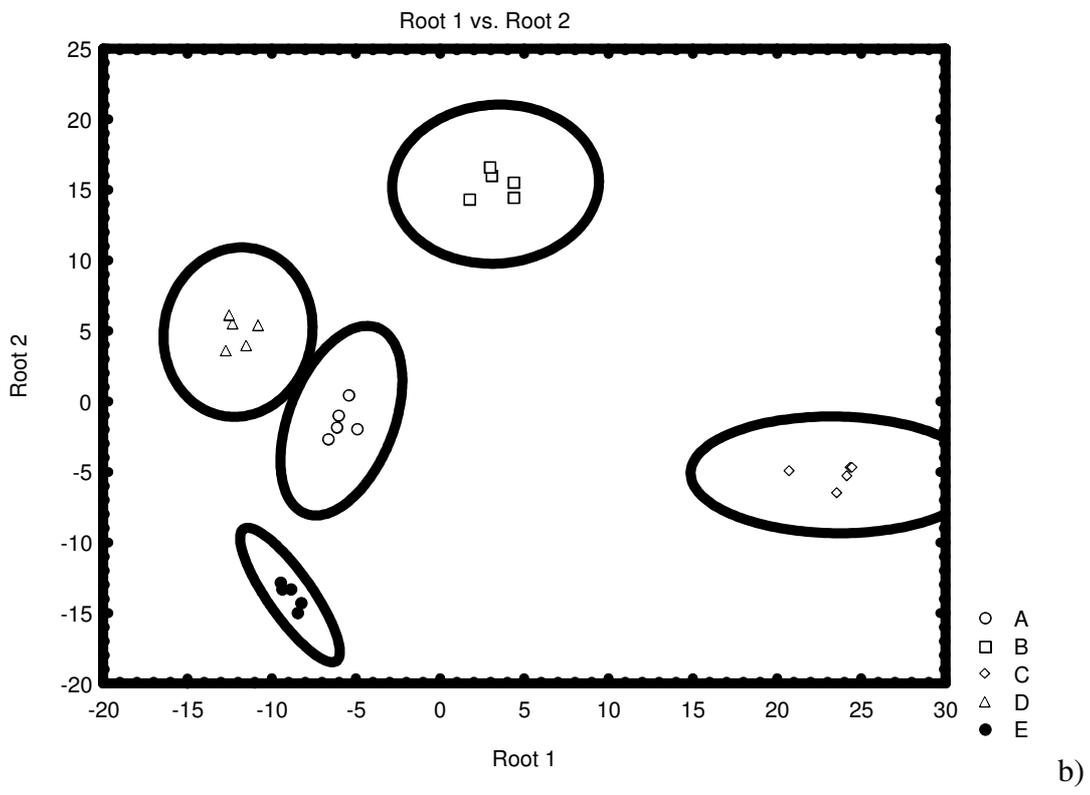
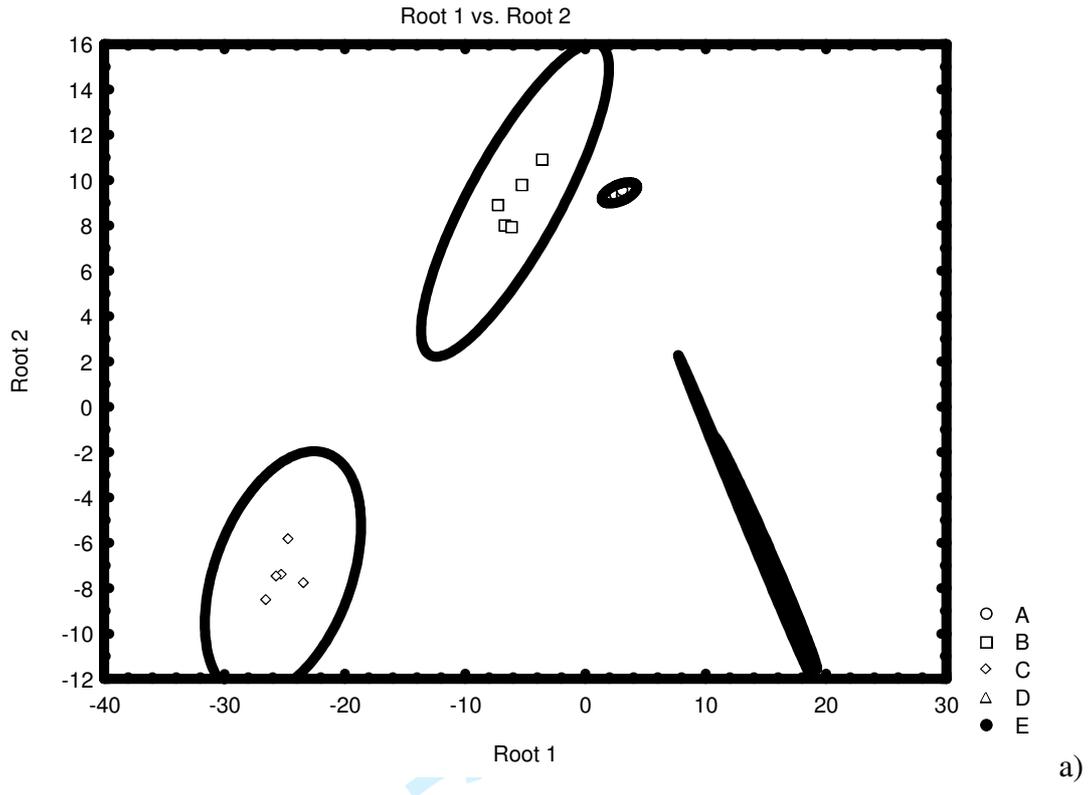


Figure 4: Linear Discriminant Analysis for marcs from 2003 (a) and 2004 (b)