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Influence of olive cultivars and period of harvest on the contents of Cu, Cd, Pb, and Zn in virgin olive oils

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Abstract

Levels of four of the major pollutant heavy metals were assessed by ICP-OES in virgin olive oil monocultivar samples. The data showed high variability within cultivars for lead and zinc, whereas, for cadmium and copper, no statistical difference was observed. The influence of the cultivar and the stage of ripening of olives on heavy metal content was assessed; zinc was the only metal with a great variability within the first and the second harvest. All olive samples were processed *with the same milling apparatus.*

Keywords: Heavy metals; Olive oil; ICP-OES

1. Introduction

The analytical determination of heavy metals, such as Cd, Cu, Pb, and Zn, in biological matrices is nowadays recognised as an important parameter concerning food hygiene, foodstuff quality and food chemistry (Payne, Alins, Gunselman, Rahimtula, & Yeats, 1998; Underwood & Mertz, 1987), although it is still not mandatory.

Heavy metals are normal constituents of earth soil and are part of all ecosystems. On the other hand, humans have dramatically modified the distribution and the amounts of these elements in the environment. Heavy metals present in ground, in water, and in air may contaminate aliments, drinking water, and consequently humans (Payne et al., 1998). Data from the UNEP classify Italy in the first position in marine pollution for Cd, Cu, Pb, and Zn levels (UNEP/MAP, 2003).

A source of contamination in agriculture is related to the use of compost of unknown origins (Amlinger, 2004). The adsorption process of metals may differ, not only between plant species, but also between cultivars within one species. Moreover, the accumulation varies in the different parts of the plant, being lower in leaves than in roots and in fruits.

Heavy metals cannot be degraded or destroyed in the environment and, to a small extent, they enter our bodies via food, drinking water, and air. As trace elements, some heavy metals are essential to maintain the metabolism of the human body (Underwood & Mertz, 1987). However, at higher concentrations they can lead to poisoning. In particular, heavy metals interfere with vital biochemical processes, such as the Krebs cycle and steroid synthesis (Reijnders & Brausseur, 1997). Free heavy metals can also attack molecular binding sites, breaking them and increasing the production of free radicals. Heavy metals are dangerous because they tend to bioaccumulate.

The list of 14 toxic metals published by Morgan and Stumm (1991) includes: antimony, arsenic, silver, bismuth, cadmium, chromium, indium, mercury, lead, copper,

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selenium, tin, thallium and zinc. In 2003, the Agency for Toxic Substances and Disease Registry (ATSDR) of the United States of America published a list of 275 organic and inorganic substances hazardous for humans and the environment (CERCLA Priority List Of Hazardous Substances, 2003). Among the 20 most dangerous compounds, 5 were heavy metals. Arsenic, lead, mercury, cadmium and chromium were at 1st, 2nd, 3rd, 7th, and 17th positions, respectively. Data from the World Health Organization (WHO), report that 60–70% of all acute and chronic diseases can be associated with heavy metal pollution (FAO/WHO, 1993).

The presence of heavy metals in foods may also affect food quality. For instance, trace levels of some heavy metals may catalyse oxidation reactions, with deleterious effects on oil flavour and storage (Fedeli, 1968). Lead is the only heavy metal having a fixed maximum residue level (MRL) for olive oil (0.10 mg/l). Dugo, Lo Curto, Lo Turco, La Torre, and Salvo (2002) studied the presence of heavy metals in Sicilian olive oils. The average level found was below the MRL (0.09 mg/l), but 8 of the 34 samples analysed exceeded the MRL, having maximum values of 0.28 mg/l. The higher levels of Pb were correlated with the use of organophosphorus pesticides. The literature on heavy metals on olives and olive oil mainly involves analytical methods for their determination in oils (Castillo, Jimenez, & Ebdon, 1999; Jimenez, Velarte, & Castillo, 2003; Jimenez, Velarte, Gomez, & Castillo, 2004; La Pera, Lo Coco, Mavrogeni, Giuffrida, & Dugo, 2002; La Pera, Lo Curto, Visco, La Torre, & Dugo, 2002; Llbet, Falco, Casa, Teixido, & Domingo, 2003; Lo Coco, Ceccon, Circolo, & Novelli, 2003; O'Connor, Rowland, & Evans, 2002; Roca, Cabrera, Lorenzo, & Lopez, 2004) but lacks in quantitation and in field experiments.

In this paper, we study the amounts of copper, cadmium, lead, and zinc on virgin olive oils, pointing out the influence of the cultivar and of the period of harvest.

2. Materials and methods

2.1. Samples

The trials were carried out in Sardinia on nine different cultivars (Bosana, Pizz'e Carroga, Mallocrina, Manna, Nera di Gonnos, Nera di Oliena, Ogliastrina, Semidana, Tonda di Cagliari). Bosana (16 samples) and Pizz'e Carroga (12 samples) were the most represented cultivars.

Samples of 300–400 kg of olives were randomly harvested in the principal olive tree vegetation zone of Sardinia. Harvest was carried out in the same olive grove at the beginning of invaiatura and after 1 month. After harvesting, all samples were processed at the Consorzio Interprovinciale di Frutticoltura di Cagliari, Oristano e Nuoro at Villasor (Cagliari, Italy), with the following procedure: the olives were cleaned of leaves, stems, dirt, rock, and sands. The olives were crushed with a hammer mill and the paste was subjected to malaxation for 30 min; then the oil was separated from the water and the feed using a dual phase centrifugal decanter. Cultivar, vegetation zone, and time of harvest for each sample are listed in Table 1.

In order to assess the merceological class, all oils were subjected to the following analyses: acidity, peroxide number, K232, ΔK (Regulation (EEC) No. 2568/91).

2.2. Chemicals

Distilled, deionized water 18 MΩ cm resistivity, obtained from a Milli-Q (Millipore, Bedford, MA) was used to prepare all solutions. Nitric acid at 69.5% (Fluka, Trace-Select), and hydrogen peroxide at 30% (Aldrich, Milan, Italy) were reagent grade solvents. Copper (10090 μg/ml in 1–2 wt % HNO_3), cadmium (10080 μg/ml in 1–2 wt % HNO_3), lead (9812 μg/ml in $\leqslant$2 wt % HNO_3) and zinc (10116 μg/ml in 1–2 wt % HNO_3) were ICP/DCP analytical standards (Aldrich, Milan, Italy). All glass and plastic ware were soaked overnight in 10% v/v nitric acid, and rinsed three times with deionized water before use.

2.3. Apparatus

A simultaneous atomic emission spectrometer ICP-OES Varian Vista-MPX (Varian Inc., Palo Alto, CA) with axial viewed plasma, was used. The plasma was operated with a concentric nebulizer coupled to a cyclone type nebulization chamber. The operating conditions were set as follows: power 1.2 kW; plasma flow gas 15 l/min; auxiliary gas flow 1.5 l/min; nebulizer gas flow 0.9 l/min. The wavelengths used for the quantification were: copper 324.754 nm; cadmium 226.502 nm; lead 217.000 nm; zinc 206.200 nm.

The limit of detection (LOD) was calculated according to Szikla (2004).

2.4. Microwave-assisted acid decomposition

To dissolve the oil sample for elemental analysis, microwave-assisted acid decomposition was performed at high pressure and temperature. The digestion was carried out as follows: 4 ml of nitric acid at 69.5%, and 2 ml of hydrogen peroxide at 30% were added to 0.25 g of oil. A CEM model MarsX Microwave-Assisted system was used. The microwave operation parameters were: power 300 W (83%) for 15 min, 600 W (75%) for 10 min, 1200 W (65%) for 15 min, 300 W (83%) for 5 min; maximum temperature was 200 °C. After digestion, samples were allowed to cool to room temperature for 12 h; the residues were taken up with nitric acid at 1% and brought to a final volume of 25 ml with bi-distilled water.

Quantitative determinations were made by the standard additions method (Gaines, 2004).

3. Results and discussion

The data listed in Table 2 indicate that all samples analysed are virgin oils (Commission Regulation (EC) No.

Table 1
Cultivar, vegetation zone, and time of harvest of the samples analysed

Vegetation zone	Time of harvest	Vegetation zone	Time of harvest
Cultivar Bosana			
Villacidro	26/11/03, 15/12/03	Villacidro	02/12/03, 22/12/03
Berchidda	28/11/03, 05/12/03	Seneghe	03/12/03, 14/01/04
Dolianova	02/12/03, 16/12/03	Villasor	04/12/03, 04/01/03
Orosei	17/12/03, 18/01/04	Alghero	17/12/03, 15/01/04
Oliena	19/12/03, 15/01/04		
Cultivar Mallocrina			
Gergei	12/11/03, 11/12/03		
Cultivar Manna			
Seneghe	03/12/03, 14/01/04		
Cultivar Nera di Gonnos			
Gonnosfanadiga	11/11/03, 03/12/03	Villasor	11/11/03, 03/12/03
Orosei	17/12/03, 17/01/04		
Cultivar Nera di Oliena			
Oliena	19/12/03, 15/01/04		
Cultivar Ogliastrina			
Lanusei	16/12/03, 17/01/04		
Cultivar Pitz'e Carroga			
Ussasai	04/11/03, 02/12/03	Dolianova	12/11/03, 04/12/03
Gonnosfanadiga	11/11/03, 03/12/03	Berchidda	13/11/03, 28/11/03
Gergei	12/11/03, 11/12/03	Dolianova	17/11/03, 10/12/03
Cultivar Semidana			
Villasor	10/12/03, 12/01/04	Oristano	09/12/03, 22/12/03
Cultivar Tonda di Cagliari			
Dolianova	12/11/03, 04/12/03	Dolianova	17/11/03, 10/12/03
Oristano	20/11/03, 09/12/03		

Table 2
Acidity (% oleic acid), peroxide number (meqO$_2$/kg), and K232, K270, and ΔK values in the oil samples studied

Cultivar	Oleic acid (%)	Peroxide number	K270	K232	ΔK
Bosana	0.26–0.56 (0.90[a])	4.02–6.67	0.15 ± 0.02	2.06 ± 0.13	−0.001 ± 0.002
Mallocrina	0.28–0.45	5.67–6.72	0.60 ± 0.01	2.30 ± 0.50	0.001 ± 0.005
Manna	0.65–(1.66[a])	4.74–4.83	0.12 ± 0.01	2.06 ± 0.09	0.001 ± 0.001
Nera di Gonnos	0.36–0.49	6.60–7.14	0.12 ± 0.02	2.12 ± 0.11	0.001 ± 0.001
Nera di Oliena	0.37–0.45	5.08–9.35	0.15 ± 0.01	1.98 ± 0.06	−0.002 ± 0.001
Ogliastrina	0.35–0.40	3.89–6.32	0.14 ± 0.00	1.94 ± 0.18	0.001 ± 0.000
Pitz'e Carroga	0.26–0.47	5.16–7.82	0.12 ± 0.03	2.17 ± 0.27	0.001 ± 0.002
Semidana	0.36–0.40	4.17–7.22	0.15 ± 0.02	2.03 ± 0.11	−0.001 ± 0.003
Tonda di Cagliari	0.31–0.66	6.14–7.15	0.11 ± 0.03	1.81 ± 002	−0.002 ± 0.001

[a] Sample with high levels of acidity %.

1989/2003 of 6 November 2003), with acidity and peroxide numbers below 2% and 20 meqO$_2$/kg, respectively, and the spectrophotometric analysis (K232, K270, and ΔK) show that they are below their legal limits. Most of the samples can be classified as extra virgin oils, the acidity and peroxide number being below 0.8%, and 10 meqO$_2$/kg, respectively. Only two samples, one from Bosana and the other from Manna, showed higher acidity values (0.90% and 1.66%, respectively) and can be classified as virgin oils. Adopting good agricultural practice (GAP), the time of harvest does not affect the levels of peroxide, the acidity of the oils or the spectrophotometric analysis detection limits (LOD) which were: 0.05 μg/l for cadmium, 0.25 μg/l for copper, 0.30 μg/l for zinc, and 3.75 μg/l for lead. The amounts of heavy metals in the samples analysed are listed in Table 3. The levels of cadmium are similar in the different cultivars, ranging from 4.8 to 9 μg/l with an average value of 5.3 μg/l. A similar conclusion can be drawn for copper, which ranged from 4.0 to 5.2 μg/l with an average value of 4.3 μg/l. The amount of lead determined showed a significant variability, both in different cultivars and in different samples from the same cultivar. The cultivar Nera di Oliena with 10.3 μg/l, showed the lowest amount, whereas the Tonda di Cagliari (36.3 μg/l) showed the highest. However, all oils showed amounts under the MRL adopted for oils and fats

Table 3
Influence of the cultivar on heavy metal amounts (μg/l ± SD) in olive oils

Cultivar	Cadmium	Copper	Lead	Zinc
Bosana	5.4 ± 0.4	4.3 ± 0.1	19.1 ± 3.7	3.0 ± 4.9
Mallocrina	5.4 ± 0.2	4.3 ± 0.0	19.6 ± 1.2	2.2 ± 2.0
Manna	5.3 ± 0.3	4.2 ± 0.2	14.7 ± 1.6	2.2 ± 2.3
Nera di Gonnos	5.3 ± 0.4	4.4 ± 0.1	17.5 ± 5.4	6.4 ± 6.2
Nera di Oliena	5.3 ± 0.5	4.4 ± 0.1	13.8 ± 4.9	1.4 ± 1.1
Ogliastrina	5.0 ± 0.3	4.3 ± 0.1	27.9 ± 1.3	0.7 ± 0.1
Pitz'e Carroga	5.8 ± 1.2	4.6 ± 0.3	17.7 ± 4.3	4.8 ± 6.6
Semidana	5.0 ± 0.2	4.3 ± 0.2	14.1 ± 1.4	1.5 ± 0.9
Tonda di Cagliari	5.3 ± 0.3	4.3 ± 0.2	22.7 ± 10.4	10.3 ± 12.8

Table 4
Influence of the time of harvest (1st, 2nd) on heavy metal amounts (μg/l ± SD) in olive oils

Cultivar	Lead		Copper		Cadmium		Zinc	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Bosana	19.3 ± 4.1	18.8 ± 3.5	4.4 ± 0.1	4.3 ± 0.2	5.4 ± 0.5	5.3 ± 0.3	4.4 ± 6.3	1.3 ± 1.2
Mallocrina	18.7 ± 3.9	20.4 ± 2.7	4.3 ± 0.0	4.3 ± 0.1	5.5 ± 0.2	5.2 ± 0.2	3.6 ± 0.1	0.8 ± 0.0
Manna	13.6 ± 2.6	15.8 ± 2.1	4.3 ± 0.1	4.0 ± 0.0	5.5 ± 0.2	5.1 ± 0.1	3.8 ± 0.1	0.5 ± 0.0
Nera di Gonnos	17.4 ± 6.1	17.6 ± 6.6	4.4 ± 0.1	4.5 ± 0.1	5.3 ± 0.5	5.2 ± 0.3	4.8 ± 5.1	4.2 ± 5.1
Nera di Oliena	10.3 ± 2.1	17.2 ± 4.3	4.3 ± 0.0	4.4 ± 0.1	4.9 ± 0.2	5.6 ± 0.4	2.2 ± 0.0	0.6 ± 0.0
Ogliastrina	29.7 ± 2.1	27.9 ± 3.4	4.2 ± 0.1	4.3 ± 0.1	4.8 ± 0.1	5.2 ± 0.3	0.6 ± 0.0	0.7 ± 0.0
Pitz'e Carroga	19.3 ± 4.5	15.6 ± 3.3	4.5 ± 0.2	4.6 ± 0.4	6.1 ± 1.5	5.4 ± 0.6	5.9 ± 8.6	3.1 ± 2.1
Semidana	13.3 ± 0.1	15.7 ± 1.9	4.3 ± 0.2	4.3 ± 0.1	5.0 ± 0.2	5.1 ± 0.2	1.1 ± 0.7	2.4 ± 0.1
Tonda di Cagliari	28.0 ± 12.8	17.4 ± 4.9	4.4 ± 0.3	4.1 ± 0.1	5.6 ± 0.3	5.1 ± 0.1	18.4 ± 14.5	2.2 ± 0.2

(100 μg/l). Also zinc showed a high variability with minimum and maximum values obtained for Bosana (0.1 μg/l) and Tonda di Cagliari (27.2 μg/l), respectively. The data reported in the literature are obtained by different methods such as ICP-MS (Castillo et al., 1999; Jimenez et al., 2003, 2004; La Pera, Lo Coco, et al., 2002; La Pera, Lo Curto, et al., 2002; Llbet et al., 2003; O'Connor et al., 2002), flame atomization atomic absorption (FAO/WHO, 1993), and potentiometric stripping (La Pera, Lo Coco, et al., 2002; La Pera, Lo Curto, et al., 2002; Lo Coco et al., 2003). Lo Coco et al. (2003) did not detect cadmium and the zinc values ranged between 26 and 60 μg/l, higher than those found in the Sardinian oils. Llbet et al. (2003) reported values of cadmium of about 8 μg/l and lead of about 30 μg/l but do not specify the number of samples analysed on the coefficient of variation so that the data cannot be comparable. Dugo et al. (2002) reported a minimum lead level of 5.82 μg/l, and maximum 280.17 μg/l with an average value of 94.13 μg/l, under the legal limit of 100 μg/l. Nevertheless 8 of the 34 samples analysed were above the legal limit. The high levels of lead in some samples were correlated with the use of organophosphorus pesticides. La Pera et al. (2002) shows levels of Pb between 32.6 and 157 μg/l, but only 4 samples were analysed. La Pera, Lo Coco, et al. (2002) analysed more samples, 36 Sicilian oils in two different years and 22 from Apulia. The oils harvested in the second year showed lower values of lead, and those picked manually had lower values than those mechanically harvested. The time of harvest did not affect significantly the contents of lead, copper and cadmium in the analysed oils (Table 4). Some samples of Tonda di Cagliari showed higher values for lead and zinc in the first harvest than in the second. Levels of zinc were, for all cultivars, higher in the first harvest than in the second, except for ogliastrina and semidana.

All Sardinian olive oils analysed showed very low contents of metals. Levels of lead were below MRLs fixed for olive oil and fats, although some of the olive grove were treated with organophosphorous pesticides (Dugo et al., 2002). Zinc was the only metal with a great variability within the first and the second harvest.

References

Amlinger, F. (2004). Heavy metals and organic compounds from waste used as organic fertilisers. In: *Joint conference of the Dutch EU Presidency and the European Commission for the environment*, 18 and 19 November.

Castillo, J. R., Jimenez, M. S., & Ebdon, L. (1999). Semiquantitative simultaneous determination of metals in olive oil using direct emulsion nebulization. *Journal of Analytical Atomic Spectrometry, 14*(9), 1515–1518.

CERCLA priority list of hazardous substances (2003).

Commission Regulation (EC) No. 1989/2003 of 6 November 2003.

Dugo, G., Lo Curto, S., Lo Turco, V., La Torre, L., & Salvo, F. (2002). Valutazione del contenuto di Cu(II), Zn (II), Cd (II) e Pb (II) in oli di oliva prodotti nella valle del belice. *Rivista Italiana Sostanze Grasse, 79*, 157–160.

FAO/WHO. (1993). Evaluation of certain food additives and contaminants. *Technical Report*, Series 837, World Health Organization, Geneva.

Fedeli, E. (1968). In E. Marcuse (Ed.), *Metals catalysed lipid oxidation* (pp. 105). Goteborg, Sweden: SIK.

Gaines, P. ICP operations. http://www.ivstandards.com/tech/icp-ops/part10.asp, 2004.

Jimenez, M. S., Velarte, R., & Castillo, J. R. (2003). On-line emulsions of olive oil samples and ICP-MS multi elemental determination. *Journal of Analytical Atomic Spectrometry, 18*(9), 1154–1162.

Jimenez, M. S., Velarte, R., Gomez, M. T., & Castillo, J. R. (2004). Multielement determination using on-line emulsion formation and ICP-MS/FAAS for the characterization of virgin olive oils by principal component analysis. *Atomic Spectroscopy, 25*(1), 1–12.

La Pera, L., Lo Coco, F., Mavrogeni, E., Giuffrida, D., & Dugo, G. (2002). Determination of copper (II), lead (II) and zinc (II) in virgin olive oils produced in Sicily and Apulia by derivative potentiometric stripping analysis. *Italian Journal of Food Science, 14*(4), 389–399.

La Pera, L., Lo Curto, S., Visco, A., La Torre, L., & Dugo, G. (2002). Derivative potentiometric stripping analysis (dPSA) used for the determination of cadmium, copper, lead, and zinc in Sicilian olive oils. *Journal of Agricultural and Food Chemistry, 50*(11), 3090–3093.

Llbet, J. M., Falco, G., Casa, C., Teixido, A., & Domingo, J. L. (2003). Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catolina, Spain. *Journal of Agricultural and Food Chemistry, 51*, 838–842.

Lo Coco, F., Ceccon, L., Circolo, L., & Novelli, V. (2003). Determination of cadmium(II) and zinc(II) in olive oils by derivative potentiometric stripping analysis. *Food Control, 14*, 55–59.

Morgan, J. J., & Stumm, W. (1991). Chemical processes in the environment, relevance of chemical speciation. In E. Merian (Ed.), *Metals and their compounds in the environment* (pp. 67–103). Weinheim: VCH.

O'Connor, G., Rowland, S. J., & Evans, E. H. (2002). Evaluation of gas chromatography coupled with low pressure plasma source mass spectrometry for the screening of volatile organic compounds in food. *Journal of Separation Science, 25*(13), 839–846.

Payne, J. F., Alins, D. C., Gunselman, S., Rahimtula, A., & Yeats, P. A. (1998). DNA oxidative damage and vitamin a reduction in fish from a large lake system in Labrador, Newfoundland, contaminated with iron-ore mine tailings. *Marine Environmental Research, 46*, 289–294.

Regulation (EEC) No. 2568/91.

Reijnders, P. J. H., & Brausseur, S. M. J. M. (1997). Xenobiotic induced hormonal and associated developmental disorders in marine organisms and related effects in humans. An overview. *Journal of Clean Technology Environmental Toxicology and Occupational Medicine, 6*, 367–380.

Roca, A., Cabrera, C., Lorenzo, M. L., & Lopez, M. C. (2004). Levels of calcium, magnesium, manganese, zinc, selenium and chromium in olive oils produced in Andalusia. *Grassa y Aceites, 51*(6), 393–399.

Szikla, I. (2004). Determination of 22 elements following US EPA guidelines with a new megapixel CCD ICP-OES. Varian ICP-OES Application Notes No. 30.

Underwood, E., & Mertz, W. (1987). Trace elements needs and tolerances. In W. Mertz (Ed.), *Trace elements in human and animal nutrition* (pp. 11–19). London: Academic Press.

UNEP/MAP. (2003). Assessment of transboundary pollution issues in the Mediterranean Sea. Athens: UNEP/MAP, Mediterranean shelved at: 153/K2003/MED.POL. p. 292.