

Derivative Potentiometric Stripping Analysis (dPSA) Used for the Determination of Cadmium, Copper, Lead, and Zinc in Sicilian Olive Oils

LARA LA PERA,[†] SIMONA LO CURTO,[†] ALESSANDRO VISCO,[‡]
LOREDANA LA TORRE,[†] AND GIACOMO DUGO^{*,†}

Dipartimento di Chimica Organica e Biologica, University of Messina, Salita Sperone 31, 98166 Messina, Italy, and Centro Analisi e Servizi, s.r.l., via U. La Malfa 18, 98051 Barcellona, P.G., Messina, Italy

Derivative potentiometric stripping analysis (dPSA) was utilized to evaluate the Cd(II), Cu(II), Pb(II), and Zn(II) content in olive oil samples produced in Sicily in the crop year 2000–2001. The repeatability of the method was attested at 86.36% for cadmium, at 94.94% for copper, at 99.00% for lead, and at 98.92% for zinc. Recovery tests were carried out, both on cleanup procedures and on extraction steps, on olive oil spiked at different levels; obtained recoveries were 84.52 ± 9.86 for cadmium, 97.34 ± 2.72 for copper, 100.68 ± 0.67 for lead and 83.35 ± 1.72 for zinc. Theoretic detection limits were 1.2 ng g^{-1} for Cd, 3.6 ng g^{-1} for Cu, 5.9 ng g^{-1} for Pb, and 14.3 ng g^{-1} for Zn. Found concentrations range were $15.94\text{--}58.51 \text{ ng g}^{-1}$ for Cu, $32.64\text{--}156.48 \text{ ng g}^{-1}$ for Pb, and $157.00\text{--}385.22 \text{ ng g}^{-1}$ for Zn. Copper, lead, and zinc were found in all samples. The main advantage of this determination consists of a not too aggressive metals extraction procedure using hydrochloric acid, which avoids losses of elements typical of sample calcinations methods.

KEYWORDS: Potentiometric analysis; metals; olive oil

INTRODUCTION

Heavy metals are present everywhere, variously distributed in nature, and are found in soil, air, water, and many alimentary matrices (1–6). Lead is considered to be one of most dangerous elements for human health and is mainly originated by combustion of carburant, industrial emissions, varnishes, and chemical colorants. Cadmium is even more dangerous because it is ~10 times more toxic than lead, and its adsorption may be carried by water, air, and food because it is utilized in industry as a battery electrode, a pigment for paints, and a stabilizer of rubbers and plastics. Other metals, such as zinc and copper, have a biological action at low doses and a toxic effect when taken at higher quantities (7). A high concentration of copper in foods is mainly originated from copper-containing fungicide residues used in agriculture and from water plumbings. Zinc is variously contained in foods and in environment and, at this moment, has unknown toxic symptomatology due to elevated assumption of this element with the diet are unknown (8). Among alimentary matrices, even in olive oil it is possible to find the presence of heavy metals (9–11), due to a variety of factors such as olive treatment processes (Cu contamination), packaging procedures (cession of Cd and Cu), or the presence of streets,

highways, or metallurgic industries near olive plantation (presence of Pb) (8). Moreover, it is important to determine trace levels of some metals, such as copper, in edible oils because it could catalyze oxidation of fatty acid chains, exerting a deleterious influence over shelf life (12). To determine metallic elements are often utilized atomic adsorption spectroscopy techniques (5, 9); the quantities found are, generally, on the order of nanograms per gram. In this work was utilized, as an alternative, derivative potentiometric stripping analysis (dPSA) to quantify Cd, Cu, Pb, and Zn in olive oil samples from Sicily. This very sensitive analytical technique is used to determine traces of metals (4, 11); it is a combination of classic potentiometric analysis and polarographic methods such as, in particular, anodic stripping voltammetry (ASV) (13). In both techniques metals are concentrated by electrolysis on a working electrode, which in potentiometric stripping analysis (PSA) is a carbon electrode coated with a mercury or gold film. The electrodeposition is managed at an unvarying potential (plating potential), and metal reoxidation is carried in the presence of a chemical oxidant, such as Hg(II). dPSA is utilized to facilitate evaluation of the analytical signal by using its derivative. Potential and time data are digitally converted into dt/dE , and E is plotted against dt/dE . The potential versus dt/dE curve has the form of a Gaussian curve, and the peak, symmetrical with respect to the abscissa, has an area normally proportional to the concentration of the analyte.

* Corresponding author (fax +39 090 676 5180; e-mail dugogia@isengard.unime.it).

[†] University of Messina.

[‡] Centro Analisi e Servizi.

Table 1. Analytical Conditions for Determination of Pb, Cu, Cd, and Zn

	units	Pb	Cu	Cd	Zn
integration range	mV	-580; -380	-380; -140	-760; -590	-1100; -750
potential range	mV	-900; -100	-900; -100	-900; -100	-1200; -100
conditioning potential	mV	50 × 5 s	50 × 5 s	50 × 5 s	50 × 5 s
plating potential	mV	-950	-950	-950	-1250
plating time	min	3	3	3	3
stripping time	s	10	10	10	10
acquisition final potential	mV	0	0	0	0
sampling time	μs	300	300	300	300
discharge potential	mV	-430	-260	-640	-950
agitation speed	turns/s	2	2	2	2
cycles		2	2	2	2
standard additions		2	2	2	2

MATERIALS AND METHODS

Reagents. Four virgin olive oil samples, produced in Sicily in the crop year 2000–2001, were analyzed. All oils were sampled in dark glass bottles, with blind nipples, and stored at 4 °C until the analyses. Ultrapure hydrochloric acid (34–37%), Hg(II) (1000 μg mL⁻¹, 1 M in hydrochloric acid), and Cd(II), Cu(II), Pb(II), Zn(II) (1000 μg mL⁻¹, 0.5 N in nitric acid) standard solutions were purchased by Panreac (Barcelona, Spain); Ga(NO₃)₃·3H₂O (5 g, 99.9%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Filtration was effected on a carbon column Supelclean ENVI-Carb SPE (0.5 g, 6 mL), purchased from Supelco (Bellefonte, PA); the column was activated by pure HPLC methanol, purchased from Carlo Erba Reagenti (Milano, Italy). Ultrapure water (18.2 Mohm cm⁻¹) was prepared at the Department of Organic and Biological Chemistry, University of Messina.

Apparatus. Analyses were carried out with a potentiometer ION³ Steroglass (Perugia, Italy); data were elaborated by Neotes 2.0.1 software. To confirm the analytical results of the potentiometric method, the oily extracts were subjected to atomic absorption spectrometry (AAS), using a Shimadzu 800 series graphite furnace atomic absorption spectrometer, equipped with autosampler ASC-6100.

Sample Cleanup Procedure. A 19.2 g (20 mL) aliquot of oil was weighed and mixed with a 20 mL volume of concentrated HCl, under magnetic stirring in a Teflon beaker at 50 °C for 45 min. The moisture was transferred in a separation funnel and was allowed to cool for ~5 min in order to favor the separation of two phases; the lower acidic one was directly collected into a 40 mL volumetric flask, and the oily one was washed with a 20 mL volume of warm ultrapure water. The aqueous phase was again recovered and added to the first extract, up to the mark with water. To purify the total extract from organic compounds, it was filtered on a carbon column, previously activated by a 2 mL volume of methanol followed by the same aliquot of ultrapure water.

Electrode Preparation (Plating). The working electrode was a glassy carbon electrode coated with an Hg film; the reference one was a KCl saturated Ag–AgCl–Cl⁻ electrode; the counter was a Pt electrode. Before the start of every analysis, the working electrode was well cleaned with absolute methanol and filter paper; then the plating was effected by putting in the electrochemical cell a 20 mL volume of a 1000 μg mL⁻¹ Hg standard solution and carrying out the electrolysis, at -950 mV, against the reference electrode, for 1 min.

Potentiometric Determination. The determination of Cd, Cu, and Pb in oily extract was executed at pH 0.5, putting in the electrochemical cell a 3 mL volume of elute, a 17 mL volume of ultrapure H₂O, and a 1 mL volume of Hg(II) standard solution as oxidant agent. Every analysis was effected in two cycles, utilizing the multiple standard addition method by adding at the end of every cycle a 0.2 mL volume of each standard. The peak area was plotted against standard concentration, obtaining a calibration straight.

Zn has a plating potential higher than those of the other studied metals, and it was quantified individually. Metals determination was managed under analytical conditions described in **Table 1**.

Repeatability Evaluation. Method and instrument repeatabilities were evaluated by extracting three times the same oil sample and quantifying three times each analyte in the same extract. The repeat-

Table 2. Repeatability Tests

	ng g ⁻¹			
	Pb	Cu	Cd	Zn
first extraction	87.81	26.77	1.25	114.51
	87.64	28.06	1.21	114.43
	87.36	27.42	1.23	116.45
mean value	87.60	27.42	1.23	115.13
SD	0.23	0.65	0.02	1.14
% SD	0.26	2.35	1.63	0.99
second extraction	80.04	24.34	1.09	117.28
	78.84	29.99	1.69	119.59
	80.64	27.84	1.47	118.44
mean value	79.84	27.39	1.42	118.44
SD	0.92	2.85	0.30	1.16
% SD	1.15	10.41	21.43	0.98
third extraction	83.92	27.95	1.24	116.98
	82.18	29.33	1.50	114.20
	81.37	28.68	1.05	114.70
mean value	82.49	28.65	1.26	115.29
SD	1.30	0.69	0.23	1.48
% SD	1.58	2.41	17.88	1.29
total mean value	83.31	27.82	1.30	116.29
mean SD	0.82	1.40	0.18	1.26
mean % SD	1.00	5.06	13.64	1.08
repeatability for each metal	99.00	94.94	86.36	98.92

ability values for each metal were calculated by subtraction from 100, as theoretical value, the mean percent standard deviation. Mean values, standard deviations, percent standard deviations, and calculated repeatability values for each element are reported in **Table 2**.

Recovery Tests. For a greater accuracy in quantification of metallic ions, the possibility of decreases or increases of their amounts, due to the extract cleanup steps, was considered. Then, all above-described steps were repeated three times on an HCl solution spiked with a 42 ng g⁻¹ aliquot of each metal. Mean obtained recoveries were as follows: 94% for cadmium, 100% for copper, 105% for lead, and 99% for zinc. Further Zn recovery tests were managed in the presence of an excess of Ga(III), spiking an aqueous acidic solution (pH values of 2.0 and 3.0) at different levels with Cu and Zn. Ga(III) was added to the sample, as a 1000 μg mL⁻¹ solution, in an amount 200 times greater than Cu concentration (**Table 3**). A recovery of metallic ions from the oily matrix was effected by adding a 23 ng g⁻¹ quantity of each metal to a 19.2 g (20 mL) aliquot of tested oil; the moisture was left under magnetic stirring for 24 h to ensure a complete dissolution of metals in oil. Then, the above-described extraction procedure was executed. These steps were repeated again two times on the same oil sample. Mean obtained recoveries were as follows: 84% for cadmium, 97% for copper, 101% for lead, and 85% for zinc (**Table 4**); thus, further significant losses of Cd and Zn, due to the extraction steps, did not occur.

Detection Limits. Theoretical instrumental detection limits were evaluated utilizing the expression $3\sigma/S$, where σ is the peak threshold (set at 200) and S is the sensitivity obtained from the calibration straight (*11*). Under the above-described analytical conditions, detection limits

Table 3. Zn Recovery Tests, with and without Ga(III), from Acidic Aqueous Solution (Mean of Three Determinations)

Zn added (ng g ⁻¹)	Cu added (ng g ⁻¹)	Zn recovery (ng g ⁻¹)	Zn recovery (%)	pH
Without Ga(III)				
10	20	10.03 ± 1.13	100.33 ± 12.66	2
25	25	24.36 ± 0.49	97.10 ± 1.56	3
50	50	49.42 ± 2.56	98.87 ± 5.32	3
100	100	98.18 ± 2.40	98.20 ± 2.38	3
With Ga(III)/Cu(II) = 200:1				
10	10	9.38 ± 3.28	93.80 ± 7.11	2
30	30	28.57 ± 4.77	94.13 ± 14.78	2
50	50	49.36 ± 9.81	97.35 ± 6.64	2

Table 4. Recovery of Metals from Oily Matrix (Mean of Three Determinations)

metal	originally present (ng g ⁻¹)	added (ng g ⁻¹)	theor value (ng g ⁻¹)	found (ng g ⁻¹ ± SD)	recovery % (mean ± SD)
Pb(II)	142.03	23.00	165.03	166.15 ± 3.56	100.68 ± 0.67
Cu(II)	129.12	23.00	152.12	148.08 ± 8.30	97.34 ± 2.72
Cd(II)	nd ^a	23.00	23.00	19.44 ± 1.71	84.52 ± 9.86
Zn(II)	203.34	23.00	226.34	192.17 ± 4.72	85.35 ± 1.72

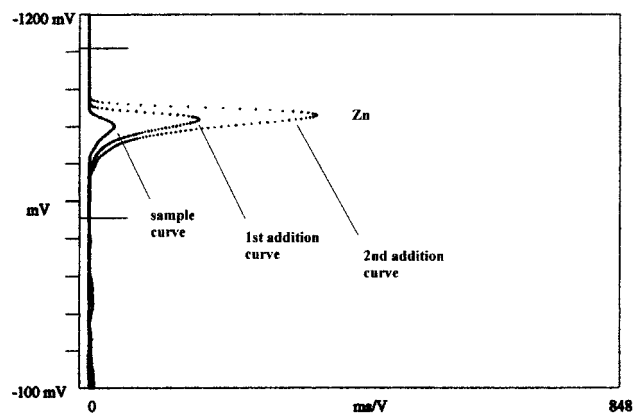
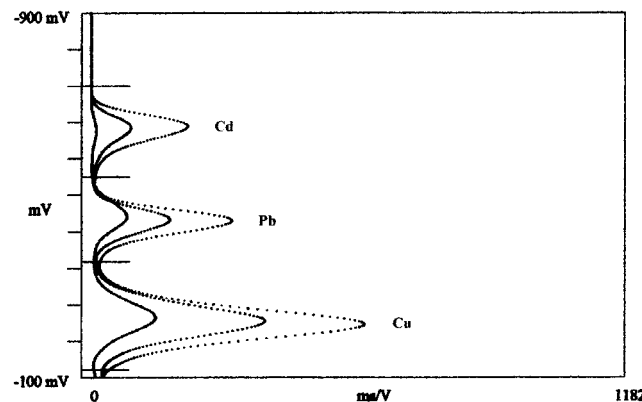
^a nd, not detectable.

were 1.2 ng g⁻¹ for Cd, 3.6 ng g⁻¹ for Cu, 5.9 ng g⁻¹ for Pb, and 14.3 ng g⁻¹ for Zn.

AAS Confirmation Analyses. The determination of lead and cadmium was carried out by adding, for each injection, 5 μL of a PdNO₃ solution (Pd concentration = 100 μg mL⁻¹), as matrix modifier. The working wavelengths were 228.8 nm for Cd, 324.8 nm for Cu, 283.3 nm for Pb, and 213.9 nm for Zn.

RESULTS AND DISCUSSION

The obtained results for the extra virgin olive oil samples are listed in **Table 5**. The presence of cadmium was not noticed in any listed sample, whereas lead, copper, and zinc were found in all samples. Cd was detected only in the oil sample utilized to carry on repeatability and detection limits tests. In **Figures 1** and **2** are reported the metals potential curves. Maximum values were 58.51 ± 1.31 ng g⁻¹ for Cu, 156.48 ± 12.06 ng g⁻¹ for Pb, and 385.22 ± 11.06 ng g⁻¹ for Zn, with minimum values of 15.94 ± 1.06, 32.64 ± 1.98, and 157.02 ± 7.56 ng g⁻¹, respectively. Zinc determination was effected at a pH value not lower than 2.0 in order to avoid H₂ developing at the working electrode, because it has an overvoltage similar to the Zn plating potential. In fact, as the Nernst equation shows (14), H₂ development increases proportionally to H⁺ concentration. To be sure that the range of potential, from -1100 to -800 mV, showed only the peak due to Zn oxidation, a blank test on ultrapure HCl solution (measured pH of 1.78) was effected; the studied range was free of any peak. Moreover, the Zn(II) plating potential (-1200 mV) is incompatible with that of Cu(II)

**Figure 1.** Zinc potentiometric stripping curve obtained under the experimental conditions specified in **Table 1**.**Figure 2.** Cadmium, lead, and copper potentiometric stripping curves obtained under the experimental conditions specified in **Table 1**.

because of Zn–Cu intermetallic compound formation on Hg film. The Zn interference with analysis of Cu can be eliminated by application of a plating potential less negative than -950 mV. The Cu interference with analysis of Zn can be avoided by the addition of an excess of Ga(III) when a pH range of 5–6 (15) is used, because the Ga–Cu compounds are more stable than Zn–Cu compounds. Nevertheless, it was observed that Ga(III) is not necessary in Zn dosage when the analysis is executed at a pH value below 3.

Data obtained from AAS as confirmation analyses are also reported in **Table 5**. No significant quantitative differences exist between the two data groups, even in consideration of the very small amounts of detected elements. The analytical method reveals a high repeatability (from 86.36% for cadmium to 99.00% for lead). Recovery tests showed that no excessive losses of metals occur during extraction and cleanup procedures. The calculated detection limits are so low that they permit a high precision of the quantitative analyses; moreover, the particularly small amounts of cadmium found, near its theoretical detection limit, motivate the repeatability for this metal, lower than others.

Table 5. Metal Concentrations (± Standard Deviations) Determined by dPSA and AAS (Each Value Is the Mean of Five Determinations)

sample	Pb (ng g ⁻¹)		Cu (ng g ⁻¹)		Cd (ng g ⁻¹)		Zn (ng g ⁻¹)	
	dPSA	AAS	dPSA	AAS	dPSA	AAS	dPSA	AAS
1	32.64 ± 1.98	34.74 ± 3.44	19.20 ± 1.14	18.63 ± 2.73	nd ^a	nd	385.22 ± 11.06	377.88 ± 18.73
2	156.48 ± 12.06	155.65 ± 18.22	15.94 ± 1.06	15.57 ± 3.14	nd	nd	207.92 ± 8.59	199.08 ± 15.34
3	43.14 ± 3.28	38.19 ± 4.47	58.51 ± 1.31	52.77 ± 2.13	nd	nd	339.78 ± 9.38	321.55 ± 16.17
4	129.31 ± 8.08	123.91 ± 11.45	45.57 ± 0.98	39.01 ± 2.87	nd	nd	157.02 ± 7.56	148.66 ± 13.07

^a nd, not detectable (<1.2 ng g⁻¹).

It has already been demonstrated (14) that the main advantage of this analytical method is the storing up of metals on the working electrode, which allows the recovery of very small quantities, on the order of nanograms per gram; however, the sensitivity of the instrument may be enhanced by lengthening metal deposition times. This method is free from electroattractive organic molecule interferences, because the working electrode, which is not polarized during the collection signal phase (in contrast to the ASV technique), remains inert toward organic compounds. The difference, compared to the other types of analytical determination by PSA on the same matrix, consists of a not too aggressive metals extraction method that keeps the oily matrix intact and, most of all, the lack of calcination of samples; this aspect is very important because metals are present in plants in a variety of structures, both in inorganic and in organometallic compounds, obviously having different physicochemical properties. Then, to avoid losses of such a small quantity of elements, we chose a simple extraction method avoiding laborious and destructive steps based on sample carbonization (9). Both the above-described sample preparation and instrumental determination are rapid enough to allow routine analyses. Furthermore, the utilized technique is cheaper than atomic absorption spectroscopy methods, with regard to both lower equipment cost and easier instrument maintenance. Work is in progress toward the determination of the minimum quantifying amount by the above-described method in certified oily matrices. It could be also interesting to investigate a great number of olive oils to find a relationship between metals concentration and the kind of pesticide treatment that the plants are subjected to, the nature of the soil, and the occurrence of irrigation. These factors could produce very different amounts of the same metal in oils from the same grouping or territory.

LITERATURE CITED

- (1) Hartyáni, Z.; Dávid, E.; Szabó, S.; Szilágyi, V.; Horváth, T.; Hargitai Tóth, Á. Determination of trace elements distribution of polluted soils in Hungary by X-ray methods. *Microchem. J.* **2000**, *67*, 195–200.
- (2) McComb, M. E.; Gesser, H. D. Analysis of trace metals in water by in-situ sample pre-concentration combined with wavelength dispersive X-ray fluorescence spectroscopy and inductively coupled plasma-optical emission spectroscopy. *Talanta* **1999**, *49*, 869–879.
- (3) Bullock, R. O. Current methods and research strategies for modeling atmospheric mercury. *Fuel Process. Technol.* **2000**, *65–66*, 459–471.
- (4) Lo Coco, F.; Monotti, P.; Fiecchi, V.; Ceccon, L. Determination of copper(II) and lead(II) in hard and soft wheat by derivative potentiometric stripping analysis. *Anal. Chim. Acta* **2000**, *409*, 93–98.
- (5) He, M.; Yang, J.; Cha, Y. Distribution, removal and chemical forms of heavy metal in polluted rice seed. *Toxicol. Environ. Chem.* **2000**, *76*, 137–146.
- (6) Carlosena, A.; Andrade, J. M.; Tomas, X.; Fernandez, E.; Prada, D. Classification of edible vegetables affected by different traffic intensities using potential curves. *Talanta* **1999**, *48*, 795–802.
- (7) Rojas, E.; Herrera, L. A.; Poirier, L. A.; Ostrosky-Wegman, P. Are metals dietary carcinogens? *Mutat. Res.* **1999**, *443*, 157–181.
- (8) Paoletti, R.; Nicosia, S.; Clementi, F.; Fumagalli, G. Chapter 8. In *Tossicologia degli Alimenti*, 1st ed.; UTET: Torino, Italy, 1999.
- (9) Karadjova, I.; Zachariadis, G.; Boskou, G.; Stratis, J. Electrothermal atomic adsorption spectrometric determination of aluminium, cadmium, chromium, copper, iron, manganese, nickel and lead in olive oil. *J. Anal. At. Spectrom.* **1998**, *13*, 201–204.
- (10) Martin-Polvillo, M.; Albi, T.; Guinda, A. Determination of trace elements in edible vegetable oils by atomic absorption spectrophotometry. *J. Am. Oil Chem. Soc.* **1994**, *71*, 347–353.
- (11) Lo Coco, F.; Monotti, P.; Rizzotti, S.; Ceccon, L. Determination of copper(II) and lead(II) in olive oils by potentiometric stripping analysis. *Ital. J. Food Sci.* **2000**, *12*, 477–483.
- (12) Calapaj, R.; Chiricosta, S.; Saija, G.; Bruno, E. Method for the determination of heavy metals in vegetable oils by graphite furnace atomic absorption. *At. Spectrosc.* **1988**, *9*, 107–109.
- (13) Sanna, G.; Pilo, M. I.; Piu, P. C.; Tapparo, A.; Seeber, R. Determination of heavy metals in honey by anodic stripping voltammetry at microelectrodes. *Anal. Chim. Acta* **2000**, *415*, 165–173.
- (14) Harris, D. C. In *Chimica Analitica Quantitativa*, 1st ed.; Zanichelli: Bologna, Italy, 1991; Chapters 15 and 18.
- (15) Jagner, D. Instrumental approach to potentiometric stripping analysis of some heavy metals. *Anal. Chem.* **1978**, *50*, 1924–1929.

Received for review October 3, 2001. Revised manuscript received February 5, 2002. Accepted February 6, 2002. This work was financially supported by Regione Siciliana in the Research Project P.O.P 94/99, Mis. 10/4, "Nuove tecnologie e proposte di innovazione della filiera olivicola".

JF0113124