

Determination of Some Heavy Metals and Selenium in Sicilian and Calabrian Citrus Essential Oils Using Derivative Stripping Chronopotentiometry

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This paper aims to bring some novelty about the concentration of some heavy metals and selenium in biological citrus essential oils (CEO) produced in Sicily and Calabria in different crop years. Derivative stripping chronopotentiometry has been used as an accurate, sensitive, and rapid technique for the determination of Cd, Cu, Mn, Ni, Pb, Zn, and Se in hydrochloric acid extracts of CEO; in the optimized electrochemical conditions, detection limits of $<1 \mu\text{g kg}^{-1}$ were obtained for all of the studied metals. In particular, the concentrations of metals were determined in biological bergamot essential oils produced in Calabria in 1999 and 2000 and in biological CEO produced in Sicily in 2003 and 2004. The obtained results provided evidence that Mn was the most abundant metal in all of the studied CEO followed by Zn, Ni, Cu, Pb, and Se; Cd concentrations were always lower than the limit of detection ($0.6 \mu\text{g kg}^{-1}$).

KEYWORDS: Citrus essential oils; heavy metals; selenium; stripping chronopotentiometry

INTRODUCTION

Citrus essential oils (CEO) are mainly used as aromatizing agents for food, beverages, and cosmetics, as antiseptics, and as additives of some pharmaceuticals; they are also widely employed for their therapeutic properties (for example, in aromatherapy). Therefore, a strict control of the presence of contaminants such as heavy metals would be recommended. All aspects regarding toxicity of essential oils are part of the scientific aims of the International Fragrance Association (IFRA) and the International Organization of the Flavour Industry (IOFI), because of the lack of specific regulations. Unfortunately, there are no recent available data about the content of heavy metals and selenium in CEO. The presence of heavy metals in CEO depends on many factors such as the nature of the soils, climatic conditions, genotype of the plant, agronomic techniques, and storage and extraction procedures (1–3). Heavy metals such as Cu(II), Zn(II), Mn(II), and Se(IV) naturally occur in citrus fruit because they are important micronutrients both for animals and for plants. These metals are essential at low concentrations but may become toxic at high levels (4, 5). Some other metals, including Cd(II), Ni(II), and Pb(II), are potentially toxic and can be found in essential oils as a result of a contamination process (2). Particularly, in the past decades great concern has been addressed to selenium and nickel. Selenium due to its antioxidant activity protects cells from oxidative

damage, thus reducing the risk of cardiovascular diseases, inflammatory pathologies, and cancer (6). Nickel is essential for plants and bacteria but not for humans; it has been recognized as a contact irritant, causing a reaction called systemic contact dermatitis (7), and for this reason its concentration has to be strictly monitored in products, such as CEO, used to make cosmetic compounds. Because CEO come daily in contact with humans through food and cosmetic products, it is important to assess the levels of heavy metals for their nutritional or toxicological properties, even though legal limits for their concentration in essential oils have not yet been established.

The purpose of this study is to use derivative stripping chronopotentiometry to obtain some information about the concentrations of Cd(II), Cu(II), Mn(II), Pb(II), Ni(II), Se(IV), and Zn(II) in different types of citrus essential oils produced in Sicily and Calabria in different crop years. In stripping chronopotentiometry a glassy carbon mercury film coated electrode (GCMFC) was used as the working electrode; this technique is based on metals electrodeposition at constant potential onto the mercury film, followed by metals redissolution (stripping). Three different chronopotentiometric mechanisms can be exploited: (i) electrolytic reduction of the metal ion followed by its spontaneous oxidation by a chemical oxidant, usually referred to as derivative potentiometric stripping analysis (dPSA), which was employed for the simultaneous determination of Cd, Cu, Pb, and Zn (8, 9) and for the separate analysis of Mn; (ii) electrolytic reduction of the metal ion followed by a further reduction by an applied constant current, called derivative

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cathodic stripping potentiometry (dCSCP), employed in this study for the analysis of selenium (10, 11); (iii) adsorption of a metal complex, such as Ni(DMG)₂, followed by the reduction of the metal by a cathodic constant current, usually referred to as derivative adsorptive cathodic stripping chronopotentiometry (AdCSCP), employed for the analysis of Ni (II) (12). In all cases the stripping step represents the signal collection phase: the time elapsed after stripping initiation is monitored versus electrode potential and E (mV) is plotted against dt/dE (ms/mV). La Pera et al. (13) described in a previous paper the use of dPSA for the simultaneous determination of Cd(II), Cu(II), Pb(II), and Zn(II) in CEO after a hydrochloric acid extraction procedure. This technique is used in this study to determine the concentration of the four metals in a large number of bergamot essential oils produced in Calabria in 1999 and 2000 crop years and in CEO produced in Sicily in 2003 and 2004 crop years. Furthermore, this paper provides an accurate description of the use of dPSA, dCSCP, and dAdSCP for the determination of trace levels of Mn(II), Se(IV), and Ni(II).

MATERIALS AND METHODS

Instruments. A potentiometric stripping analyzer PSA ION 3 (Steroglass, Perugia, Italy), equipped with a conventional three-electrode cell, was used for the analysis of Cd(II), Cu(II), Mn(II), Ni(II), Pb(II), Se(IV), and Zn(II) in essential oil samples. The working electrode was a glassy carbon electrode coated with a thin mercury film; the reference electrode was an Ag/AgCl electrode (3 M KCl), and a platinum wire auxiliary electrode was used (9–13). To confirm the analytical results of derivative stripping chronopotentiometry, the acid extracts of the essential oils were subjected to atomic absorption spectrometry (AAS), using a Shimadzu 800 series graphite furnace atomic absorption spectrometer, equipped with an ASC-6100 autosampler.

Reagents. Ultrapure hydrochloric acid (34–37%), Hg(II) (1000 mg kg⁻¹, 1 M in HCl), Cd(II), Cu(II), Mn(II), Ni(II), Pb(II), Se(IV), and Zn(II) (1000 mg kg⁻¹, 0.5 M in HNO₃), and Pd(II) (100 μg mL⁻¹, 0.5 M in HNO₃) standard solutions, the 0.5 M ammonia buffer, and the 0.05 M ethanolic solution of dimethylglyoxime were obtained from Panreac (Barcelona, Spain). Ga(NO₃)₃·3H₂O (5 g, 99.9%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). A certified reference oily matrix, CertIPUR, containing 100 mg kg⁻¹ of Cd(II), Cu(II), Mn(II), Ni(II), Pb(II), and Zn(II), was purchased from Merck (Darmstadt, Germany). To carry out the recovery test, oily solutions containing 25.0, 150, 250, and 550 μg kg⁻¹ of each metal were prepared for dilution with *n*-hexane (Carlo Erba, Milano, Italy) of the certified reference oily matrix. Filtration of the acid extracts 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 methanol, purchased from Carlo Erba Reagenti (Milano, Italy).

Samples. Cd(II), Cu(II), Pb(II), Zn(II), Mn(II), Ni(II), and Se(IV) concentrations were determined in 26 samples of bergamot essential oils from 1999 and 29 samples from 2000; all of the samples were produced from bergamot fruit grown in the same field in the province of Reggio Calabria (Calabria, Italy) and extracted in the same firm. The content of these metals was also evaluated in different types of biological CEO extracted in the same firm from citrus fruit harvested in different fields in the province of Messina (Sicily, Italy); in particular, 35 samples from 2003 and 31 samples from 2004 were analyzed (Table 1). All of the studied essential oils from Calabria and Sicily were produced from citrus fruit harvested by using the method of biological control, which involves the use of natural enemies and pathogens for the control of the pests in order to limit or avoid the use of chemical products. Moreover, all of the samples were extracted by means of “slow folding” or *sfumatura*, which consists of superficial rasping of the whole fruits, employing a mechanism that largely reproduces the manual sponge process (3). All of the samples were stored in glass bottles at 4 °C until the analysis.

Sample Extraction Procedure. A 5.0 g aliquot of each citrus oil sample and 5.0 mL of 36% ultrapure hydrochloric acid were introduced

Table 1. Biological Citrus Essential Oils Samples Produced in Sicily in the Crop Years 2003–2004

oil	no. of samples	
	2003	2004
bergamot	4	4
citron	4	0
clementine	4	4
lemon	6	6
mandarin	6	6
blond orange	6	6
red orange	5	5
total	35	31

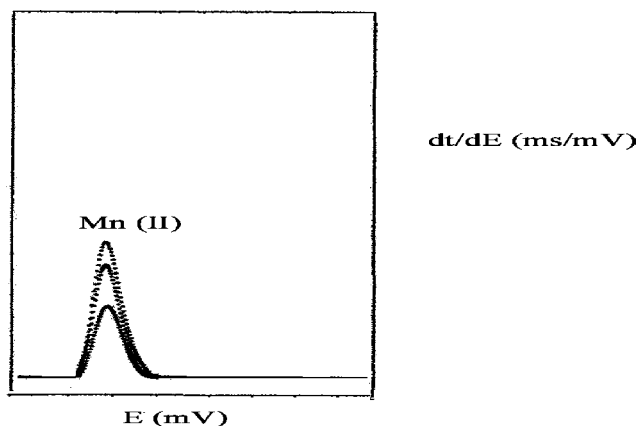


Figure 1. Mn(II) chronopotentiometric curve.

into a Teflon beaker. The extraction was carried out for ~30 min under magnetic stirring at the temperature of 80 °C. The mixture was transferred in a separating funnel: the acid phase was taken apart in a 10.0 mL flask, whereas the organic layer was extracted twice with 2.5 mL of concentrated hydrochloric acid for 10 min in the conditions described earlier (13). The treatment with concentrated HCl allowed the conversion of all selenium to the electroattractive Se(IV) species (10, 11, 14). The collected acid phases were filtered on a carbon column previously activated by 2.0 mL of methanol followed by 2.0 mL of ultrapure water. Sample preparation took ~50 min.

Electrode Preparation: Plating. The electrodes were stored in ultrapure water; before the start of each analysis, they were well cleaned with pure absolute methanol and filter paper. Before each analysis, the plating of the working electrode was executed as described in previous papers (9–13).

Analysis of Metals by Derivative Stripping Chronopotentiometry. Simultaneous Determination of Cd(II), Cu(II), Pb(II), and Zn(II). The electrochemical conditions for the simultaneous determination of Cd, Cu, Pb, and Zn in CEO by dPSA are described in a previous paper (13).

Analysis of Mn(II) by dPSA. For the quantification of Mn(II) in CEO, 0.05 mL of the acid extract was placed into the electrochemical cell together with 10 mL of ultrapure water, 1 mL of Hg(II) as the chemical oxidant, and 1 mL of ammonia buffer (pH 9.5); before the analysis, the solution was deoxygenated for 5 min by N₂ purging. The electrodeposition potential was fixed at -1600 mV versus an Ag/AgCl reference electrode, and the electrolysis time was 120 s; the solution was stirred at 1000 rpm. The manganese stripping peak was registered around -1460 mV (Figure 1), and the final acquisition potential was -500 mV (14). The quantitative analysis was done by using the multiple-point standard additions method. Optimum precision and accuracy were obtained with the addition of two 0.03 mL aliquots of a 1.0 μg L⁻¹ standard solution of Mn(II) and by performing the measurements four times (15). Calibration curves with $r \geq 0.995$ and a linear concentration range of 0–2000 μg L⁻¹ were obtained. In the optimized electrochemical conditions, the manganese analysis lasted 25 min.

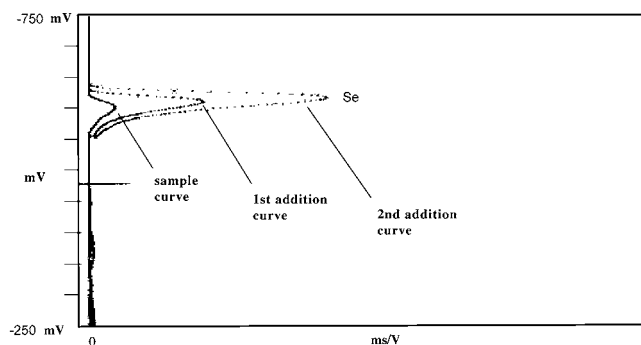


Figure 2. Se(IV) chronopotentiometric curve

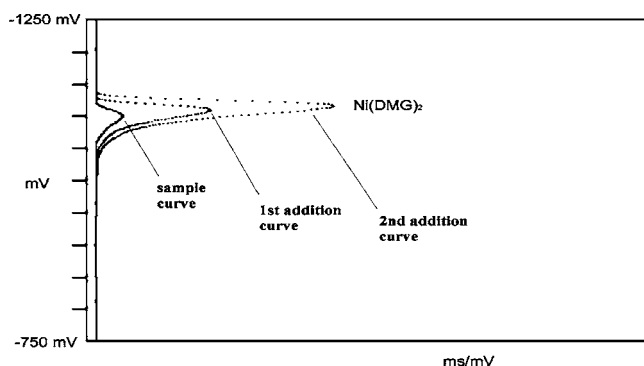


Figure 3. Ni(II) chronopotentiometric curve.

Analysis of Se(IV) by dCSCP. For the quantification of Se(IV) in CEO, 0.5 mL of the acid extract was placed into the electrochemical cell together with 10 mL of ultrapure water. The preconcentration of Se(IV) occurred at -150 mV versus the Ag/AgCl reference electrode, and the electrolysis time was 120 s. The deposited Hg–Se was further reduced to Se(–II) during the stripping step by a -30 μ A cathodic constant current, whereas the potential decreased to -750 mV. The peak of selenium appeared at -580 mV (Figure 2), and the final acquisition potential was -780 mV (10, 11, 16). The quantitative analysis was executed as described earlier by executing two 0.05 mL standard additions of 2.5 μ g mL^{-1} of Se(IV) standard solution and performing each measurement four times; calibration curves with $r > 0.995$ and a linear concentration range of 0 – 1000 μ g L^{-1} were obtained. Each analysis lasted 25 min.

Analysis of Ni(II) by dAdSCP. Ni(II) was determined as its complex with dimethylglyoxime, by placing into the electrochemical cell 0.5 mL of acid extract, 0.2 mL of DMG, 1 mL of ammonia buffer, and 10 mL of ultrapure water. The Ni(DMG)₂ complex was preconcentrated onto the working electrode at the adsorption potential of -500 mV for 120 s, while the agitation speed was 1000 rpm; then the stirrer was stopped, and the potential was scanned toward the final acquisition potential fixed at -1400 mV; the sampling time was 300 μ s. The nickel stripping peak was registered around -1060 mV (Figure 3). The quantitative analysis was executed as described earlier by executing two 0.1 mL standard additions of 2.5 μ g mL^{-1} of Ni(II) standard solution and performing each measurement four times; calibration curves with $r \geq 0.995$ and a linear concentration range of 0 – 2000 μ g L^{-1} were obtained. Each analysis lasted 25 min.

Analytical Performances. The analytical performances obtained for the simultaneous determination of Cd(II), Cu(II), Pb(II), and Zn(II) in CEO are reported in a previous paper (13). Precision and repeatability for Mn(II), Se(IV), and Ni(II) were evaluated by extracting each citrus oil sample in triplicate and quantifying the four times in the same extract. The instrument precision was indicated as mean relative standard deviation percent (RSD%), and method repeatability was represented by the total RSD%. The possibility of decreases or increases of metal amounts, due to the extraction procedure or to the electrodeposition step, was explored. Aliquots of 5.0 g of a lemon essential oil sample were separately spiked at different levels with 10.0 μ g mL^{-1} Mn(II), Se(IV), and Ni(II) standard solutions. To homogenize the obtained mixture, it was left under magnetic stirring overnight (9, 13).

Table 2. Analytical Performances Obtained for the Analysis of Mn(II), Se(IV), and Ni(II) by dPSA, dCSCP, and dAdSCP, Respectively

	Mn(II)	Se(IV)	Ni(II)
precision (%)	2.1 ± 0.3	3.0 ± 0.2	2.8 ± 0.2
repeatability (%)	2.6 ± 0.2	6.5 ± 0.5	6.0 ± 0.4
mean recovery (%)	96.0 ± 1.0	92.5 ± 1.0	94.1 ± 0.8
sensitivity ($\text{ms kg } \mu\text{g}^{-1}$)	24500	12000	4800
σ (ms)	490	200	80
LOD ($\mu\text{g kg}^{-1}$)	0.06	0.05	0.05

Table 3. Spike-and-Recovery Test on Certified Reference Oily Matrix^a

	certified ($\mu\text{g/kg}$)	found ($\mu\text{g/kg}$)	recovery (%)
Mn	25	23.5 ± 1.0	94.0
Ni	25	24.1 ± 0.9	96.4
Se	25	22.8 ± 0.8	91.2
Cd	25	24.4 ± 0.7	96.0
Cu	25	23.5 ± 1.1	94.0
Pb	25	23.9 ± 1.0	95.6
Zn	25	24.1 ± 1.3	96.4
Mn	150	145 ± 5.0	96.6
Ni	150	141 ± 5.5	94.0
Se	150	144 ± 6.1	96.0
Cd	150	148 ± 7.1	98.7
Cu	150	145 ± 3.9	96.6
Pb	150	142 ± 5.2	94.7
Zn	150	148 ± 4.4	98.7
Mn	250	239 ± 9.1	95.6
Ni	250	237 ± 8.5	94.8
Se	250	241 ± 5.5	96.4
Cd	250	240 ± 7.6	96.0
Cu	250	239 ± 6.4	95.6
Pb	250	236 ± 7.0	94.4
Zn	250	237 ± 6.3	94.8
Mn	550	525 ± 15.4	95.4
Ni	550	520 ± 12.6	94.5
Se	550	532 ± 14.0	96.7
Cd	550	519 ± 12.5	94.4
Cu	550	525 ± 16.3	95.4
Pb	550	518 ± 11.7	94.2
Zn	550	520 ± 13.2	94.5

^a Reliability is indicated as the mean recovery. Each value is the mean of three determinations and is referred to dry matter.

Then, the extraction procedure described earlier was executed. Both spiked and unspiked samples of each type of oil were analyzed by stripping chronopotentiometry, and the mean recoveries were calculated. Detection limits were obtained according to the $3\sigma/S$ criterion, where σ was the peak threshold (set at 490 mV s^{-1} for Mn, 200 mV s^{-1} for Se, and 80 mV s^{-1} for Ni) and S was the sensitivity represented by the slope of the calibration curves. All of the performance parameters are given in Table 2.

Reliability. To assess the reliability of the method described, ensuring that analyte loss or sample contamination did not occur during either the extraction procedure or the chronopotentiometric analysis, recovery tests from the certified reference oil were performed. The certified oil, which contained 100 mg kg^{-1} of each metal, was diluted with *n*-hexane to obtain standard oily matrices respectively containing 25, 150, 250, and 550 $\mu\text{g kg}^{-1}$ of Mn(II), Ni(II), Se(II), Cd(II), Cu(II), Pb(II), and Zn (II). The dilution was performed to obtain concentrations similar to those actually found in oils. The certified reference oil and the standard oily solutions were subjected to the extraction procedure described above; each extract was analyzed three times. The obtained results are given in Table 3.

GF AAS Confirmation Analysis. The reliability of the chronopotentiometric methods was evaluated by performing confirmation analysis with GF AAS. The citrus oil samples extracted as previously described were analyzed in triplicate both by Zeeman GF AAS and by stripping chronopotentiometry for Mn(II), Se(IV), and Ni(II) determinations. The spectroscopic analysis was carried out by adding, for each 20.0 μL injection, 5.0 μL of a Pd(NO₃)₂ solution (Pd concentration = 100 μg

Table 4. Confirmation Analysis Using GF AAS^a

	CP	AAS	% difference
Manganese			
lemon	883 ± 18	866 ± 23	+2.6
blond orange	371 ± 8	375 ± 12	-1.1
mandarin	596 ± 12	595 ± 16	+0.2
Nickel			
lemon	118 ± 4	116 ± 7	-2.5
blond orange	186 ± 6	179 ± 9	-4.0
mandarin	685 ± 23	705 ± 25	+2.6
Selenium			
lemon	8.5 ± 0.3	8.6 ± 0.9	+1.2
blond orange	10.2 ± 0.4	10.1 ± 1.1	-1.3
mandarin	19.5 ± 0.7	20.1 ± 2.3	+3.2

^a All values (mg kg⁻¹) are the mean of three determinations.

Table 5. Heavy Metals Concentration Ranges (Mean ± SD, Expressed in Micrograms per Kilogram), in Bergamot Essential Oils from 1999 and 2000

	1999	2000
Cd(II)	nd ^a	nd ^a
Cu(II)	75.7–440 (150 ± 62)	90.0–310.1 (171 ± 97)
Pb(II)	40.0–346.9 (147 ± 83)	59.2–282.3 (128 ± 55)
Zn(II)	110–807 (323 ± 223)	120–800 (307 ± 187)
Mn(II)	960–1550 (1178 ± 403)	935–1440 (1187 ± 396)
Ni(II)	225–380 (281 ± 79)	216–401(313 ± 90)
Se(IV)	nd ^b –29.2 (12 ± 9)	nd ^b –23.7 (9.9 ± 8)

^a Not detectable, <0.6 µg kg⁻¹. ^b Not detectable, <0.05 µg kg⁻¹.

mL⁻¹), as matrix modifier. The working wavelengths were 279.5 nm for Mn, 196.0 nm for Se, and 232.0 nm for Ni(II) with a band-pass of 0.2 nm. The obtained reliability data are illustrated in **Table 4**. Confirmation analysis results for Cd(II), Cu(II), Pb(II), and Zn(II) are reported in a previous paper (13).

RESULTS AND DISCUSSION

Analytical Methods. The determination of ultratrace concentration (<1 ppb) of heavy metals in complex matrices, such as citrus essential oils, has always been an important analytical challenge. A previous paper (13) has demonstrated that derivative stripping chronopotentiometry is well suited for the precise and reliable analysis of trace concentrations of Cd(II), Cu(II), Pb(II), and Zn(II) in various types of citrus essential oils. This study provides evidence that chronopotentiometry allows the detection of concentrations of Mn(II), Se(IV), and Ni(II) of <0.06 µg kg⁻¹ in CEO with a precision that ranges from 2.1 to 3.0%, expressed as mean RSD of the measurements. The

obtained recoveries, from 92.5 to 96.0%, confirmed that no significant metal losses occurred during the extraction and electrodeposition procedures (**Table 2**). The reliability of the chronopotentiometric methods was evaluated using a certified mixture of hydrocarbons; the obtained results provide evidence that the reliability ranged from 91.2 to 97.8% for all of the studied metals (**Table 3**). Confirmation analyses were performed by GF AAS, and the results of the two methods agreed within 3.2% (**Table 4**).

Heavy Metals in CEO. In recent years a great importance has been placed on the monitoring of alimentary products for contamination by heavy metals, mainly as a result of a requirement of the consumers, who are much more concerned today about the quality and integrity of foodstuffs than they were in the past. Heavy metal ions may represent a risk for human health not only for their ingestion but are also as contact sensitizers, and the most common is nickel; a notable proportion of nickel allergic patients are also reactive to other metals such as copper. CEO are not consumed as such, but small amounts are used as flavors for food, beverages, and cosmetics; consequently, it would be quite difficult to estimate both heavy metals concentrations that are ingested by humans through alimentary products which contain essential oils and heavy metals amounts that come in contact with the skin through the use of cosmetic products. Even so, considering the wide diffusion of CEO, it is of great concern to assess metal levels of nutritional or toxicological interest. This research has brought some novelty about the concentration of some heavy metals and selenium in CEO produced in Calabria and Sicily in different crop years, because there is a total lack of recent data concerning this argument. Bergamot essential oil produced in Calabria represents 90% of the world production. For its uniqueness and its wide use, it was interesting to determine the concentrations of Cu(II), Zn(II), Pb(II), Cd(II), Mn(II), Ni(II), and Se(IV) in oils from two different crop years: 26 samples from 1999 and 29 from 2000. **Table 5** provides evidence that Mn was the most abundant metal, followed by Zn, Ni, Cu, and Pb in the samples from both crop years; Se is present in very small amounts, whereas Cd levels were lower than the detection limits in all of the studied bergamot oils. Pb and Zn mean concentrations are similar to those reported in the literature (3) for lemon essential oils (150 and 150 µg kg⁻¹, respectively), and Cu levels are lower (380 µg kg⁻¹).

The concentrations of Cd(II), Cu(II), Pb(II), Zn(II), Mn(II), Se(IV), and Ni(II) were evaluated in biological CEO produced in the same firm in the province of Messina in 2003 and 2004. **Table 6** shows that Mn(II) was the most abundant metal,

Table 6. Heavy Metals Concentration Ranges (Micrograms per Kilogram) in Citrus Essential Oils from 2003 and 2004

oil	Cd(II)	Cu(II)	Pb(II)	Zn(II)	Mn(II)	Ni(II)	Se(IV)
2003							
bergamot	nd ^a	110–203	35.5–107	815–1050	1021–1950	175–285	nd ^b
citron	nd	155–221	46.5–112	535–658	312–610	nd ^a –375	nd–21.6
clementine	nd	119–245	45.2–78.5	566–770	733–1195	130–170	nd
lemon	nd	75.5–145	96.3–135	350–780	538–1403	45.0–156	nd–16.2
mandarin	nd	186–251	33.4–85.1	1010–1350	171–894	650–1099	nd–23.4
blond orange	nd	106–203	101–196	915–1225	458–1401	nd–217	nd–13.6
red orange	nd	85.2–191	65.5–180	612–799	371–998	nd–768	nd–8.1
2004							
bergamot	nd	77.2–198	26.9–102	650–1026	255–512	600–100	nd
clementine	nd	107–202	39.6–58.4	490–701	128–595	17.0–45.2	25.6–64.3
lemon	nd	60.2–103	70.2–101	300–790	260–625	33.1–71.3	2.5–9.7
mandarin	nd	116–224	40.2–80.5	970–1302	710–1114	150–950	2.3–12.1
blond orange	nd	77–161	65.1–105	832–1235	213–512	58.2–106	nd
red orange	nd	58.5–129	77.9–201	550–819	123–289	28.2–73.5	nd

^a Not detectable, <0.6 µg kg⁻¹. ^b Not detectable, <0.05 µg kg⁻¹.

followed by Zn(II), Ni(II), Cu(II), Pb(II), and Se(IV), in both 2003 and 2004 essential oils; Cd levels were lower than the limit of detection (LOD) in all samples. All of the studied citrus essential oils from 2003 showed higher Cu, Pb, and Zn levels than oils from 2004 ($p < 0.05$, t test). Among samples from 2003 and 2004, mandarin oils showed the highest mean amounts of Cu, 210 ± 45 and $165 \pm 46 \mu\text{g kg}^{-1}$, respectively, and Zn, 1225 ± 178 and $1177 \pm 122 \mu\text{g kg}^{-1}$, respectively, whereas the highest Pb mean amounts were detected in blond orange samples from 2003 ($159 \pm 51 \mu\text{g kg}^{-1}$) and red orange samples from 2004 ($129 \pm 83 \mu\text{g kg}^{-1}$). Bergamot, clementine, lemon, and blond and red orange essential oils from 2003 showed higher Mn concentrations than samples from the following crop year; in particular, bergamot and red and blond orange essential oils presented the most significant differences ($p \leq 0.05$, t test); on the contrary, mandarin essential oils from 2004 showed a remarkably higher amount of Mn ($p < 0.05$, t test) than oils from 2003. Bergamot essential oils from 2003 presented the highest mean amount of Mn ($1529 \pm 477 \mu\text{g kg}^{-1}$) and mandarin the lowest ($411 \pm 328 \mu\text{g kg}^{-1}$); among samples from the following crop year, clementine oils had the highest ($797 \pm 205 \mu\text{g kg}^{-1}$) and red orange oils the lowest ($209 \pm 100 \mu\text{g kg}^{-1}$) mean content of Mn. Bergamot, clementine, lemon, and blond and red orange essential oils from 2003 presented higher Ni mean levels than samples from the following crop year, and clementine oils presented the most significant difference ($p < 0.001$, t test). Among samples from 2003, mandarin essential oils had the highest mean concentration of Ni ($505 \pm 460 \mu\text{g kg}^{-1}$) and blond orange the lowest ($115 \pm 100 \mu\text{g kg}^{-1}$); mandarin essential oils from 2004 presented the highest average content of Ni ($918 \pm 190 \mu\text{g kg}^{-1}$) and clementine oils the lowest ($30 \pm 12 \mu\text{g kg}^{-1}$). The greatest part of all the studied samples from 2003 and 2004 presented Se levels lower than the detection limits; moreover, the concentration of Se was highly variable both in samples of the same type and in samples from different citrus fruit: as was noted in a previous study concerning olive oils from different zones and different varieties (17), this variability might depend on the provenance of fruits and on the genotype of the plants. The highest Se mean concentrations were found in citron essential oils ($11.8 \pm 11.7 \mu\text{g kg}^{-1}$) from 2003 and in clementine essential oils ($35 \pm 25 \mu\text{g kg}^{-1}$) from 2004. Of all the studied trace elements, plant nutrients such as manganese followed by zinc, nickel, and copper were the most abundant in CEO from both crop years. Plants usually absorb the elements they need for growth from the soil, which are then translocated to the edible part of the plant. In particular, manganese is usually present in vegetable products such as CEO, in concentrations $> 1 \text{ mg kg}^{-1}$ because it plays an important role in regulating nitrogen metabolism and photosynthesis in plants (14, 18). Nickel levels in processed products such as CEO can be increased by pick-up from stainless steel extraction equipments and containers (1, 2). Furthermore, very low levels of selenium, which is essential for humans but not for citrus plants, were observed in CEO. Toxic elements such as cadmium and lead, which are present in foodstuffs mainly as a result of contamination processes (soil contamination, air pollution, food processing), were found in the studied CEO in very low concentrations. On the whole, even though legal limits for metals concentration in essential oils have not yet been established, the obtained results have shown very low heavy metals contamination levels in Sicilian and Calabrian biological citrus oils from 2003 and 2004; therefore, their use does not represent a risk for human health.

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