

## SUPPLEMENTARY MATERIAL

### Multiple analytical approaches for the organic and inorganic characterization of *Origanum vulgare* L. samples

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## Abstract

*Origanum vulgare* L. samples, marketed in different geographic locations, were characterized by their organic and inorganic chemical composition. A total of 35 commercial samples were collected from various sites and analyzed to determine the qualitative and quantitative profile of essential oils, phenolic compounds and some inorganic elements. The variation in the content and composition of the essential oil was assessed by GC and GC-MS analyses, the phenolic fraction was investigated by UPLC<sup>®</sup>/PDA, and the inorganic elements were determined by ICP-MS. The Principal Component Analysis (PCA) was applied with the aim to sort out the *Origanum vulgare* L. samples with different composition according to the different belonging origins. The results showed appreciable qualitative and quantitative differences among samples from different geographic origin.

**Keywords:** Food analysis, Food composition, *Origanum vulgare* L., Oregano, Phenol, Essential oil, Mineral analysis, Statistical analysis, Geographic origin

## MATERIALS AND METHODS

### Chemicals

LC-MS grade acetonitrile, methanol, and formic acid were purchased from Sigma-Aldrich (Milan, Italy). Ultrapure water ( $< 5 \text{ mg L}^{-1}$  TOC) was obtained from a Barnstead Smart2pure 12 purification system (Thermo Scientific, Milan, Italy). Nitric acid ( $\text{HNO}_3$ , 69% v/v) for ICP-MS analysis was supra-pure grade (J.T. Baker, Mallinckrodt Baker, Milan, Italy). Stock standard solutions ( $1000 \text{ mg L}^{-1}$  in  $\text{HNO}_3$  2%) of each inorganic element under investigation were purchased from Fluka, (Milan, Italy); only Hg was from Merck (Darmstadt, Germany).

All other chemical reagents and standards were purchased from Sigma-Aldrich (Milan, Italy), Fluka (Milan, Italy) and Extrasynthese (Genay, France). Argon (99,9990 % purity) and helium (99,9995 % purity) were supplied by Rivoira S.p.A (Milan, Italy).

### Samples

The study was carried out on 35 *O. vulgare* L. commercial samples collected in various countries in 2016. Each sample was directly purchased from the international market; in particular, 5 commercial packets from Calabria (Italy-A), Sicily (Italy-B), Istanbul (Turkey), Monterrey (Mexico), Sofia (Bulgaria), London (UK) and New York (USA) were purchased. None of the

packaging showed the exact taxonomic characterization of subspecies; the label mentioned only that it was *O. vulgare* L. and indicated the production area that coincided with the area where the product was purchased.

The 35 packages were opened directly in our laboratory; subsequently, from each of them three aliquots were taken and homogenized. Each portion was separately subjected to essential oil extraction, phenolic isolation and mineralization.

### **Isolation and GC-MS analyses of the essential oil**

Essential oils were obtained by hydro-distillation of air dried plant material (50–100 g) for 3 hours. The obtained oils were dried on Na<sub>2</sub>SO<sub>4</sub> anhydrous and, after filtration, stored into dark glass bottles under nitrogen at -18°C, until analysis. The qualitative and quantitative fraction composition was studied by multidimensional GC/MS analyses that were carried out by means of a Shimadzu (Kyoto, Japan) MDGC/GCMS system, composed of two GC ovens which were coupled to a FID and a quadrupole MS detector, according to the method proposed by (Costa et al. 2018).

The compounds identification was based on their GC retention index, MS spectral data matching with those from NIST MS libraries, (NIST1998) the comparison of the obtained fragmentation patterns with those reported in literature (Adams 2007) and other homemade databases. The quantitative composition, for each compound, was calculated by the peak area normalization method, considering the response factor for each component equal to 1. The data here presented are obtained as average values of triplicate analysis. The coefficient of variation of the three analyses was always lower than 5%. percentages of compounds were determined from their peak areas.

### **Extraction of phenolics and UPLC<sup>®</sup>/PDA determination**

The extraction was carried out in a similar manner to the methods reported for *Origanum vulgare* L. and *rosmarinus officinalis* L. (Kotsiou et al. 2010; Santos et al. 2012). After extraction, the samples were filtered through vacuum filtration and methanol was evaporated in-vacuum at room temperature. The residues were stored at -18°C and kept away from light. Before UPLC<sup>®</sup>/PDA analysis the residues were dissolved in acetonitrile (1 mL) and filtered through a 0.2 µm PTFE filters.

UPLC<sup>®</sup>/PDA analysis was carried out using an Acquity UPLC<sup>®</sup> Waters liquid chromatography system equipped with a column heater, a photodiode detector ACQ-PDA, a quaternary solvent manager ACQ-QSM and a sample manager ACQ-FTN, controlled by Waters<sup>®</sup> Empower<sup>TM</sup> chromatographic software. An Acquity UPLC<sup>®</sup> Waters BEH-C18 column of 1.7 µm (2.1 x 50

mm), protected by 0.2  $\mu\text{m}$  stainless steel In-Line Filter with a Holder Waters, was used. The mobile phase was a gradient prepared from 0.15 % (v/v) acetic acid in water (solvent A) and 0.15 % (v/v) acetic acid in methanol (solvent B): 0-7 min, 2-10% B; 7-10.5 min, 10-17% B; 10.5-16 min, 17% B isocratic; 16-23 min 17-100% B; 23-26 min, 100% B isocratic; 26-26.2 min, 100-2% B; 26.2-30 min, 2% B isocratic (four minute of equilibration was required before the next injection). Analyses were run at 35°C. The injection volume was 2  $\mu\text{L}$  and the flow rate was 0.35  $\text{mL min}^{-1}$ . During UPLC<sup>®</sup>/PDA analysis the absorption spectra were recorded on-line from 200 to 600 nm. For the analytic determination the PDA detection was conducted at the wavelength corresponding to the maximum absorbance of each phenolic compound.

The compounds were identified by comparing both retention times and UV-vis spectra with those of pure standards analyzed under identical analytical conditions. Phenolic compounds were quantified by the external standard method. Each sample was analyzed in triplicate and the results were expressed as milligram per 1 kilogram of dry weight of *Origanum vulgare L.*

### Mineral Analysis

The minerals concentrations were estimated by ICP-MS after a preventive sample digestion, applying a method already used for the minerals determination in other matrices. (Salvo et al. 2016; Salvo et al 2018) All samples were dried to constant weight at 50°C; then, each one (~0.25 gr) was weighed, transferred into Teflon vessel and added with 1 mL of internal standard (Re, 0.8  $\text{mg L}^{-1}$ ) and 10 mL  $\text{HNO}_3$  65%. Mineralization was performed using a microwave digestion system (Ethos1, Milestone, Bergamo, Italy) and was carried out in two steps with a constant microwave power (1000 W). Firstly, the temperature was increased to 180°C in 10 minutes and then held at 180°C for 10 minutes. After cooling down at room temperature, the digested samples were diluted with ultrapure deionized water and stored at 4°C. Subsequently the mineral analysis was carried out by ICP-MS. Blanks were also prepared according to the same digestion procedure to check for any loss or cross contamination.

ICP-MS analysis of the mineral compounds in *O. vulgare L.* samples was performed following a procedure that has been validate according the EURACHEM guidelines, considering sensitivity, linearity, accuracy and repeatability parameters. (EURACHEM/CITAC 2012)

### Statistical Analysis

Statistical analysis was performed in R Project for Statistical Computing, version 3.3.3. Statistical methods were carried out starting by building the multivariate matrix where variables were the concentrations of 47 chemical species (15 inorganic ions, 12 polyphenols and 20 essential oils

compounds, respectively) and 35 analyzed *O. vulgare* L. samples. The data were subdivided into seven groups according to the oregano samples origin: 5 samples from each geographic area (Italy-A-Calabria, Italy-B-Sicily, Turkey, Bulgaria, USA, Mexico and UK).

Due to the nature of data and to the aim of research, the statistical analysis has been developed in three steps. Kruskal-Wallis test (one-way ANOVA on ranks) was applied to test differences among the seven oregano varieties for each component. Considering the small number of samples we used the p-value based on Monte Carlo approximation (with 100.000 sampling) that generate an extremely accurate estimate of the exact p-value. In a second step, post-hoc multiple comparison through the Mann Whitney test was performed to investigate which pairs of groups differ significantly. (Tables 2S, 4S and 6S)

Principal Component Analysis (PCA) was applied in order to identify the more important compounds (phenolic fraction, essential oil compounds and inorganic ions) that contributed to the differentiation of the *O. vulgare* L. samples from different geographic origin and to establish the relationships among samples and variables. Considering the data feature, we applied a robust PCA based on singular value decomposition (SVD).

## ***2.1 Chemical profile of the essential oils (detailed discussion)***

Considering that EOEOVLs from different countries display a variable profile, in order to evaluate any correlation among the various components, we classified the samples taking into account the concentrations of the most representative identified compounds. Almost all the analyzed samples were characterized by high percentage of phenols, especially those from USA, UK and Bulgaria whose total phenol content (sum of carvacrol, thymol and their derivatives) represents on average 53.39, 46.09 and 44.15 % of the oil, respectively. The EOEOVLs from Turkey, showing a good content of phenolic compounds (average total phenol content 27.93 %), were clearly distinguishable from the others by their higher content of total monoterpene hydrocarbons (33.35 %). Particularly, whilst EOEOVLs from USA and Mexico had, on average, comparable percentage of carvacrol and thymol, almost all the other samples can be classified as thymol/carvacrol chemotype. Moreover EOEOVLs from Italy-A-Calabria and Italy-B-Sicily were quite rich or almost pure in thymol and never showed thymol methyl ether; however, for their specific  $\alpha$ -terpineol content could be classified as thymol/ $\alpha$ -terpineol chemotype.

From a qualitative point of view, all EOEOVLs contained a large predominance of monoterpenes both in the hydrocarbon and oxygenated forms; however, Italy-B ones showed the peculiar, even though tiny, presence of *p*-cymene-8-ol.

The total contents of monoterpene hydrocarbons into the EOEOVLs demonstrated a wide range of variability, moving from a country to another, and ranged from an average of 33.35 % for Turkish samples to 23.65 % of UK ones. Thymol, that is a monoterpenoid phenol, was the main component in all the analyzed samples, except for the Turkish ones which resulted quite rich in  $\beta$ -terpineol. Compared to these samples, all the other analyzed EOEOVLs were rather poor in this monoterpene alcohol. Generally, individual EOEOVLs were found to be considerably rich in carvacrol, mainly UK and Mexico samples. On the other hand, EOEOVLs from Italy-A and B showed the lowest amount of carvacrol and were particularly rich in thymol. *p*-Cymene, representing with  $\gamma$ -terpinene the biosynthetic precursor of monoterpenoids phenols (thymol and carvacrol), was observed throughout the samples in large percentage, being in EOEOVLs from USA constantly more represented than in the other samples. Also  $\gamma$ -terpinene was present in all of the analyzed EOEOVLs, and it was generally found in much lower amounts than *p*-cymene; however, these two compounds reached rather comparable levels in the Italian samples. These data are consistent with other studies concerning the *O. vulgare* L. ssp. *hirtum*. (De Martino et al. 2009)

Low proportion of sabinene and related sabinyl compounds, were detected in almost all the samples. Particularly, sabinene was not found in all Turkish and Bulgarian *O. vulgare* L. samples while the *cis* and *trans* isomers of sabinene hydrate were never identified into USA and UK samples. We observed that, for any EOEOVL sample, low proportions of sabinyl compounds are strictly related to low sesquiterpenes concentrations and occurred with essential oils compounds primary made up of monoterpenes. These results are supported by other studies regarding European *O. vulgare* and samples cymil compound poor/sesquiterpene rich. (Lukas et al. 2015)

Among the sesquiterpenes hydrocarbons,  $\beta$ -bisabolene was the only one constantly present in all the analyzed samples, while the oxygenated sesquiterpene, found in most of the essential oils, was  $\beta$ -caryophyllene oxide. All the other sesquiterpenes hydrocarbons were found in small amounts and often were specific for geographic belonging.

The oxygenated monoterpenes showed a large variability; indeed, they were predominant in all the EOEOVLs from Turkey (particularly  $\beta$ -terpineol) representing on average 35.48 % of the extract, whereas, samples from Bulgaria and USA exhibited a moderate percentage of these compounds. Particularly, in these samples, were never detected *p*-cymene-8-ol, *cis*-pinene hydrate, *trans*-linalool oxide and 1,8-cineole.

## **2.2 Polyphenols Composition**

The highest concentrations ( $\geq$  total median) of phenolic acids and flavonoids were found in *Origanum vulgare* L. samples from Italy-A-Calabria, Italy-B-Sicily and Mexico. The difference among these samples and those from other countries were likely due to environmental conditions (latitude location, climate, temperature) and harvest time.

For the main phenolic acids, *o*-coumaric acid turned out to be the most predominant phenolic compound in all *O. vulgare* L. samples examined. The highest concentrations of this hydroxycinnamic acid were determined in samples from Italy-B-Sicily and Italy-A-Calabria and these values were almost over two times higher than those from USA and UK.

Considerable variation was found in chlorogenic acid contents. A comparison of the data reported in Table 3S highlighted that while for Sicilian, Calabrian and Mexican samples the chlorogenic acid levels were proximate to those of *o*-coumaric and rosmarinic acid, for the samples from Bulgaria the mean chlorogenic acid content was close to the rosmarinic acid but four times less the *o*-coumaric acid.

The results regarding rosmarinic acid showed that this despite was predominant in samples from Italy-A-Calabria and Italy-B-Sicily, two region of Southern Italy, geographically very close. Also vanillic acid was determined at highest concentrations in Italy-B-Sicily and Italy-A-Calabria samples so that its content was well four times higher than that of Bulgaria and USA samples.

Gallic acid occurred in small amount respect to the phenolic acid considered before and this trend was common to all the samples. Once more, samples coming from Italy-B-Sicily and Italy-A-Calabria showed the highest content while the samples from USA and Bulgaria the lowest.

As regards all the other phenolic acids, their average contents were below 1 mg kg<sup>-1</sup>, only syringic acid appeared relatively more abundant in samples from Italy-B-Sicily and Italy-A-Calabria.

With respect to flavonoids, Italy-B-Sicily samples presented the highest apigenin, luteolin and rutin mean contents; at the same time for quercetin, the maximum mean amount occurred in Italy-A-Calabria samples.

To the best of our knowledge, this is the first quantitative determination on *Origanum vulgare* L. concerning the phenolic compounds listed in Table 3S; therefore, it is difficult to compare these results to the literature ones. Specifically, studies on *Origanum dictamnus* did not detect the mentioned phenolic species, (Proestos et al. 2006) whereas on *Origanum onites* and *Origanum indicendes* were just detected caffeic and rosmarinic acids. (Pizzale et al. 2002) Particularly, the results of quantitative analysis of phenolic acids in medicinal plants belonging to the *Lamiaceae* family evidenced that these compounds are present in different qualitative and quantitative proportions depending on the plant species. (Wojdyło et al. 2007; Zgórka et al. 2001) These data confirm that the plant kingdom offers a wide range of natural antioxidant; moreover, they support

the hypothesis according to which the composition could be “territorial specificity” and that same species growing in different area could exhibit an impressive diversity in phenol content; moreover, the climatic conditions could be an important driving force for phenols evolution and variation. (Ignat et al. 2011)

### 2.3 Minerals

The average content of the other minor non-toxic elements was generally following the order: Fe>Ba>Zn>Cu>Mn>Al. Bulgarian samples are on average featured by high levels of Fe, Ba and Zn; this pattern is reminding some previously analysed Romanian samples. (Antal et al. 2015) Samples from Italy-B-Sicily display high levels of Fe, while the low level of Ba detected in USA samples is surprising. This last result is probably due to the poor Ba content of the soil in the north-America territories where the plants were grown. Indeed, plant's composition of essential and non-essential elements are related to the plant's physiology, soil, water source composition, use of fertilizers and pesticides. (Watanabe et al. 2007)

The mean Mn content resulted remarkably highest in samples from USA and showed a great variability among the other samples. Literature data on *O. vulgare* L. presented much higher values (Antal et al. 2015) but our actual detection is comparable to the values found in some *Origanum Majorana* samples. (Khalil et al. 2012) The Zn levels of samples from the Mediterranean basin, Mexico and USA showed similar values, whereas it was found in higher level in the Bulgarian and British samples. The literature reference showed for Romanian samples, higher Zn amounts respect to all our analysed samples. (Antal et al. 2015) Copper content, found to be different among the seven marketing places, is lower than expected; (Antal et al. 2015) it is probably dependent by both, selectivity of the mineral accumulation process and phylogenetic plant evolutionary factors. (Watanabe et al. 2007)

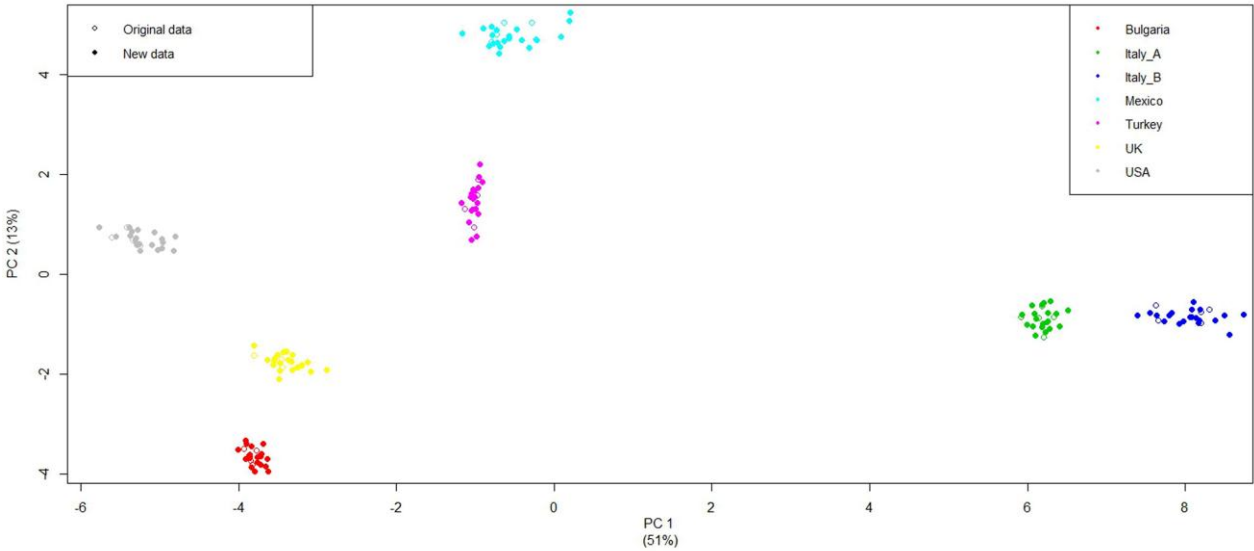
Samples from the London market showed minor Al concentration, respect to the others, while the fifteen samples from Mexico, Turkey and Italy-B-Sicily exhibited the highest and similar content of this element; generally Al content was lower than expected from other reference. (Antal et al. 2015) The level of potentially toxic elements As, Cr, Pb, Hg and Ni is specifically crucial for the great concern on the possible effects exerted against the human health even in small amounts. The actual law regulation establishes legal limits for As, Hg and Pb in spices, while Cr and Ni do not have a fixed limit. In Europe, the maximum residue levels is regulated by the European directive applied to flavouring products used to impart odour and/or taste to food, as well as flavouring and foodstuffs imported into the European Community. (European Commission. Council Directive 88/388/EEC) The more restricted roles in Italy fix the maximum levels of As, Pb and Hg in aromas to 3, 10 and 1



mg·kg<sup>-1</sup> respectively. (Legislative Decree (L.D.) n° 107. 1992) Nevertheless FAO/WHO established international limits for spice and assessed the values of 1, 3 and 10 mg·kg<sup>-1</sup> for As, Hg, and Pb, respectively. (FAO/WHO 1984)

For the analysed samples, the general quantitative decreasing order for toxic-metals was Ni>Pb>Cr>Hg>As. The residual level of Pb, Ni and Cr displayed highest values for Bulgarian samples; whereas the Italy-B-Sicily samples used in this study looked the safest ones as all the non-toxic metals were below the instrumental detection limits for any sample, very far below the regulation limits. Turkish samples were the only group issuing an instrumental record for the As, even if these were still below the safety limits set by L.D. n° 107 (3 mg·kg<sup>-1</sup>) and FAO/WHO (1 mg·kg<sup>-1</sup>). New York samples showed the highest Hg mean content, still respecting the national and international legal limits (1 mg·kg<sup>-1</sup>). These values are followed by those of the Mexican samples (around 0.13 mg·kg<sup>-1</sup>), which are still much more than those detected for any other sample. Except for the Italy-B-Sicily samples, Pb was always detected being the Bulgarian samples the Pb richest ones, still in compliance with the legal limits (10 mg·kg<sup>-1</sup>). Measured mean Pb concentrations were twice higher than those usually reported in similar analyses, and samples from UK, USA and Mexico displayed quantities close to those found in South-West Romania samples; (Antal et al. 2015) on the other hand these values were very low for Turkish and Italy-A-Calabria samples. The Cr levels, again not detected for Italy-B-Sicily and USA samples, were highest for Bulgarian samples and these values are comparable to precedent results found for Romanian samples, suggesting a soil similarity explained by the neighbouring provenience lands. (Antal et al. 2015)

**Figure 1S.** Prediction of PCs for validation dataset.



1 **Table 1S** Percentage composition of 38 compounds identified in essential oils from 35 *Origanum vulgare* L. commercial samples collected in various countries.

Compounds	Turkey		Bulgaria		USA		Mexico		UK		Italy A		Italy B	
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD
hexanal <sup>#</sup>	0.09 – 0.15	0.12 $\pm$ 0.02	0.15 – 0.19	0.17 $\pm$ 0.02	0.19 – 0.26	0.23 $\pm$ 0.03	0.12 – 0.17	0.15 $\pm$ 0.02	0.16 – 0.20	0.18 $\pm$ 0.02	0.08 – 0.13	0.10 $\pm$ 0.02	0.04 -0.11	0.07 $\pm$ 0.03
$\alpha$ -thujene <sup>†</sup>	0.30 – 0.37	0.34 $\pm$ 0.03	0.48 – 0.55	0.52 $\pm$ 0.03	0.19 – 0.25	0.22 $\pm$ 0.02	1.09 – 1.18	1.14 $\pm$ 0.04	1.16 – 1.22	1.19 $\pm$ 0.02	1.80 - 1.86	1.83 $\pm$ 0.02	1.97 –3.09	2.59 $\pm$ 0.43
$\alpha$ -pinene <sup>†</sup>	0.46 – 0.54	0.51 $\pm$ 0.03	1.58 – 1.69	1.64 $\pm$ 0.04	1.09 – 1.30	1.20 $\pm$ 0.09	1.85 – 1.91	1.87 $\pm$ 0.02	0.86 – 1.03	0.93 $\pm$ 0.07	0.49 – 0.63	0.56 $\pm$ 0.05	0.31 – 0.42	0.36 $\pm$ 0.04
camphene <sup>†</sup>	0.13 – 0.24	0.19 $\pm$ 0.04	0.15 – 0.25	0.20 $\pm$ 0.04	0.12 – 0.19	0.16 $\pm$ 0.03	0.28 – 0.38	0.34 $\pm$ 0.04	0.18 – 0.25	0.22 $\pm$ 0.03	0.10 – 0.15	0.13 $\pm$ 0.02	0.07 – 0.15	0.11 $\pm$ 0.03
sabinene <sup>†</sup>	n.d.		n.d.		0.10 – 0.15	0.12 $\pm$ 0.02	1.89 – 2.51	2.31 $\pm$ 0.14	n.d.		3.58 – 5.02	4.34 $\pm$ 0.53	3.73 – 5.05	4.61 $\pm$ 0.46
3- octanone <sup>#</sup>	0.07 – 0.15	0.10 $\pm$ 0.03	0.19 – 0.38	0.30 $\pm$ 0.04	n.d.		0.08 – 0.16	0.14 $\pm$ 0.02	0.06 – 0.13	0.10 $\pm$ 0.02	n.d.		n.d.	
$\beta$ -myrcene <sup>†</sup>	n.d.		1.30 – 1.58	1.49 $\pm$ 0.11	1.98 – 2.30	2.18 $\pm$ 0.12	n.d.		1.95 – 2.18	2.07 $\pm$ 0.08	2.00 – 2.42	2.21 $\pm$ 0.07	2.54 – 3.32	3.01 $\pm$ 0.21
3-octanol <sup>#</sup>	0.06 - 0.15	0.10 $\pm$ 0.02	n.d.		n.d.		0.35 – 0.52	0.40 $\pm$ 0.06	0.08 – 0.18	0.13 $\pm$ 0.03	n.d.		n.d.	
$\beta$ -phellandrene <sup>†</sup>	6.48 – 7.95	7.43 $\pm$ 0.45	n.d.		n.d.		n.d.		1.98 – 2.32	2.15 $\pm$ 0.09	n.d.		n.d.	
$\alpha$ -phellandrene <sup>†</sup>	0.47 – 0.62	0.54 $\pm$ 0.05	n.d.		n.d.		n.d.		0.14 – 0.18	0.16 $\pm$ 0.02	0.20 – 0.32	0.29 $\pm$ 0.02	n.d.	
$\alpha$ -terpinen <sup>†</sup>	0.18 – 0.28	0.23 $\pm$ 0.04	0.23 – 0.37	0.31 $\pm$ 0.05	0.17 – 0.28	0.22 $\pm$ 0.04	0.29 – 0.38	0.33 $\pm$ 0.03	0.07 – 0.18	0.12 $\pm$ 0.04	0.61 – 0.80	0.70 $\pm$ 0.07	0.64 – 0.75	0.69 $\pm$ 0.04
<i>p</i> -cymene <sup>†</sup>	13.12 – 14.55	13.61 $\pm$ 0.56	12.10 – 14.15	13.35 $\pm$ 0.77	18.62 – 19.38	19.09 $\pm$ 0.30	11.97 – 13.99	13.21 $\pm$ 0.81	8.83 – 9.38	9.09 $\pm$ 0.23	5.67 – 5.97	5.82 $\pm$ 0.14	3.02 – 3.45	3.19 $\pm$ 0.17
limonene <sup>†</sup>	1.65 – 1.80	1.71 $\pm$ 0.06	0.72 – 0.93	0.83 $\pm$ 0.09	1.29 – 1.51	1.40 $\pm$ 0.09	1.64 – 1.80	1.72 $\pm$ 0.07	1.54 – 1.74	1.63 $\pm$ 0.08	2.47 – 3.05	2.90 $\pm$ 0.12	2.62 – 3.32	2.95 $\pm$ 0.25
1,8-cineole <sup>‡</sup>	n.d.		n.d.		n.d.		2.86 – 3.25	3.12 $\pm$ 0.22	4.98 – 5.54	5.32 $\pm$ 0.23	n.d.		n.d.	
$\beta$ -Z-ocimene <sup>†</sup>	3.50 – 4.68	4.07 $\pm$ 0.42	2.68 – 3.95	3.57 $\pm$ 0.51	1.69 – 1.91	1.80 $\pm$ 0.08	3.26– 3.85	3.61 $\pm$ 0.22	2.01 – 2.18	2.11 $\pm$ 0.06	4.50 – 5.05	4.83 $\pm$ 0.21	4.33 – 5.11	4.76 $\pm$ 0.28
$\beta$ -E-ocimene <sup>†</sup>	2.98 – 3.79	3.47 $\pm$ 0.31	2.88 – 3.57	3.33 $\pm$ 0.27	2.08 – 2.34	2.22 $\pm$ 0.10	2.38 – 2.64	2.51 $\pm$ 0.10	1.81 – 1.98	1.89 $\pm$ 0.07	4.95 – 5.62	5.26 $\pm$ 0.26	3.70 – 4.02	3.84 $\pm$ 0.12
$\gamma$ -terpinene <sup>†</sup>	1.19 – 1.34	1.25 $\pm$ 0.06	2.20 – 2.88	2.54 $\pm$ 0.25	1.40 – 1.58	1.49 $\pm$ 0.07	1.68 – 1.83	1.73 $\pm$ 0.06	1.97 – 2.23	2.09 $\pm$ 0.10	3.03 – 3.32	3.18 $\pm$ 0.11	4.40 – 5.02	4.67 $\pm$ 0.23
<i>cis</i> -sabinene hydrate <sup>†</sup>	0.24 – 0.39	0.32 $\pm$ 0.06	2.01 – 2.48	2.23 $\pm$ 0.19	n.d.		1.73 – 1.92	1.82 $\pm$ 0.07	n.d.		3.96 – 4.33	4.20 $\pm$ 0.15	3.56 – 3.94	3.79 $\pm$ 0.16
<i>trans</i> -linalool oxide <sup>‡</sup>	n.d.		n.d.		n.d.		0.09 – 0.13	0.11 $\pm$ 0.01	0.12 – 0.18	0.16 $\pm$ 0.02	0.44 – 0.69	0.61 $\pm$ 0.09	0.39 – 0.56	0.44 $\pm$ 0.07
linalool <sup>‡</sup>	0.58 – 0.77	0.65 $\pm$ 0.08	n.d.		1.32 – 1.63	1.56 $\pm$ 0.13	2.58 – 2.99	2.87 $\pm$ 0.16	0.49 – 0.69	0.61 $\pm$ 0.08	3.21 – 3.79	3.52 $\pm$ 0.21	3.98 – 4.99	4.75 $\pm$ 0.39
<i>trans</i> -sabinene hydrate <sup>†</sup>	0.32 – 0.51	0.41 $\pm$ 0.07	1.56 – 1.88	1.77 $\pm$ 0.13	n.d.		0.83 – 1.03	0.93 $\pm$ 0.08	n.d.		3.24 – 3.72	3.44 $\pm$ 0.19	2.84 – 3.19	3.05 $\pm$ 0.13
borneol <sup>‡</sup>	0.06 – 0.23	0.15 $\pm$ 0.07					0.07 – 0.18	0.12 $\pm$ 0.05	0.09 – 0.20	0.13 $\pm$ 0.04	2.46 – 2.72	2.62 $\pm$ 0.10	2.86 – 3.14	3.02 $\pm$ 0.12
terpinen-4-ol <sup>‡</sup>	0.50 – 0.75	0.68 $\pm$ 0.10	0.85 – 1.12	0.90 $\pm$ 0.12	0.51 – 0.73	0.66 $\pm$ 0.10	0.50 – 0.67	0.62 $\pm$ 0.06	0.61 – 0.83	0.70 $\pm$ 0.08	0.74 – 0.96	0.80 $\pm$ 0.10	0.78 – 1.19	0.88 $\pm$ 0.17
<i>p</i> -cymene-8-ol <sup>‡</sup>	n.d.		n.d.		n.d.		n.d.		n.d.		n.d.		0.05 – 0.14	0.10 $\pm$ 0.03
<i>cis</i> -pinene hydrate <sup>†</sup>	n.d.		n.d.		n.d.		0.08 – 0.14	0.12 $\pm$ 0.02	n.d.		0.18 – 0.23	0.20 $\pm$ 0.02	0.12 – 0.19	0.16 $\pm$ 0.03
$\beta$ -terpineol <sup>‡</sup>	24.31 – 28.96	27.01 $\pm$ 1.69	1.58 – 1.78	1.68 $\pm$ 0.08	1.41 – 1.63	1.53 $\pm$ 0.09	2.92 – 3.19	3.05 $\pm$ 0.12	7.84 – 8.52	8.18 $\pm$ 0.24	3.24 – 3.85	3.51 $\pm$ 0.25	5.81 – 7.20	6.72 $\pm$ 0.54

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Compounds	Turkey		Bulgaria		USA		Mexico		UK		Italy A		Italy B	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
α-terpineol †	5.98 – 6.47	6.21 ± 0.19	2.25 – 2.39	2.32 ± 0.06	3.39 – 3.64	3.51 ± 0.09	4.99 – 5.73	5.50 ± 0.30	2.37 – 2.93	2.60 ± 0.21	1.54 – 1.73	1.64 ± 0.07	0.73 – 0.92	0.81 ± 0.08
dihydrocarvone †	0.06 – 0.16	0.13 ± 0.04	0.24 – 0.38	0.32 ± 0.05	0.62 – 0.94	0.88 ± 0.13	0.98 – 1.37	1.26 ± 0.16	0.24 – 0.40	0.32 ± 0.06	n.d.		n.d.	
thymol methyl ether	0.45 – 0.61	0.52 ± 0.06	0.49 – 0.70	0.61 ± 0.08	0.65 – 0.88	0.76 ± 0.09	0.20 – 0.29	0.25 ± 0.04	n.d.		n.d.		n.d.	
linalyl acetate †	0.58 – 0.67	0.65 ± 0.03	0.49 – 0.62	0.53 ± 0.06	n.d.		n.d.		3.99 – 5.71	5.29 ± 0.73	n.d.		n.d.	
thymol §	17.99 – 21.31	19.66 ± 1.40	29.78 – 32.30	31.37 ± 0.96	21.06 – 25.10	23.16 ± 1.44	14.16 – 18.55	16.50 ± 1.57	27.59 – 30.28	29.34 ± 1.02	31.58 – 37.12	34.38 ± 2.17	34.45 – 37.42	35.50 ± 1.20
carvacrol §	4.73 – 5.62	5.32 ± 0.36	7.82 – 9.46	8.54 ± 0.60	22.34 – 31.27	26.33 ± 3.31	11.73 – 13.12	12.47 ± 0.55	13.23 – 16.48	14.64 ± 1.20	1.44 – 1.65	1.54 ± 0.08	0.89 – 1.30	1.04 ± 0.16
thymol acetate §	2.24 – 2.56	2.43 ± 0.13	3.39 – 3.87	3.63 ± 0.17	2.88 – 3.29	3.14 ± 0.17	3.10 – 3.48	3.33 ± 0.15	1.93 – 2.25	2.12 ± 0.12	3.95 – 4.44	4.19 ± 0.18	5.64 – 6.08	5.88 ± 0.16
α-ylangene ¶	n.d.		0.52 – 0.84	0.74 ± 0.12	n.d.		0.33 – 0.60	0.52 ± 0.11	n.d.		0.18 – 0.26	0.21 ± 0.03	n.d.	
α-copaene ¶	n.d.		n.d.		n.d.		n.d.		n.d.		0.31 – 0.59	0.47 ± 0.10	n.d.	
aromadendrene ¶	n.d.		2.96 – 3.91	3.58 ± 0.35	n.d.		n.d.		n.d.		n.d.		n.d.	
β-bisabolene ¶	0.12 – 0.29	0.21 ± 0.07	1.64 – 1.81	1.71 ± 0.07	0.26 – 0.45	0.34 ± 0.07	4.34 – 4.85	4.64 ± 0.19	0.59 – 0.72	0.65 ± 0.05	2.37 – 2.56	2.45 ± 0.08	0.08 – 0.14	0.11 ± 0.02
caryophyllene oxide †			4.32 – 4.79	4.57 ± 0.17	3.69 – 3.84	3.76 ± 0.06	6.52 – 7.37	7.00 ± 0.33	1.74 – 1.98	1.84 ± 0.09	2.84 – 3.23	3.02 ± 0.15	n.d.	
Phenols		27.93		44.15		53.39		32.55		46.09		40.11		42.42
Monoterpene hydrocarbons		33.35		27.78		29.98		26.46		23.65		27.71		26.17
Oxygenated monoterpenes		35.48		5.75		8.18		16.77		23.60		12.90		16.17

Compounds are listed in order of elution. n.d.= not detected.

† monoterpene hydrocarbon; ‡ oxygenated monoterpene; § monoterpenoid phenol; ¶ sesquiterpene hydrocarbon; # non-terpenoidic compound.

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**Table 2S.** Kruskal Wallis test results and significant differences in *post hoc* analysis for essential oil compounds (grey *p*-value <0.05)

	<i>p</i> -value	HEX	α-THU	α-PIN	CAM	α-TER	<i>p</i> -CY	LIM	β-Z-OC	β-E-OC	γ-TER	β-TEOH	α-TEOH	THY	CAR	THYAC	β-BIS	CAOX	BOR	<i>t</i> -SAB	<i>c</i> -SAB
Turkey-Bulgaria	0.00																				
Turkey-USA	0.00																				
Turkey-Mexico	0.00																				
Turkey-UK	0.00																				
Turkey-Italy-A	0.00																				
Turkey-Italy-B	0.00																	*			
Bulgaria-USA	0.00																		-		
Bulgaria-Mexico	0.00																				
Bulgaria-UK	0.00																				
Bulgaria-Italy-A	0.00																				
Bulgaria-Italy-B	0.00																				
USA-Mexico	0.00																				
USA-UK	0.00																			-	-
USA-Italy-A	0.00																				
USA-Italy-B	0.00																				
Mexico-UK	0.00																				
Mexico-Italy-A	0.00																				
Mexico-Italy-B	0.00																				
UK- Italy-A	0.00																				
UK-Italy-B	0.00																				
Italy-A-Italy-B	0.00																				

Abbreviations: HEX: hexanal, α-THU: α-thujene, α-PIN: α-pinene, CAM: camphene, α-TER: α-terpinen, *p*-CY: *p*-cymene, LIM: limonene, β-Z-OC: β-Z-ocimene, β-E-OC: β-E-ocimene, γ-TER: γ-terpinene, β-TEOH: β-terpineol, α-TEOH: α-terpineol, THY: thymol, CAR: carvacrol, THYAC: thymol acetate, β-BIS: β-bisabolene, CAOX: caryophyllene oxide, *t*-SAB: *trans*-sabinene hydrate, *c*-SAB: *cis*-sabinene hydrate.

\* – Pair comparison do not conducted.

19     **Table 3S.** Occurrence of polyphenols identified in 35 *Origanum vulgare* L. commercial samples collected in various countries

Compound	Total Sample			Turkey			Bulgaria			USA			Mexico			UK			Italy-A			Italy-B			p-value
	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	
GA	5.60	6.75	3.42	5.60	5.45	0.45	4.00	3.96	0.13	3.68	3.62	0.14	6.04	6.09	0.17	4.25	4.27	0.12	11.78	11.81	0.42	12.00	12.02	0.44	0.00
HPA	0.70	0.60	0.26	0.78	0.76	0.05	0.80	0.80	0.04	0.61	0.61	0.02	0.71	0.71	0.04	0.87	0.88	0.04	0.20	0.20	0.02	0.21	0.21	0.02	0.00
VA	15.37	21.51	11.88	13.20	13.40	0.63	10.05	10.05	0.13	10.12	10.18	0.38	25.65	25.53	0.73	15.37	14.94	0.78	35.84	35.74	1.44	40.40	40.71	1.06	0.00
CA	3.10	3.35	1.71	1.89	1.87	0.10	1.58	1.66	0.14	1.77	1.76	0.17	3.65	3.69	0.12	3.10	3.13	0.10	4.80	4.86	0.19	6.50	6.47	0.30	0.00
CHA	26.00	30.06	14.12	20.89	21.44	2.75	9.51	9.30	0.91	19.65	20.30	1.64	36.66	36.67	1.21	25.64	26.05	1.50	46.10	45.57	1.32	51.08	51.07	0.88	0.00
SA	0.63	0.66	0.39	0.44	0.43	0.03	0.20	0.20	0.01	0.30	0.31	0.02	0.63	0.62	0.04	0.70	0.69	0.03	0.98	1.00	0.08	1.35	1.37	0.10	0.00
RA	19.55	26.48	14.53	19.54	18.93	2.47	9.63	9.79	0.44	10.04	10.81	1.09	39.11	38.89	0.67	18.33	18.53	0.58	45.03	45.14	0.62	43.00	43.23	0.88	0.00
o-CA	46.21	48.01	11.48	48.68	50.33	5.93	40.65	40.19	3.72	33.16	33.98	2.75	47.31	47.27	0.91	38.44	38.35	1.29	60.04	59.47	1.16	65.89	66.45	4.85	0.00
RU	13.12	13.03	4.01	13.12	12.98	1.19	8.51	8.66	0.61	7.78	7.91	0.78	16.92	16.20	2.03	10.52	10.60	0.32	16.20	16.31	0.69	18.30	18.55	1.13	0.00
LUT	26.51	25.11	11.14	26.66	26.71	3.11	12.04	12.08	0.16	15.69	16.03	1.90	28.68	28.50	1.38	13.21	13.26	0.30	38.00	38.16	0.57	41.06	41.03	0.73	0.00
API	30.85	29.54	20.48	30.85	30.42	1.75	2.50	2.50	0.71	15.37	15.77	1.78	45.65	45.73	1.44	6.54	6.60	0.40	48.12	48.34	0.79	57.61	57.45	2.00	0.00
QUE	31.65	27.52	13.14	33.89	34.07	1.11	10.02	10.15	0.29	22.17	22.60	2.42	31.65	31.63	0.46	9.47	9.47	0.17	44.87	45.02	1.02	39.85	39.68	1.28	0.00

20     Abbreviations: Ga: gallic acid, HPA: 3,4-dihydroxyphenylacetic acid, VA: vanillic acid, CA: caffeic acid, CHA: chlorogenic acid, SA: syringic acid, RA: rosmarinic acid, o-CA: o-coumaric acid, RU: rutin, LUT: luteolin, API: apigenin,  
21     QUE: quercetin.

22     Median and Mean values are expressed in mg kg<sup>-1</sup>. SD = standard deviation.

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32 **Table 4S.** Kruskal Wallis test results and significant differences in *post hoc* analysis for polyphenols (grey *p*-value <0.05)

	<i>p</i> -value	GA	HPA	VA	CA	CHA	SA	RA	<i>o</i> -CA	RU	LUT	API	QUE
Turkey-Bulgaria	0.00												
Turkey-USA	0.00												
Turkey-Mexico	0.00												
Turkey-UK	0.00												
Turkey-Italy-A	0.00												
Turkey-Italy-B	0.00												
Bulgaria-USA	0.00												
Bulgaria-Mexico	0.00												
Bulgaria-UK	0.00												
Bulgaria-Italy-A	0.00												
Bulgaria-Italy-B	0.00												
USA-Mexico	0.00												
USA-UK	0.00												
USA-Italy-A	0.00												
USA-Italy-B	0.00												
Mexico-UK	0.00												
Mexico-Italy-A	0.00												
Mexico -Italy-B	0.00												
UK- Italy-A	0.00												
Uk-Italy-B	0.00												
Italy-A-Italy-B	0.00												

33 Abbreviations: Ga: gallic acid, HPA: 3,4-dihydroxyphenylacetic acid, VA: vanillic acid, CA: caffeic acid, CHA: chlorogenic acid, SA: syringic acid, RA: rosmarinic acid, *o*-CA: *o*-coumaric acid, RU: rutin, LUT: luteolin, API: apigenin, QUE:  
34 quercetin.

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37 **Table 5S.** Occurrence (median, mean and standard deviation) of nontoxic and potentially toxic elements in 35 *Origanum vulgare* L. commercial samples collected in various  
38 countries

Elements	Total Sample			Turkey			Bulgaria			USA			Mexico			UK			Italy-A			Italy-B			
	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	<i>p-value</i>
Al	1.81	1.86	0.59	2.46	2.47	0.22	1.85	1.84	0.13	1.33	1.31	0.16	2.67	2.54	0.41	1.07	1.08	0.10	1.48	1.47	0.06	2.38	2.34	0.09	0.00
Ba	20.62	20.56	12.25	26.86	26.21	1.29	39.51	39.51	0.72	0.82	0.83	0.03	20.31	20.31	0.61	29.56	29.15	0.73	20.45	20.08	0.78	7.80	7.79	0.20	0.00
Ca	1460.81	2262.24	1866.41	1460.81	1446.55	30.09	6600.37	6547.94	142.43	1734.30	1721.26	29.32	1200.81	1201.82	18.46	1100.00	1109.50	116.19	1007.50	1003.80	14.94	2783.44	2804.83	52.81	0.00
Cu	4.63	4.66	1.26	5.18	5.37	0.54	4.11	4.24	0.38	3.26	3.33	0.30	2.88	2.86	0.13	6.10	6.10	0.25	6.10	6.20	0.35	4.51	4.49	0.24	0.00
Fe	34.64	32.38	5.22	35.23	34.24	2.37	34.71	34.98	0.71	24.10	24.28	0.57	36.07	36.14	0.74	27.77	28.13	1.35	29.12	28.95	0.88	39.88	39.93	0.95	0.00
K	3138.20	3915.43	2549.32	1596.61	1596.66	4.54	1283.44	1270.65	40.16	6200.01	6147.77	196.23	8114.13	8122.86	33.11	1521.82	1539.09	53.36	5711.06	5579.18	339.32	3138.20	3151.78	113.94	0.00
Mg	1642.63	1742.96	494.41	1275.45	1281.18	18.70	2166.53	2166.93	25.49	2640.20	2623.08	68.38	1482.70	1479.15	64.34	1900.00	1901.53	31.50	1152.23	1135.92	56.24	1642.63	1612.90	63.99	0.00
Mn	3.80	5.26	4.04	3.80	4.18	0.76	5.97	6.32	0.59	13.10	13.56	1.24	1.66	1.75	0.18	7.00	7.01	0.24	2.75	2.65	0.46	1.34	1.33	0.08	0.00
Na	1583.41	2357.47	1521.62	2452.16	2411.75	107.41	1583.41	1582.48	30.07	1197.43	1196.99	17.18	4993.80	4984.35	85.85	4192.40	4215.26	188.63	1085.20	1085.31	77.61	1000.45	1026.13	48.64	0.00
Zn	15.46	17.81	5.69	13.02	12.67	0.96	25.20	25.45	0.80	16.55	16.61	0.25	13.80	13.54	0.84	27.73	27.22	0.93	13.24	13.39	0.80	15.46	15.79	0.86	0.00
Pb	0.50	0.60	0.49	0.20	0.20	0.01	1.47	1.45	0.08	0.86	0.85	0.06	0.97	0.99	0.09	0.50	0.51	0.06	0.23	0.23	0.05	0.00	0.00	0.00	0.00
As	0.00	0.01	0.03	0.08	0.08	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ni	0.30	0.89	0.91	0.25	0.26	0.03	2.41	2.40	0.05	0.04	0.04	0.01	0.30	0.29	0.03	1.69	1.69	0.06	1.50	1.57	0.16	0.00	0.00	0.00	0.00
Cr	0.24	0.31	0.35	0.24	0.23	0.02	0.97	0.97	0.06	0.00	0.00	0.00	0.31	0.31	0.04	0.05	0.05	0.01	0.66	0.64	0.05	0.00	0.00	0.00	0.00
Hg	0.05	0.15	0.28	0.05	0.04	0.01	0.05	0.05	0.01	0.91	0.74	0.37	0.14	0.13	0.03	0.05	0.05	0.01	0.04	0.04	0.01	0.00	0.00	0.00	0.00

39 Median and Mean values are expressed in mg kg<sup>-1</sup>. SD = standard deviation.

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**Table 6S.** Kruskal Wallis test results and significant differences in *post hoc* analysis for minerals (grey *p*-value <0.05)

	<i>p</i> -value	Al	Ba	Ca	Cu	Fe	K	Mg	Mn	Na	Zn	Pb	As	Ni	Cr	Hg
Turkey-Bulgaria	0.00															
Turkey-USA	0.00															
Turkey-Mexico	0.00															
Turkey-UK	0.00															
Turkey-Italy-A	0.00															
Turkey-Italy-B	0.00															
Bulgaria-USA	0.00															
Bulgaria-Mexico	0.00															
Bulgaria-UK	0.00															
Bulgaria-Italy-A	0.00															
Bulgaria-Italy-B	0.00															
USA-Mexico	0.00															
USA-UK	0.00															
USA-Italy-A	0.00															
USA-Italy-B	0.00															
Mexico-UK	0.00															
Mexico-Italy-A	0.00															
Mexico -Italy-B	0.00															
UK- Italy-A	0.00															
UK-Italy-B	0.00															
Italy-A-Italy-B	0.00															

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