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Traceability of Croatian extra virgin olive oils to the provenance soils by multielement and carbon isotope composition and chemometrics

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Abstract:	A capacity to determine the provenance of high-value food products is of high scientific and economic interest. With the aim to develop a tool for geographical traceability of Croatian extra virgin olive oils (EVOO), multielement composition and 13C/12C isotope ratio in EVOO as well as the geochemistry of the associated soils were analysed in samples collected from three regions along the Croatian Adriatic coast. Soil geochemistry was shown to influence the transfer and elemental composition of EVOO. The most discriminating variables to distinguish EVOO from different regions were S, Mo, Rb, Mg, Pb, Mn, Sn, K, V and δ13C. The predictive models achieved high sensitivity and specificity, especially when carbon isotope composition was added. The results suggest that interregional geographical traceability of Croatian EVOO is possible based on matching their multielement composition with that of the soils in the provenance area.
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Dr. Paul Finglas
Editor-in-Chief
Food Chemistry

March 20, 2023

Dear Dr. Finglas,

I would like to submit the manuscript entitled **"Traceability of Croatian extra virgin olive oils to the provenance soils by multielement and carbon isotope composition and chemometrics"** for publication consideration in Food Chemistry.

The manuscript consists of 5964 words and a total of 6 figures and tables.

In the manuscript, we formulated and confirmed the hypothesis that soil geochemistry is one of the most important factors influencing the elemental composition of extra virgin olive oil (EVOO) and a direct link between soils and EVOO was determined. Traceability of EVOO was possible when the samples were separated according to the different parent materials with contrasting soil geochemical signatures.

We believe this manuscript is a valuable contribution to the existing knowledge on EVOO traceability and fits the aim and scope of the journal Food Chemistry.

This paper has not been published previously, it is not under consideration for publication elsewhere, and it is approved by all co-authors. We have no conflicts of interest to disclose.

We thank you in advance for your consideration and look forward to your response.

Kind regards in the name of all co-authors,

Mavro Lučić

Mavro Lučić
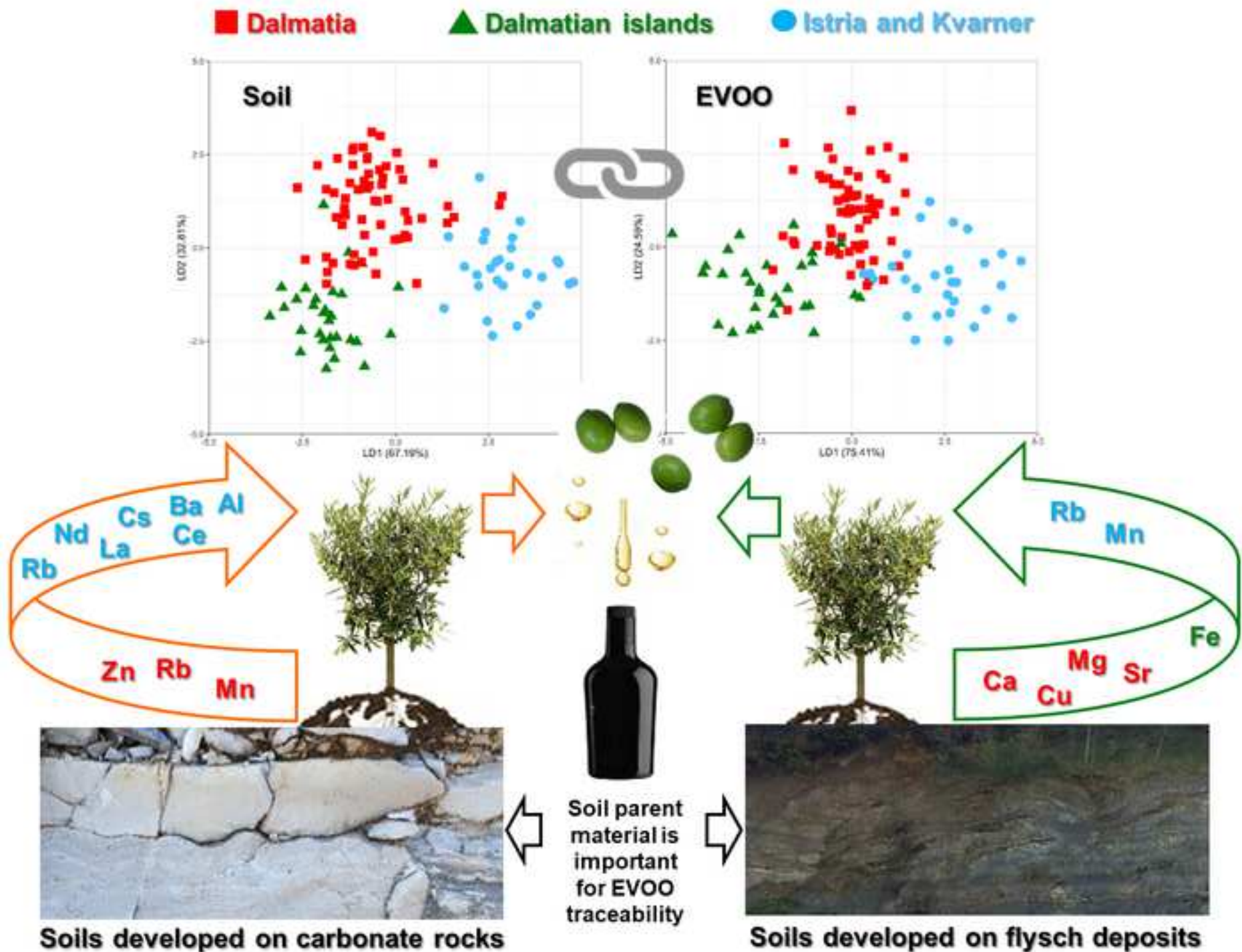
Highlights

- Soil geochemical fingerprints influence transfer and elemental composition of EVOO

- Direct link between elements in provenance soil and EVOO was proven

- Multielement and C isotopic composition can be used for authentication of EVOO

1　**Traceability of Croatian extra virgin olive oils to the provenance soils**

2　**by multielement and carbon isotope composition and chemometrics**

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27

28   **Abstract**

29   A capacity to determine the provenance of high-value food products is of high scientific and

30   economic interest. With the aim to develop a tool for geographical traceability of Croatian extra

31   virgin olive oils (EVOO), multielement composition and $^{13}C/^{12}C$ isotope ratio in EVOO as well as

32   the geochemistry of the associated soils were analysed in samples collected from three regions

33   along the Croatian Adriatic coast. Soil geochemistry was shown to influence the transfer and

34   elemental composition of EVOO. The most discriminating variables to distinguish EVOO from

35   different regions were S, Mo, Rb, Mg, Pb, Mn, Sn, K, V and $\delta^{13}C$. The predictive models

36   achieved high sensitivity and specificity, especially when carbon isotope composition was

37   added. The results suggest that interregional geographical traceability of Croatian EVOO is

38   possible based on matching their multielement composition with that of the soils in the

39   provenance area.

40

41   Keywords: Traceability; Soil; Olive oil; Multielement composition; Carbon isotope ratio;

42   Chemometrics

43

1. Introduction

45   Extra virgin olive oil (EVOO) is one of the high-quality high-price food products that has

46   achieved strong growth in the world market in recent years; correspondingly, it is the subject of

47   intensive research to improve existing practices and to identify new methods to protect against

48   various forms of fraud (Tahir et al., 2022). At the level of categorisation and authenticity, the

49   analytical and sensory methods are defined in the international trade standards of the

50 International Olive Council (IOC) and the European Union (EU) (EEC, 1991). On the other hand,

51 the quality of the EVOO category is quite broad, and there is a market interest in the capacity to

52 single out the best of the best. In this sense, oils can be highlighted by their nutritional quality

53 through the nutrition and/or health claims (EFSA NDA Panel, 2011), but also protected as PDO

54 (Protected Designation of Origin) or PGI (Protected Geographical Indication) based on the

55 region where they are produced. The latter reflects consumer interest in foods that are strongly

56 identified with a place of origin in contrast to the globalisation of the food industry. EVOO with

57 clear regional identity are produced according to a set of specific rules established by the holder

58 of a designation in a specification document, regulating aspects such as the olive varieties used

59 and the cultivation, harvesting and processing conditions, as well as the oil physicochemical and

60 sensory characteristics (Lukić et al., 2020). Therefore, the methods for determining the origin of

61 EVOO in the main olive-growing countries have become a matter of great scientific interest in

62 the last decade (Beltrán, Sánchez-Astudillo, Aparicio, & García-González, 2015; Camin et al.,

63 2010; Damak et al., 2019; Dimitrakopoulou & Vantarakis, 2021; Nasr et al., 2022a,b; Portarena,

64 Gavrichkova, Lauteri & Brugnoli, 2014; Tahir et al., 2022).

65 Although the chromatographic, NMR and infrared spectroscopy methods are becoming

66 increasingly popular for EVOO authentication, two approaches (the multielement and isotopic

67 composition of the oil) have shown the greatest potential in determining the origin (Drivelos &

68 Georgiou, 2012; Nasr et al., 2022b; Tahir et al., 2022). The first encouraging studies on the use

69 of elemental composition of olive oil for their geographical characterisation were based on

70 determination of a small number of elements by atomic spectrometry (e.g., Zeiner, Steffan &

71 Cindric, 2005). However, the development of analytical techniques (inductively coupled plasma

72 mass spectrometry – ICPMS) for measurement of metals in environmental matrices enabled

73 determination of many elements (macro, micro, trace and rare earth elements), providing an

74 insight into the complex multielement composition of oil (Camin et al., 2010; Pošćić et al., 2019).

75 Multielement fingerprinting of olive oils in combination with sophisticated chemometric analysis

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76 has been used successfully for determination of geographic origin of olive oils in different

77 Mediterranean countries (Beltrán, Sánchez-Astudillo, Aparicio, & García-González, 2015;

78 Damak et al., 2019; Nasr et al, 2022a,b).

79   Although the basis for using trace elements in geographical traceability of food is an

80 assumption that soil geochemistry is the most important factor influencing elemental

81 composition of plants and plant products (Antoniadis et al., 2017; Kelly, Heaton & Hoogewerff,

82 2005; Nasr et al., 2022b; Tyler & Olsson, 2001), there are only few publications investigating

83 simultaneously multielement composition of olive oils and associated soils. A Spanish group

84 (Beltrán, Sánchez-Astudillo, Aparicio, & García-González, 2015) studied the elemental

85 composition of olive grove soils, olive pomace and EVOO and found some similarities in the

86 selection of elements in these matrices, suggesting a potential usefulness of the method in oil

87 traceability. A study of the elemental fingerprinting of olive oils and associated soils from Tunisia

88 (Damak et al., 2019) provided good classification results, and although no clear correlation was

89 found between elements in soils and oils, a group of discriminating elements (Fe, Rb, Mg and

90 Pb) was detected. The most recent study (Nasr et al., 2022a) found correlations of Mg, Mn, Ni

91 and Sr in oils and corresponding soil samples from Tunisia. These few studies have shown that

92 it is not easy to establish a direct correlation between element concentrations in olive oils and

93 their associated soils, but they have demonstrated the importance of such an approach to

94 define distinguishing elements for traceability and to explain why they may vary in different

95 geographical areas.

96   Stable isotope ratios of EVOO have been used widely to verify oil authenticity, either on

97 their own (Bontempo et al., 2019; Chiocchini, Portarena, Ciolfi, Brugnoli & Lauteri, 2016;

98 Jiménez-Morillo, Palma, Garcia, Dias & Cabrita, 2020; Portarena, Gavrichkova, Lauteri &

99 Brugnoli, 2014) or in combination with oil multielement composition (Camin et al., 2010; Gumus,

100 Celenk, Tekin, Yurdakul & Ertas, 2017). It was shown that the stable isotope ratios of H, C and

101 O in olive oils are correlated with the climatic (temperature) and geographical (latitude and

4

102   distance from the coast) characteristics of the provenance sites (Camin et al., 2010), as well as

103   with annual seasonal changes due to variations in the stable isotope ratios of fatty acids during

104   fruit ripening (Portarena, Gavrichkova, Lauteri & Brugnoli, 2014). Thus, the use of multielement

105   composition and stable isotope data of olive oils underpins the links with both lithological and

106   geographical (climatic) characteristics of the area from where oils are originating, and provides

107   an ideal tool for olive oil traceability. Furthermore, the above analytical techniques combined

108   with the application of chemometric approaches such as linear discriminant analysis and k-

109   nearest neighbour analysis may have a great potential for solving the authentication problems in

110   food analysis (Granato et al., 2018).

111   To the best of our knowledge, there is no study in the literature that combines the

112   analysis of elements and isotopes in EVOO with the geochemical analysis of the associated

113   soils. However, the methods to enable geographical traceability are important for producers

114   whose oil quality is excellent, judged by an increasing number of national and international

115   recognition and awards. Croatian producers have therefore started to follow the European

116   trends and highlight the origin of their oil through a Protected Designation of Origin or a Croatian

117   national label "Croatian Island Product". Differentiation based on specific links between EVOO

118   and the characteristics of the area of origin, thus establishing the provenance of the product,

119   would offer an incentive for a more profitable use of EVOO, the main agricultural product of the

120   Mediterranean Croatia.

121   The main aim of the present work was to characterise the application of multielement

122   and isotopic composition coupled with chemometrics in interregional geographic traceability of

123   Croatian EVOO. The hypothesis is that the geochemistry of provenance soils is one of the most

124   important factors influencing the elemental composition of EVOO. Specific objectives were: (i) to

125   elucidate the link between soil and EVOO samples in the studied regions based on the two

126   parent materials (limestone and flysch deposits) influencing soil geochemistry and (ii) to

5

127   evaluate the performance of multielement and isotopic composition in combination with

128   chemometrics in predicting the geographical authenticity of Croatian EVOO.

129

130   **2. Materials and methods**

131   *2.1. Description of the study area and sampling locations*

132   *2.1.1. Geographical characteristics*

133   The study area geographically and geomorphologically belongs to the Coastal Croatia,

134   and geologically to the Inner Adriatic zone of the Dinaric Karst, which includes the islands and

135   the coastal mainland zone. The climate along the Croatian coast and in the adjacent hinterland

136   is Mediterranean and sub-Mediterranean (Csa and Cfa types) (Zaninović, 2008). The Istrian

137   peninsula is characterised by rainy winters and hot, dry summers with an average temperature

138   of 13°C and rainfall of about 900 mm/year. Similar values are found in the northern part of

139   Dalmatia, while slightly higher average temperatures of about 16.5°C and precipitation of more

140   than 1500 mm/year are observed in the southern part. The Dalmatian islands have

141   temperatures averaging 16°C and rainfall between 1000 and 1200 mm/year (Zaninović, 2008).

142

143   *2.1.2. Geological setting and lithology*

144   The geology of the area studied includes platforms of different ages, types and

145   palaeogeography (Hasan et al., 2020). The lithology consists mainly of a thick sequence of

146   carbonate rocks deposited between the Middle Permian and the Eocene/Oligocene, whose

147   succession ends with the deposition of flysch (Fig. 1). The studied soils on the Istrian peninsula

148   and Kvarner are developed on Jurassic and Cretaceous limestones and dolostones as well as

149   on the flysch deposits consisting of interbedded siliciclastic sandstones, marlstones and

150   calciturbidites (Durn, Perković, Stummeyer, Ottner & Mileusnić, 2021; Halamić, Peh, Miko,

151   Galović & Šorša, 2012; Hasan et al., 2020). Similar lithological units are also found in the

152   regions of Dalmatia and Dalmatian islands. The parent rock is mainly carbonate rock consisting

153 of Cretaceous rudist limestones and thick stratified dolostones of Late Jurassic and Cretaceous

154 age, which characterise the southernmost part of the studied area. The flysch sequences in

155 Dalmatia are represented by various rock types, including limestone breccias, breccia

156 conglomerates, sandstones, siltstones and clayey siltstones and marls with varying amounts of

157 calcium carbonate (Hasan et al., 2020). This distribution and spreading of the lithological

158 members on the investigated terrain thus indicates a basic subdivision into two main units,

159 namely carbonate rocks and flysch deposits (Table A1).

160

161 *2.1.3. Soil types*

162 The major soil types (Table A1) include Rhodic Cambisols, Rendzic Leptosols, and

163 anthropogenically modified Lithosols (IUSS Working Group WRB, 2015). Rhodic Cambisols,

164 also called Terra Rossa, are red polygenetic relict soils rich in clay and developed on limestone

165 or dolomite, typically found in regions with a Mediterranean climate (Durn, Perković,

166 Stummeyer, Ottner & Mileusnić, 2021). Rendzic Leptosols, also known as Rendzinas, are soils

167 formed on flysch, i.e. they usually form on soft marls and weakly consolidated calcareous

168 sandstones. Anthropogenically modified Lithosols are undeveloped soils with low water-holding

169 capacity. These soils are formed by agro-technical procedures and stone crushing to provide

170 land suitable for agricultural production (Romić et al., 2020).

171

172 *2.2. Sampling of soils and olives*

173 A total of 38 olive groves from three major geographic regions distinguished by their

174 specific lithological, pedological and climatic conditions [i.e., Istria and Kvarner, Dalmatia

175 (encompassing northern, central and southern Dalmatia) and the Dalmatian islands] (Fig. 1)

176 were sampled for soils in February and for olives in October. Number of olive groves and

177 samples collected in each region (separated by parent materials and soil types in these olive

178 groves) are listed in Table A1. In each olive grove, the soil and olive samples were collected in

179   triplicate (at three locations) within the grove. Each soil sample (topsoil, 0-30 m) consisted of

180   three soil cores (40 mm diameter) collected around the clusters of three olive trees. To reduce

181   the effect of the variety, the study was conducted only on the 'Oblica' variety, which is the

182   predominant variety in Croatia (Jović, Smolić, Jurišić, Meić & Hrenar, 2013). Olive fruit sampling

183   followed the same scheme as soil sampling; in each of the 38 olive groves, three batches of

184   olive fruit were harvested by hand from trees under which soil samples were taken. Each batch

185   of olive fruits consisted of 2-3 kg of healthy fruits from three olive trees sampled from the four

186   sides (SE, NE, SW and NW) of each tree; samples were then taken to the laboratory for

187   processing into oil.

188

189   *2.3. Sample preparation and analysis*

190   *2.3.1. Olive processing and oil extraction*

191   Olive fruits were processed within 48 h after harvesting. Prior to processing, olives were

192   rinsed with tap water, placed in 1% v/v acetic acid for 5 min, and finally rinsed 2 x 5 min with

193   deionised water. Olive fruits were milled in a hammer crusher, and then the olive paste was

194   kneaded at 26 ± 2°C in a thermo beater for 35 min. Between each batch of olives, the laboratory

195   equipment was washed with hot tap water, laboratory soap, and kitchen sponge, and then

196   rinsed several times with deionized water. After vertical centrifugation at 1370 g for 70 s and

197   decantation, the obtained oil samples were stored in plastic bottles (previously washed in 10%

198   v/v HNO_3 and rinsed with Milli-Q® water) in dark at 18 ± 2 °C.

199

200   *2.3.2. Soils and EVOO preparation*

201   Soil samples were air-dried and passed through a 2-mm sieve. For soil elemental

202   analyses, total digestion of samples (0.05 g) was performed in a microwave oven (Microwave

203   3000, Anton Paar, Graz, Austria) using a 6 mL of acid mixture 4:1:1 of HNO_3 (65% v/v pro

204   analysis, Kemika, Zagreb, Croatia): HCl (30% v/v Suprapur® Merck, Darmstadt, Germany): HF

205   (47–51% v/v Fluka TraceSELECT®, Steinheim, Germany) followed by addition of 6 mL of 0.65

206   M H_3BO_3 (Fluka, Steinheim, Germany) (Fiket, Mikac & Kniewald, 2017).

207   To remove pomace residues from oil before extraction, EVOO samples were centrifuged

208   at 3500 g for 5 min, and then 15 g of EVOO supernatant was transferred carefully (without

209   disturbance of the precipitated pomace residue) into 50 mL polypropylene (PP) falcon bottles

210   and subjected to the ultrasonic acid extraction with 15 mL of acid solution consisting of 0.2% v/v

211   HCl and 2% v/v HNO_3 (prepared from 69% v/v HNO_3, Fluka TraceSELECT®, Steinheim,

212   Germany). The upper oil phase was removed by aspiration, and acid solution was transferred to

213   clean PP vials for ICP-MS analysis (Poščić et al., 2019).

214

215   *2.3.3. Multielement analysis*

216   Multielement analysis of soil digests and EVOO extracts was performed by a high

217   resolution inductively coupled plasma mass spectrometer (HR ICP-MS) Element 2 (Thermo,

218   Bremen, Germany). The HR ICP-MS instrument conditions, measurement parameters,

219   measured isotopes, standards for multi-element analysis and other details of the analytical

220   procedures were reported in Fiket, Mikac & Kniewald (2017) for soil and in Poščić et al. (2019)

221   for EVOO. Parameters of the validation of the multielement analysis of soil after a microwave

222   digestion were obtained by the analysis of certified reference materials of soil NCS DC 77302

223   (China National Analysis Centre for Iron and Steel, Beijing, PR China), which was analysed

224   simultaneously with each group of samples. The recoveries for measured elements in soils

225   varied between 91-95 %, with coefficients of variation between 5 and 8 % (Fiket, Mikac &

226   Kniewald, 2017). Validation of the method used for multielement analysis of EVOO after

227   ultrasonic acid extraction was described in detail in Poščić et al. (2019). The recoveries of

228   measured elements in olive oils (obtained by analysis of pomace, as certified reference material

229   for olive oil is not available) varied in the range 70-108 %, with coefficient of variation of 1-20 %

230   (depending on the element and concentration level); the limit of detection (LOD) for trace

231   elements varied between 0.001 and 0.2 ng/g EVOO.

232

233   *2.3.4. Carbon isotope ratio analysis*

234   The carbon isotope ratio ($^{13}C/^{12}C$) analysis was performed using an isotopic ratio mass

235   spectrometer (IRMS) Delta V Plus (Thermo Fischer Scientific, Bremen, Germany) coupled to

236   Elemental Analyzer Flash EA^{TM}1112 Series (EA) using a dosing interface (Conflo III, Thermo

237   Fischer Scientific) and an autosampler (MAS 200 R, Thermo Fischer Scientific). Instruments

238   were controlled by the Isodat 3.0 software (Thermo Fischer Scientific). Chemicals used for filling

239   an EA combustion reactor for conversion of the sample to carbon dioxide were copper (II) oxide,

240   silvered cobaltous/cobaltic oxide and chromium (III) oxide (Thermo Fischer Scientific).

241   The samples were weighed (0.1 mg) in tin capsules and measured against the certified

242   reference material [NBS 22, International Atomic Energy Agency (IAEA), Vienna, Austria]. The

243   isotopic ratio of $^{13}C/^{12}C$ is expressed in the delta notation, $\delta^{13}C$, as part per thousand (‰) versus

244   V-PDB (Vienna-Peedee Belemnite). The repeatability of sample measurements was 1.5 %

245   expressed as relative standard deviation (RSD) of oil sample measured six times. The quality of

246   results was controlled by measurements of the matrix-matched reference material (oil sample

247   from interlaboratory comparison; Eurofins Analytics, Nantes, France), showing stable within-lab

248   reproducibility (0.13 ‰ or 0.5 % RSD).

249

250   *2.4. Data processing and statistics*

251   Because the data were not normally distributed (Shapiro-Wilk normality test), a Kruskal-

252   Wallis test was performed for the soil and EVOO samples to examine whether element

253   concentrations differ significantly among the geographical regions (at $\alpha = 0.05$). A post-hoc

254   comparison adjusted by the Benjamini-Hochberg method was then carried out to highlight the

255   region differences.

256   To determine a grouping of samples based on soil parent materials in a reduced

257   dimension space and to visualise elements with similar relationships in soil and EVOO samples,

258   we applied principal component analysis (PCA). To classify the samples according to their

259   origin, two supervised chemometrics approaches were used: the linear discriminant analysis

260   (LDA) and k-nearest neighbours (*k*-NN). Before the multivariate analyses, the data matrix was

261   standardised because the element concentrations were of different orders of magnitude. The

262   linear discriminant analysis is a linear classification tool based on generating a number of

263   orthogonal linear discriminant functions (LDs). The method maximises between-group variance

264   and minimises within-group variance. The k-nearest neighbour is one of the simplest classifiers

265   that does not use a distributional assumption (Granato et al., 2018). The method is based on

266   determining the distances between an unknown object and each of the objects in the training

267   set. Then, the smallest distance is selected for a group membership assignment. The optimal

268   number of the k neighbours was selected by a cross-validation procedure. Before performing

269   pattern recognition, we eliminated parameters that were non-informative regarding geographical

270   classification by applying forward stepwise LDA. A forward stepwise variable selection algorithm

271   uses a Wilks' lambda as the selection criterion and an F-statistics to determine the significance

272   of lambda changes when assessing the influence of a new variable (the threshold F-values

273   were 2 (to enter) and 0.5 (to remove)). Therefore, only variables with a high discriminatory

274   power were selected for modelling.

275   The results of a classification method applied to a dataset were evaluated to determine

276   its performance. For this purpose, a random split (using 10-fold cross-validation, repeated 100

277   times and averaged) into the training (70%) and test (30%) sets was required. The training

278   dataset was used to optimise the parameters required for each method, and the test dataset

279   was used to evaluate the performance of the model using the receiver operating characteristic

280   curves (ROC). The ROC curves are commonly used to evaluate the compromise between

281   sensitivity (samples belonging to the class and correctly classified in that class) and specificity

282   (samples not belonging to the modelled class and correctly classified as not belonging) as a

283   function of the probability threshold for class assignment (Oliveri, 2017). A ROC curve lying on

284   the diagonal line represents a random classifier, whereas a perfect classifier shows a vertical

285   rise with an inflection at the point [0,1]. The area under the ROC curve (AUC) was used to

286   evaluate the performance of the classifier, with an ideal classifier resulting in an AUC of 1.

287

288   **3. Results and discussion**

289   *3.1. Multielement and isotopic composition of soils and EVOO*

290   The results of the descriptive statistics of the chemical composition of EVOO were

291   presented in Table 1 and those of the soils in Table A2. Sodium was the only element in the soil

292   with statistically different concentrations ($p \leq 0.05$) in Istria and Kvarner from the other two

293   regions (Table A2). There were statistically significant differences in Co, Cr, Ni, Sr and Zn in the

294   comparison between Istria and Kvarner and Dalmatia. Cerium, Cs, La, Mg, Nd, S and Y

295   statistically differed in the Dalmatian islands compared to the other two regions. For Al, there

296   was a difference only between Dalmatia and the Dalmatian islands. Cadmium showed

297   differences among all regions studied. These elemental associations could be attributed to the

298   lithological diversity of the studied area and various mechanisms and sources such as

299   weathering, pedogenesis, erosion, anthropogenic input, etc. that cause their variable distribution

300   across the soils (Halamić, Peh, Miko, Galović & Šorša, 2012; Hasan et al., 2020).

301   Based on the median values, K was the most abundant element in the EVOO samples,

302   followed by S, Ca, P and Na (Table 1). Eight out of 30 parameters (Cd, Mn, Mo, P, S, Sn, V and

303    $\delta^{13}C$) revealed significant differences ($p \leq 0.05$) between the medians of at least one pair of

304   origins in EVOO samples. However, there were some elements that did not differ statistically but

305   showed high variability in the studied regions, such as Ca, K and Mg. Manganese, S and Sn

306   exhibited significant differences in the Dalmatian islands compared to the other two regions.

307   Dalmatia had statistically different concentrations of Cd and V compared to Istria and Kvarner,

308   and Mo compared to both other regions. For P there were significant differences between

309   Dalmatia and the Dalmatian islands.

310   The concentrations of most elements determined in Croatian EVOO (Table 1) are in the

311   lower range of concentrations determined in Mediterranean EVOO (Camin et al., 2010; Damak

312   et al., 2019; Nasr et al., 2022a,b; Poščić et al., 2019). The plant macronutrients Ca, Mg, K, P

313   and S and the beneficial element Na had the highest median concentrations, ranging from 11.3

314   μg/kg for Mg to 140 μg/kg for K, which is consistent with other data on the distribution of

315   elements in EVOO (Beltrán, Sánchez-Astudillo, Aparicio, & García-González, 2015; Nasr et al.,

316   2022a). Micronutrients such as Cu, Fe, Mn and Zn were present at lower concentrations (up to

317   1 μg/kg) than macronutrients, but higher than trace elements not involved in plant metabolism

318   (median concentrations mostly below 0.1 μg/kg). An exception is Al (the beneficial element for

319   some plant species), whose concentrations were similar to those of the micronutrients

320   (Bojórquez-Quintal, Escalante-Magaña, Echevarría-Machado & Martínez-Estévez, 2017). With

321   respect to the quality criteria for olive oil set by the International Olive Council (IOC, 2019) which

322   define the maximum permissible concentrations of the two metals in olive oil (Fe ≤ 3000 μg/kg

323   and Cu ≤ 100 μg/kg), all 123 EVOO samples analysed in this study had concentrations well

324   below these thresholds.

325   The median values of measured stable isotope $\delta^{13}C$ in EVOO were -30.02 ‰ in Istria

326   and Kvarner, -29.01 ‰ in Dalmatia and -27.84 ‰ in Dalmatian islands (Table 1), which is

327   consistent with the ranges of $\delta^{13}C$ values found in other Mediterranean olive oil-producing

328   countries such as Italy, Portugal, France, Turkey, Greece and Spain (Camin et al., 2010;

329   Jiménez-Morillo, Palma, Garcia, Dias & Cabrita, 2020; Nasr et al., 2022b; Portarena,

330   Gavrichkova, Lauteri & Brugnoli, 2014). The value of $\delta^{13}C$ (-28.6 ±0.6) for Croatian olive oils in

331   the research of Chiavaro et al. (2011) is also within the range obtained in this study. Statistical

332   evaluation showed the significant difference (Table 1) among $\delta^{13}C$ values in EVOOs originating

333 from the three investigated Croatian regions. This was probably influenced by specific local

334 climatic parameters (temperatures and precipitation levels) affecting the olive trees and

335 consequently the $\delta^{13}C$ in EVOO (Chiocchini, Portarena, Ciolfi, Brugnoli & Lauteri, 2016).

336

337 *3.2. Grouping of elements according to lithology and link between soil and EVOO*

338   To gain insight into the provenance of the elements in the soil and EVOO samples, we

339 performed principal component (PCA) and linear discriminant (LDA) analyses. Single

340 correlations were tested to investigate the relationship between the elements in EVOO and the

341 corresponding soil samples. We focused here on the two main parent materials (carbonate

342 rocks or flysch deposits) that produced contrasting geochemical signatures in soils.

343   The results of PCA are presented as plots of the first two principal components for soil

344 (Fig. 2A) and EVOO samples (Fig. 2B). For soils, the first principal component (PC1) accounted

345 for ~50% of the total variability and was characterised by strong negative loadings of Sr and Ca

346 and positive loadings of Al, Fe, V, Ti, rare earth elements (La, Nd, Ce and Y), etc. This shows

347 the dissimilarity between soils rich in carbonate minerals developed on flysch deposits and

348 those rich in aluminosilicates formed from the insoluble residues of carbonate rocks and the

349 influence of various external materials, including aeolian dust, volcanic debris and clastic

350 sedimentary particles (Durn, Perković, Stummeyer, Ottner & Mileusnić, 2021; Hasan et al.,

351 2020).

352   The PC2 axis contained 11.8% of the total variability and was represented by negative

353 scores corresponding to high concentrations of Cd and S and with positive scores for high

354 concentrations of Cr and Ni. The latter probably reflects the sources of flysch deposits from

355 mafic and ultramafic rocks of the Dinarides, whereas the enrichment in Cd and S is mainly

356 related to their natural accumulation in soils and binding to Fe hydroxides, although Cd in soils

357 may also be due to anthropogenic sources (Halamić, Peh, Miko, Galović & Šorša, 2012; Hasan

358 et al., 2020).

14

359   When considering EVOO samples, the two PCs explained ~48% of the variability of the

360   system. The PC1 (~33%) showed positive loadings for the majority of the elements (Figure 2B).

361   The PC2 accounted for ~15.5% of the total variability; Rb, K, P, Mn and Cs were the dominant

362   variables in the positive part of the axis, whereas rare earth elements (REEs) were the most

363   important variables in the negative PC2 axis. The elements along the positive part of the axis

364   can be considered nutrients (with the exception of Cs), which means that plants have evolved

365   specific mechanisms to take them up (Rengel & Marschner, 2005; Tyler & Olsson, 2001).

366   Because PCA could not separate the sample groups based on the two main soil parent

367   materials (carbonate rocks and flysch deposits), we performed linear discriminant analysis

368   (LDA). The linear discriminant function (LD) for soils and olive oils explained 100% of the total

369   variance (Fig. A1), and the significance of the variables was given in Table A3. The area under

370   the receiver-operating characteristic curve (AUC) was calculated by the cross-validation

371   procedure to evaluate the classification performance. In the soil samples, the AUC value was

372   very high at over 0.98, whereas in the EVOO samples it was lower at 0.74, but still satisfactory.

373   This shows that it is possible to distinguish soil and EVOO samples according to two main

374   parent materials, which is a good starting point for traceability purposes.

375   Given that most soil samples were correctly classified into the two main parent materials

376   by LDA, we tested the correlations of elements between soil and EVOO samples in the studied

377   regions based on these two parent materials (carbonate rocks and flysch deposits) that

378   influence soil geochemistry (Table 2). In the first group (soils formed on carbonate rocks), the

379   region of Istria and Kvarner was characterised by a significant positive correlation between soil

380   and EVOO samples for Rb (0.67, $p \leq 0.01$), Ce (0.66, $p \leq 0.01$), Cs (0.65, $p \leq 0.01$), Nd (0.63, p

381   ≤ 0.01), La (0.58, $p \leq 0.05$), Ba (0.49, $p \leq 0.05$) and Al (0.43, $p \leq 0.05$), whereas in Dalmatia a

382   significant positive correlation was detected for Rb (0.48, $p \leq 0.01$), Mn (0.41, $p \leq 0.05$) and Zn

383   (0.40, $p \leq 0.05$). Rubidium and Cs have similar geochemical behaviour in soils and are mainly

384   associated with the pedogenic clay minerals and organic matter. There are numerous

385 observations of their high concentrations in reddish Mediterranean soils (Navarro, Ortiz-Villajos,

386 Jimenez & Ballesta, 2011; Vingiani, Di Iorio, Colombo & Terribile, 2018) and evidence of their

387 availability for uptake by plants (Nasr et al., 2022a; Tyler & Olsson, 2001). For REEs (La, Ce,

388 Nd), most of their correlations can be explained by their accumulation in reducible

389 (ferromanganese oxides) and oxidisable (organic matter and sulfides) fractions of Istrian soils

390 (Durn, Perković, Stummeyer, Ottner & Mileusnić, 2021). The changes in redox conditions and

391 soil acidity could influence strongly the availability of REEs and concentrations in leaves and

392 pomace (Poščić et al., 2020). Although there are no clear correlations, similar results and

393 matches of REEs in soil and EVOO samples were also observed by Aceto, Calà, Musso, Regalli

394 & Oddone (2019). Manganese and Zn are two essential micronutrients for plant growth. It is not

395 uncommon in soils developed on carbonate lithology that large proportions of these two

396 elements are in the exchangeable and reducible fractions (Durn, Perković, Stummeyer, Ottner &

397 Mileusnić, 2021) that are available for plant uptake. Nasr et al. (2022a) also found a positive

398 correlation of Mn between the available soil fraction and Tunisian EVOO. For Dalmatian islands,

399 no significant correlation between soil and EVOO samples was found for any element.

400   The second group (soils developed on flysch deposits) showed a significant positive

401 correlation for Rb (0.62, $p \leq 0.05$) and Mn (0.51, $p \leq 0.05$) in Istria and Kvarner. In Dalmatia

402 there was a significant positive correlation for Ca (0.53, $p \leq 0.01$), Mg (0.46, $p \leq 0.01$), Cu (0.42,

403 $p \leq 0.05$) and Sr (0.39, $p \leq 0.05$). This is interesting because Ca, Mg and Sr are the main

404 components of the carbonate minerals that are abundant in the rendzinas soils on the flysch

405 deposits, typical of the Dalmatian region. Rendzinas, as poorly developed soils on flysch

406 material represented by marls and weakly consolidated calcareous sandstones with low

407 accumulation of aluminosilicates and elevated levels of carbonate minerals, develop due to poor

408 soil drainage and leaching (Hasan et al., 2020). In such substrates, elements such as Ca, Sr

409 and Mg can easily be extracted by plants. For the Dalmatian islands, a significant positive

410 correlation was found only for Fe (0.83, $p \leq 0.05$) as an essential micronutrient. Elements that

16

411 have not retained the elemental signature of the parent soil in EVOO are influenced by the

412 complex soil-plant interactions and various agricultural and climatic factors of the area studied,

413 but also by the processes of olive oil production, refining and storage (Camin et al., 2010; Nasr

414 et al., 2022b). These results indicate the importance of soil geochemistry in the transfer of

415 elements into EVOO, which confirms our hypothesis and could be a basis for the geographical

416 traceability of EVOO in the Croatian coastal area.

417

418 *3.3. Geographical authentication of EVOO using classification models*

419 To confirm whether we can determine the geographical origin of the soil and EVOO

420 samples, we first performed a linear discriminant analysis (Fig. 3), considering three

421 geographical regions. The two linear discriminant functions (LDs) for both types of samples

422 explained 100% of the total variance, and good separation of the three groups was observed

423 (Fig. 3; Table A3). The classification procedure and the construction of the models for

424 authenticating EVOO according to its geographical origin were carried out using two different

425 supervised classification techniques, namely linear discriminant analysis (LDA) and k-nearest

426 neighbour analysis (*k*-NN). It is advantageous to use both models, mainly to ensure better

427 accuracy, but also because *k*-NN makes no assumptions about the data distribution, and it is

428 good for systems with a small number of samples (Granato et al., 2018).

429 Multicollinearity between variables and geographically unrelated variability is a source of

430 noise in the data, so reducing variables to the most predictive ones is always desirable. To this

431 end, we first performed a stepwise linear discriminant analysis (S-LDA) before applying the

432 classifiers. Thus, only 10 variables (S, Mo, Rb, Mg, Pb, Mn, Sn, K, V and δ^{13}C) were retained

433 through the stepwise procedure and used as input for further classification (Table A4). These 10

434 variables agree very well with the variables selected by the multiple group comparison using the

435 Kruskal-Wallis test (Table 1) and with the variables showing the highest discriminatory power in

436 LDA (Table A3). Furthermore, the classifiers such as the k-nearest neighbour (*k*-NN) required

17

437   the adjustment of additional input parameters throughout the 10-fold cross-validation technique,

438   namely the number of neighbours k.

439   Model performance was assessed using receiver-operating characteristic curves (ROC)

440   (Oliveri, 2017; Bontempo et al., 2019). The ROC curves were plotted by featuring the 1-

441   specificity as the horizontal axis and sensitivity as the vertical axis at different thresholds. The

442   chosen trade-off between sensitivity and specificity and the area under the curve (AUC) as a

443   measure of the model performance are presented in Fig. 4 and Table A5. The models were

444   applied to selected elements and to combination of elements and δ^{13}C isotopic ratio. The LDA

445   generally gave higher averaged AUC values than the *k*-NN, and the best possible trade-off

446   between sensitivity and specificity was better for the LDA than for the *k*-NN. This means that the

447   LDA classifier detected a larger number of samples belonging to the target class, i.e. provided

448   higher sensitivity, which was our goal. When considering multielement composition, both

449   classifiers obtained the highest AUC for Dalmatian islands (0.888 for LDA and 0.872 for *k*-NN),

450   followed by Istria and Kvarner (0.875 for LDA and 0.839 for *k*-NN) and Dalmatia (0.811 for LDA

451   and 0.761 for *k*-NN). The LDA provided the highest sensitivity for Dalmatian islands (88.8%),

452   whereas *k*-NN provided the highest sensitivity for Istria and Kvarner (75.0%).

453   The addition of isotopic composition (δ^{13}C) to the multielemental composition

454   significantly improved the classification results. The highest AUC value of 0.973 and sensitivity

455   of 100% using the LDA classifier were obtained for the Istria and Kvarner region. The *k*-NN

456   classifier also achieved the highest AUC value of 0.933 for Istria and Kvarner, but the highest

457   sensitivity was for Dalmatian islands (88.8%). These results suggest that isotopic composition is

458   an important variable for the traceability of Croatian olive oils, as it reflects the climatic

459   conditions and geographical characteristics of the area where the olives are grown (Camin et

460   al., 2010; Gumus, Celenk, Tekin, Yurdakul & Ertas, 2017). Therefore, the elements analysed

461   and the δ^{13}C isotope composition have demonstrated their suitability for classification of olive

462　oils from the Croatian Adriatic coast according to their geographical origin, with the determining

463　factors being nine chemical elements (S, Mo, Rb, Mg, Pb, Mn, Sn, K and V) and δ^{13}C.

464

465　**4. Conclusions**

466　　　We analysed the multielement and δ^{13}C isotope composition in EVOO and the

467　geochemistry of the associated soils from three regions along the Croatian Adriatic coast. The

468　geographical traceability of EVOO was achieved by linking the multielement composition to the

469　provenance area with characteristic geochemical signatures of the soil. Rubidium and Mn were

470　the only elements that showed a direct link between soil and EVOO on both types of parent

471　materials, while Al, Ba, Cs, Mn and REEs were positively correlated to aluminosilicate-rich

472　provenance and Ca, Mg, Sr and Cu to carbonate-rich provenance. Chemometric approaches

473　(LDA and k-NN) with high sensitivity and specificity demonstrated the potential of multielement

474　and δ^{13}C isotope composition for the correct classification of EVOO, providing a reliable and

475　robust basis for the authentication of its geographical origin.

476

477　**CRediT authorship contribution statement**

478　**Mavro Lučić**: Methodology, Formal analysis, Writing – original draft, Writing - Review & Editing,

479　Visualization. **Maja Jukić Špika**: Methodology, Investigation, Writing – original draft, Writing -

480　Review & Editing. **Nevenka Mikac**: Methodology, Writing – original draft, Writing - Review &

481　Editing. **Filip Pošćić**: Methodology, Investigation, Writing - Review & Editing. **Zed Rengel**:

482　Conceptualization, Writing - Review & Editing, Supervision. **Marija Romić**: Investigation, Writing

483　- Review & Editing. **Helena Bakić Begić**: Investigation. **Željka Fiket**: Writing - Review & Editing.

484　**Martina Furdek-Turk**: Investigation. **Niko Bačić**: Investigation. **Renata Leder**: Formal analysis,

485　Investigation, Writing – review & editing. **Ivana Vladimira Petric**: Investigation, Writing –

486　original draft. **Branimir Urlić**: Formal analysis, Investigation. **Marko Runjić**: Formal analysis,

487 Investigation. **Gabriela Vuletin Selak**: Investigation. **Elda Vitanović**: Investigation. **Tatjana**

488 **Klepo**: Investigation. Methodology. **Jakša Rošin**: Investigation. **Slavko Perica**:

489 Conceptualization, Supervision, Project administration, Funding acquisition.

490

Declaration of Competing Interest

492 The authors declare that they have no known competing financial interests or personal

493 relationships that could have appeared to influence the work reported in this paper.

494

Data availability

496 Data will be made available upon reasonable request.

497

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505

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647   *81(*2), 171–176. https://doi.org/10.1016/j.microc.2004.12.002.

648 **Table 1** Basic statistics (minimum, median and maximum) for the multielement compositions
649 and $^{13}C/^{12}C$ isotope ratio of extra virgin olive oil (EVOO) samples from the three geographical
650 regions of the Croatian Adriatic coast. The non-parametric Kruskal-Wallis test was used for the
651 region comparison: non-significant (ns); $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$. For pairwise
652 comparisons, Dunn's multiple comparison adjusted by the Benjamini-Hochberg method was
653 used. Median values with different letters in the same row are significantly different ($p \leq 0.05$).

| Element (µg/kg)	Istria and Kvarner			Dalmatia			Dalmatian islands			
	min	median	max	min	median	max	min	median	max	p-value
Al	<0.2	**0.33 a**	1.45	<0.2	**0.36 a**	2.83	<0.2	**<0.2 a**	6.41	ns
Ba	<0.01	**0.013 a**	0.205	<0.01	**0.022 a**	0.287	<0.01	**0.01 a**	0.211	ns
Ca	<5	**10.2 a**	211	<5	**18.2 a**	145	<5	**13.1 a**	153	ns
Cd	<0.001	**0.001 b**	0.011	<0.001	**0.001 a**	0.013	<0.001	**0.001 ab**	0.004	*
Ce	<0.001	**0.001 a**	0.037	<0.001	**0.001 a**	0.033	<0.001	**0.001 a**	0.021	ns
Co	<0.005	**<0.005 a**	0.075	<0.005	**<0.005 a**	0.01	<0.005	**<0.005 a**	0.061	ns
Cr	<0.001	**<0.001 a**	0.004	<0.001	**<0.001 a**	0.004	<0.001	**<0.001 a**	0.001	ns
Cs	<0.001	**<0.001 a**	0.002	<0.001	**<0.001 a**	0.003	<0.001	**<0.001 a**	0.005	ns
Cu	0.09	**0.236 a**	1.98	<0.05	**0.247 a**	2.4	<0.05	**0.191 a**	0.725	ns
Fe	<0.1	**0.50 a**	2.57	<0.1	**0.47 a**	3.72	<0.1	**0.32 a**	4.35	ns
K	3.1	**140 a**	1666	<2	**123 a**	1940	<2	**81.4 a**	243	ns
La	<0.001	**<0.001 a**	0.024	<0.001	**0.001 a**	0.013	<0.001	**<0.001 a**	0.007	ns
Mg	<2	**6.89 a**	99.2	<2	**9.66 a**	90.5	<2	**11.3 a**	77.2	ns
Mn	0.016	**0.102 a**	7.18	<0.01	**0.150**	4.252	<0.01	**0.06 b**	0.312	**
Mo	<0.003	**<0.002 b**	0.009	<0.003	**0.004 a**	0.021	<0.003	**0.003 b**	0.008	**
Na	<1	**5.43 a**	12.5	<1	**5.10 a**	27	<1	**4.22 a**	26.1	ns
Nd	<0.001	**<0.001 a**	0.011	<0.001	**0.001 a**	0.012	<0.001	**0.001 a**	0.006	ns
Ni	<0.05	**0.059 a**	1	<0.05	**0.054 a**	0.675	<0.05	**<0.03 a**	0.23	ns
P	1.03	**15.3 ab**	367	<1	**14.3 a**	340	<1	**7.80 b**	31.9	*
Pb	<0.01	**0.01 a**	0.229	<0.01	**0.02 a**	0.426	<0.01	**0.023 a**	0.215	ns
Rb	<0.009	**0.098 a**	1.81	<0.009	**0.075 a**	1.34	<0.009	**0.055 a**	0.183	ns
S	17.1	**75.9 a**	151	7.9	**102 a**	399	18	**44.2 b**	80.0	***
Sb	<0.002	**0.002 a**	0.005	<0.002	**0.003 a**	0.007	<0.002	**0.003 a**	0.005	ns
Sn	<0.02	**0.037 a**	0.148	<0.02	**0.032 a**	0.111	<0.02	**0.01 b**	0.066	**
Sr	<0.01	**0.013 a**	0.168	<0.01	**0.022 a**	0.163	<0.01	**<0.01 a**	0.311	ns
Ti	<0.01	**<0.01 a**	0.043	<0.01	**<0.01 a**	0.107	<0.01	**0.007 a**	0.104	ns
V	<0.002	**0.002 b**	0.005	<0.002	**0.002 a**	0.011	<0.002	**<0.002 ab**	0.015	*
Y	<0.001	**<0.001 a**	0.009	<0.001	**<0.001 a**	0.009	<0.001	**<0.001 a**	0.004	ns
Zn	<0.2	**0.588 a**	5.61	<0.2	**0.978 a**	4.08	<0.2	**0.421 a**	3.94	ns
δ^{13}C (‰)	-31.46	**-30.02 c**	-28.48	-30.26	**-29.01 a**	-27.20	-30.37	**-27.84 b**	-26.92	***

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656 **Table 2** Spearman's correlation coefficient between the concentrations of elements in paired
657 soil and extra virgin olive oil (EVOO) samples from the three geographical regions on the
658 Croatian Adriatic coast within the two soil parent materials. The number of samples is given in
659 parentheses. Significant positive correlations at $*p \le 0.05$, $**p \le 0.01$ and $***p \le 0.001$ were
660 indicated in bold.

	Soils developed on carbonate rocks			Soils developed on flysch deposits		
	Istria and Kvarner (n = 17)	Dalmatia (n = 32)	Dalmatian islands (n = 24)	Istria and Kvarner (n = 12)	Dalmatia (n = 32)	Dalmatian islands (n = 6)
Al	**0.43***	0.05	0.05	-0.09	-0.11	0.54
Ba	**0.49***	-0.15	0.03	-0.15	-0.06	0.00
Ca	-0.30	0.15	-0.34	-0.02	**0.53****	-0.28
Cd	-0.76***	-0.25	-0.02	0.11	-0.37*	-0.44
Ce	**0.66****	-0.33*	0.18	-0.32	-0.32*	-0.14
Co	-0.09	0.17	0.23	-0.10	0.08	-0.12
Cr	-0.13	0.14	0.21	0.15	-0.03	0.65
Cs	**0.65****	0.16	0.19	0.50	-0.35*	0.00
Cu	-0.40	0.07	-0.04	-0.37	**0.42***	0.02
Fe	0.38	0.22	0.00	0.19	-0.21	**0.83***
K	-0.06	0.13	0.02	-0.43	-0.05	-0.37
La	**0.58***	-0.05	-0.12	-0.25	-0.50**	0.13
Mg	-0.39	-0.33*	0.00	0.00	**0.46****	0.08
Mn	-0.34	**0.41***	0.26	**0.51***	0.03	-0.14
Mo	0.09	0.28	-0.14	-0.24	-0.59***	-0.94**
Na	-0.21	0.10	0.14	-0.34	0.00	-0.03
Nd	**0.63****	-0.15	0.09	-0.34	-0.51**	-0.27
Ni	0.29	-0.44*	-0.05	-0.09	0.14	-0.54
P	-0.36	0.28	0.16	-0.64*	0.13	-0.02
Pb	-0.22	-0.39*	0.11	-0.05	-0.02	-0.37
Rb	**0.67****	**0.48****	0.10	**0.62***	-0.08	-0.20
S	-0.24	0.22	0.17	0.49	0.05	-0.77
Sb	-0.33	-0.10	0.09	-0.31	-0.41*	-0.29
Sn	0.03	-0.26	-0.05	-0.14	-0.01	0.67
Sr	0.00	-0.38*	0.20	0.41	**0.39***	-0.85*
Ti	-0.12	-0.43*	-0.01	-0.09	-0.16	-0.03
V	0.34	-0.31*	0.16	-0.19	0.09	0.54
Y	0.31	-0.14	-0.12	0.19	-0.44*	-0.41
Zn	-0.36	**0.40***	-0.40*	-0.07	-0.14	-0.75*

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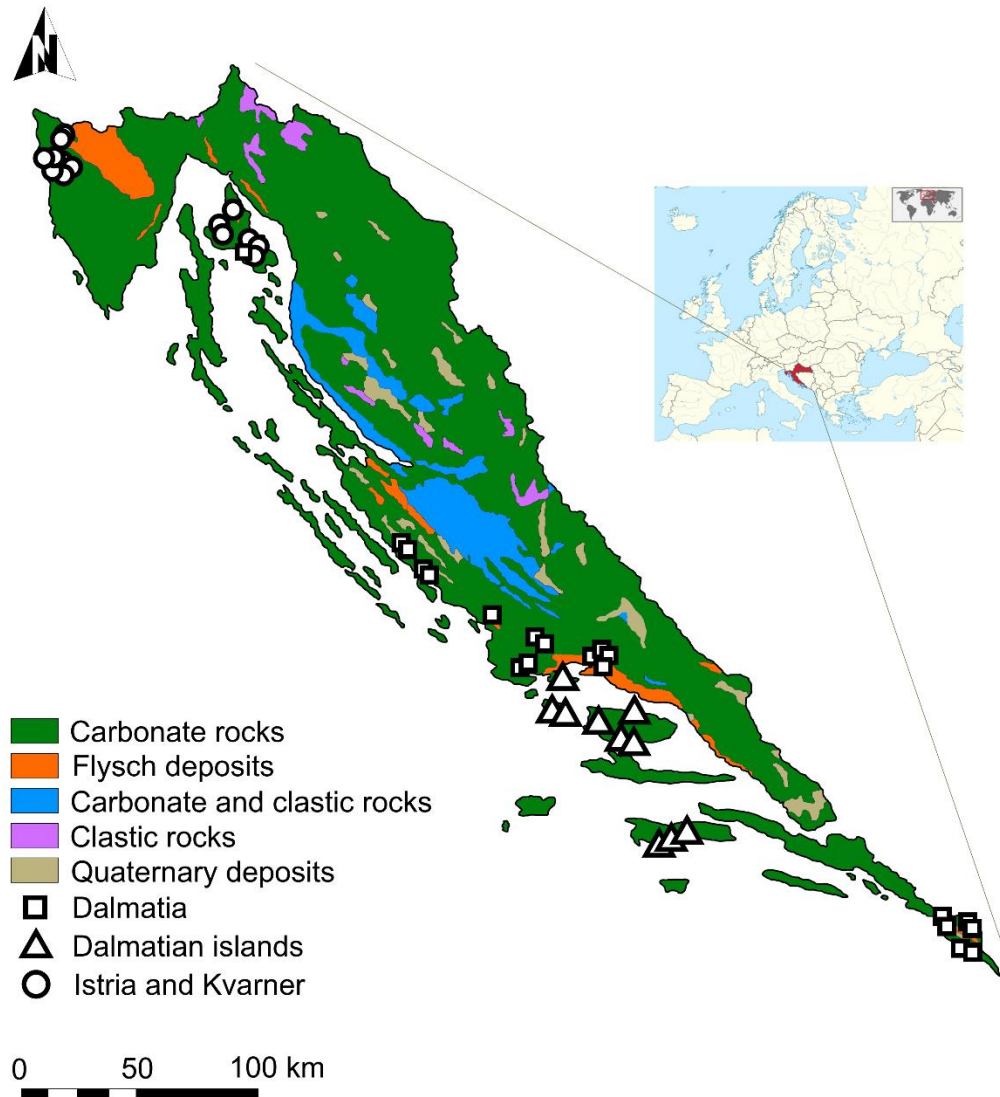
Fig. 1. Simplified lithological map of the study area. The locations of the sampled olive groves within three geographical regions were also shown.
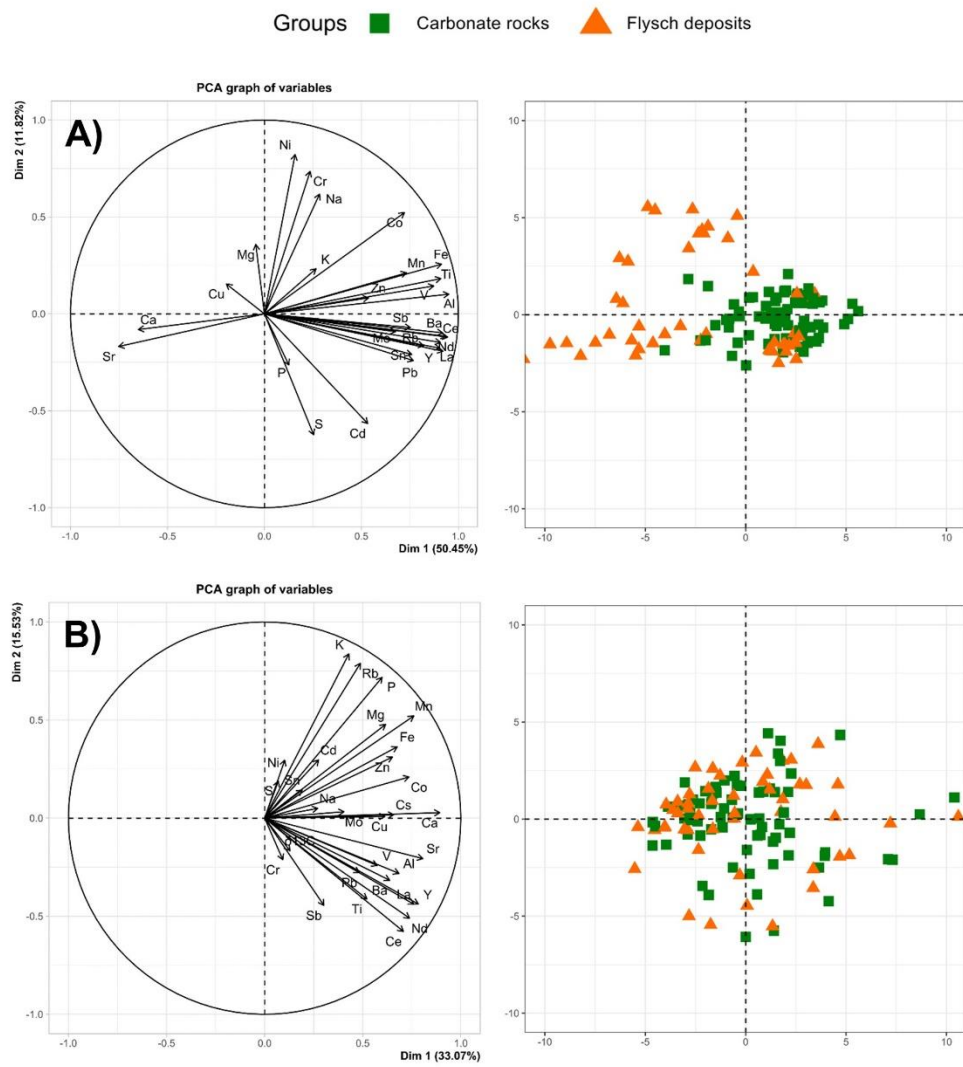
Fig. 2. Plots of the first principal component (PC1) versus the second principal component (PC2) for the (A) soil and (B) EVOO samples based on the main soil parent materials.
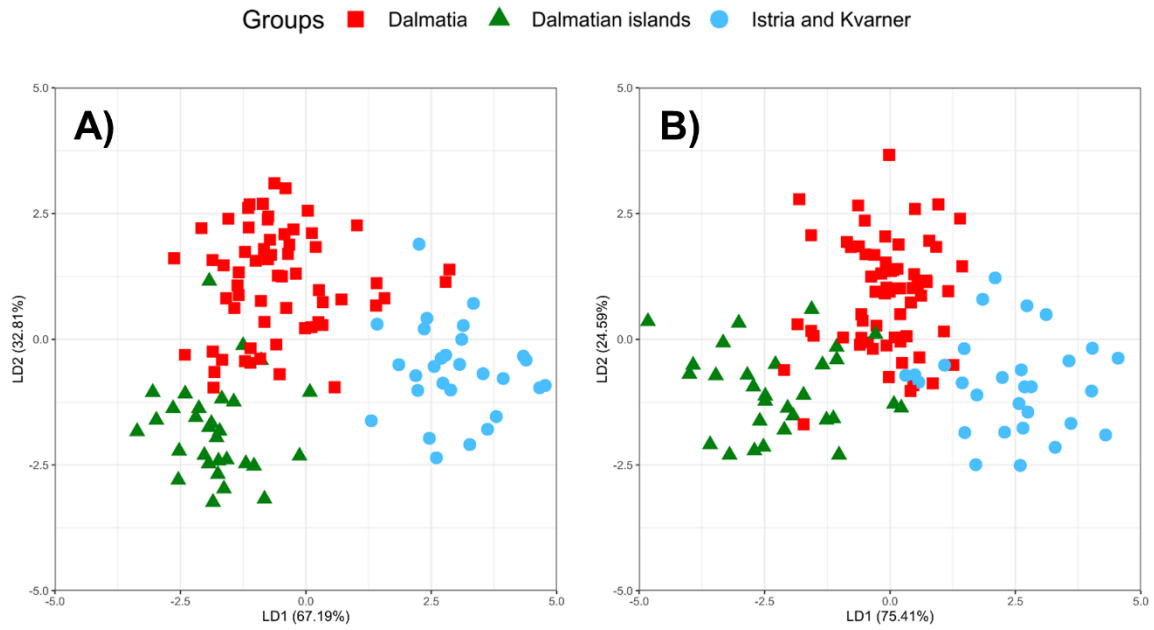
Fig. 3. Linear discriminant analysis of soil (A) and EVOO samples (B) according to their geographical origin.
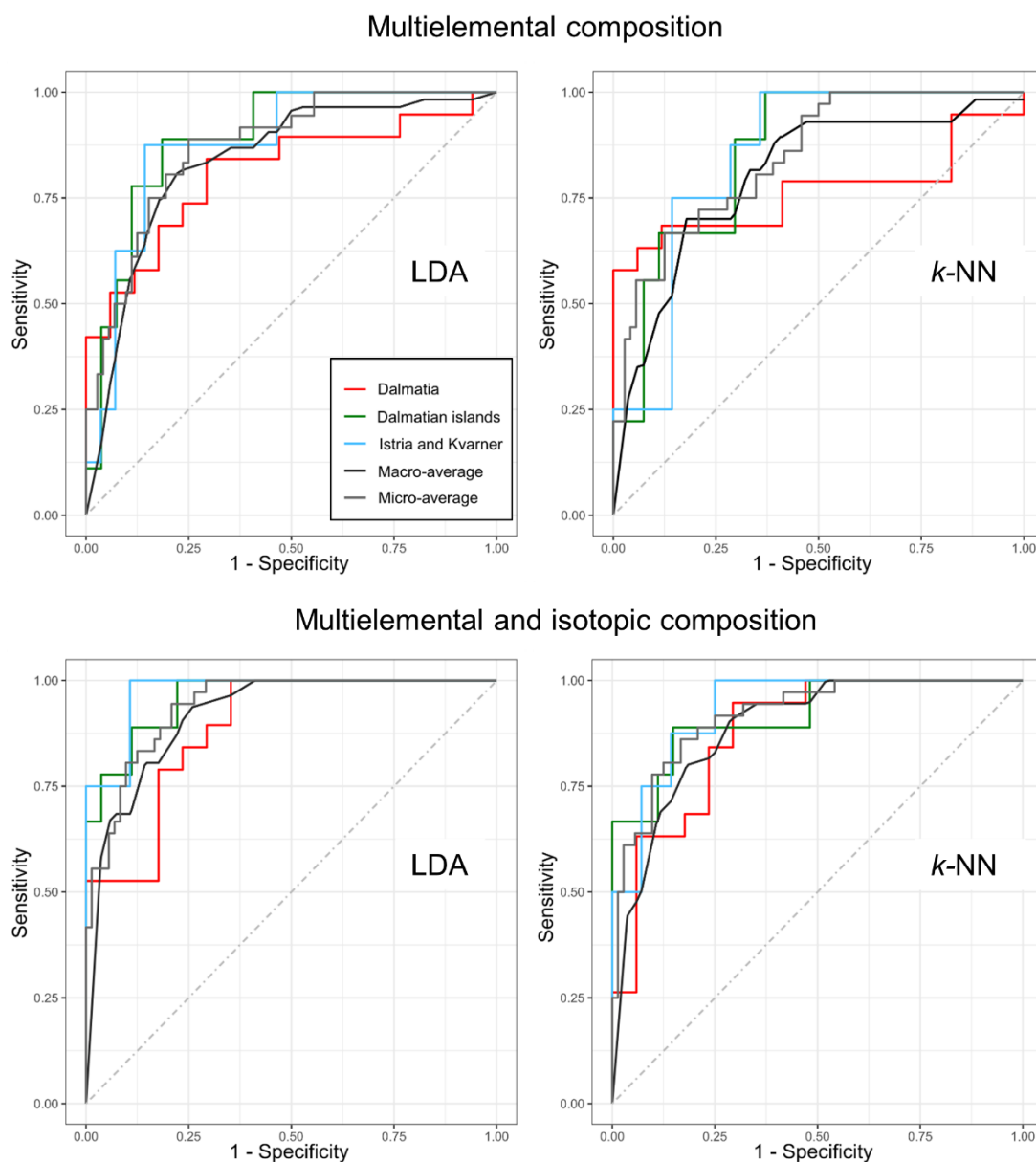
Fig. 4. Receiver-operating characteristic (ROC) curves of the extra virgin olive oil (EVOO) obtained with two classifiers: LDA – Linear discriminant analysis and *k*-NN – k-nearest neighbour. The multielement composition consists of the elements S, Mo, Rb, Mg, Pb, Mn, Sn, K and V without and with the isotopic composition $\delta^{13}C$. The area under the ROC curve (AUC) was used to validate the predictive reliability of the models. The macro-averaged AUC was calculated separately for each class and then averaged. The micro-averaged AUC represents the summed contributions of all classes to calculate the average metric and is preferable in case of imbalance between classes.

Table A1 Number of sampled olive groves and collected soil and extra virgin olive oil (EVOO) samples (total number and the split between the two prevailing bedrocks and among the three soil types) in the three geographical regions of the Croatian Adriatic coast.

Geographical region	Olive groves	Samples	Prevailing bedrock[†]		Type of soil[‡]		
			C	F	TR	RK	RED
Istria and Kvarner	10	29	17	12	11	6	12
Dalmatia	18	64	32	32	19	25	20
Dalmatian islands	10	30	24	6	9	18	3

[†] C = Carbonate rocks, F = Flysch deposits
[‡] TR = terra rossa, RED = rendzina; RK = reclaimed karst

Table A2 Basic statistics (minimum, median and maximum) for the total element concentrations in soil samples from the three geographical regions of the Croatian Adriatic coast. The non-parametric Kruskal-Wallis test was used for the group comparison: not significant (ns); * $p <$ 0.05; ** $p <$ 0.01; *** $p <$ 0.001. For pairwise comparisons, Dunn's multiple comparison adjusted by the Benjamini-Hochberg method was used. Values with different letters in the same row are significantly different ($p \leq 0.05$).

Element (unit)	Istria and Kvarner			Dalmatia			Dalmatian islands			p-value
	min	median	max	min	median	max	min	median	max	
Al (g/kg)	40.5	**70.9 ab**	106	16.9	**68.2 a**	102	34.0	**77.5 b**	101	*
Ba (mg/kg)	178	**285 b**	423	103	**326 ab**	454	143	**351 a**	492	*
Ca (g/kg)	4.80	**25.6 a**	258	1.60	**48.1 a**	267	3.20	**10.4 a**	259	ns
Cd (mg/kg)	0.218	**0.365 c**	1.30	0.172	**0.731 a**	2.71	0.278	**0.970 b**	2.80	***
Ce (mg/kg)	32.6	**74 a**	151	17.4	**88.2 a**	171	31.0	**107 b**	144	**
Co (mg/kg)	13.7	**22.4 b**	36.0	7.42	**18.0 a**	36.8	13.3	**20.3 ab**	27.8	***
Cr (mg/kg)	82.2	**171 b**	240	66.2	**142 a**	431	112	**142 ab**	258	*
Cs (mg/kg)	4.15	**7.54 a**	13.3	2.54	**8.31 a**	16.3	4.55	**11.5 b**	13.8	***
Cu (mg/kg)	26.3	**50.0 a**	222	23.8	**58.7 a**	253	23.8	**64.5 a**	110	ns
Fe (g/kg)	24.6	**43.8 b**	52.7	12.7	**35.8 a**	55.0	25.3	**42.3 b**	57.3	**
K (g/kg)	8.95	**14.8 b**	40.5	5.46	**12.1 a**	44.5	9.18	**14.3 b**	36.0	***
La (mg/kg)	16.5	**34.2 a**	67.9	9.65	**42.5 a**	80.6	15.2	**51.2 b**	70.1	***
Mg (g/kg)	2.82	**5.53 a**	8.05	3.20	**5.11 a**	47.4	4.08	**6.57 b**	21.9	***
Mn (mg/kg)	659	**1132 a**	1909	279	**1046 a**	2049	750	**1239 a**	1798	ns
Mo (mg/kg)	0.353	**1.63 a**	12.6	0.493	**1.85 a**	11.4	0.531	**1.50 a**	3.54	ns
Na (g/kg)	2.03	**3.24 b**	9.61	0.865	**2.80 a**	5.80	1.92	**2.86 a**	4.71	**
Nd (mg/kg)	15.5	**35.8 a**	58.7	8.45	**38.5 a**	71.2	15.8	**43.9 b**	58.9	*
Ni (mg/kg)	48.8	**96.7 b**	152	43.2	**64.1 a**	201	42.8	**79.8 ab**	162	***
P (g/kg)	0.436	**0.697 a**	1.88	0.392	**0.701 a**	5.79	0.350	**0.960 a**	1.36	ns
Pb (mg/kg)	13.0	**40.3 a**	117	13.1	**40.7 a**	168	20.5	**47.8 a**	59.2	ns
Rb (mg/kg)	51.0	**85.3 b**	137	33.8	**103 ab**	183	66.6	**120 a**	171	**
S (g/kg)	0.140	**0.268 a**	0.845	0.202	**0.396 a**	1.08	0.192	**0.462 b**	1.02	**
Sb (mg/kg)	0.575	**1.80 a**	2.95	0.408	**1.37 a**	9.98	0.658	**1.54 a**	2.32	ns
Sn (mg/kg)	1.70	**4.14 a**	6.11	1.65	**4.44 a**	9.08	1.81	**4.27 a**	8.48	ns
Sr (mg/kg)	57.6	**78.8 b**	281	60.2	**96.9 a**	384	63.5	**82.2 ab**	239	*
Ti (mg/kg)	2678	**5208 a**	7263	1345	**4524 a**	7594	2254	**4895 a**	7172	ns
V (mg/kg)	95.1	**160 a**	239	50.3	**134 a**	302	81.8	**144 a**	206	ns
Y (mg/kg)	12.9	**24.1 a**	33.6	8.68	**26.7 a**	44.9	12.6	**29.4 b**	41.7	**
Zn (mg/kg)	83.6	**114 b**	180.1	41.8	**93.0 a**	166	67.3	**103 ab**	189	***

Table A3 Coefficients of linear discriminants for element concentrations in soil and extra virgin olive oil (EVOO) samples based on the two main soil parent materials and the three geographical regions of the Croatian Adriatic coast.

| | Soil parent materials | | Geographical regions | | | |
| | Soil | EVOO | Soil | | EVOO | |
	LD1	LD1	LD1	LD2	LD1	LD2
Al	2.22	0.05	1.51	-0.09	0.13	-0.51
Ba	-0.07	-0.16	-0.78	2.38	0.04	0.07
Ca	1.23	-0.02	0.31	-0.59	-0.53	0.17
Cd	0.48	-0.18	-1.28	-1.07	-0.03	0.09
Ce	-1.99	-0.28	-3.18	-0.10	-0.8	1.38
Co	-1.50	0.46	0.22	1.68	0.18	0.10
Cr	0.54	-0.11	-0.84	1.04	0.10	-0.30
Cs	-0.71	-0.17	-2.78	-1.83	-0.53	-0.68
Cu	-0.07	0.03	-0.03	0.13	0.11	-0.18
Fe	-0.91	0.47	1.03	-2.54	0.05	0.05
K	0.02	0.01	-0.24	-0.22	-0.61	-0.04
La	2.30	-0.56	-0.47	-3.91	0.10	-1.50
Mg	-0.65	-0.70	-0.25	-0.21	-0.74	-0.35
Mn	1.82	-1.08	-0.11	-0.68	0.84	-1.00
Mo	-0.09	0.05	-0.77	0.15	-0.28	0.58
Na	0.38	-0.43	0.24	-1.00	0.09	0.06
Nd	-3.03	0.98	3.63	1.42	0.34	-0.05
Ni	0.02	0.12	-0.73	-0.72	0.08	-0.17
P	0.20	1.11	0.10	0.27	0.12	1.30
Pb	0.61	0.29	0.36	0.71	-0.10	0.38
Rb	1.35	-0.43	-0.04	1.20	0.86	0.14
S	-0.87	0.79	0.04	0.48	-0.06	0.93
Sb	0.01	-0.16	-0.32	0.23	-0.10	-0.11
Sn	-0.10	0.28	0.24	-0.57	0.27	-0.08
Sr	0.28	0.45	-0.27	0.56	0.60	0.56
Ti	0.38	-0.10	0.38	-1.09	0.01	-0.07
V	0.45	-0.28	1.75	2.20	-0.63	0.07
Y	-0.80	-0.06	-0.39	2.14	0.42	0.03
Zn	-0.71	0.05	0.73	-0.93	-0.04	0.30
δ¹³C		-0.49			-1.01	0.08

Table A4 The most discriminating variables selected by applying a stepwise linear discriminant analysis using a Wilks' lambda as the selection criterion and an F-statistics to determine the significance of changes in lambda when assessing the influence of a new variable (the threshold F-values were 2 (to enter) and 0.5 (to remove)).

	Wilks' λ	F-value	p-value
Δ^{13}C/^{12}C	0.445	37.0	0.0000
S	0.341	15.5	0.0000
Mo	0.313	9.74	0.0001
Rb	0.297	6.39	0.002
Mg	0.299	6.91	0.0015
Pb	0.288	4.60	0.012
Mn	0.290	4.89	0.0093
Sn	0.286	3.84	0.025
K	0.284	3.83	0.025
V	0.256	3.17	0.046

Table A5 The discrimination results of the two classification models for the test sets: linear discriminant analysis (LDA) and k-nearest neighbour analysis (*k*-NN) using multielemental composition of EVOO without or with δ^{13}C. Sensitivity (Sens) and specificity (Spec) are expressed as percentages. The values of micro- and macro-average area under the curve (AUC; with 95% bootstrap confidence intervals) are measures of model performance.

Geographical region	Multielemental composition						Multielemental and isotopic composition					
	LDA			**_k_-NN** (k=5)[a]			**LDA**			**_k_-NN** (k=7)[a]		
	Sens	Spec	AUC	Sens	Spec	AUC	Sens	Spec	AUC	Sens	Spec	AUC
Istra and Kvarner	87.5	85.8	0.875	75.0	89.0	0.839	100.0	89.3	0.973	87.5	85.5	0.933
Dalmatia	84.2	70.6	0.811	68.7	91.0	0.761	88.8	88.9	0.958	83.4	76.5	0.876
Dalmatian islands	88.8	81.5	0.888	66.7	92.6	0.872	78.9	82.4	0.888	88.8	84.5	0.917
Macro-average (95% CI)	0.836 (0.693-0.906)			0.801 (0.698-0.903)			0.918 (0.805-0.953)			0.887 (0.748-0.948)		
Micro-average (95% CI)	0.870 (0.768-0.944)			0.845 (0.746-0.925)			0.939 (0.857-0.976)			0.917 (0.810-0.976)		

[a] *k*: number of k neighbours
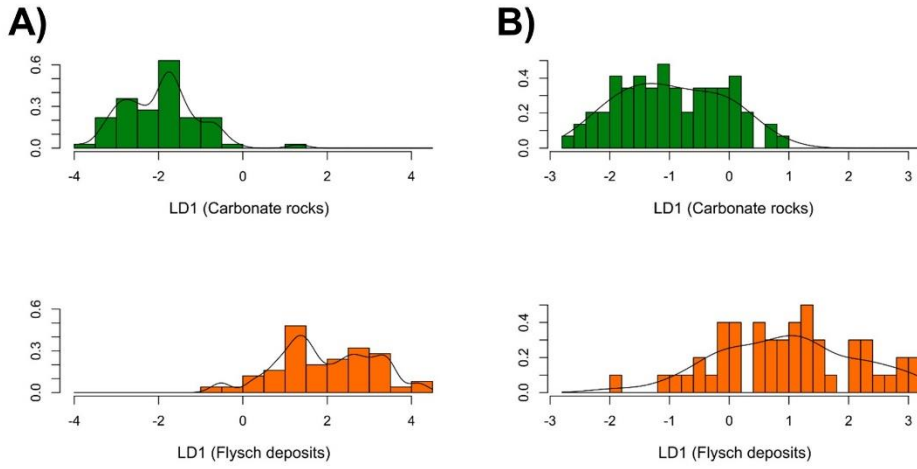
Fig. A1. Linear discriminant analysis of soil (A) and extra virgin olive oil (EVOO) samples (B) according to their parent materials.

Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: