

Communication

# Isotopic Characterization of Italian Industrial Hemp (*Cannabis sativa* L.) Intended for Food Use: A First Exploratory Study

Marco Calvi <sup>1,2,\*</sup> , Luana Bontempo <sup>3</sup> , Sarah Pizzini <sup>1,†</sup> , Lorenzo Cucinotta <sup>3,4</sup> , Federica Camin <sup>3,5,‡</sup>   
and Barbara Stenni <sup>1</sup> 

<sup>1</sup> Department of Environmental Sciences, Informatics and Statistics, Ca' Foscari University of Venice, Via Torino, 155, 30172 Venice Mestre, Italy; sarah.pizzini@unive.it (S.P.); barbara.stenni@unive.it (B.S.)

<sup>2</sup> Certottica S.c.r.l.—Italian Institute of Certification of Optical Products, Villanova Industrial Area, 32013 Longarone, Italy

<sup>3</sup> Traceability Unit, Research and Innovation Center, Edmund Mach Foundation (FEM), Via E. Mach, 1, 38098 San Michele all'Adige, Italy; luana.bontempo@fmach.it (L.B.); lorenzo.cucinotta@unime.it (L.C.); federica.camin@unitn.it (F.C.)

<sup>4</sup> Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Annunziata, 98168 Messina, Italy

<sup>5</sup> Center Agriculture Food Environment (C3A), University of Trento, Via E. Mach, 1, 38098 San Michele all'Adige, Italy

\* Correspondence: marco.calvi@unive.it; Tel.: +39-041-234-8637

† Current affiliation: Institute for Marine Biological Resources and Biotechnology, National Research Council (CNR-IRBIM), Largo Fiera della Pesca, 2, 60125 Ancona, Italy.

‡ Current affiliation: International Atomic Energy Agency (IAEA), Vienna International Center, P.O. Box 100, 1400 Vienna, Austria.



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**Abstract:** In this study, Italian industrial hemp (*Cannabis sativa* L.) intended for food use was isotopically characterized for the first time. The stable isotope ratios of five bioelements were analyzed in different parts of the plant (i.e., roots, stems, inflorescences, and seeds) sampled in eight different regions of Italy, and in five hemp seed oils. The values of  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{34}\text{S}$  differed according to the latitude and, therefore, to the geographical origin of the samples and the climate conditions of plant growth, while the  $\delta^{15}\text{N}$  values allowed us to distinguish between crops grown under conventional and organic fertilization. The findings from this preliminary study corroborate the reliability of using light stable isotope ratios to characterize hemp and its derived food products and contribute to the creation of a first isotopic database for this plant, paving the way for future studies on authentication, traceability, and verification of organic labeling.

**Keywords:** hemp; *Cannabis sativa*; H, C, N, O, S stable isotopes; Isotope Ratio Mass Spectrometry (IRMS); geographical origin; climate conditions

## 1. Introduction

Hemp is the common name of *Cannabis sativa* (Linnaeus, 1753), an annual and principally dioecious tall weed based on the C3 photosynthetic cycle [1], belonging to the family *Cannabaceae* [2]. It has a large number of traditional and innovative applications in different sectors, encompassing, among others, fuel, textiles, green building, and foodstuff, resulting in its global distribution as an emerging high-value specialty crop [3,4]. Particular interest has been aroused by hemp essential oils and bioactive compounds (e.g., non-psychoactive cannabinoids, flavonoids, terpenes, etc.), as well as by their cosmetic, nutraceutical, and pharmaceutical formulations, prompting several reviews summarizing traditional and innovative technologies (e.g., supercritical CO<sub>2</sub>) to effectively extract them from the plant while preserving their biological properties [5–8].

Nowadays, thanks to the increasing attention paid to healthy food and alternative diets (e.g., gluten-free, vegan, and vegetarian), the consumption of hemp flour-based products, hemp seeds, and oil is spreading widely. Hemp has a long history of cultivation in Italy [9]. It is noteworthy that Italy was the second world's largest hemp producer until the Second World War [10] and in the 1950s, it was still one of the most important global hemp industries [11]. After years of planting ban, the Italian Law 242/2016 [12] has legalized the cultivation of industrial hemp again, so long as it has a content of delta-9-Tetrahydrocannabinol ( $\Delta^9$ -THC) lower than 0.2%. The introduction of this law led to an increase of hemp-cultivated fields, whose extension increased from 400 hectares in 2013 to 4000 hectares in 2018 [13].

Since the Italian market for hemp and its derived food products is becoming increasingly important [9,14], in 2021, the Italian hemp Federation has established the first guidelines for hemp cultivation (for plants intended for both the production of extracts and food use). The future aim is to define a proper rulebook, thus enhancing the product value and counteracting emerging cases of adulteration, especially arising from the use of foreign hemp seed oils of doubtful origin and quality [15,16].

In the literature, one of the most widely used methods for agro-product authentication and traceability is the stable isotope ratio analysis of bioelements [17–19]. Isotopic ratio measurements have indeed proven to be a valid tool to discriminate between biological and environmental processes and therefore provide a specific fingerprint of a product that is directly linked to its geographical and ecological origin [20]. Studies based on stable isotopes of *C. sativa* are mainly focused on defining the routes of marijuana illicit traffic and use the light stable isotopes (i.e., H, C, N, O) to track the origin of drug plantations [21–24], not considering the others multiple applications of hemp. To date, the potential of stable isotope ratio analysis of Italian industrial hemp intended for food use has not been explored.

The aim of this exploratory study was to determine the specific value ranges of five light stable isotope ratios (i.e.,  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{34}\text{S}$ ) in Italian industrial hemp intended for food use, thus contributing to create a first isotopic database for this plant and paving the way for future studies on the authentication and traceability of food products derived from hemp, such as hemp flour-based foodstuffs, and the verification of their possible organic labeling, safeguarding the product and the producers against emerging cases of adulteration.

## 2. Materials and Methods

### 2.1. Sampling and Cultivation Sites

Eighty-four samples of hemp grown in open fields (8 roots, 26 stems, 38 inflorescences, and 12 seeds) and 5 hemp seed oil samples were hand-collected in 8 different regions of Italy between 2018 and 2019 (Figure 1). Udine sampling site in Friuli Venezia Giulia region (NE Italy; point No. 14 in Figure 1), has been the subject of a specific focus as all the parts of the hemp plant (roots, stem, inflorescences, and seeds) were sampled from two different cultivations: one subjected to synthetic fertilization (nitrogen-phosphorus-potassium, NPK), and one subjected to manure fertilization.

Every analyzed sample consisted of a pool of five-six plants separated into their different parts, which were then stored in polyethylene bags and kept at  $-20\text{ }^\circ\text{C}$  until treatment. Details related to the location of the sampling sites, hemp cultivars, method of cultivation (conventional or organic), and extension of hemp fields are reported in Supplementary Material Table S1.

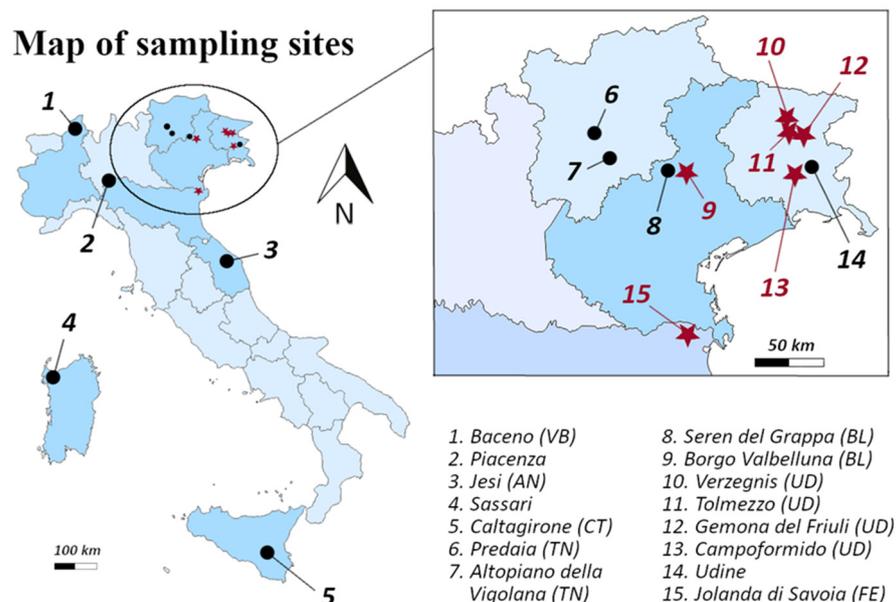


Figure 1. Map of the 2018 (red stars) and 2019 (black circles) sampling sites.

### 2.2. Stable Isotope Ratio Analysis

Isotopic analysis was carried out on freeze-dried, ground, and homogenized samples, except for oils, which were directly analyzed. Details on the pre-treatment procedure and decontamination process are reported in the Supplementary Material.

About  $0.25 \pm 0.05$  mg of duplicate powdered sample were weighed into silver capsules for hydrogen ( $^2\text{H}/^1\text{H}$ ) and oxygen ( $^{18}\text{O}/^{16}\text{O}$ ) isotopic ratio analysis and introduced into a High-Temperature Conversion Elemental Analyzer (TC/EA), coupled with a Delta Plus XP Isotope Ratio Mass Spectrometer (IRMS; Thermo Fisher Scientific GmbH, Bremen, Germany). For the analyses of carbon ( $^{13}\text{C}/^{12}\text{C}$ ), nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ), and sulfur ( $^{34}\text{S}/^{32}\text{S}$ ) isotopic ratios, about 2.5 mg of triplicate powdered sample were weighed into tin capsules and introduced into a vario EL cube EA, coupled with an isoprime precisION IRMS (Elementar Analysensysteme GmbH, Langenselbold, Germany).

The isotopic ratios were reported in delta notation and calculated according to Brand and Coplen [25]:

$$\delta^i E = \frac{iR_{\text{sample}} - iR_{\text{standard}}}{iR_{\text{standard}}}$$

where the superscript  $i$  indicates the mass number of the heavier isotope of element  $E$  (e.g.,  $^{18}\text{O}$ ), and  $R$  indicates the isotopic ratio of  $E$  (e.g.,  $^{18}\text{O}/^{16}\text{O}$ ) in the sample and in an internationally recognized standard (Vienna-Standard Mean Ocean Water (V-SMOW) for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , Vienna-Pee Dee Belemnite (V-PDB) for  $\delta^{13}\text{C}$ , air nitrogen for  $\delta^{15}\text{N}$ , and Vienna-Canyon Diablo Troilite (V-CDT) for  $\delta^{34}\text{S}$ ). The delta values were multiplied by 1000 and expressed in ‰.

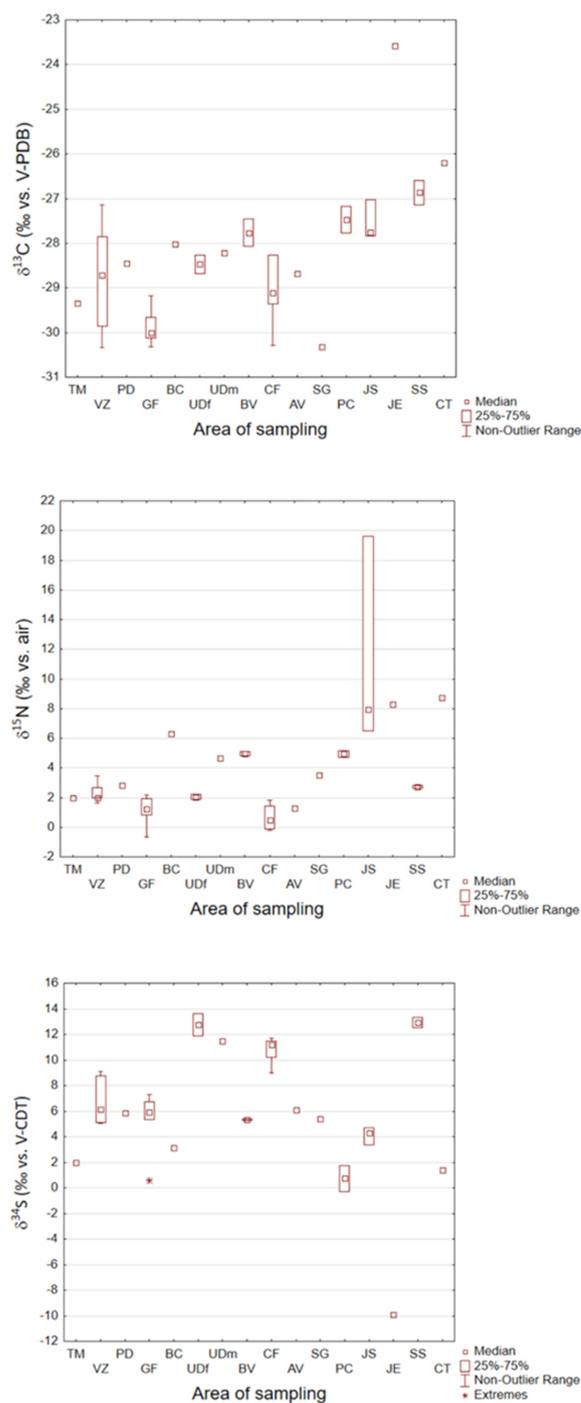
The isotopic values for  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  were calculated against the United States Geological Survey (USGS) international reference materials USGS54 (Canadian lodgepole pine;  $\delta^2\text{H} = -150.4\text{‰}$ ,  $\delta^{18}\text{O} = +17.79\text{‰}$ ) and USGS56 (South African red ivory wood;  $\delta^2\text{H} = -44.0\text{‰}$ ,  $\delta^{18}\text{O} = +27.23\text{‰}$ ). The isotopic values for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  were calculated against in-house working standards, which were themselves calibrated against the International Atomic Energy Agency (IAEA) and USGS international reference materials IAEA-CH-6 (sucrose;  $\delta^{13}\text{C} = -10.449\text{‰}$ ), NBS22 (mineral oil;  $\delta^{13}\text{C} = -30.031\text{‰}$ ), USGS40 (L-glutamic acid;  $\delta^{13}\text{C} = -26.39\text{‰}$ ,  $\delta^{15}\text{N} = -4.52\text{‰}$ ), IAEA-NO-3 (potassium nitrate;  $\delta^{15}\text{N} = +4.7\text{‰}$ ), IAEA-SO-5 (barium sulfate;  $\delta^{34}\text{S} = +0.5\text{‰}$ ), NBS127 (barium sulfate;  $\delta^{34}\text{S} = +20.3\text{‰}$ ), USGS42 (Tibetan human hair;  $\delta^{34}\text{S} = +7.84\text{‰}$ ), and USGS43 (Indian human hair;  $\delta^{34}\text{S} = +10.46\text{‰}$ ). Reports with certified/recommended val-



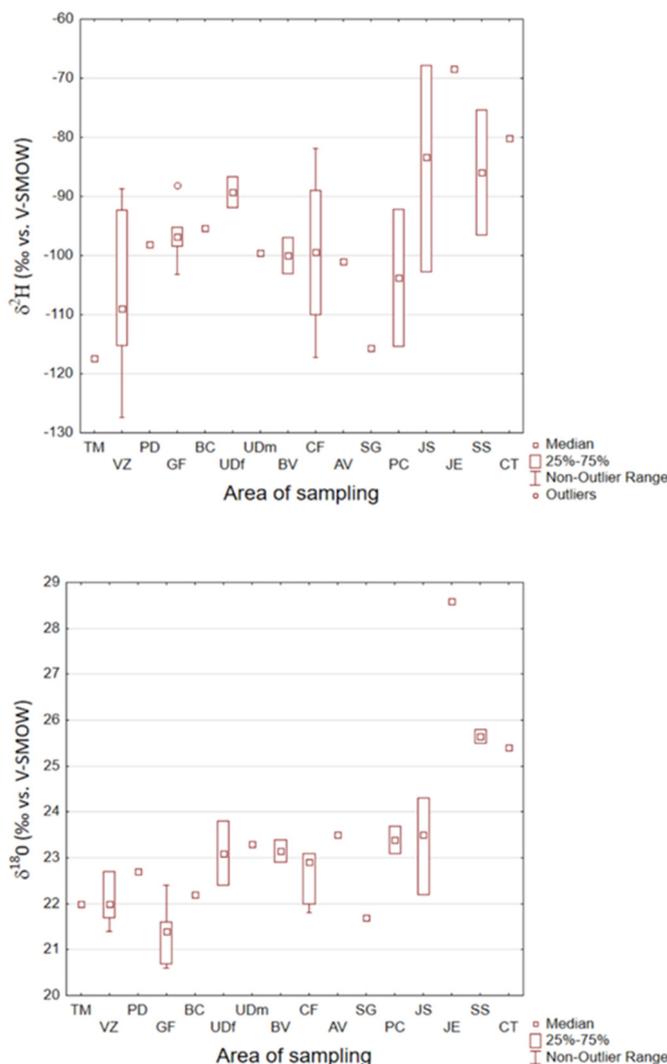
**Table 1.** *Cont.*

Samples		$\delta^2\text{H}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{18}\text{O}$		$\delta^{34}\text{S}$		
Area	Type *	n	Mean	S.Dev	Mean	S.Dev	Mean	S.Dev	Mean	S.Dev	Mean	S.Dev
Borgo Valbelluna (BL)	I	2	−100	4	−27.8	0.4	4.9	0.2	23.2	0.4	5.3	0.1
Campoformido (UD)	I	6	−103	10	−29.1	0.8	0.6	0.8	22.6	0.6	10.8	1.0
	S	6	−103	8	−28.7	0.5	1.1	0.5	23.2	0.3	8.6	1.6
	R	1	−117		−28.6		1.9		23.9		8.7	
Altopiano della Vigolana (TN)	SD	1	−141		−29.4		2.6		22.3		6.3	
	I	1	−101		−28.7		1.3		23.5		6.1	
Seren del Grappa (BL)	SD	1	−138		−30.9		3.6		21.5		4.5	
	I	1	−116		−30.3		3.5		21.7		5.4	
Piacenza	SD + I	2	−104	16	−27.5	0.4	5.0	0.3	23.4	0.4	0.7	1.4
	S	2	−84	2	−26.7	0.1	4.7	0.8	24.5	0.4	−1.2	0.8
Jolanda di Savoia (FE)	O	1	−200						20.9			
	I	3	−85	18	−27.5	0.4	11.3	7.2	23.3	1.1	4.1	0.7
Jesi (AN)	O	1	−181						22.4			
	SD	1	−118		−28.5		10.7		23.7		2.8	
	I	1	−68		−23.6		8.3		28.6		−9.9	
Sassari	O	1	−179						24.5			
	SD	2	−99	7	−27.8	0.4	3.1	0.7	27.3	0.2	10.5	0.2
	I	2	−86	15	−26.9	0.4	2.7	0.1	25.7	0.2	12.9	0.6
Caltagirone (CT)	O	1	−173						25.6			
	SD	1	−104		−25.8		7.3		28.7		1.4	
	I	1	−80		−26.2		8.7		25.4		1.4	
	S	1	−88		−27.6		7.9		26.5		0.5	
	R	1	−93		−27.3		7.1		27.8		2.6	
<i>Minimum value</i>			−200		−30.9		0.6		17.9		−9.9	
<i>Maximum value</i>			−68		−23.6		11.3		28.7		12.9	

\* Samples are labeled as follow: O = Oils, SD = Seeds, I = Inflorescences, S = Stems, R = Roots. Only for Udine sampling area: *f* = grown under synthetic fertilization, *m* = grown under bovine manure fertilization.



**Figure 2.** Box-plots of  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values, expressed in ‰, of Italian industrial hemp inflorescence samples (*Cannabis sativa* L.) ordered according to the latitude of the sampling areas (from the highest to the lowest). V-PDB = Vienna-Pee Dee Belemnite, V-CDT = Vienna-Canyon Diablo Troilite, TM = Tolmezzo (UD), VZ = Verzegnis (UD), PD = Predaia (TN), GF = Gemona del Friuli (UD), BC = Baceno (VB), UD = Udine (*f* = grown under synthetic fertilization, *m* = grown under bovine manure fertilization), BV = Borgo Valbelluna (BL), CF = Campofornido (UD), AV = Altopiano della Vigolana (TN), SG = Seren del Grappa (BL), PC = Piacenza, JS = Jolanda di Savoia (FE), JE = Jesi (AN), SS = Sassari, CT = Caltagirone (CT).



**Figure 3.** Box-plots of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values, expressed in ‰, of Italian industrial hemp inflorescence samples (*Cannabis sativa* L.) ordered according to the latitude of the sampling areas (from the highest to the lowest). V-SMOW = Vienna-Standard Mean Ocean Water, TM = Tolmezzo (UD), VZ = Verzegnis (UD), PD = Predaia (TN), GF = Gemona del Friuli (UD), BC = Baceno (VB), UD = Udine (*f* = grown under synthetic fertilization, *m* = grown under bovine manure fertilization), BV = Borgo Valbelluna (BL), CF = Campoformido (UD), AV = Altopiano della Vigolana (TN), SG = Seren del Grappa (BL), PC = Piacenza, JS = Jolanda di Savoia (FE), JE = Jesi (AN), SS = Sassari, CT = Caltagirone (CT).

### 3.1. Carbon, Nitrogen, and Sulfur Stable Isotope Ratios in Hemp

The carbon stable isotope ratio is strictly related to the photosynthetic cycle of the plant species considered but, on a lesser extent, it is also related to the climatic conditions of the place of growth of the plant [26]. In this study, as expected, the mean  $\delta^{13}\text{C}$  values were within the typical range of C3 plants [27], varying from  $-30.9$  to  $-23.6$ ‰ (Table 1). These values are in line with those reported in other literature studies aimed at tracking the routes of drug illicit traffic for seized marijuana samples grown outdoor in different regions of Brazil (from  $-32$  to  $-24$ ‰ in Shibuya et al. [23]) and the USA ( $-28.2$ ‰ as the mean outdoor value in West et al. [24]). Nevertheless, the paucity of literature data on stable isotope ratios of *C. sativa* makes a comparison with previous studies on this matter very difficult, especially considering that most of the research in this field has dealt with marijuana samples grown in indoor environment [21,22], characterized by a pronounced  $^{13}\text{C}$ -depletion (minimum  $\delta^{13}\text{C}$  values up to  $-53.8$ ‰ in Booth et al. [21];  $-51.8$ ‰ in West et al. [24]), thus exceeding the range characteristic of C3 plants [27]. Our data showed

a clear increasing trend as the latitude decreased (Figure 2), with the most negative values belonging to samples collected from Northern Italy, in agreement with the geographical and climatic conditions of the place of growth of hemp.

The nitrogen stable isotope ratio has been proposed as a marker to differentiate plants grown under conventional and organic regimes [28], since it reflects the type of fertilizer used for their growth. The  $\delta^{15}\text{N}$  values can vary from 0.3 to 14.6‰ in crops grown under organic fertilization and from  $-4.0$  to 8.7‰ in those grown under conventional fertilization [29]. An exception to the above-mentioned ranges is related to crops organically grown with a fertilization strategy based on nitrogen-fixing plants, which leads to lower  $\delta^{15}\text{N}$  values, within the range of conventionally grown crops [26]. In this study, the mean  $\delta^{15}\text{N}$  values ranged between 0.6 and 11.3‰, which corresponded to the hemp inflorescences collected at Campoformido (Friuli Venezia Giulia) and Jolanda di Savoia (Emilia Romagna), respectively (Table 1). Both samples were labeled as organically grown. However, the mean  $\delta^{15}\text{N}$  values in samples collected at Campoformido, Seren del Grappa (Veneto), and Sassari (Sardinia), all labeled as organically grown, fell into the conventional range (Table 1; Figure 2). This may be due to a fertilization strategy based on leguminous plants, which affected the nitrogen values of the samples, lowering them [26]. Worthy of note is the mean  $\delta^{15}\text{N}$  value (10.7‰) of seed sample collected at Jesi (the Marche; point No. 3 in Figure 1; Table 1). Although this was labeled as a cultivation area under conventional regime, its  $\delta^{15}\text{N}$  values fell into the organic range, maybe because of the use of organic fertilizers.

As reported in the literature, the sulfur stable isotope ratio is affected by different factors, such as distance from the sea, microbial processes, kind of soil, fertilizers used, and anthropogenic inputs [30–32]. Most of the samples analyzed in this study showed positive mean  $\delta^{34}\text{S}$  values ranging between 0.5 and 12.9‰, with the only exception of samples from Piacenza (Emilia Romagna) and Jesi sampling sites, which showed negative mean values ( $-1.2$  and  $-9.9$ ‰, respectively; Table 1). This range of values agrees with those reported by Paolini [30] for plants. Since plants present a limited process of isotopic fractionation for sulfur [27], their  $\delta^{34}\text{S}$  values have been related to those found in the soil, and therefore, to the geographical origin of the considered samples. It should be noted that the inflorescence samples of (1) Udine and Sassari and (2) Piacenza and Caltagirone (Sicily) showed similar mean  $\delta^{34}\text{S}$  values (12.7 and 12.9‰, 0.7 and 1.4‰, respectively; Table 1; Figure 2), even if these sampling sites are geographically far from each other (Figure 1). This could be explained by the common nature of the bedrocks: carbonate for the former, and clay/calcareous for the latter [33]. The sampling area of Jesi represents an exception here too, with the inflorescence sample that presented a quite negative mean  $\delta^{34}\text{S}$  value ( $-9.9$ ‰; Table 1; Figure 2), strongly different from that of all the other samples. This aspect is probably attributable to the geology of this area of Central Italy, where the Messinian evaporite formation may outcrop [33].

### 3.2. Hydrogen and Oxygen Stable Isotope Ratios in Hemp

The hydrogen and oxygen stable isotope ratios are influenced by climatic and geographical factors, which can deplete or enrich their values [34]. Furthermore, it is important to consider that the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of plant organic matter reflect the plant's primary source of water (irrigation or soil) and undergo an enrichment process during evapotranspiration and cellulose synthesis by the plant [27]. In this study, the mean  $\delta^2\text{H}$  values ranged from  $-200$  to  $-68$ ‰, with the lower values detected in all the five hemp seed oil samples considered (see, Section 3.3), while the mean  $\delta^{18}\text{O}$  values ranged from 17.9 to 28.7‰ (Table 1).

Considering the inflorescence samples, increasing mean  $\delta^{18}\text{O}$  values were observed when moving from North to South (mean difference over 15‰; Figure 3), according to the meteorological conditions and the dynamic fractionation in the hydrological cycle [34]. The highest values were found in the sampling sites of Central and Southern Italy (i.e., Jesi, Sassari, and Caltagirone; Table 1; Figure 3), and this aspect can be related to two main factors: (i) the precipitation  $\delta^{18}\text{O}$  values [35] and (ii) the effects of warmer climates on

evapotranspiration processes, which further enrich hydrogen and oxygen isotopic ratios. A similar, although less evident, positive tendency was shown also by the mean  $\delta^2\text{H}$  values, which are strongly influenced, in organic matter, by the isotopic composition of the water taken up by the plant [36] and by photosynthetic metabolism of cellulose [37]. Also in this case, the sample collected at Jesi represented an outlier, and its less negative mean  $\delta^2\text{H}$  value ( $-68\text{‰}$ ; Table 1; Figure 3) could be related to precipitation values characteristic of coastal areas [35].

### 3.3. Hydrogen and Oxygen Stable Isotope Ratios in Hemp Seed Oil

In this work, we briefly investigated the hydrogen and oxygen stable isotope ratios in hemp seed oil bulk samples, collected in the following five different sampling sites, reported from North to South: Predaia, Jolanda di Savoia, Jesi, Sassari, and Caltagirone (Figure 1). As already reported in Section 3.2, the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values are generally affected by the climatic and geographical conditions of the considered sampling areas [38]. This aspect was highlighted also in the mean  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values detected in this study in hemp seed oil samples (Table 1). Indeed, the mean  $\delta^{18}\text{O}$  values showed a clear trend, with increasing values in samples from Predaia (Trentino-South Tyrol, NE Italy;  $17.9\text{‰}$ ) to Caltagirone (Sicily, S Italy;  $25.6\text{‰}$ ) sampling sites. The same tendency was observed for the mean  $\delta^2\text{H}$  values (Predaia,  $-197\text{‰}$ ; Caltagirone,  $-173\text{‰}$ ), with the only exception of Jolanda di Savoia (Emilia Romagna, N Italy) sampling site, whose sample presented the lowest mean value detected in this study ( $-200\text{‰}$ ; Table 1).

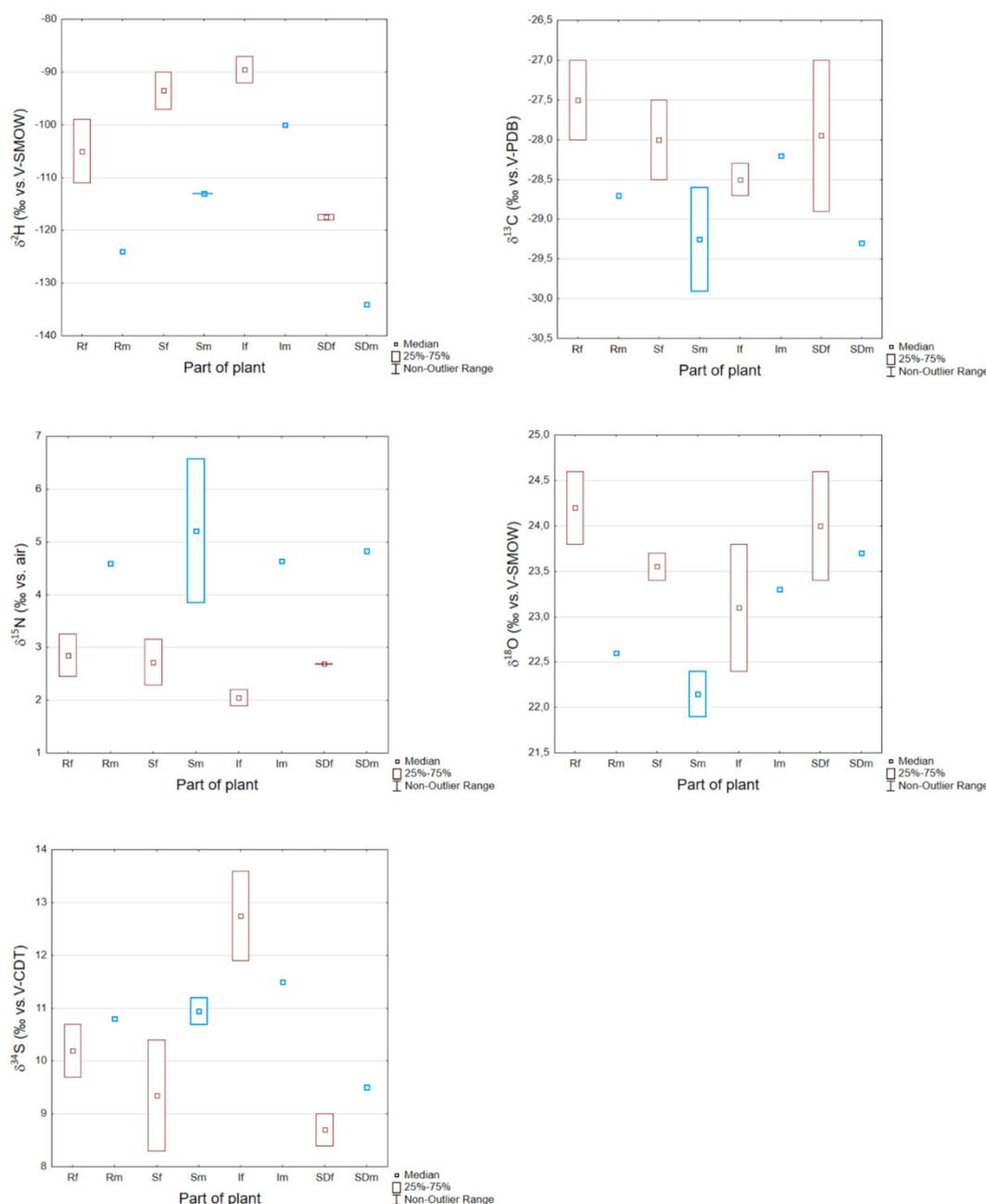
Marked differences were observed, as expected, in the mean  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values moving from seed to oil samples, with a mean decrease of  $65\text{‰}$  for the hydrogen isotopic ratio and of  $2.6\text{‰}$  for the oxygen isotopic ratio. Indeed, this decreasing trend reflects the depletion of the cellulosic component in favor of the lipidic one, which is largely  $^2\text{H}$ -depleted relative to the bulk of the organic matter [39].

Notwithstanding the limited number of hemp seed oil samples considered in our study, a comparison with the literature data was hampered by the lack of  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  data on this matrix. To the best of our knowledge, the only research on stable isotope ratio analysis in hemp-derived oils was focused on  $\delta^{13}\text{C}$  determination in terpenes isolated from hemp inflorescence essential oils [40].

### 3.4. Insight on Stable Isotope Ratios in Hemp Collected at Udine Sampling Site

A specific focus was placed on the Udine sampling area in Friuli Venezia Giulia region (NE Italy; point No. 14 in Figure 1), whose samples were thoroughly analyzed, examining all parts of the hemp plant, from roots to seeds. Moreover, the samples were collected from two different cultivations: one grown under synthetic fertilization (NPK), and one under bovine manure fertilization.

This last aspect affected mainly the mean  $\delta^{15}\text{N}$  values, for which a marked enrichment (mean increase of  $2.3\text{‰}$ ) was observed in all parts of the hemp plant grown under organic fertilization (Figure 4). Conversely, all parts of the hemp plant grown under organic fertilization showed lower mean  $\delta^2\text{H}$  values than those from the conventionally fertilized cultivation, a tendency shown, although less evidently, also by the carbon and oxygen stable isotope ratios, except for the inflorescence samples (Figure 4). These trends have already been noticed for other kinds of plants such as wheat [41–43] and were hypothesized to be related to a higher transpiration and water evaporative loss in conventionally grown plants, and with differences in cultivation practices, such as plant density and growth rates, which can affect plant respiration, water uptake, and evapotranspiration. Lastly,  $\delta^{34}\text{S}$  exhibited more positive mean values in organically fertilized crops, with the exception of the inflorescence samples (Figure 4).



**Figure 4.** Box-plots of  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ , and  $\delta^{34}\text{S}$  values, expressed in ‰, of Italian industrial hemp samples (*Cannabis sativa* L.) collected at the Udine sampling site in Friuli Venezia Giulia region (NE Italy), ordered according to the part of plant and the fertilization strategy (conventional in red, organic in blue). V-SMOW = Vienna-Standard Mean Ocean Water, V-PDB = Vienna-Pee Dee Belemnite, V-CDT = Vienna-Canyon Diablo Troilite, R = Roots, S = Stems, I = Inflorescences, SD = Seeds, *f* = grown under synthetic fertilization, *m* = grown under bovine manure fertilization.

Moving from the roots to the inflorescences, increasing mean  $\delta^2\text{H}$  values were observed, while a pronounced depletion was registered for the  $\delta^2\text{H}$  values in the inflorescences compared to the seeds (mean decrease of 32%; Table 1), likely due to the lower cellulose content and a higher content of lipids in seeds [39]. This pattern was observed in both organically and conventionally fertilized cultivations (Figure 4). When considering conventionally grown hemp, slightly decreasing mean  $\delta^{13}\text{C}$  values were observed moving from the roots to the inflorescences, while slightly increasing values were measured in the seed samples. This variability was not mirrored in organically grown hemp (Figure 4).

No noticeable difference was registered in the mean  $\delta^{15}\text{N}$  values moving from the roots to the seeds, regardless of the fertilization strategy (Figure 4). Instead, in conventionally grown hemp, slightly decreasing mean  $\delta^{18}\text{O}$  values were observed moving from the roots to the inflorescences, with a further slight enrichment in the seed samples. A different pattern was highlighted in organically grown hemp, with a decrease from the roots to the stems, and a further increase from the inflorescences to the seeds (Figure 4). The different trends between  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values could be related to additional biochemical or source effects [37], linked to water evaporation in the leaves or to organic matter synthesis [44]. No clear pattern was observed for the mean  $\delta^{34}\text{S}$  values, regardless of the fertilization strategy (Figure 4). However, even if the plants present a limited process of fractionation for sulfur [27] (Section 3.1), the mean  $\delta^{34}\text{S}$  values in hemp inflorescence samples were higher than those detected in all the other parts of the plant (Figure 4).

#### 4. Conclusions

This exploratory study allowed us to gather important data on the isotopic composition of Italian industrial hemp intended for food use, which will be useful in future studies to address authenticity and origin issues of this still little-investigated high-value emerging crop. The stable isotope ratios detected in this work showed peculiar characteristics that may be linked to samples' geographical origin, fertilization practices, and climatic conditions of the plant growth areas. In particular,  $\delta^{13}\text{C}$  and, to a lesser extent,  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$ , showed values clearly dependent on climatic conditions and latitudinal distribution of the investigated areas,  $\delta^{15}\text{N}$  values reflected conventional or organic fertilization practices, while  $\delta^{34}\text{S}$  values clustered different samples, probably according to the common nature of the bedrocks. A marked depletion in the mean  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values was observed in hemp seed oil bulk samples compared to seed samples, likely driven by the high content of lipids in oil.

A wider sampling, with more representative areas and carried out under different climatic conditions, is needed to achieve a more robust isotopic tracing of the origin of hemp and to better understand the behavior of light stable isotopes in the biogeochemical processes of the hemp plant, exploitable also in other relevant industrial sectors, such as the textile, cosmetic, nutraceutical, and pharmaceutical branches.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/separations9060136/s1>, Table S1: Sampling locations with altitude and extension of the hemp fields considered in this study, method of cultivation employed, varieties cultivated, and year of sampling. It should be noted that it was not possible to collect the whole hemp plant in each area, and the sampling period differed from Southern to Northern Italy, according to the stage of maturation of the crops. Sample pre-treatment procedure and decontamination process.

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