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From Waste to Resource: Mineral and Biochemical Characterization of Hemp By-Products in the Fiber and Seed Supply Chain

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Abstract: Industrial hemp (*Cannabis sativa* L.) is a versatile and sustainable multipurpose plant for agroecology services and a zero-waste circular economy. While the focus has traditionally been on primary products like fiber and seeds, nowadays there is an increasing awareness of the potential value of the by-products generated during hemp cultivation and processing. This article explores various methods of valorizing industrial hemp wastes, focusing on their mineral and biochemical composition, highlighting the benefits of utilizing what was once considered a mere by-product. The apical and the basal leaves of 12 industrial hemp varieties, six monoecious, and six dioecious, representing the main by-product of fiber supply chain, were assessed for their mineral (N, K, Na, Ca; Mg, Cu, Mn, Fe, and Zn), chlorophyll, carotenoids, and total soluble phenols contents, as well as for their antioxidant activity. The same parameters were also evaluated in the inflorescences; the main waste was derived from both hemp fiber and seed harvesting, which were collected at three stages of flower development for four selected genotypes, together with the yield and chemical composition of their essential oils. Differences in the evaluated parameters among genotypes and tissues were highlighted, showing the potential for diversifying the utilization of industrial hemp wastes. The possible uses of these residual biomasses are discussed based on their composition.

Keywords: *Cannabis sativa*; minerals; polyphenols; chlorophylls; carotenoids; antioxidant activity

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1. Introduction

Hemp (*Cannabis sativa* L.) belongs to Cannabaceae, a small family comprising nine genera [1], most of which shared the presence of inconspicuous unisexual flowers [2]. Hemp male and female flowers typically grow on separate dioecious plants. Nevertheless, monoecious genotypes have also been selected during hemp breeding programs [3,4]. This species is characterized by high genetic and phenotypic variability representing an important opportunity for breeders to produce new varieties for different purposes [2].

In fact, hemp is a valuable multipurpose crop, used for many industrial applications. Moreover, it is a sustainable crop [5], perfectly fitting with the objectives of the European Green Deal. In recent years, the industrial hemp sector has gained particular attention due to its versatility and sustainability. The global hemp market was valued at approximately USD 5.49 billion in 2023 and is expected to grow at a compound annual growth rate (CAGR) of 17.5% from 2024 to 2030, reflecting a growing demand for eco-friendly bio-based materials [6].

Industrial hemp has a wide range of uses. The fiber, obtained from the plant stems [5,7], is used for several applications, spanning from the textile industry to paper production and the construction sector [8]. Hemp plants grown for their fiber are harvested at the stage of fiber maturity, usually at the onset of flowering (10–20% of flowering plants). However, in some cases, harvesting occurs later, between the bloom and seed-set, e.g., between the time of panicles harvest for cannabidiol (CBD) extraction and seed harvesting [7].

Additionally, hemp is widely cultivated for its seeds, which are rich in oil, proteins, carbohydrates, and fibers, as well as in vitamins and minerals [7]. They are used as food for human consumption, or as feed for animals [5,8], as well as for obtaining the hempseed oil, which is highly appreciated for its pleasant nutty flavor and for its nutritional properties [7]. Seeds are harvested when about 70% of seeds are mature and cleaned by removing flowers, green material (leaves, bracts), and immature seeds.

While fiber and seeds are the main bioproducts from industrial hemp, hurds, leaves, and inflorescences, they are considered a waste of low economic value, also described as hemp biomass residue. These products can be re-employed into innovative and sustainable resources, or used for extracting bioactive molecules pursuing a zero waste/circular [9]. In particular, female inflorescences, which are considered waste products of the hemp seed supply chain, stood out as valuable by-products, which are exploitable for their content in bioactive compounds, both volatile and non-volatile [10,11]. Besides them, leaves also represent an important waste material and are exploitable for their content in bioactive compounds, as well as in minerals [12]. Recent studies have demonstrated that hemp leaves are a significant source of important molecules, including phytocannabinoids, terpenes, polyphenols, and flavonoids, whose extraction and utilization could represent a zero-waste strategy for the hemp industry [13]. Among those bioactive molecules, phytocannabinoids and terpenes are the main chemical classes found in hemp essential oil (EO) [14], which, in recent years, has attracted increasing attention mainly thanks to its potential industrial applications [15–19]. EOs are complex mixtures of volatile compounds produced by plants in specialized secretory tissues through different biosynthetic pathways of the secondary metabolism [20]. Their chemical composition is affected by many intrinsic and extrinsic factors [21]. In our previous works, among the endogenous factors, we highlighted the strong influence of genotype, year of cultivation, and plant phenological stage on EO composition [14,22]. Also, phytocannabinoid and flavonoid content have been reported to be affected by the genotype, cultivation site, and phenological stage [11,23–25].

Furthermore, the residual materials could also be employed for their mineral compositions to improve soil fertility, thus reducing the need for chemical fertilizers [26]. Indeed, hemp is reported to be an excellent source of calcium (Ca), which is essential for plant growth [27], as it is able to enhance soil structure, such as potassium (K), which is implicated in water balance regulation and photosynthetic efficiency in plants, and phosphorus (P), which is crucial for root development and fruit formation [28]. The role of minerals in hemp waste products is multifaceted, contributing to soil health, environmental remediation, sustainable construction, and resource efficiency. By leveraging these minerals, hemp industry can enhance its sustainability and integrate more deeply into circular economy frameworks, turning waste into valuable inputs for various sectors [29]. Furthermore, hemp

cultivation-derived leaf residues can be exploitable for their content in phytochemicals like chlorophylls, carotenoids, and polyphenols, which are known antioxidant compounds [30].

The present work deals with mineral and biochemical characterization of the apical and the basal leaves of 12 industrial hemp genotypes, six monoecious, and six dioecious cultivated in an open field. Moreover, four genotypes were selected to investigate the evolution of these parameters, as well as the EO yield, chemical composition, and inflorescences during plant growth.

2. Materials and Methods

2.1. Plant Material

Field cultivation was conducted in Rovigo, Italy (Lat 45°04′45.4″ N; Long 11°45′57.3″ E, 3 m asl) in 2021 for 12 hemp varieties (Table S1), six monoecius (Carmaleonte, Codimono, Fedora 17, Felina 32, Futura 75, and Santhica 27), and six dioecious (Felsinea, Carmagnola, CS, Eletta Campana, Fibranova, and Fibrante). Sowing was conducted on April 26 in three replicated plots of 25 m^2 each using 20 kg/ha seed density. Fertilization was applied only before sowing with 40 units of nitrogen. The average temperature between April and October was 19 °C, and the total amount of rainfall in the same period was 289.4 mm. No additional water was supplied by irrigation. For each variety, apical and basal leaves were collected from 18 different plants (6 for each parcel) for each variety on August 4th, and four pools were prepared: two of "apical leaves" and two of "basal leaves". Following the BBCH scale codification and description in *Cannabis sativa* (Mishchenko et al., 2017) [31], at this collection time, monoecious plants were in BBCH stages 65 (Full flowering)–71 (10% of fruits have reached final size and coloration), while dioecious plants were at BBCH stage 39 (stem elongation) or 51 (first individual flower buds of male flowers visible). After collection, leaves were air-dried in the dark and at room temperature. For only four out of the twelve genotypes (Carmaleonte, Futura 75, Eletta Campana, and Carmagnola), the inflorescences were also collected at three distinct BBCH stages: 67, 69, and 87 during growth stages 6 (Flowering) and 8 (Ripening of the Fruit). At each sampling time, apical parts of about 30 cm both from the main stem and lateral branches of nine different plants (three from each plot) were harvested and air-dried in the dark at room temperature to avoid photo-oxidation reactions and loss of volatile compounds. These samples included inflorescences, floral bracts, and apical leaves, which were manually separated from stems and seeds, using a 2 mm-diameter sieve. Both leaves and inflorescences were analyzed for the mineral content, total soluble phenol content, total flavonoids, and antioxidant activity, as reported in Sections 2.3 and 2.4. For inflorescences, the EOs were obtained and analyzed, as in Section 2.5.

2.2. Chemicals and Reagents

Analytical grade methanol and n-butanol used for extract preparation were purchased from Merck (Darmstadt, Germany). UHPLC grade methanol, formic acid, and water were supplied from Romil–Deltek (Pozzuoli, Italy). DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), aluminium trichloride ($AlCl_3$), and Folin–Ciocalteau reagent were purchased by Merck (Darmstadt, Germany).

2.3. Mineral Content

Dried samples were digested with a mixture (5:2) of nitric acid (65%) and perchloric acid (35%) at 240 °C for 1 h, and mineral elements were determined as follows: Ca, Cu, Fe, Mg, K, Mn, Na, and Zn by atomic absorption spectrophotometry. The content of organic nitrogen was determined using the Kjeldahl method [32]. The content of each mineral is expressed either as g/Kg dry weight (DW) or mg/Kg DW, depending on its concentration.

2.4. Biochemical Analyses

The concentration of photosynthetic pigments (chlorophylls and carotenoids) was evaluated in 100 mg of dried samples. Pigments were, overnight, extracted in 5 mL of pure methanol at 4 °C in the dark. The absorbance of the extracts was measured at 665, 652, and 470 nm with a UV-VIS spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan), as described by Lichtenthaler (1987) [33]. Leaf contents of chlorophylls and carotenoids were expressed as mg/g DW.

Other dried samples (100 mg) were pulverized and homogenized in a mortar with 1 mL of 70% (*v*/*v*) methanol. After 30 min of incubation at 4 °C, the samples were centrifuged at 15,000× *g* for 10 min, then the supernatants were utilized for further analyses [34]. Total soluble phenol content (TPC) was assayed with the method based on Folin–Ciocalteau's phenolic reagent (Singleton, Rossi 1965) [35]. The absorbance of samples was spectrophotometrically detected at 765 nm [34], and the data were expressed as mg of gallic acid equivalents per g DW (mg GAE/g DW).

The antioxidant activity was determined by using the stable 2,2-diphenyl-1-picrylhyd razyl radical (DPPH)-scavenging method and the 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay [30,36]. For DPPH, the activity was measured as a decrease in absorbance at 517 nm after 30 min of incubation at room temperature. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula: % inhibition = $(A_{blank} - A_{sample}/A_{blank}) \times 100$, where A_{blank} is the absorbance of the DPPH radical without the antioxidant and A_{sample} is the absorbance of the samples. The results of the DPPH free-radical scavenging activity were expressed as mM of Trolox equivalent antioxidant capacity (TE) for g dry weight (mM TE/g DW).

For ABTS, the activity was assessed according to the Re et al. method [37]. The oxidization of the ABTS with potassium persulfate produced the green/blue radical cation, ABTS+, and ABTS+ was reduced, in turn, by the presence of hydrogen-donating antioxidants, determining a decolorization of the green/blue chromogen. The reaction is followed spectrophotometrically at 734 nm. Trolox was used as a control (2.5 mM). The results were expressed as mM of Trolox equivalents (TE) for g dry weight (mM TE/g DW).

2.5. EO Hydrodistillation and GC-MS Analysis

The EOs were obtained from the air-dried and shredded inflorescences and floral bracts of the four analysed genotypes. In detail, 100 g of plant material of each genotype were separately subjected to hydrodistillation with a Clevenger standard apparatus for 2 h. The obtained EOs were then collected and suddenly analysed by means of Gas Chromatography, coupled with Mass Spectrometry (GC-MS), after being diluted to 5% in HPLC-grade n-hexane. GC-MS analyses were performed as reported in our previous studies [13].

2.6. Statistical Analyses

All the measurements were accomplished in triplicates. For the leaves, mineral composition and biochemical parameters were subjected to one-way analysis of variance (ANOVA) to evaluate the presence of significant differences among the different genotypes, considering the same plant material. Conversely, *t*-test was accomplished only on mineral content to assess the significant difference between basal and apical leaves. Furthermore, the influence of genotype, type of leaf, and their interaction was assessed by two-way ANOVA. For this analysis, averages were separated by Tukey's post-hoc test, using a $p < 0.05$ to assess the significance of differences between means.

For the inflorescences, one-way ANOVA was used to evaluate significant differences of the mineral composition and the biochemical parameters, comprising also the components

of the essential oils (EOs). Additionally, a two-way ANOVA was conducted to assess the effects of genotype, sampling time, and their interaction (genotype × sampling time) on the relative content of these components. For this analysis, averages were separated by Tukey's post-hoc test, using a $p < 0.05$ to assess the significance of differences between means.

Statistical analyses were performed using the JMP Pro 14.0.0 software package (SAS Institute, Cary, NC, USA).

3. Results and Discussions

Major by-products of the hemp supply chain are hemp leaves and inflorescences, which are discarded after fiber and seed harvesting. Indeed, these two materials were studied in this work and finely characterized for their mineral and biochemical composition to gain new scientific knowledge for their valorization.

3.1. Mineral and Chemical Characterization of Hemp Leaves

3.1.1. Mineral Composition of Hemp Leaves

The mineral content of hemp leaves, which are the principal by-product of the fiber industry, were analyzed in this work for their mineral content, which resulted to be influenced by both the genotype and the position of the leaf along the stem, as well as by their interaction, as evidenced by the two-way ANOVA (Table 1). The only elements that were not affected by leaf age were K and Mn, while Zn was the only one whose content was not influenced by genotype x leaf stage interaction.

Table 1. Two-way analysis of variance evaluating the effect of genotype, leaf age, and their interaction on the mineral composition of the leaves.

Elements	Genotype	Leaf Age	Genotype × Leaf Age
N	***	***	***
K	***	n.s.	***
Na	***	***	***
Ca	***	***	***
Cu	***	**	***
Mg	***	***	***
Mn	***	n.s.	***
Fe	***	***	*
Zn	**	**	n.s.

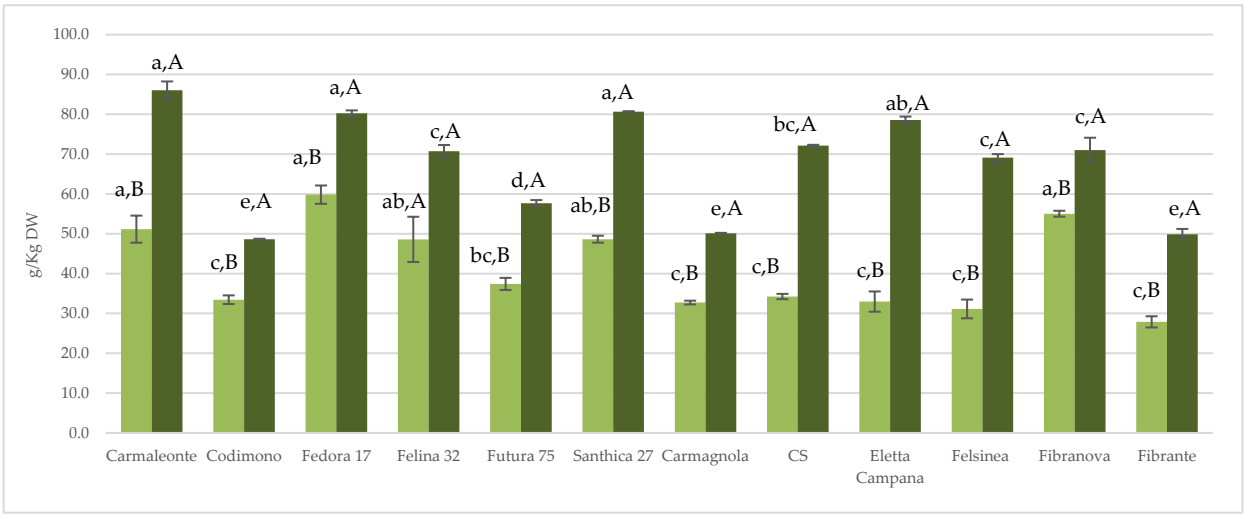
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$; n.s.: not significative.

The major component of hemp leaves was found to be Ca (from 27.9 to 86.4 g/Kg DW), followed by N (28.9–47.5 g/Kg DW), K (10.8–27.4 g/Kg DW), Mg (3.3–10.8 g/Kg DW), and Na (0.2–0.8 g/Kg DW). Fe was present at 105.4–369.6 mg/Kg DW, while Zn, Mn, and Cu were below 100 mg/Kg DW. Cu was found at the lowest concentration in hemp leaves, ranging from 1.99 to 9 mg/Kg DW. The hemp leaf residues could be exploited to enrich soils with N, Ca, and K. Moreover, this mineral composition makes hemp a new attractive crop for agricultural systems like intercropping, contributing to sustainable agriculture and reducing the reliance on synthetic fertilizers. Ca is an important element for plant growth but also an important mineral for the human diet. As hemp leaves have become very popular as food ingredients for preparation infusions, their composition was evaluated in comparison with the literature on other herbal teas. The mineral composition was similar to those of herbal and green teas from *Lippia multiflora* [38] but was different from those reported for other traditional teas by Oliver et al. (2012) [39] like black and green tea, Matè, Coca, and Rooibos, which contains more K than Ca. Interestingly, cistolithyc trichomes,

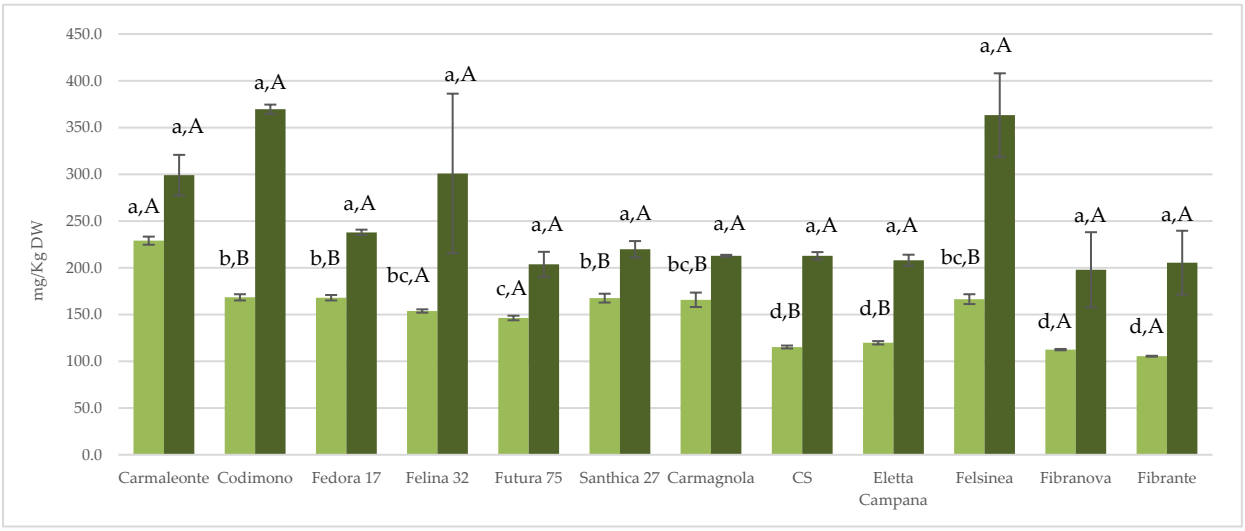
which are short, non-glandular hairs containing basal deposits of calcium carbonate, are present on the adaxial surface of *Cannabis sativa* leaves [40].

In general, some elements were accumulated differently according to leaf age without distinction based on the sexual behavior of the plants, while others showed differences among monoecious and dioecious varieties and not according to leaf age. Mn, K, and Cu showed a genotype-associated trend (Figure S1).

In detail, as clearly shown in the histogram reported in Figure 1A–C and evidenced by one-way ANOVA and *t*-test differences in Ca, Mg, and Fe contents were observed according to the leaf age, with higher amounts in the basal leaves compared to the apical ones and without distinction between monoecious and dioecious varieties.



A



B

Figure 1. *Cont.*

C

Figure 1. (**A**) Calcium (Ca, g/Kg DW); (**B**): Iron (Fe, mg/Kg DW), and (**C**): Magnesium (Mg) content (g/Kg DW) of the apical (light green) and basal (dark green) leaves of the analysed hemp genotypes. Lowercase letters (ANOVA analysis) indicate statistically significant differences among different genotypes considering the same leaf type. Uppercase letters (*t*-test) indicate statistically significant differences between apical and basal leaves per genotype.

Conversely, an opposite accumulation trend, higher in apical leaves compared to the basal ones, was observed for Na and Zn contents (Figure 2), although genotype-specific exceptions were found in Futura 75, Santhica 27, Fibranova, and Fibrante for Na (Figure 2A), as well as in Felina 32 and Felsinea for Zn. Indeed, Na content ranged from 0.2 to 0.8 g/Kg DW for apical leaves and from 0.2 to 0.3 g/Kg DW for basal ones, while Zn content was far lower.

A

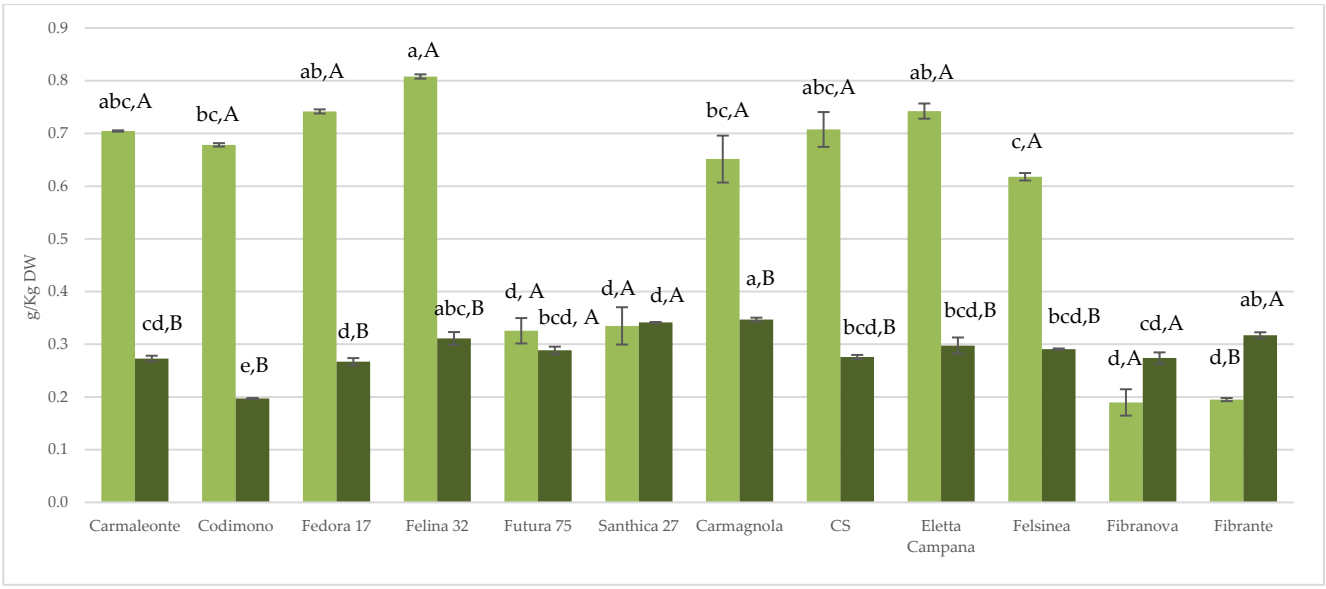
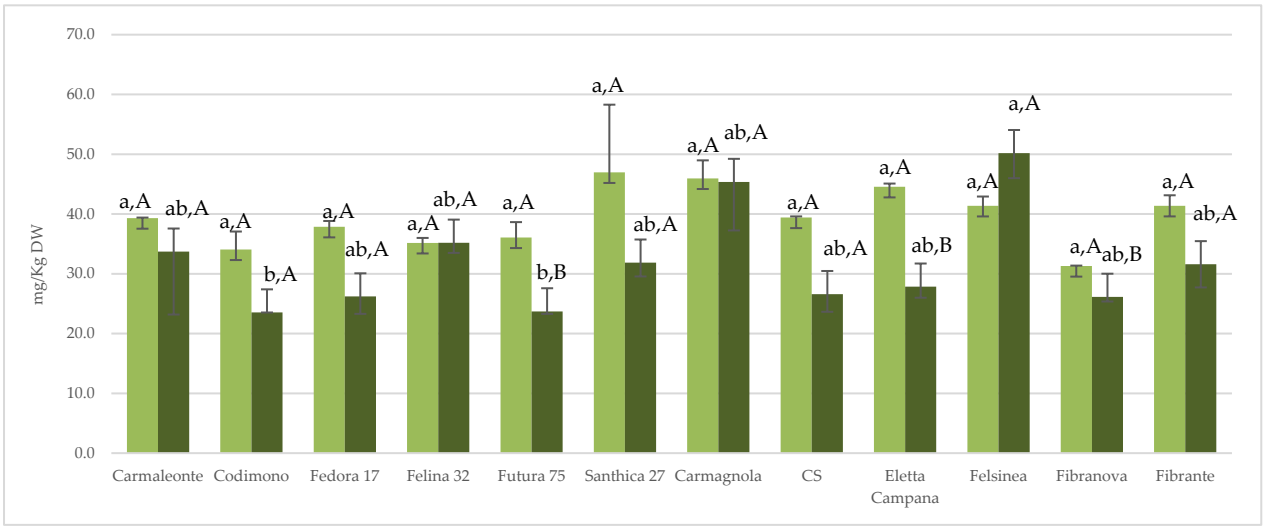


Figure 2. *Cont.*



B

Figure 2. (**A**): Sodium (Na, g/Kg DW); (**B**): Zinc (Zn) content (mg/Kg DW) of the apical (light green) and basal (dark green) leaves of the analysed hemp genotypes. Lowercase letters (ANOVA analysis) indicate statistically significant differences between different genotypes considering the same leaf type. Uppercase letters (*t*-test) indicate statistically significant differences between apical and basal leaves per genotype.

As reported in the histograms of Figure 3, differences in the organic nitrogen content were clearly shown according to sexual behavior. Indeed, N concentration was, in general, significantly higher in the apical leaves of the dioecious varieties, while no differences were revealed for monoecious ones. The maximum concentration was found in the apical leaves of Felsinea (47.5 g/Kg DW), while the minimum was found in basal leaves of Fibrante (28.9 g/Kg DW).

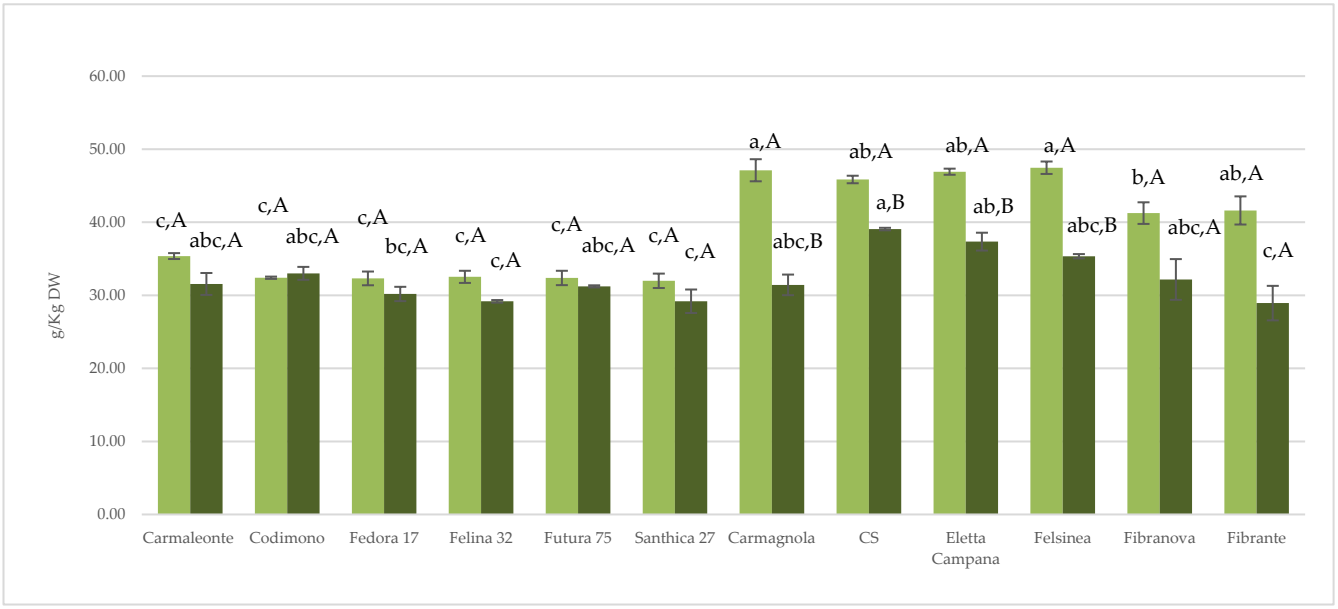


Figure 3. Organic nitrogen (N) (g/Kg DW) of the apical (light green) and basal leaves (dark green) of the analysed hemp genotypes. Lowercase letters (ANOVA analysis) indicate statistically significant differences between different genotypes considering the same leaf type. Uppercase letters (*t*-test) indicate statistically significant differences between apical and basal leaves per genotype.

3.1.2. Chlorophyll, Carotenoids, Total Polyphenols Content, and Antioxidant Activity of Hemp Leaves

The total chlorophyll content differed significantly according to both genotype and leaf position, as well as to their interaction, as clearly evidenced by two-way ANOVA (Table 2). In general, dioecious plants revealed higher concentrations than monoecious, except for Codimono, which showed values similar to those of CS. The highest amount was detected in basal leaves of cv Fibrante (7.83 mg/g DW), followed by Fibranova (7.67 mg/g DW in basal and 7.19 mg/g DW in apical leaves). Carmaleonte showed, by far, the lowest content in total chlorophylls, with significant differences between apical (1.76 mg/g DW) and basal ones (1.11 mg/g DW) that could be attributed to diverse photosynthetic rate [40]. Furthermore, carotenoid content was found to be lower in Carmaleonte with respect to most of the tested varieties, with CS showing content at least 10-fold higher than the others in the apical leaves. Looking at the literature data on carotenoid content in leaves, a similar content, considered on average, was found in *Amaranthus* [41]. Total polyphenol content (TPC) differed significantly among genotypes only in basal leaves: The highest value was recorded in Felina 32 (300.391 mg/g DW), and the lowest was recorded in Carmaleonte (174.55 mg/g DW). Besides the high variability found in the literature concerning the adopted extraction methods, solvents, and analyzed fraction, a comparison with the soluble leaf fractions of other plant species commonly used in tea or infusion preparations was done. Taken as whole apical and basal leaves data, TPC values of *Cannabis sativa* varieties were higher than methanolic extracts from *Amaranthus lividus*, *Urtica dioica*, and *Mentha* spp. [42] and were lower than the average TPC content in *Moringa oleifera* [43] and *Camellia sinensis* leaves [44–46], with the latter indeed known for its antioxidant properties. The antioxidant activity was measured using both DPPH and ABTS assays. Diverse correlations with chlorophylls, carotenoids, and TPC are generally observed when referring to DPPH and ABTS in studies underlying the biochemical composition of plant-derived extracts [36,47,48] In this work, DPPH agreed with pigment composition and TPC content, particularly in basal leaves. The highest radical-scavenging activity based on the DPPH assay was found in Fibrante basal leaves (13.37 mmol TE/g DW) and in Carmaleonte apical leaves (12.39 mmol TE/g DW), while the lowest in the apical leaves of CS (4.76 mmol TE/g DW), with similar activity in both apical and basal leaves (except for Carmaleonte and CS). Considering ABTS results, the basal leaves showed a remarkable radical scavenging activity, which is higher for Fibrante (8.43 mmol TE/g DW) and Futura 75 (8.29 mmol TE/g DW) and lower for Carmaleonte (5.72 mmol TE/g DW). In the apical leaves, values ranged from 6.8 mmol/g DW in Fibrante to 2.71 mmol TE/g DW in Fedora 17. Taken together, these data suggested a potential alternative use of hemp leaves for beverage and food preparations due to their content in phytochemical and antioxidant capacity.

Table 2. Total chlorophylls (Chl tot), carotenoids, total phenolic content (TPC), and antioxidant activity (DPPH assay and ABTS assay) on apical and basal leaves of 12 hemp varieties.

Type	Genotype	Chl Tot (mg/g DW)	Carotenoids (mg/g DW)	TPC (mg GAE/g DW)	Antioxidant Activity DPPH Assay (mM TE/g DW)	Antioxidant Activity ABTS Assay (mM TE/g DW)
			Apical leaves			
Monoecious	Carmaleonte	$1.76 \pm 0.04^{C,a}$	$0.59 \pm 0.01^{F,a}$	$209.61 \pm 28.43^{A,a}$	$12.39 \pm 0.38^{A,a}$	$4.88 \pm 0.63^{A,a}$
	Codimono	$6.52 \pm 0.03^{AB,b}$	$1.22 \pm 0.00^{A,b}$	$240.29 \pm 38.11^{A,a}$	$11.27 \pm 0.32^{AB,a}$	$6.39 \pm 0.69^{A,a}$
	Fedora 17	$4.89 \pm 0.10^{AB,a}$	$0.92 \pm 0.03^{E,a}$	$268.61 \pm 41.27^{A,a}$	$11.64 \pm 1.86^{B,a}$	$2.71 \pm 0.37^{A,a}$
	Felina 32	$5.69 \pm 0.22^{AB,a}$	$1.09 \pm 0.02^{CD,a}$	$234.88 \pm 36.47^{A,a}$	$10.69 \pm 1.08^{AB,a}$	$5.25 \pm 0.57^{A,a}$
	Futura 75	$5.59 \pm 0.24^{AB,a}$	$1.05 \pm 0.03^{D,b}$	$184.48 \pm 19.51^{A,a}$	$9.76 \pm 1.65^{AB,a}$	$5.50 \pm 0.81^{A,a}$
	Santhica 27	$4.24 \pm 0.16^{B,a}$	$1.18 \pm 0.00^{AB,a}$	$176.24 \pm 20.59^{A,a}$	$7.37 \pm 1.15^{AB,a}$	$5.53 \pm 0.56^{A,a}$

Table 2. *Cont.*

Type	Genotype	Chl Tot (mg/g DW)	Carotenoids (mg/g DW)	TPC (mg GAE/g DW)	Antioxidant Activity DPPH Assay (mM TE/g DW)	Antioxidant Activity ABTS Assay (mM TE/g DW)
Dioecious	Felsinea	$4.44 \pm 1.6^{B,a}$	$1.19 \pm 0.07^{AB,a}$	$207.83 \pm 35.97^{A,a}$	$11.45 \pm 0.60^{A,a}$	$5.87 \pm 0.77^{A,a}$
	Carmagnola	$6.32 \pm 0.06^{AB,a}$	$1.14 \pm 0.00^{BC,a}$	$195.49 \pm 27.60^{A,a}$	$10.74 \pm 0.45^{A,a}$	$6.64 \pm 0.61^{A,a}$
	CS	$7.10 \pm 0.06^{A,a}$	$11.97 \pm 0.01^{AB,a}$	$283.40 \pm 40.29^{A,a}$	$4.76 \pm 0.49^{A,a}$	$6.76 \pm 0.74^{A,a}$
	Fibrante	$6.93 \pm 0.04^{A,b}$	$1.19 \pm 0.05^{AB,a}$	$208.11 \pm 31.39^{A,a}$	$10.07 \pm 0.59^{AB,a}$	$6.80 \pm 0.85^{A,a}$
	Eletta Campana	$5.95 \pm 0.02^{AB,b}$	$1.05 \pm 0.00^{D,a}$	$191.08 \pm 26.75^{A,a}$	$10.08 \pm 1.13^{AB,a}$	$5.98 \pm 0.91^{A,a}$
	Fibranova	$7.19 \pm 0.07^{A,b}$	$1.16 \pm 0.01^{AB,b}$	$202.39 \pm 27.24^{A,b}$	$7.79 \pm 1.07^{AB,a}$	$4.84 \pm 0.69^{A,a}$
			Basal leaves			
Monoecious	Carmaleonte	$1.11 \pm 0.01^{G,b}$	$0.46 \pm 0.00^{C,b}$	$174.55 \pm 19.79^{DE,a}$	$8.86 \pm 1.47^{A,b}$	$5.72 \pm 1.30^{A,a}$
	Codimono	$7.03 \pm 0.03^{B,a}$	$1.26 \pm 0.00^{AB,a}$	$242.22 \pm 15.30^{ABCDE,a}$	$8.70 \pm 0.61^{A,a}$	$6.99 \pm 1.45^{A,a}$
	Fedora 17	$4.54 \pm 0.08^{F,a}$	$1.03 \pm 0.00^{B,a}$	$272.18 \pm 11.64^{BCDE,a}$	$10.75 \pm 1.06^{A,a}$	$7.51 \pm 1.35^{A,a}$
	Felina 32	$5.17 \pm 0.02^{E,a}$	$1.13 \pm 0.00^{AB,a}$	$300.39 \pm 4.14^{BCDE,a}$	$6.91 \pm 1.66^{A,a}$	$7.55 \pm 1.44^{A,a}$
	Futura 75	$4.41 \pm 0.05^{F,b}$	$1.40 \pm 0.00^{A,a}$	$223.87 \pm 29.44^{ABC,a}$	$11.38 \pm 1.33^{A,a}$	$8.29 \pm 1.22^{A,a}$
	Santhica 27	$4.20 \pm 0.04^{F,a}$	$0.94 \pm 0.01^{B,b}$	$273.97 \pm 7.15^{CDE,a}$	$8.52 \pm 0.81^{A,a}$	$7.57 \pm 1.62^{A,a}$
Dioecious	Felsinea	$5.95 \pm 0.08^{D,a}$	$0.26 \pm 0.00^{C,b}$	$188.02 \pm 8.35^{E,a}$	$7.48 \pm 0.80^{A,a}$	$6.51 \pm 1.07^{A,a}$
	Carmagnola	$5.93 \pm 0.06^{D,b}$	$1.15 \pm 0.04^{AB,a}$	$239.33 \pm 10.42^{ABCD,a}$	$10.46 \pm 0.11^{A,b}$	$6.92 \pm 1.13^{A,a}$
	CS	$6.75 \pm 0.14^{BC,a}$	$1.24 \pm 0.02^{AB,a}$	$228.04 \pm 7.08^{AB,a}$	$9.59 \pm 1.35^{A,a}$	$8.13 \pm 1.56^{A,a}$
	Fibrante	$6.49 \pm 0.02^{C,a}$	$0.96 \pm 0.12^{B,a}$	$215.52 \pm 10.02^{A,a}$	$8.96 \pm 0.97^{A,a}$	$8.23 \pm 1.20^{A,a}$
	Eletta Campana	$7.83 \pm 0.08^{A,a}$	$0.45 \pm 0.08^{C,b}$	$265.08 \pm 1.88^{BCDE,a}$	$13.37 \pm 0.89^{A,a}$	$8.43 \pm 1.38^{A,a}$
	Fibranova	$7.67 \pm 0.10^{A,a}$	$0.32 \pm 0.17^{C,a}$	$202.01 \pm 5.05^{AB,a}$	$8.39 \pm 0.50^{A,a}$	$6.69 \pm 1.08^{A,a}$

Columns with different letters indicate a statistically significant difference in the means ± standard error (SE). Superscript letters (A–G) indicate statistically significant differences between the same leaf type of the different genotypes; lowercase letters (a,b) indicate statistically significant differences among apical and basal leaves of the same genotype. The statistical significance was determined by Tukey's post-hoc test, with $p \leq 0.05$. GAE, gallic acid equivalent; TE, trolox equivalents.

3.2. Mineral and Chemical Characterization of Hemp Inflorescences

3.2.1. Mineral Composition of Hemp Inflorescences

The mineral composition of hemp inflorescences was quite similar to that of leaves. In fact, the results, reported in Table 3, indicate a prevalence of Ca (from 42.8 to 65.2 g/Kg DW), followed by N (29.9–39.9 g/Kg DW), K (from 14.4 to 19.3 g/Kg DW), Mg (5.4–7.0 g/Kg DW), and Na (0.2–0.9 g/Kg DW). Among microelements, Fe is the most abundant in hemp inflorescences (from 192.0 to 300.0 mg/Kg DW), followed by Zn at 45–59 mg/Kg DW, Mn at 44–62 mg/Kg DW, and Cu between 14 and 20 mg/Kg DW. The two-way analysis of variance (Table 4) evidenced that genotype, sampling time, and their interaction have no significant influence on Ca, Mg, Na, and Fe concentration in the inflorescences. Conversely, K and N were significantly influenced by the genotype, while Cu, Mn, and Zn were influenced by the sampling time.

In detail, significant differences in accumulation trends of K and N were found only in Eletta Campana (Table 3): K was significantly lower in T3 than T2 and T4, while for N, a clear decreasing trend with the minimum at T4 was found. An N decreasing trend during time was also observed for Carmaleonte and Carmagnola, even though the differences were not significative at all. Moreover, the results of the analysis of variance among genotypes for each sampling time indicated that only K and Cu were differently accumulated by genotypes at T2.

Table 3. Amount, expressed in g (macroelements) or mg (microelements) per kg of dry weight of mineral elements in the hemp inflorescences of four different varieties at three sampling times (T2, 3, and 4).

Variety	T	N (g)	K (g)	Na (g)	Ca (g)	Mg (g)	Cu (mg)	Mn (mg)	Fe (mg)	Zn (mg)
Carmaleonte	2	39.2 ± 0.63 ^{A,a}	18.3 ± 0.31 ^{A,a}	0.6 ± 0.00 ^{A,a}	63.1 ± 2.01 ^{A,a}	6.9 ± 0.11 ^{A,a}	14.0 ± 0.00 ^{A,b}	46.0 ± 0.00 ^{A,a}	192.0 ± 16.00 ^{A,a}	49.0 ± 1.00 ^{A,a}
	3	39.7 ± 0.94 ^{A,a}	16.7 ± 1.75 ^{A,a}	0.8 ± 0.01 ^{A,a}	51.3 ± 13.51 ^{A,a}	5.8 ± 0.96 ^{A,a}	16.0 ± 2.00 ^{A,a}	52.0 ± 10.00 ^{A,a}	233.0 ± 51.00 ^{A,a}	55.0 ± 5.00 ^{A,a}
	4	36.7 ± 1.67 ^{A,a}	17.1 ± 1.67 ^{A,a}	0.9 ± 0.23 ^{A,a}	65.2 ± 1.09 ^{A,a}	6.0 ± 0.20 ^{A,a}	18.0 ± 2.00 ^{A,a}	62.0 ± 8.00 ^{A,a}	241.0 ± 29.00 ^{A,a}	58.0 ± 4.00 ^{A,a}
Futura 75	2	35.9 ± 0.80 ^{A,a}	14.4 ± 0.30 ^{A,b}	0.3 ± 0.01 ^{A,a}	46.0 ± 5.30 ^{A,a}	5.4 ± 0.38 ^{A,a}	15.0 ± 1.00 ^{A,b}	45.0 ± 5.00 ^{A,a}	202.0 ± 44.00 ^{A,a}	45.0 ± 3.00 ^{A,a}
	3	36.3 ± 0.57 ^{A,a}	16.3 ± 0.99 ^{A,a}	0.5 ± 0.26 ^{A,a}	62.0 ± 1.99 ^{A,a}	5.9 ± 0.34 ^{A,a}	17.0 ± 3.00 ^{A,a}	59.0 ± 9.00 ^{A,a}	281.0 ± 89.00 ^{A,a}	45.0 ± 3.00 ^{A,a}
	4	37.7 ± 0.11 ^{A,a}	15.8 ± 0.50 ^{A,a}	0.3 ± 0.07 ^{A,a}	64.1 ± 3.89 ^{A,a}	6.7 ± 0.10 ^{A,a}	19.0 ± 1.00 ^{A,a}	59.0 ± 3.00 ^{A,a}	218.0 ± 4.00 ^{A,a}	54.0 ± 0.00 ^{A,a}
Carmagnola	2	34.7 ± 0.38 ^{A,a}	15.3 ± 0.00 ^{A,b}	0.4 ± 0.17 ^{A,a}	53.8 ± 3.52 ^{A,a}	5.4 ± 0.43 ^{A,a}	14.0 ± 0.00 ^{A,b}	49.0 ± 1.00 ^{A,a}	239.0 ± 1.00 ^{A,a}	46.0 ± 0.00 ^{A,a}
	3	36.2 ± 0.11 ^{A,a}	16.3 ± 0.98 ^{A,a}	0.6 ± 0.26 ^{A,a}	62.1 ± 5.94 ^{A,a}	6.0 ± 0.46 ^{A,a}	16.0 ± 0.00 ^{A,a}	61.0 ± 7.00 ^{A,a}	300.0 ± 110.00 ^{A,a}	50.0 ± 0.00 ^{A,a}
	4	29.9 ± 1.96 ^{A,a}	15.5 ± 1.38 ^{A,a}	0.3 ± 0.06 ^{A,a}	56.7 ± 2.39 ^{A,a}	6.6 ± 0.15 ^{A,a}	17.0 ± 1.00 ^{A,a}	58.0 ± 4.00 ^{A,a}	214.0 ± 0.00 ^{A,a}	54.0 ± 0.00 ^{A,a}
Eletta Campana	2	39.9 ± 0.17 ^{A,a}	18.3 ± 0.12 ^{A,a}	0.4 ± 0.01 ^{A,a}	60.3 ± 0.96 ^{A,a}	7.0 ± 0.30 ^{A,a}	18.0 ± 0.00 ^{A,a}	47.0 ± 1.00 ^{A,a}	206.0 ± 6.00 ^{A,a}	53.0 ± 1.00 ^{A,a}
	3	35.8 ± 0.26 ^{AB,a}	14.7 ± 0.08 ^{B,a}	0.2 ± 0.00 ^{A,a}	42.8 ± 2.88 ^{A,a}	5.4 ± 0.77 ^{A,a}	16.0 ± 0.00 ^{A,a}	44.0 ± 4.00 ^{A,a}	208.0 ± 8.00 ^{A,a}	47.0 ± 7.00 ^{A,a}
	4	34.2 ± 0.72 ^{B,a}	19.3 ± 0.53 ^{A,a}	0.7 ± 0.28 ^{A,a}	50.8 ± 3.88 ^{A,a}	5.7 ± 0.47 ^{A,a}	20.0 ± 2.00 ^{A,a}	60.0 ± 8.00 ^{A,a}	246.0 ± 8.00 ^{A,a}	59.0 ± 5.00 ^{A,a}

Columns with different letters indicate a statistically significant difference in the means ± standard error (SE). Uppercase letters indicate the presence of significant differences among the different sampling times of each genotype, while lowercase letters indicate the presence of significant differences among the same sampling times of the different genotypes.

Table 4. Two-way analysis of variance evaluating the effect of genotype and sampling time on the mineral composition of the inflorescences.

Elements	Genotype	Sampling Time	Genotype × Sampling Time
K	*	n.s.	n.s.
Ca	n.s.	n.s.	n.s.
Mg	n.s.	n.s.	n.s.
Na	n.s.	n.s.	n.s.
Cu	n.s.	*	n.s.
Mn	n.s.	*	n.s.
Fe	n.s.	n.s.	n.s.
Zn	n.s.	*	n.s.
N	*	n.s.	n.s.

* $p < 0.05$; n.s.: not significative.

3.2.2. Chlorophyll, Carotenoids, Total Polyphenols Content, and Antioxidant Activity of Hemp Inflorescences

The chlorophyll content was significantly different among genotypes and sampling times. Carmagnola inflorescences had the highest content, followed by Eletta Campana, Futura 75, and Carmaleonte. The content decreased during time, reaching the minimum at T4, except for Futura 75 and Carmaleonte (T3 and T4). In Carmaleonte inflorescences, the chlorophyll content was by far lower than the other varieties and was halved in T3 and T4. TPC (Table 5) was lower in Carmaleonte (spanning from 25.8 to 30.8 mg GAE/g DW) and higher in Futura 75 (from 32.2 to 34.7) according to previous evaluations (Beleggia et al., 2023) [49], suggesting that, besides environmental cues (site of cultivation and growing season), the TPC composition is quite stable in these varieties at the inflorescence level. Indeed, sampling time did not affect TPC content. Two-way ANOVA analysis indicates a significant effect of the genotype and the interaction between genotype and sampling

time on polyphenols, while DPPH and ABTS were not significantly affected. Interestingly, the TPC content in the leaves of the 13 selected hemp varieties was higher compared to that of the inflorescences. Furthermore, the latter was lower than the reported values in *Hibiscus rosa-sinensis* L. flowers [50], usually employed for the preparation of herbal infusions. Carotenoids were present from 0.3 to 0.8 mg/g DW and followed the same accumulation trend observed for the total chlorophylls, reaching the minimum at T4 in all genotypes. Interestingly, on average, the total content of both chlorophylls and carotenoids in hemp inflorescences was higher compared to *Hibiscus rosa-sinensis* L. flowers.

Table 5. Total chlorophylls (Chl tot), carotenoids, total phenolic content (TPC), DPPH assay, and ABTS assay on inflorescences of Carmaleonte, Futura 75, Carmagnola, and Eletta Campana, collected at T2, T3, and T4.

Type	Variety	T	Chl Tot (mg/g DW)	Carotenoids (mg/g DW)	TPC (mg GAE/g DW)	Antioxidant Activity DPPH Assay (mM TE/g DW)	Antioxidant Activity ABTS Assay (mM TE/g DW)
Monoecious	Carmaleonte	2	$19.1 \pm 1.27^{A,c}$	$0.5 \pm 0.04^{A,a}$	$30.8 \pm 0.57^{A,ab}$	$4.8 \pm 0.37^{A,a}$	$5.9 \pm 0.47^{A,a}$
		3	$8.0 \pm 0.24^{B,c}$	$0.3 \pm 0.02^{A,c}$	$25.8 \pm 1.74^{A,b}$	$4.8 \pm 0.50^{A,a}$	$5.8 \pm 0.65^{A,a}$
		4	$10.3 \pm 1.24^{B,a}$	$0.4 \pm 0.08^{A,c}$	$30.0 \pm 0.23^{A,b}$	$4.4 \pm 0.51^{A,a}$	$5.9 \pm 0.71^{A,a}$
	Futura 75	2	$52.1 \pm 1.65^{A,a}$	$0.8 \pm 0.08^{A,a}$	$32.2 \pm 0.40^{A,a}$	$6.1 \pm 0.51^{A,a}$	$5.5 \pm 0.82^{A,a}$
		3	$31.1 \pm 0.62^{B,a}$	$0.5 \pm 0.05^{A,a}$	$34.7 \pm 0.67^{A,a}$	$6.0 \pm 0.47^{A,a}$	$7.1 \pm 0.61^{A,a}$
		4	$33.3 \pm 1.67^{C,a}$	$0.5 \pm 0.07^{A,b}$	$32.7 \pm 0.39^{A,a}$	$5.1 \pm 0.51^{A,a}$	$5.6 \pm 0.95^{A,a}$
Dioecious	Carmagnola	2	$64.3 \pm 3.56^{A,ab}$	$0.9 \pm 0.12^{A,a}$	$31.8 \pm 0.33^{A,ab}$	$5.1 \pm 0.53^{A,a}$	$6.8 \pm 0.61^{A,a}$
		3	$46.7 \pm 1.59^{B,a}$	$0.8 \pm 0.07^{AB,ab}$	$32.8 \pm 0.43^{A,a}$	$4.8 \pm 0.50^{A,a}$	$6.9 \pm 1.01^{A,a}$
		4	$20.5 \pm 1.71^{C,a}$	$0.5 \pm 0.05^{B,a}$	$28.3 \pm 0.28^{B,c}$	$4.0 \pm 0.48^{A,a}$	$6.7 \pm 1.09^{A,a}$
	Eletta Campana	2	$54.5 \pm 1.31^{A,b}$	$0.8 \pm 0.08^{A,a}$	$29.2 \pm 0.65^{B,b}$	$5.1 \pm 0.73^{A,a}$	$5.1 \pm 0.57^{A,a}$
		3	$42.3 \pm 2.46^{B,b}$	$0.6 \pm 0.08^{A,bc}$	$33.6 \pm 0.36^{A,a}$	$5.1 \pm 0.54^{A,a}$	$7.2 \pm 0.96^{A,a}$
		4	$27.4 \pm 0.63^{B,a}$	$0.4 \pm 0.05^{A,a}$	$33.1 \pm 0.22^{A,a}$	$4.5 \pm 0.45^{A,a}$	$6.1 \pm 0.57^{A,a}$

Uppercase letters indicate the presence of significant differences among the different sampling times of each genotype, while lowercase letters indicate the presence of significant differences among the same sampling times of the different genotypes. GAE, gallic acid equivalent; TE, trolox equivalents.

Two-way ANOVA analysis (Table 6) indicates a significant effect of the genotype, sampling time, and their interaction on total chlorophylls and carotenoids. As already observed for the leaves, the radical scavenging activity profile determined with ABTS agrees with polyphenolic content, according to Beleggia et al. [49], and to the nature of this assay [51], while the profile determined with DPPH better correlates with the carotenoid one. Remarkably, the TPC content in inflorescences resulted to be lower than in leaves, suggesting a diverse potential application of hemp-derived biomass.

Table 6. Two-way analysis of variance evaluating the effect of genotype and sampling time on pigments, polyphenol content, and antioxidant activity of the inflorescences.

Pigments	Genotype	Sampling Time	Genotype × Sampling Time
CHL tot	***	***	***
Carotenoids	***	***	**
Polyphenols	***	n.s.	***
DPPH assay	n.s.	n.s.	n.s.
ABTS assay	n.s.	n.s.	n.s.

** $p < 0.01$, *** $p < 0.0001$, n.s. not significative.

3.2.3. Essential Oil Yield and Chemical Composition of Hemp Inflorescences

The yield and chemical composition of the EOs obtained from the air-dried inflorescences of the analysed hemp genotypes collected in the three consecutive sampling times is reported in Table S2.

The hydrodistillation yield showed an increase (Figure 4), peaking at T4 for all genotypes; this result was expected since the last developmental stages correspond to the period of maturation and is, thus, of greater productivity of the inflorescences glandular trichomes [22]. Besides the sampling time, the genotype and the interaction between the genotype and sampling time were also responsible for significant differences in the EO hydrodistillation yield (Table 7). The highest yield was, in fact, found for the variety Eletta Campana (0.22%) at T4.

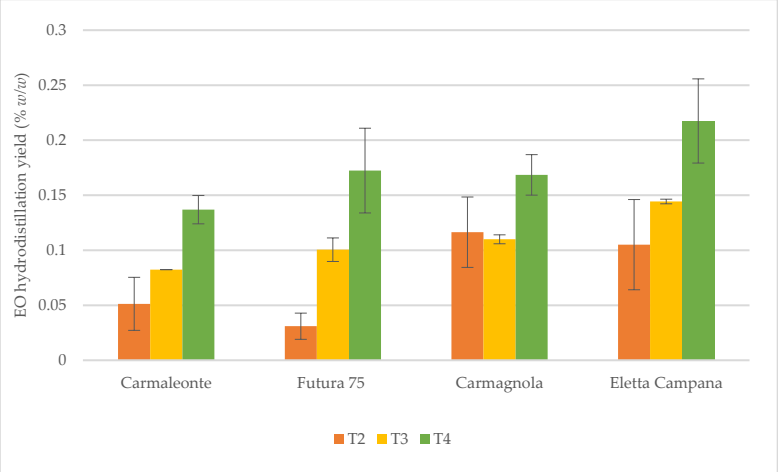


Figure 4. Trend of EO productivity during plant growth.

Table 7. Two-way analysis of variance evaluating the effect of genotype and sampling time on the EO chemical classes and hydrodistillation yield.

Chemical Classes	**Genotype**	**Sampling Time**	**Genotype × Sampling Time**
Monoterpene hydrocarbons (mh)	***	***	**
Oxygenated monoterpenes (om)	***	***	***
Sesquiterpene hydrocarbons (sh)	*	**	***
Oxygenated sesquiterpenes (os)	***	***	***
Diterpene hydrocarbons (dh)	***	***	***
Oxygenated diterpenes (od)	***	***	***
Apocarotenoids (ac)	***	***	***
Other non-terpene derivatives (nt)	***	***	***
Phytocannabinoids (cann)	*	n.s.	***
Phenylpropanoids (pp)	***	***	**
EO Hydrodistillation Yield	**Genotype**	**Sampling Time**	**Genotype × Sampling Time**
EO hydrodistillation yield (%)	***	***	**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$; n.s.: not significative.

As already observed before [16,23], the analysed EOs were characterized by a prevalence of sesquiterpenes in both the hydrocarbon and the oxygenated forms, as well as in phytocannabinoids, whose relative amounts showed a non-homogeneous trend.

Indeed, in the EOs obtained from Carmaleonte and Eletta Campana, these major chemical classes showed a progression during the plant development, as evidenced in the previous work [22]. In detail, sesquiterpene hydrocarbons decreased on behalf of oxygenated sesquiterpenes, which instead increased along with the plant growth, while phytocannabinoids remained stable in Carmaleonte and increased from T2 to T3 in Eletta Campana. Conversely, in Carmagnola, sesquiterpene hydrocarbon levels resulted greatest

at the middle stage, while oxygenated sesquiterpenes remained constant and phytocannabinoids varied in time, with the lowest amount at T3.

Finally, in Futura 75, the major classes did not show significant differences among the three investigated phenological stages (Figure 5).

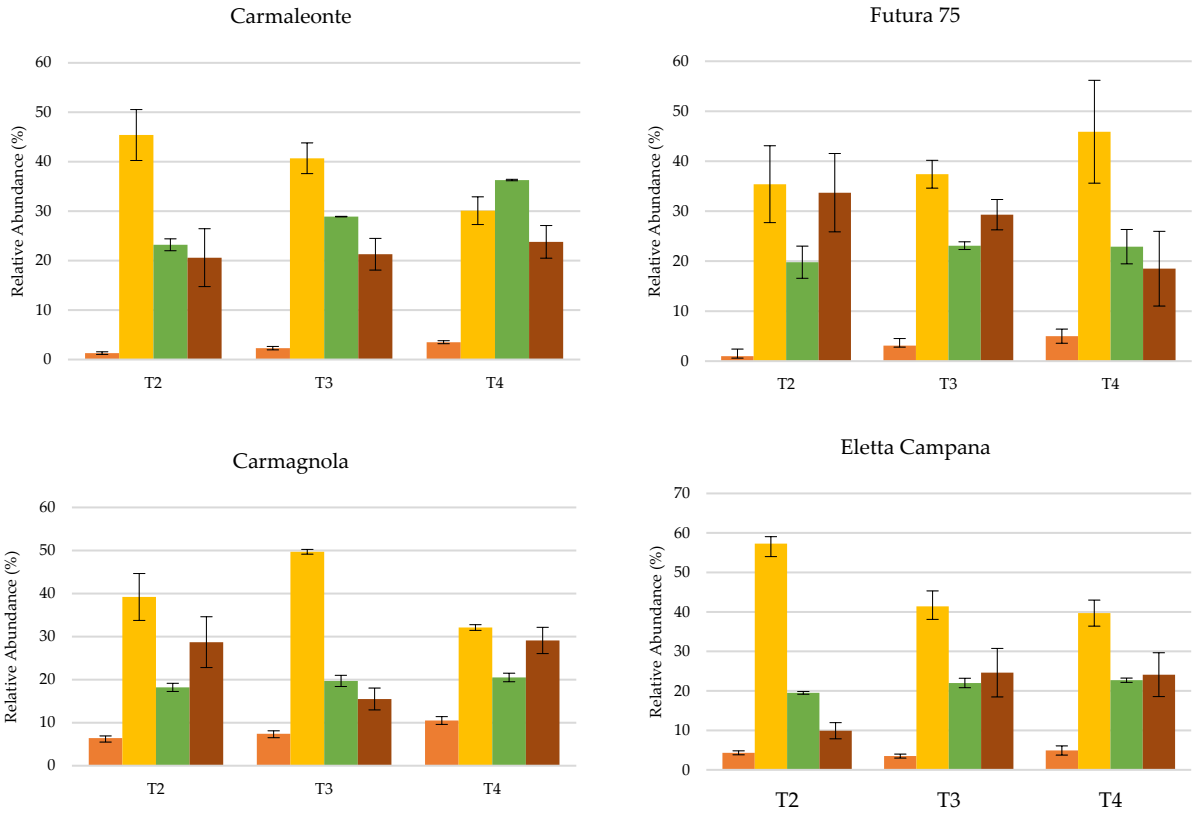


Figure 5. Trend of the major chemical classes (monoterpene hydrocarbons, orange; sesquiterpene hydrocarbons, yellow; oxygenated sesquiterpenes, green; phytocannabinoids, brown) from T2 to T4. Data are expressed as means ± standard deviation (n = 3).

The two-way ANOVA, reported in Table 7, evidenced a strong influence of both genotype and sampling time, as well as their interaction, on the relative abundance of all the detected chemical classes, with the only exception of phytocannabinoids, whose content, instead, was not affected by the sampling time.

Considering the single components, β-caryophyllene, α-humulene, and selina-3,7(11)-diene were the major compounds belonging to the class of sesquiterpene hydrocarbons, but while the former two showed the typical reduction during the plant growth, the latter remained almost constant. Conversely, caryophyllene oxide and humulene oxide II, chief compounds of the class of oxygenated sesquiterpenes, as expected, increased in their relative abundance passing from T2 to T4 in all the genotypes.

Within the class of terpenes, diterpene hydrocarbon phytol has been detected in good amounts in the EOs obtained from the first sampling time, mainly of the monoecious plants, whose content tended to reduce along with the plant development.

Finally, cannabidiol, cannabichromene, and Δ^9-tetrahydrocannabinol represented the only components belonging to phytocannabinoids, but while the latter two did not exceed 1.5%, the former reached great percentages up to almost 30% of the EO composition.

Indeed, although the EO yield is quite low compared to other officinal plants, the variable composition of the EOs among genotypes and sampling time makes attractive the possibility that they could have different biological activities. A prevalence of β-

caryophyllene at T2, and of caryophyllene oxide at T4, for example, suggest different applications of the EOs collected at different times thanks to the different bioactivities of the major compounds. Indeed, to bring some examples, it has been widely demonstrated that essential oils characterized by a prevalence of oxygenated terpenes are associated with a higher antimicrobial activity [52,53], while those rich in conjugated double-bond compounds are responsible for a higher free-radical scavenging activity [54].

4. Conclusions

Hemp is notable for its remarkable abundance of biomolecules, which are attracting growing interest from both scientific and industrial sectors. The plant provides a wide array of bioactive compounds with potential applications in areas such as food pharmaceuticals, bioplastics, and innovative textiles.

The revitalization and enhancement of the industrial hemp supply chain are crucial for the sustainable development of this crop. Although hemp fiber and seeds have long been acknowledged as the main marketable products, it is equally crucial to explore and utilize the by-products generated at each stage of production. The valorization of such by-products has the potential to significantly increase the overall value of the hemp supply chain, opening new industrial opportunities in different sectors across high-tech, biomedicine, and eco-friendly materials sectors. Inflorescences remaining after fiber or seed harvesting can provide a variety of bioactive molecules such as cannabidiol and terpenes (especially sesquiterpenes), with potential applications in the pharmaceutical and cosmeceutical sector, as well as in the development of products for plant protection. Moreover, the leaves, which are lesser-explored by-products of the hemp value chain, hold significant potential for utilization within a circular economic framework. These materials are especially valuable due to their mineral content and non-volatile compounds, including antioxidants, making them suitable for a range of industrial applications. Their different characteristics allow for use across sectors from food to manufacturing industry as evidenced in this study. Hemp residues like leaves and inflorescences can be proposed as bioresource material to sustain soil health due to their mineral composition rich in Ca, N, K, and Mg. Moreover, hemp leaves can be used as food ingredients for the preparation of herbal teas, either as hemp-only tea or as component of mixtures, while hemp flower extracts and infusions can also be used for aromatizing beverages, mainly due to their persistent volatile compounds, which are mainly represented by sesquiterpenes. Despite the numerous advantages, some challenges remain, such as the establishment of quality standards that can facilitate the entry into these innovative products into global markets. Future research should focus on optimizing by-product valorization and deepening our understanding of hemp's wide-ranging applications, ensuring its central role in the sustainable economy of the future.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15030564/s1, Table S1: Description of some morpho-physiological parameters of 12 hemp varieties cultivated in the experimental field of Rovigo. Plant dry weight (g) and the weight of leaves, flowers, and bracts after seed harvest (expressed in g/Mq) are collected at the stage "Ripening of the Fruit, BBCH 87". Figure S1: A: Manganese (Mn); B: Potassium (K); and C: Copper (Cu) content (mg/Kg D.W.) of the apical and basal leaves of the analysed hemp genotypes. Lowercase letters (ANOVA analysis) indicate statistically significant differences among different genotypes considering the same leaf type. Uppercase letters (*t*-test) indicate statistically significant differences between apical and basal leaves per genotype. Table S2. Complete chemical composition of the essential oils obtained from the inflorescences of four *Cannabis sativa* genotypes harvested at three different phenological stages.

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References

1. World Flora Online—WFO. Available online: http://www.worldfloraonline.org/ (accessed on 5 February 2023).
2. Schilling, S.; Dowling, C.A.; Shi, J.; Ryan, L.; Hunt, D.J.L.; O'Reilly, E.; Perry, A.S.; Kinnane, O.; McCabe, P.F.; Melzer, R. The Cream of the Crop: Biology, Breeding, and Applications of Cannabis sativa. *Annu. Plant Rev. Online* **2021**, *4*, 471–528. [CrossRef]
3. Bonini, S.A.; Premoli, M.; Tambaro, S.; Kumar, A.; Maccarinelli, G.; Memo, M.; Mastinu, A. *Cannabis sativa*: A comprehensive ethnopharmacological review of a medicinal plant with a long history. *J. Ethnopharmacol.* **2018**, *227*, 300–315. [CrossRef] [PubMed]
4. Chandra, S.; Lata, H. *Cannabis sativa L.—Botany and Biotechnology*; Chandra, S., Lata, H., ElSohly, M.A., Eds.; Springer International Publishing: Cham, Switzerland, 2017; pp. 79–100, ISBN 978-3-319-54563-9.
5. Ahmed, A.T.M.F.; Islam, M.Z.; Mahmud, M.S.; Sarker, M.E.; Islam, M.R. Hemp as a potential raw material toward a sustainable world: A review. *Heliyon* **2022**, *8*, e08753. [CrossRef] [PubMed]
6. Industrial Hemp Market Size & Share, Industry Report. 2030. Available online: https://www.grandviewresearch.com/industry-analysis/industrial-hemp-market# (accessed on 13 December 2024).
7. Small, E. *Cannabis—A Complete Guide*; CRC Press: Boca Raton, FL, USA, 2016; ISBN 9781498761642.
8. European Commission Hemp—Agriculture and Rural Development. Available online: https://agriculture.ec.europa.eu/farming/crop-productions-and-plant-based-products/hemp_en (accessed on 8 February 2023).
9. Moscariello, C.; Matassa, S.; Esposito, G.; Papirio, S. From residue to resource: The multifaceted environmental and bioeconomy potential of industrial hemp (*Cannabis sativa* L.). *Resour. Conserv. Recycl.* **2021**, *175*, 105864. [CrossRef]
10. Orlando, G.; Adorisio, S.; Delfino, D.; Chiavaroli, A.; Brunetti, L.; Recinella, L.; Leone, S.; D'Antonio, M.; Zengin, G.; Acquaviva, A.; et al. Comparative Investigation of Composition, Antifungal, and Anti-Inflammatory Effects of the Essential Oil from Three Industrial Hemp Varieties from Italian Cultivation. *Antibiotics* **2021**, *10*, 334. [CrossRef]
11. Bassolino, L.; Fulvio, F.; Cerrato, A.; Citti, C.; Cannazza, G.; Capriotti, A.L.; Alberti, I.; Terracciano, I.; Pecchioni, N.; Paris, R. Metabolic characterization and transcriptional profiling of polyphenols in *Cannabis sativa* L. inflorescences with different chemical phenotypes. *Planta* **2024**, *260*, 76. [CrossRef]
12. Rehman, M.; Fahad, S.; Du, G.; Cheng, X.; Yang, Y.; Tang, K.; Liu, L.; Liu, F.-H.; Deng, G. Evaluation of hemp (*Cannabis sativa* L.) as an industrial crop: A review. *Environ. Sci. Pollut. Res.* **2021**, *28*, 52832–52843. [CrossRef]
13. Stasiłowicz-Krzemień, A.; Sip, S.; Szulc, P.; Cielecka-Piontek, J. Determining Antioxidant Activity of *Cannabis* Leaves Extracts from Different Varieties—Unveiling Nature's Treasure Trove. *Antioxidants* **2023**, *12*, 1390. [CrossRef]
14. Pieracci, Y.; Ascrizzi, R.; Terreni, V.; Pistelli, L.; Flamini, G.; Bassolino, L.; Fulvio, F.; Montanari, M.; Paris, R. Essential Oil of *Cannabis sativa* L: Comparison of Yield and Chemical Composition of 11 Hemp Genotypes. *Molecules* **2021**, *26*, 4080. [CrossRef]
15. Fiorini, D.; Molle, A.; Nabissi, M.; Santini, G.; Benelli, G.; Maggi, F. Valorizing industrial hemp (*Cannabis sativa* L.) by-products: Cannabidiol enrichment in the inflorescence essential oil optimizing sample pre-treatment prior to distillation. *Ind. Crops Prod.* **2019**, *128*, 581–589. [CrossRef]

16. Benelli, G.; Pavela, R.; Petrelli, R.; Cappellacci, L.; Santini, G.; Fiorini, D.; Sut, S.; Dall'Acqua, S.; Canale, A.; Maggi, F. The essential oil from industrial hemp (*Cannabis sativa* L.) by-products as an effective tool for insect pest management in organic crops. *Ind. Crops Prod.* **2018**, *122*, 308–315. [CrossRef]
17. Ascrizzi, R.; Iannone, M.; Cinque, G.; Marianelli, A.; Pistelli, L.; Flamini, G. "Hemping" the drinks: Aromatizing alcoholic beverages with a blend of *Cannabis sativa* L. flowers. *Food Chem.* **2020**, *325*, 126909. [CrossRef] [PubMed]
18. Pellegrini, M.; Palmieri, S.; Ricci, A.; Serio, A.; Paparella, A.; Lo Sterzo, C. In vitro antioxidant and antimicrobial activity of *Cannabis sativa* L. cv 'Futura 75' essential oil. *Nat. Prod. Res.* **2021**, *35*, 6020–6024. [CrossRef]
19. Majidiyan, N.; Hadidi, M.; Azadikhah, D.; Moreno, A. Protein complex nanoparticles reinforced with industrial hemp essential oil: Characterization and application for shelf-life extension of Rainbow trout fillets. *Food Chem. X* **2022**, *13*, 100202. [CrossRef]
20. Bicchi, C.; Maffei, M. The Plant Volatilome: Methods of Analysis. In *High-Throughput Phenotyping in Plants: Methods and Protocols, Methods in Molecular Biology*; Normanly, J., Ed.; Methods in Molecular Biology; Humana Press: Totowa, NJ, USA, 2012; Volume 918, pp. 351–362. ISBN 978-1-61779-994-5.
21. Moghaddam, M.; Mehdizadeh, L. Chemistry of Essential Oils and Factors Influencing Their Constituents. In *Soft Chemistry and Food Fermentation*; Elsevier: Amsterdam, The Netherlands, 2017; pp. 379–419. ISBN 9780128114124.
22. Pieracci, Y.; Fulvio, F.; Isca, V.; Pistelli, L.; Bassolino, L.; Montanari, M.; Moschella, A.; Flamini, G.; Paris, R. The phenological stage of hemp inflorescences affects essential oil yield and its chemical composition. *Ind. Crops Prod.* **2023**, *197*, 116605. [CrossRef]
23. Cerrato, A.; Citti, C.; Cannazza, G.; Capriotti, A.L.; Cavaliere, C.; Grassi, G.; Marini, F.; Montone, C.M.; Paris, R.; Piovesana, S.; et al. Phytocannabinomics: Untargeted metabolomics as a tool for *Cannabis* chemovar differentiation. *Talanta* **2021**, *230*, 122313. [CrossRef]
24. Fulvio, F.; Mandolino, G.; Citti, C.; Pecchioni, N.; Cannazza, G.; Paris, R. Phytocannabinoids biosynthesis during early stages of development of young *Cannabis sativa* L. seedlings: Integrating biochemical and transcription data. *Phytochemistry* **2023**, *214*, 113793. [CrossRef]
25. Ferri, E.; Russo, F.; Vandelli, M.A.; Paris, R.; Laganà, A.; Capriotti, A.L.; Gallo, A.; Siciliano, A.; Carbone, L.; Gigli, G.; et al. Analysis of phytocannabinoids in hemp seeds, sprouts and microgreens. *J. Pharm. Biomed. Anal.* **2024**, *245*, 116181. [CrossRef]
26. Wogiatzi, E.; Gougoulias, N.; Giannoulis, K.D.; Kamvoukou, C.-A. Effect of Irrigation and Fertilization Levels on Mineral Composition of *Cannabis sativa* L. Leaves. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2019**, *47*, 1073–1080. [CrossRef]
27. Kleinhenz, M.D.; Magnin, G.; Ensley, S.M.; Griffin, J.J.; Goeser, J.; Lynch, E.; Coetzee, J.F. Nutrient concentrations, digestibility, and cannabinoid concentrations of industrial hemp plant components. *Appl. Anim. Sci.* **2020**, *36*, 489–494. [CrossRef]
28. Farinon, B.; Molinari, R.; Costantini, L.; Merendino, N. The Seed of Industrial Hemp (*Cannabis sativa* L.): Nutritional Quality and Potential Functionality for Human Health and Nutrition. *Nutrients* **2020**, *12*, 1935. [CrossRef] [PubMed]
29. Visković, J.; Zheljazkov, V.D.; Sikora, V.; Noller, J.; Latković, D.; Ocamb, C.M.; Koren, A. Industrial Hemp (*Cannabis sativa* L.) Agronomy and Utilization: A Review. *Agronomy* **2023**, *13*, 931. [CrossRef]
30. Lanzoni, D.; Mercogliano, F.; Rebucci, R.; Bani, C.; Pinotti, L.; Di Lorenzo, C.; Savoini, G.; Baldi, A.; Giromini, C. Phenolic profile and antioxidant activity of hemp co-products following green chemical extraction and ex vivo digestion. *Ital. J. Anim. Sci.* **2024**, *23*, 651–663. [CrossRef]
31. Mishchenko, S.; Mokher, J.; Laiko, I.; Burbulis, N.; Kyrychenko, H.; Dudukova, S. Phenological growth stages of hemp (Cannabis sativa L.): Codification and description according to the BBCH scale. *Žemės Ūkio Moksl.* **2017**, *24*, 31–36. [CrossRef]
32. Kirk, P.L. Kjeldahl Method for Total Nitrogen. *Anal. Chem.* **1950**, *22*, 354–358. [CrossRef]
33. Lichtenthaler, H.K. Chlorophylls and Carotenoids: Pigments of Photosynthetic Biomembranes. *Methods Enzymol.* **1987**, *148*, 350–382. [CrossRef]
34. Bretzel, F.; Benvenuti, S.; Pistelli, L. Metal contamination in urban street sediment in Pisa (Italy) can affect the production of antioxidant metabolites in *Taraxacum officinale* Weber. *Environ. Sci. Pollut. Res.* **2014**, *21*, 2325–2333. [CrossRef]
35. Singleton, V.L.; Rossi, J.A.J. Colorimetry to total phenolics with phosphomolybdic acid reagents. *Am. J. Enol. Vinic.* **1965**, *16*, 144–158. [CrossRef]
36. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]
37. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
38. Christine, E.A.; Albert, Y.-K.; Séraphin, K.-C. Determination of the Minerals of the Herbal Tea and Tea Green from *Lippia multiflora*. *Am. J. Plant Sci.* **2017**, *08*, 2608–2621. [CrossRef]
39. Olivier, J.; Symington, E.A.; Jonker, C.Z.; Rampedi, I.T.; Van Eeden, T.S. Comparison of the mineral composition of leaves and infusions of traditional and herbal teas. *S. Afr. J. Sci.* **2012**, *108*, 1–7. [CrossRef]
40. Dayanandan, P.; Kaufman, P.B. Trichomes of *Cannabis sativa* L. (Cannabaceae). *Am. J. Bot.* **1976**, *63*, 578. [CrossRef]
41. Šesták, Z. Changes in the chlorophyll content as related to photosynthetic activity and age of leaves. *Photochem. Photobiol.* **1963**, *2*, 101–110. [CrossRef]

42. Brown, N.; John, J.A.; Shahidi, F. Polyphenol composition and antioxidant potential of mint leaves. *Food Prod. Process. Nutr.* **2019**, *1*, 1. [CrossRef]
43. Rastogi, S.; Farswan, T.S.; Pandey, M.M. Seasonal variation in the phytoconstituents and antioxidant activity in *Moringa oleifera* Lam. leaves of North India. *S. Afr. J. Bot.* **2024**, *166*, 492–502. [CrossRef]
44. Atak, M.; Yılmaz Kutlu, E.; Çavuş, D.; Çomoğlu, M. Handmade green tea: Antioxidant content and activity. *Appl. Food Res.* **2024**, *4*, 100626. [CrossRef]
45. Flórez, M.; Cazón, P.; Vázquez, M. Antioxidant Extracts of Nettle (*Urtica dioica*) Leaves: Evaluation of Extraction Techniques and Solvents. *Molecules* **2022**, *27*, 6015. [CrossRef]
46. Sarker, U.; Oba, S.; Daramy, M.A. Nutrients, minerals, antioxidant pigments and phytochemicals, and antioxidant capacity of the leaves of stem amaranth. *Sci. Rep.* **2020**, *10*, 3892. [CrossRef]
47. BLOIS, M.S. Antioxidant Determinations by the Use of a Stable Free Radical. *Nature* **1958**, *181*, 1199–1200. [CrossRef]
48. Plank, D.W.; Szpylka, J.; Sapirstein, H.; Woollard, D.; Zapf, C.M.; Lee, V.; Chen, C.-Y.O.; Liu, R.H.; Tsao, R.; Düsterloh, A.; et al. Determination of Antioxidant Activity in Foods and Beverages by Reaction with 2,2′-Diphenyl-1-Picrylhydrazyl (DPPH): Collaborative Study First Action 2012.04. *J. AOAC Int.* **2012**, *95*, 1562–1569. [CrossRef] [PubMed]
49. Beleggia, R.; Menga, V.; Fulvio, F.; Fares, C.; Trono, D. Effect of Genotype, Year, and Their Interaction on the Accumulation of Bioactive Compounds and the Antioxidant Activity in Industrial Hemp (*Cannabis sativa* L.) Inflorescences. *Int. J. Mol. Sci.* **2023**, *24*, 8969. [CrossRef] [PubMed]
50. Pieracci, Y.; Pistelli, L.; Lari, M.; Iannone, M.; Marianelli, A.; Ascrizzi, R.; Pistelli, L.; Flamini, G. *Hibiscus rosa-sinensis* as Flavoring Agent for Alcoholic Beverages. *Appl. Sci.* **2021**, *11*, 9864. [CrossRef]
51. Platzer, M.; Kiese, S.; Herfellner, T.; Schweiggert-Weisz, U.; Miesbauer, O.; Eisner, P. Common Trends and Differences in Antioxidant Activity Analysis of Phenolic Substances Using Single Electron Transfer Based Assays. *Molecules* **2021**, *26*, 1244. [CrossRef]
52. Guimarães, A.C.; Meireles, L.M.; Lemos, M.F.; Guimarães, M.C.C.; Endringer, D.C.; Fronza, M.; Scherer, R. Antibacterial Activity of Terpenes and Terpenoids Present in Essential Oils. *Molecules* **2019**, *24*, 2471. [CrossRef]
53. Bassolé, I.H.N.; Juliani, H.R. Essential Oils in Combination and Their Antimicrobial Properties. *Molecules* **2012**, *17*, 3989–4006. [CrossRef]
54. Wojtunik-Kulesza, K.A.; Cieśla, Ł.M.; Waksmundzka-Hajnos, M. Approach to Determination a Structure—Antioxidant Activity Relationship of Selected Common Terpenoids Evaluated by ABTS + Radical Cation Assay. *Nat. Prod. Commun.* **2018**, *13*, 295–298. [CrossRef]