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# Cultivation of Potted Sea Fennel, an Emerging Mediterranean Halophyte, Using a Renewable Seaweed-Based Material as a Peat Substitute

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**Abstract:** Sea fennel (*Crithmum maritimum* L.), an emerging halophyte species, represents a nutritious and refined food product. In this study, the effect on yield and quality of potted sea fennel grown on three posidonia (*Posidonia oceanica* (L.) Delile)-based composts (a municipal organic solid waste compost, a sewage sludge compost and a green compost) and a peat-based substrate was analyzed. Composts were used both pure and mixed with peat at a dose of 50% on a volume basis. We hypothesized that the halophytic nature of this plant might overcome the limitations of high-salinity compost-based growing media. The growth parameters, color traits and trace metals content (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) of the edible parts were compared. Independently of the substrates, the average total and edible yields were 51 and 30 g plant<sup>-1</sup>, respectively, while the average waste portion was about 41%. The use of posidonia-based compost did not affect the color traits of sea fennel plants as compared with samples grown on the commercial peat-based substrate. In general, potted sea fennel grown on both posidonia-based composts and commercial peat-based substrate appeared a good source of essential micronutrients. Only a weak reduction of Fe and Mn concentrations was observed in plants grown on posidonia-based composts, especially when used at the highest dose. Independently of the growing medium, the content of potentially hazardous trace elements (Cd and Pb) in the edible parts of sea fennel was always below the maximum admissible limits fixed by the European legislation. Results indicate that posidonia-based composts can be used as a sustainable peat substitute for the formulation of soilless mixtures to grow potted sea fennel plants, even up to a complete peat replacement.

**Keywords:** *Crithmum maritimum* L.; domestication; food safety; heavy metal; *Posidonia oceanica* (L.) Delile; growing substrate

## 1. Introduction

Sea fennel (*Crithmum maritimum* L.) is a halophyte species belonging to the *Apiaceae* family, also known as crest marine, marine fennel and rock samphire because of its habit to grow as a wild plant on maritime rocks, breakwaters and sandy beaches. It is used as an ingredient of many dishes for its interesting sensory attributes like taste, odor and color [1,2]. The fresh leaves can be used to prepare salads, soups and sauces, or they are pickled in vinegar similarly to capers; this latter food preparation is listed as an item in the ‘List of Traditional Agri-Food Products’ of the Italian

Department for Agriculture [2], confirming the typical use of this plant in the Mediterranean tradition. However, also in British Isles, “Rock Samphire Hash” is a traditional recipe based on stems and leaves of *C. maritimum* L., mixed with pickled cucumbers and capers. Apart from the food use, sea fennel has been largely considered also for its nutritional and healthy properties [3]. In ancient times, this plant was used in traditional medicine for its stimulating, diuretic and vermifuge effects. In Italy, the sea fennel decoction was used against cystitis, prostatitis and colics, while the infusion was used in case of digestive diseases [4]. Despite the above mentioned notable characteristics, and the recent general interest for the recovery of traditional uses of plants, this species is currently considered an underutilized crop for commercial cultivation. Some Authors highlight benefits from cultivation and food use of a wide availability of genetic resources like wild edible plants, especially for their potential beneficial elements content [5–7]. To our best knowledge, the literature lacks information with regard to the sea fennel as a potential source of mineral elements in the daily diet. Moreover, only few information is available in the literature with regard to domestication and technical aspects of sea fennel growing techniques. Therefore, more information is needed in the view of a large-scale production of sea fennel, aimed to the optimal crop performance and, ultimately, to a sustainable exploitation of this emerging halophyte [2].

Like several other herbs (basil, parsley, chives), sea fennel may be suitable to be grown and potentially marketed as potted plant. In general, commercial production of potted plants implies the use of growing substrates other than real soil (soilless cultivation).

In southern Europe, the most used component for potting soilless substrates is peat, because of its good chemical and physical properties. However, peat use is becoming more and more problematic for a number of reasons, mainly related to its high costs (especially in Mediterranean countries, where peat is imported from extraction areas in Northern and Central Europe), and recently to the environmental concerns on peat extraction. Peat is a non-renewable material and its extraction can degrade wetland ecosystems, so that European policy strongly encourages the use of peat alternatives [8,9]. A recent EU Commission decision states that growing media (including soil improvers) containing peat material cannot receive the European Union “eco-label”, thus encouraging the use of organic matter derived from the processing and/or reuse of wastes [10]. Among the organic materials proposed as alternatives to peat, different organic sources (e.g., municipal sewage sludge, organic fraction of urban solid wastes, food industry and wood processing residues and byproducts, agricultural residues) subjected to appropriate stabilization treatments, such as composting, are arising interest as components of soilless growing substrates. A number of experimental evidences demonstrate that, if properly formulated, compost-based growing substrates may offer interesting possibilities for the reduction of peat use, although it is generally recommended not to exceed a 50% rate of compost in the mixture, in order to avoid a reduction of the crop performance due to reduced plant growth or possible phytotoxicity problems [11]. On the other hand, several investigations focused on the possible risks for human health of potentially toxic elements (PTEs) accumulation in vegetables, associated with the use of compost-based growing substrates [12,13].

*Posidonia* (*Posidonia oceanica* (L.) Delile) is the most important endemic marine phanerogam in the Mediterranean Sea [14]. As a part of its natural life cycle, this plant loses its senescent parts, which accumulate in large quantities along the coasts, often originating problems related to the management of this organic material. Composting may be a viable alternative to landfill disposal for this material [15]. The *posidonia*-based compost has been proposed as a promising soilless growing media component [16]. *Posidonia* compost-based substrates have been successfully tested to produce tomato [17–19], lettuce [12,20], potted basil [13], and to grow transplant seedlings of lettuce [21], melon and tomato [22]. However, no information is available about the use of *posidonia*-based composts as growing substrate for potted sea fennel cultivation.

Moving from these considerations, the objective of the present study was to ascertain the potential use of *posidonia* compost-based substrates for potted sea fennel production. We focused on: (i) the evaluation of the yield and quality of sea fennel grown on different *posidonia*-based compost soilless

substrates, using a commercial peat-based substrate as a reference; and (ii) the accumulation of PTEs in the edible parts of the plant.

## 2. Materials and Methods

### 2.1. Starting Materials for Growing Media Mixtures

A commercial peat-based substrate (**P**) and three different compost-based materials (**Gr C**, **SS C** and **MOW C**) containing posidonia residues in their formulation, were used as components for the preparation of the growing media tested in the present study (see Section 2.2 for a detailed description of the mixtures). '**P**' was a commercial horticultural substrate based on a mix of different peats enriched with 1 kg m<sup>-3</sup> PG-MIX 14-16-18 fertilizer (Brill Type 3 Special). '**MOW C**', hereafter referred to as municipal organic solid waste compost was obtained by mixing posidonia residues (≈16% on a fresh weight basis), organic fraction of municipal solid wastes (≈29%), urban green wastes (≈27%), and agro-industrial sludge from tomato, grape and olive processing (28%). '**SS C**', hereafter referred to as sewage sludge compost, was obtained by mixing posidonia residues (≈16%), urban sewage sludge (≈29%), urban green wastes (pruning and yard trimming residues) (≈27%), and agro-industrial sludge (28%). '**Gr C**', hereafter referred to as green compost, was obtained by mixing posidonia residues (≈20% of the composting pile, on a fresh weight basis), green residues resulting from a greenhouse tomato cultivation (≈40%), and pruning residues of an olive grove (≈40%). Composts were produced in a local industrial plant according to the Italian regulations on compost production. The production process was carried out in piles over three months. Piles were mechanically turned in accordance with temperature evolution. The principal chemical features of the materials are reported in Table 1.

### 2.2. Plant Material and Experimental Conditions

The trial was conducted in a polymethacrylate greenhouse located in Mola di Bari (Bari, Italy) at 'La Noria' experimental farm of the CNR-ISPA (17°04' E, 41°03' N, 24 m a.s.l.). Average daily mean, minimum and maximum air temperatures inside the greenhouse over the experiment were 19.2, 11.9 and 29.7 °C, respectively. Average daily mean, minimum and maximum air relative humidity values were 65, 36 and 87%, respectively.

Seven mixtures were prepared starting from the above described materials, and tested as growing media. Each of the posidonia-based composts (Gr C, SS C and MOW C) was used both pure, at a rate of 100%, and mixed with P at rate of 50% on a volume basis. A control treatment, consisting only of pure peat-based commercial substrate (P), was also included. The resulting seven treatments under comparison in the study were the following: (i) P, (ii) MOW C, (iii) MOW C + P, (iv) SS C, (v) SS C + P, (vi) Gr C, (vii) Gr C + P. The electrical conductivity (EC) and pH of the seven substrates, both measured on the 1:10 (*w/v*) aqueous extracts, were: P (EC = 0.3 dS m<sup>-1</sup>; pH = 6.8); MOW C (EC = 2.9 dS m<sup>-1</sup>; pH = 7.8), MOW C + P (EC = 1.5 dS m<sup>-1</sup>; pH = 7.5), SS C (EC = 2.3 dS m<sup>-1</sup>; pH = 7.6), SS C + P (EC = 1.6 dS m<sup>-1</sup>; pH = 7.5), Gr C (EC = 1.7 dS m<sup>-1</sup>; pH = 8.3), Gr C + P (EC = 1.1 dS m<sup>-1</sup>; pH = 7.4).

Seeds of *C. maritimum* L. were harvested from wild plants along the shoreline in Mola di Bari. Ascorbic acid at 40 mM was used as pre-treatment to improve sea fennel seed germination, according to the procedure described by Meot-Duros and Magné [23]. The seedlings were produced in polystyrene plug trays (160 cells per tray with diameter of 2.5 cm and volume of 21 mL) filled with peat. The seeds, three per cell, were covered with vermiculite. Seven days after emergence, the number of plants was reduced to one seedling per cell. After growing in conventional plug trays for 30 days, seedlings were transferred into 10 cm diameter plastic containers (0.5 L) filled with each of the seven growing media. The pots were placed on benches and grown in a closed cycle ebb and flow hydroponic system. The experimental treatments were organized in a fully randomized design with three replications for each treatment, each replication constituted by nine pots, for a total of 189 pots in the experiment (27 per treatment).

**Table 1.** Main chemical features of the materials used for the preparation of the growing media mixtures.

	C/N	P	Ca	K	Mg	Na	Fe	Cu	Mn	Ni	Zn	B	Cd	Cr	Cr(VI)	Pb	Hg
	Ratio	g kg <sup>-1</sup> DW										mg kg <sup>-1</sup> DW					
<b>P</b> <sup>1</sup>	-	1.41	22.0	1.27	1.59	0.28	1.13	6.7	72	5.0	9	14	0.07	1.4	-	2.6	-
<b>MOW C</b>	11.6	4.23	17.6	7.58	1.92	1.21	2.58	52.9	156	4.4	120	24	<0.50	6.8	<0.50	7.3	<0.50
<b>SS C</b>	12.5	5.08	71.0	5.14	3.18	1.19	5.97	89.7	235	11.5	170	94	<0.50	18.9	<0.50	18.8	<0.50
<b>Gr C</b>	11.8	0.75	16.6	1.39	1.91	1.49	1.31	8.9	29	3.9	43	158	<0.50	1.4	<0.50	2.2	<0.50
<b>Maximum Limits According to Regulations</b>																	
<b>Italy</b> <sup>2</sup>	25	-	-	-	-	-	-	230		100	500		1.5	-	0.5	140	1.5
<b>European Union</b> <sup>3</sup>	-	-	-	-	-	-	-	100		50	300		1.0	100	-	100	1.0

P: Peat-based commercial substrate; MOW C: Municipal organic solid waste compost; SS C: Sewage sludge compost; Gr C: Green compost. <sup>1</sup> Adapted from Mininni et al. [13]. <sup>2</sup> Italian directive on fertilizers (D.L. 75/2010, Annex 2). <sup>3</sup> Ecological criteria for EU eco-label [10].

Plants were grown for 60 days with nutrient solution (NS) containing N (140 mg L<sup>-1</sup>, NO<sub>3</sub>-N:NH<sub>4</sub>-N 80:20), P (50 mg L<sup>-1</sup>), K (200 mg L<sup>-1</sup>), Mg (40 mg L<sup>-1</sup>) and Ca (100 mg L<sup>-1</sup>), as macronutrients, whereas micronutrients were supplied according to Johnson et al. [24]. The NS pH was adjusted to 5.5–6.0 using 1 M H<sub>2</sub>SO<sub>4</sub>. The well water used to prepare the NS had the following characteristics: pH 7.1, EC 1.1 dS m<sup>-1</sup>, 1.0 M Ca<sup>2+</sup>, 0.4 M Mg<sup>2+</sup>, 6.0 M Na<sup>+</sup> and 5.7 M Cl<sup>-</sup>. The chemical composition of well water was taken into account for the preparation of the NS. Benches were flooded with the NS three times per day. The NS level in the benches was raised up to about 3 cm from the bottom of the pots, allowing the substrates to absorb the NS for about 10 min per irrigation event before the NS was discharged and collected for subsequent irrigations.

### 2.3. Plant Growth and Color Parameters Measurements

At harvest, yield and leaf area were measured on six plants per replicate. Yield was expressed in terms of both total and edible (after the removal of waste portion) fresh weight of shoots per plant. The waste portion consisted of older leaves and stems that are generally removed during the normal food use, and was expressed as percentage of the total shoots biomass. Leaf area was measured with a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA). For each sample, a portion of the plant material was dried in a forced draft oven at 105 °C until reaching a constant weight for the determination of the dry matter content, which was expressed as g 100 g<sup>-1</sup> fresh weight (FW).

Color measurements were performed using a colorimeter (CR-400, Konica Minolta, Osaka, Japan) equipped with illuminant D65, operating in reflectance mode and with the CIE *L* (lightness) *a*<sup>\*</sup> (redness) *b*<sup>\*</sup> (yellowness) color scale, according to the procedure described by Montesano et al. [25]. The colorimeter was preliminary calibrated with a standard reference material characterized by *L*, *a*<sup>\*</sup> and *b*<sup>\*</sup> values of 97.55, 0.52 and 1.45, respectively. Hue angle ( $h^\circ = \arctg b^*/a^*$ ) and saturation ( $C = [a^{*2} + b^{*2}]^{1/2}$ ) were then calculated from primary *L*, *a*<sup>\*</sup> and *b*<sup>\*</sup> readings.

### 2.4. Elemental Analysis

A representative aliquot of each plant sample was oven-dried at 65 °C and finely ground using a cutting-grinding mill (IKA MF 10B, Labortechnik, Staufen, Germany), then a subsample of 0.3 g was pre-digested overnight with 7 mL HNO<sub>3</sub> (69%) and 1 mL H<sub>2</sub>O<sub>2</sub> (30%) (TraceSELECT<sup>®</sup>, trace analysis reagents, Sigma Aldrich, St. Louis, MO, USA) in a PTFE-TFM liner. Sample digestion was performed using a microwave oven (Multiwave 3000, Anton Paar, Graz, Austria), according to the procedure described by Gattullo et al. [12]. Digested samples were diluted with deionised water up to 25 mL, then filtered through Whatman<sup>®</sup> 42 filter paper and stored at 4 °C until analysis. All glassware was cleaned using 5% HNO<sub>3</sub> solution, and then abundantly rinsed with deionised water. Total concentrations of Fe, Mn, Cu, Zn, Cd, Cr, Co, Ni and Pb were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; Thermo iCAP 6000 series, Thermo Fisher Scientific Inc., Waltham, MA, USA), following the method described by Gattullo et al. [12]. A two-point calibration was adopted using the blank (HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> 7:1, v/v) as zero point, and a multi-element calibration standard (Certipur<sup>®</sup> ICP Multi-element standard solution IV; Merck Millipore, Burlington, MA, USA) at the concentration of 2 mg L<sup>-1</sup> (prepared in the blank acidic solution). Instrument detection limits were calculated for each element as three times the standard deviation of ten replicates of the blank.

### 2.5. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA). Treatment means were separated by LSD test when there was a significant effect at the  $p < 0.05$  level. The statistical software STATISTICA 10.0 (StatSoft, Tulsa, OK, USA) was used for the analysis.

### 3. Results and Discussion

#### 3.1. Yield, Leaf Area, Dry Matter and Color Parameters

The average total and edible yields were 51 and 30 g plant<sup>-1</sup>, respectively, while the average waste portion was about 41%, without any significant difference between the growing substrates under comparison (Table 2). However, although not statistically significant, a certain decrease in yield parameters was observed in MOW C treatment. No significant differences were found between the substrates also for the leaf area and dry matter content, with an average value of 296 cm<sup>2</sup> plant<sup>-1</sup> and 10.7 g 100 g<sup>-1</sup> FW, respectively (Table 2). No visual symptoms of nutritional deficiencies or toxicities were observed on plants during the overall growing cycle. These results are in agreement with Parente et al. [16], and reveal that posidonia-based composts may be suitable materials for the formulation of soilless mixtures to grow containerized sea fennel plants, without any negative effect on growth parameters. A similar growth response was obtained using composts both at 100% and 50% rate in mixture with peat (Table 2), in contrast with the general recommendation to not exceed a 50% rate [21,22]. One of the main problems related to the use of composts is generally their high salt content, as outlined also for posidonia-based composts [18]. Indeed, the composts used in this study presented a higher salt content in comparison with the traditional peat-based commercial substrate. Despite of this, the three composts did not reduce sea fennel growth even when used at the higher dose (Table 2), possibly because of the halophytic behavior of this plant species [26]. This result further supports the use of posidonia-based composts as a potential peat substitute for the containerized sea fennel production, even up to a complete peat replacement. By considering the general interest on alternative soilless materials, and the particular attention devoted to organic materials derived from waste streams [27], the findings of this study may represent an important scientific background to develop a sustainable sea fennel production. In particular, the use of composted posidonia residues as a renewable, low-cost and locally available material, is especially feasible in areas where sea fennel cultivation may represent an opportunity for growers, as the coastal areas of Mediterranean countries [16]. Moreover, the sustainability of the cultivation process is further increased by using the ebb and flow technique, a typical soilless closed-cycle subirrigation system, which prevent pollution, while reducing water and fertilizer use [28]. Among the different soilless systems based on the closed-cycle management of the NS, subirrigation for containerized crops has been recognized as a practical and cost-effective method to achieve efficient water use in the Mediterranean greenhouse industry, due to the relative simplicity of the subirrigation techniques [29].

**Table 2.** Total shoot fresh weight (FW), edible yield, waste portion, leaf area and dry matter of sea fennel grown on seven substrates composed of peat and three different composts containing posidonia residues. A peat-based commercial substrate was used as control. The compost materials were tested alone or in mixture with peat (at a rate of 50% on a volume basis).

	Total FW	Edible Yield	Waste Portion	Leaf Area	Dry Matter
		g plant <sup>-1</sup>		cm <sup>2</sup> plant <sup>-1</sup>	g 100 g <sup>-1</sup> FW
<b>P</b>	58.6 ± 9.8	35.1 ± 6.0	40.0 ± 3.1	343 ± 59	11.4 ± 1.1
<b>MOW C</b>	34.5 ± 13.0	21.0 ± 7.4	38.4 ± 3.1	216 ± 8	10.5 ± 0.3
<b>MOW C + P</b>	47.5 ± 5.6	27.2 ± 4.9	42.9 ± 3.5	278 ± 32	10.7 ± 0.8
<b>SS C</b>	45.0 ± 3.6	26.3 ± 2.5	41.6 ± 3.5	258 ± 36	10.7 ± 0.9
<b>SS C + P</b>	54.3 ± 9.8	31.5 ± 6.1	40.6 ± 6.9	302 ± 70	10.3 ± 0.6
<b>Gr C</b>	54.5 ± 15.2	30.4 ± 6.7	43.5 ± 4.2	307 ± 70	10.2 ± 0.8
<b>Gr C + P</b>	61.9 ± 7.5	36.4 ± 5.3	41.3 ± 2.7	369 ± 37	11.0 ± 1.4
<b>Significance</b>	NS	NS	NS	NS	NS

P: Peat-based commercial substrate; MOW C: Municipal organic solid waste compost; SS C: Sewage sludge compost; Gr C: Green compost. NS = not significant. Values are the mean of three replications (six subsamples per replication), ± standard deviation.



On the basis of the shoots dry matter, the total average sea fennel biomass production was about 5.4 g dry weight (DW) plant<sup>-1</sup> (Table 2). These results are in agreement with Hamed et al. (2004) who reported a potted sea fennel biomass production between 1 and 13 g DW plant<sup>-1</sup> and a leaf area between about 100 and more than 600 cm<sup>2</sup> plant<sup>-1</sup>, at NaCl concentration in the NS ranging from 0 to 300 mM. A lower sea fennel biomass production, however, was found in other studies, with values ranging from 2.1 to 3.0 g DW plant<sup>-1</sup> [26,29].

As regard the color analysis of the sea fennel leaves, no significant differences were found between the different treatments (Table 3) with an average *L* (lightness), *a*<sup>\*</sup> (redness), *b*<sup>\*</sup> (yellowness), *h*<sup>°</sup> (Hue angle) and *C* (saturation) values of 41.5, −11.5, 16.2, 125.3 and 19.9, respectively. This indicates that the color of sea fennel grown on different posidonia-based composts is similar to that obtained by using commercial peat-based substrates. These results are in agreement with Renna et al. [2], who found similar color parameters on wild sea fennel collected along sea shoreline, which is the natural habitat of this species. Color is among the first quality parameters catching the attention of consumers, and exerts a strong influence on consumers' choice and opinion about the food quality [30]. Moreover, color is one of the most important quality traits of the sea fennel [1,2].

**Table 3.** Color parameters [*L* (lightness), *a*<sup>\*</sup> (redness), *b*<sup>\*</sup> (yellowness), *h*<sup>°</sup> (Hue angle) and *C* (saturation)] of sea fennel grown on seven substrates composed of peat and three different composts containing posidonia residues. A peat-based commercial substrate was used as control. The compost materials were tested alone or in mixture with peat (at a rate of 50% on a volume basis).

	<i>L</i>	<i>a</i> <sup>*</sup>	<i>b</i> <sup>*</sup>	<i>h</i> <sup>°</sup>	<i>C</i>
<b>P</b>	41.8 ± 1.2	−11.4 ± 0.5	16.2 ± 0.8	125.3 ± 0.2	19.8 ± 0.9
<b>MOW C</b>	41.7 ± 0.8	−11.6 ± 0.1	16.3 ± 0.4	125.2 ± 0.4	19.9 ± 0.3
<b>MOW C + P</b>	41.4 ± 0.6	−11.6 ± 0.3	16.5 ± 0.4	125.1 ± 0.2	20.1 ± 0.6
<b>SS C</b>	41.3 ± 0.5	−11.5 ± 0.4	16.3 ± 0.6	125.2 ± 0.4	19.9 ± 0.7
<b>SS C + P</b>	41.5 ± 0.6	−11.2 ± 0.5	15.9 ± 0.2	125.2 ± 0.8	19.5 ± 0.5
<b>Gr C</b>	41.1 ± 0.3	−11.7 ± 0.4	16.4 ± 0.7	125.5 ± 0.5	20.2 ± 0.8
<b>Gr C + P</b>	41.7 ± 1.2	−11.5 ± 0.2	16.2 ± 0.6	125.4 ± 0.6	19.8 ± 0.6
Significance	NS	NS	NS	NS	NS

P: Peat-based commercial substrate; MOW C: Municipal organic solid waste compost; SS C: Sewage sludge compost; Gr C: Green compost. NS = not significant. Values are the mean of three replications (six subsamples per replication), ± standard deviation.

### 3.2. Trace Metals Content

Table 4 shows the concentration of some trace metal elements in potted sea fennel grown on different substrates. Cadmium, Co, Ni and Pb concentrations in plant tissues were below the instrument detection limits of 0.002 (Cd), 0.015 (Co), 0.193 (Ni) and 0.063 (Pb) mg kg<sup>-1</sup> FW (data not shown). Among these elements, Cd and Pb are considered highly toxic to humans, therefore the European Commission fixed the maximum admissible concentration in vegetables at 0.1 and 0.05 mg kg<sup>-1</sup> FW for Pb and Cd, respectively [10]. Cadmium intake through food can disturb the activity of several enzymes and proteins due to Cd inactivation of sulfhydryl groups, and cause kidney and liver dysfunction, as well as osteoporosis and other pathologies [31,32]. Lead is a neurotoxic element, and its excess in human body may also induce cardiovascular problems, nephrotoxicity, as well as carcinogenicity and genotoxicity [32,33]. In this study, the levels of Cd and Pb in sea fennel plants grown on posidonia-based compost substrates were below the limits imposed by the legislation and therefore safe for human consumption. In fact, the three composts used for sea fennel growth complied with the maximum limits fixed by the European and Italian legislation for PTEs in fertilizers (Table 1) and therefore, in this respect, they were safe for plant growth.

**Table 4.** Trace metals tissue concentration of sea fennel grown on seven substrates composed of peat and three different composts containing posidonia residues. A peat-based commercial substrate was used as control. The compost materials were tested alone or in mixture with peat (at a rate of 50% on a volume basis).

	Cr	Cu	Fe	Mn	Zn
	mg kg <sup>-1</sup> DW				
P	0.22 ± 0.05	2.33 ± 0.43	57.05 ± 11.29 a	26.07 ± 3.38 a	13.40 ± 3.24
MOW C	0.19 ± 0.03	2.77 ± 0.17	40.21 ± 3.31 b	18.11 ± 1.44 cd	17.62 ± 5.69
MOW C + P	0.17 ± 0.05	2.45 ± 0.87	49.50 ± 8.05 ab	21.49 ± 1.59 bc	12.69 ± 5.14
SS C	0.18 ± 0.05	2.92 ± 0.66	42.22 ± 2.75 b	18.73 ± 0.78 cd	15.58 ± 2.52
SS C + P	0.18 ± 0.02	2.72 ± 0.39	48.52 ± 1.11 ab	25.43 ± 0.76 ab	15.80 ± 2.06
Gr C	0.22 ± 0.08	2.52 ± 0.37	42.11 ± 2.92 b	23.83 ± 5.26 ab	16.59 ± 3.97
Gr C + P	0.18 ± 0.03	2.17 ± 0.30	45.58 ± 3.12 b	15.48 ± 1.21 d	15.64 ± 4.21
Significance	NS	NS	*	**	NS

P: Peat-based commercial substrate; MOW C: Municipal organic solid waste compost; SS C: Sewage sludge compost; Gr C: Green compost. \*, \*\* significant at  $p \leq 0.01$ , and  $p \leq 0.001$ , respectively; NS = not significant. Values are the mean of three replications (six subsamples per replication),  $\pm$  standard deviation. For each column different letters indicate statistically significant differences at  $p = 0.05$ .

The levels of total Cr in sea fennel plants were not influenced by the growing substrate (Table 4). Chromium is not an essential nutrient for plants, whereas it is an important micronutrient for humans, as it regulates the metabolism of glucose and lipids. Despite the lack of law restrictions for Cr content in vegetables, high concentrations of this element are not desired due to the carcinogenic effect of its hexavalent form [32]. In this study, the values of total Cr concentrations were similar to those reported for other vegetables grown on compost-based substrates, such as lettuce [12] and basil [13], or cultivated on soil [34]. Moreover, as reported in Table 1, Cr(VI) concentrations in the three composts were below the legislation limit, thus it can be expected that Cr was accumulated in plants as Cr(III), which is the non-toxic form.

With regard to micronutrients, the growing substrate influenced only the accumulation of Fe and Mn, whereas Cu and Zn were not affected (Table 4). Iron concentration in sea fennel grown on P was significantly higher with respect to plants grown on MOW C, SS C, Gr C and Gr C + P, but not significantly different from plants grown on MOW C + P and SS C + P (Table 4). As regard Mn, its concentration in sea fennel grown on P was significantly higher respect to all other treatments with the exception of SS C + P and Gr C treatments (Table 4). Similarly to our findings, a general higher concentration of Fe and Mn was observed in basil [13] and lettuce [12] grown on peat compared to plants grown on substrates containing posidonia-based composts. Despite the total Fe and Mn content in MOW C and SS C was much higher than in P (Table 1), the higher pH and salinity of the two compost-based substrates possibly reduced the uptake and accumulation of these nutrients by plants, compared to the control (P). However, when MOW C and SS C were used at 50% rate, the overall substrate salinity declined producing beneficial effects on element accumulation in plant shoots (with the exception of Mn for the treatment MOW C + P). This beneficial effect was not observed in plants grown on Gr C + P, possibly because the total content of Fe and Mn in Gr C was not high enough to balance the negative effect induced by pH and salinity.

### 3.3. Potential Human Intake of Essential Micronutrients

In recent years, there is a growing interest for the evaluation of mineral element content in vegetables due to their nutritional properties and health effects [35]. Some of these elements occur in human body at trace concentrations (milligrams or micrograms per kilogram of tissue) and are considered “essential” since a deficient intake may consistently result in an impairment of biological functions from optimal to suboptimal [36]. To the best of our knowledge, the literature lacks information with regard to sea fennel as a potential source of mineral elements in the daily diet.



Therefore, the intake of some essential trace elements for a serving size of potted sea fennel has been evaluated, like previously done for other domesticated and wild edible plants used to prepare dishes [5].

Regarding Cr, there is no formal Recommended Dietary Allowance (RDA). Nevertheless, the US Food and Nutrition Board [37] derived Adequate Intakes (AI) of 35 and 25  $\mu\text{g day}^{-1}$  for 19–50 year old men and women, respectively. For a serving size of 100 g FW, independently of the used growing media, a portion of sea fennel could supply, on average, 2.1  $\mu\text{g Cr}$  that is about the 6% and 8.4% of Cr daily AI for men and women, respectively. Anderson et al. [38] reported for other vegetables a Cr content between 0.4 and 1.6  $\mu\text{g } 100 \text{ g}^{-1}$  FW. Therefore, potted sea fennel may be considered a good source of this essential element.

The content of Cu in potted sea fennel was about 28  $\mu\text{g } 100 \text{ g}^{-1}$  FW, independently of the used growing media. Copper is an essential trace element for living organisms, including humans, being a vital component of several enzymes and proteins [39]. The results of the present study show a very low Cu content in potted sea fennel, considering its recommended dietary intake of 900  $\mu\text{g day}^{-1}$  [40].

Regarding Fe content, 100 g FW of sea fennel grown on P contains 0.64 mg of this element, supplying about 7% of the recommended dietary intake [37], while in all other cases a serving size contains, on average, 0.47 mg Fe  $100 \text{ g}^{-1}$  FW (about 5% of the recommended dietary intake), independently of the used growing media. Iron is an important trace element for human body, since it acts as oxygen carrier in hemoglobin in blood and myoglobin in muscle. Iron deficiency is the only nutrient deficiency which is also significantly prevalent in industrialized countries [41]. It is important to highlight that the main Fe sources in the human diet are meat and other animal products, which provide more bioavailable Fe than vegetables. However, some leafy vegetables such as spinach can moderately contribute to human Fe intake. The results of the present study show that the Fe content in sea fennel is lower respect to spinach but similar or higher in comparison with other vegetables, such as chicory and iceberg lettuce, respectively [42].

As for Mn, potted sea fennel contains between 0.2 and 0.3 mg Mn  $100 \text{ g}^{-1}$  FW depending on the growing media. Manganese plays an important role in several physiological functions of the human body, and the Food and Nutrition Board [37] recommended an adequate intake of about 2.0 mg  $\text{day}^{-1}$ . Therefore, a serving size of potted sea fennel could supply between 10 and 15% of the Mn daily intake.

Zinc content in potted sea fennel was about 0.16 mg  $100 \text{ g}^{-1}$  FW, independently of the used growing media. Zinc is an essential trace element for humans since it takes part in many enzymatic reactions, and plays an important role in growth and development, immune response, neurological function, and reproduction [43]. Considering that the European Population Reference Intake (PRI) for Zn is 9.5 and 7.0 mg  $\text{day}^{-1}$  for adult males and females, respectively [40], the results of the present study show a low Zn content in potted sea fennel, independently of the used growing media. On the other hand, it is important to highlight that the Zn content in vegetables is usually below 1 mg  $100 \text{ g}^{-1}$  FW and in many types of vegetables, such as cabbage, cardoon, celery and lettuce, its content is similar to what found in potted sea fennel [42].

#### 4. Conclusions

In this study the potential use of posidonia compost-based substrates for potted sea fennel production was evaluated. Our results show the possibility to use these substrates without any negative effect on the sea fennel growth in comparison with a commercial peat substrate. The halophytic nature of this plant may have played a role in overcoming the limitations posed by the high-salinity of compost-based growing substrates. Also for the color parameters, which are among the most important quality traits of the sea fennel, no difference was observed between plants grown on posidonia-based composts and a commercial peat-based substrate. The accumulation of elements potentially toxic for human health in the edible parts of the plant was far below the limits imposed by legislation and therefore safe for human consumption. At the same time, potted sea fennel grown on both posidonia-based composts and commercial peat-based substrate may be considered a

good source of some essential micronutrients for humans. Therefore, posidonia–based composts can be used as sustainable peat substitutes for the formulation of soilless mixtures to grow potted sea fennel plants, even up to a complete peat replacement. The use of composted posidonia residues as a renewable and low–cost material could be feasible especially in coastal areas of Mediterranean countries. Finally, these results can serve as a basis for the cultivation of other emerging halophyte species on posidonia–based compost substrates.

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