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Synthetic Zeolite A as Zinc and Manganese Fertilizer in Calcareous Soil

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ABSTRACT

The Leccino olive cultivar was grown in pots contained calcareous rendzina soil amended with NPK fertilizer (control treatment) or with further addition of Zeolite A (ZA) containing either zinc (Zn^{2+}) or manganese (Mn^{2+}) (Zn-ZA and Mn-ZA). After one year, vegetative growth and root morphological parameters were measured and elemental analysis was performed on the root, stem, and leaves. Compared to other treatments, the Zn-ZA fertilizer enhanced Zn concentration and total quantity in the root, stem, and leaves, as well as the root diameter. The root volume was greater using Zn-ZA treatment than control treatment. The total quantity of Mn in root and the root length were enhanced when using Mn-ZA compared to control or Zn-ZA treatment. According to the results of this study, it appears that zeolites containing Zn provide favorable conditions for Zn uptake in calcareous soils, while both Zn and Mn zeolites have a positive impact on olive root morphology.

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Leccino; nutrient uptake; olive; root morphology; tissues

Introduction

The world's olive production is mostly situated in the Mediterranean basin (IOOC 2003). Olives are one of the leading tree crops in the Croatian Mediterranean region of Dalmatia and are often grown on alkaline, calcareous rendzina soil (ACRS) with pH values reaching up to 8.5. On such soil, the pH has a strong impact on the soil availability of zinc and manganese nutrients. Concentrations of Zn^{2+} and Mn^{2+} in the soil solution may be reduced by up to 100 times with an increase for each pH unit (Imas 2000; Rengel 1995). In general, quantities of minerals in plants, their nutritional status, and productivity are affected by cultivar, soil conditions, and weather conditions during the growing season, as well as by the use of fertilizer (Ekholm et al. 2007). Applications of the most common inorganic zinc (Zn) and manganese (Mn) fertilizers (sulfates and their oxides) have a limited efficiency on ACRS soil (Imas 2000).

The role of Zn and Mn as essential micronutrients in some processes of olive growth has been previously documented (Connor and Fereres 2005). For example, their role in the synthesis of olive phenolic compounds has been reported (Botia et al. 2001) and which may be essential for pigmentation, resistance to pathogens, etc. (Lattanzio, Lattanzio, and Cardinali 2006). However, there is insufficient data on Zn and Mn absorption and distribution within olive tissues or on the influence of Zn and Mn fertilization on the nutritional status of olive trees (Chatzistathis, Therios, and Alifragis 2009). A lack of data on Zn and Mn levels in olive may be due to the general opinion

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that these two micronutrients tend to be present in olive leaves at adequate levels (Fernadez-Escobar 2007). Yet, this is not always true (Başar and Gürel 2016; Chatzistathis et al. 2010), and generally, scientific research dedicated to this subject is noticeably lacking.

The use of agricultural zeolite soil is based on the fact that it acts as a slow release of fertilizer. The exchangeable (mobile) cations ensure a stable and constant supply of plants for several vegetation seasons (Puschenreiter and Horak 2003). As hydrated microcrystalline aluminosilicates, zeolites are made from $[SiO_4]^{4-}$ tetrahedrons linked through common oxygen atoms, thus forming a strict three-dimensional network, with caves and channels. Framework Si atoms can be isomorphously replaced with Al atoms, causing a negative charge of the zeolite framework, which is compensated with hydrated mobile cations (Breck 1964). Clinoptilolite with K^+ and NH_4^+ is the most widely used zeolite in agriculture due to its noticeable selectivity for K^+ and NH_4^+ over Ca^{2+}, Mg^{2+}, and Na^+. Thus, adding clinoptilolite to soil has been reported to increased yields of carrots, apples, wheat, eggplants, and rice by 63%, 13–38%, 13–15%, 19–55%, and 17%, respectively (Mumpton 1999; Valente et al. 1982). However, there are other zeolites with selectivity to other cations (e.g., Zeolite A for Zn^{2+} and Mn^{2+}) and which have the potential to act as slow-release fertilizers, whereas research on their application has been limited (Ming and Allen 2001). So far, in olive mineral nutrition experiments, only the use of mixed zeolite fertilizer with Zn^{2+}, Mn^{2+}, and Fe^{2+} (Fe^{3+}) cations has been reported (Pasković et al. 2012).

Zeolite A is the first synthesized zeolite which does not have natural structural analog (LTA type). Crystals are cubic, a = 2.461 nm, and the pore opening of the 8 members ring is 0.41 nm. ZA belongs to low silica (or high Al) zeolites with maximal cation exchange capacity (CCA 5.5 mmol/g), and it is mainly used in the detergent industry, as a water softener.

Thus, the hypothesis of this research was based on the premise that a single modified Zeolite A fertilizer, charged with Zn^{2+} or Mn^{2+} cations, has a positive impact on the Zn and Mn content in different olive tissues and enhances the growth of young olive trees grown on alkaline, calcareous soil.

Materials and methods

Plant growth and treatments

At the beginning of the experiment, three-month-old, self-rooted plants of Leccino olive cultivar were potted in 3.6 L pots filled with only rendzina soil. The soil characteristics are listed in Table 1. The soil was mixed with 5.4 g of a 7:14:21 NPK fertilizer (control treatment) per pot or filled as given above with the same amount of 7:14:21 NPK fertilizer but with the addition of: (i) 7.2 g of Zn-enriched Zeolite A per pot (Zn-ZA treatment) or (ii) 7.2 g of Mn-enriched Zeolite A per pot (Mn-ZA treatment). The quantity of zeolite was based on our previous experimental data (Pasković et al. 2013a).

Table 1. Physicochemical properties of the soil used in this study.

Parameter	Value
pH (H_2O)	8.44
pH (KCl)	7.40
$CaCO_3$ (%)	45.8
CaO (%)	17.9
Organic matter (%)	1.7
Total N (%)	0.11
P (mg/100g)	1.2
K (mg/100g)	25
Sand (%)	29.2
Silt (%)	33.1
Clay (%)	37.7

Produced Zeolite A (ZA) is modified by a specifically developed ion exchange method in a thin zeolite layer. Zeolites in which the original compensating cation (Na^+) is completely replaced with Zn^{2+} (Zn-ZA) and Mn^{2+} (Mn-ZA) ions were obtained (Biškup and Subotić 1999, 2000).

The trial was conducted at the Institute of Adriatic Crops and Karst Reclamation, Split, Croatia (lat. 43° 31′ N; long. 16° 27′ E). In the beginning, plants were grown in a greenhouse from 23 February–25 July at natural temperatures (3–50°C), subject to light and a photoperiod. Due to extremely high temperatures in a greenhouse, the trial was continued in a vegetative chamber from 25 July – 24 February in the next season subject to a temperature range of 21–25°C, relative humidity 50–70%, day-night period 16–8 (h), and an illumination of 1,000 l×.

The plants were irrigated with 200 ml of tap water twice a week from 23 February to 20 April, three times a week from 21 April to 24 May, five times a week from 25 May to 25 July, and twice a week from 26 July until the end of the experiment.

Soil properties

The chemical and physical properties of the selected soil were determined as follows: soil reaction (pH) based on HRN ISO 10390 (2005), total nitrogen (TN) based on HRN ISO 11261 (2004), organic matter based on ISO 14235 (1998), total carbonate content using the volumetric method based on HRN ISO 10693 (2004), active lime using the Galet method (Galet 1951), plant-available phosphorus (P) and potassium (K) using the Egner-Riehm-Domingo method (Egner, Riehm, and Domingo 1960), while soil mechanical composition was determined in line with HRN ISO 11277 (2011) (Table 1).

Plant preparations and root analysis

Leaves, stems, and roots were collected for chemical analysis at the end of the experiment, and then, the shoot length was measured for each plant.

Plant fractions were taken to the laboratory and carefully rinsed with tap water and after that rinsed using a six-pot-system containing 5 L pots as follows: (a) tap water with 0.1% detergent; (b) and (c) only tap water; (d) and (e) deionized water; and (f) perforated pot for 5 min draining (Perica 1996).

After washing them, root samples were placed in a Plexiglas vessel (200 × 300 mm) filled with deionized water, in order to minimize their overlapping and then scanned by Epson Perfection V700 scanner (Seiko Epson Corporation, Nagano, Japan). The morphological parameters of the root were measured using WinRhizo software (2005a, Quebec City, Quebec, Canada).

After scanning the roots, the other collected plant fractions were dried at 80°C for 48 hours and the dry weight was measured. Finally, samples were grounded to a fine powder to determine the nutrients (Gomez-Casero et al. 2007).

Analysis of nutrient concentrations

Fine powder (0.5 g) from each sample was digested using concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a microwave system (Milestone 1200 Mega Microwave Digester). After dissolution, plant extracts were diluted and analyzed for total calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) using an atomic adsorption spectroscopy (AAnalyst 200, Perkin Elmer, Waltham, Massachusetts, USA) according to AOAC (1995). The same samples were analyzed spectrophotometrically for total P, and a flame photometer used to determine K. The total quantities of nutrients for each plant were calculated by multiplying the dry weights of plant parts with the corresponding element concentration.

Statistical analysis

The experiment was set up as a random block design in three replications. The total number of plants was thirty-six. An analysis of variance (ANOVA) was performed for all the data. Multiple comparisons of means were based on Tukey's test. Statistical analysis was performed using Statistica 8 (StatSoft®, Palo Alto, CA).

Results and discussion

Zinc-enriched Zeolite A application (Zn-ZA) showed a significant influence on Zn concentration and total quantity (TQ) of Zn in the olive roots (Table 2). In both cases, the content of Zn in Zn-ZA treatment (181 mg/kg; TQ 384 μg root DW) was significantly higher than the control treatment (61 mg/kg; TQ 120.4 μg root DW) or Mn-ZA treatment (71 mg/kg; TQ 159.1 μg root DW). Thus, soil fertilization with zinc Zeolite A in this study may be suitable for enhancing Zn olive status. Swietlik (2001) reported that soil applications are generally ineffective in correcting Zn deficiency in fruit crops.

The root concentration of Zn in this experiment (61–181 mg/kg root DW) was higher than those reported by Chatzistathis, Therios, and Alifragis (2009) and Chatzistathis et al. (2010), who reported that root concentration of Zn in their studies depended on olive cultivar or soil type. This is consistent with Lindsay (1972), who reported that different cultivars of the same species have different abilities to uptake Zn as well as the fact that plant roots usually have a higher mineral concentration of Zn than aboveground plant parts.

Leaf and stem concentrations of Zn in this research ranged from 11 to 24 mg/kg DW and were higher for Zn-ZA treatment compared to control or Mn-ZA, whether expressed as a concentration or total quantity (Table 2). Given the above, the results confirmed that Zn-ZA efficiency enhances Zn content in olive tissues, which is consistent with previous research into synthetic Zeolite A applications on chicory growth (Pasković et al. 2013a). Furthermore, a significant increase of Zn content in spinach, wheat grain, and stalks after applying modified zeolite with Cu and Zn was reported by Puschenreiter and Horak (2003).

Zinc concentration values in stems in this study (11 to 24 mg/kg stem DW) (Table 2) are higher compared to those reported by Chatzistathis, Therios, and Alifragis (2009), who found that they ranged from 7 to 14 mg/kg stem DW. The average Zn concentration in dry olive leaf matter in Mediterranean countries is 23.5 mg/kg leaf DW (Bouat 1968, cited in Lasram and Tnani 1992) which was greater than in the control treatment but close to the Zn-ZA treatment (22 mg/kg leaf DW) (Table 2). In general, the results of this study (11–22 mg/kg leaf DW) (Table 2) correspond to the amount of Zn reported by various authors (Chatzistathis, Therios, and Alifragis 2009; Chatzistathis et al. 2010; El-Fouly et al. 2007; Fernandez-Escobar, Moreno, and Garcia-Creus 1999; Soyergin, Genc, and Fidan 2002). Despite the fact that this experiment was conducted under alkaline soil conditions in which the availability of Zn is greatly reduced (Imas 2000), the amount of Zn,

Table 2. The concentration and the total quantity of Zn in root, stem, and leaves dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

	Zn concentration (mg/kg DW)						Zn quantity (μg/plant)					
Treatment	Root		Stem		Leaf		Root		Stem		Leaf	
Control	61 ± 4[z]	c[y]	15 ± 1	b	11 ± 0	b	120 ± 12	b	80 ± 4	b	86 ± 1	b
Zn-ZA	181 ± 2	a	24 ± 2	a	22 ± 2	a	384 ± 35	a	136 ± 10	a	192 ± 18	a
Mn-ZA	71 ± 3	b	11 ± 0	b	11 ± 1	b	159 ± 7	b	63 ± 1	b	96 ± 8	b
F	22.490		23.681		15.038		47.455		38.159		22.023	
P	<0.001		0.006		0.014		0.002		0.002		0.007	

[z]Mean values ± SE, n = 3.
[y]Mean values followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test.

regardless of the fertilization treatment, did not fall below the limit of 10 mg/kg leaf DW which is the deficiency level in olives (Connor and Fereres 2005; Freeman, Uriu, and Hartmann 2005; Therios 2009). The distribution of Zn in olive tissues shows that it was mostly retained in the roots (up to 50%), followed by the leaves and stems (Table 2). Similar results for Zn distribution in olive plants were reported by Chatzistathis, Therios, and Alifragis (2009).

The potential of zeolites as slow-release fertilizers are described using ion exchange mechanisms and a combination of ion exchange and mineral dissolution (Ming and Allen 2001). The possibility of zeolite dissolution using olive organic acids cannot be excluded, given that root exudation is suggested as one of the major pathways for the nutrient acquisition mechanisms in calcareous soils (Ström et al. 2005).

The concentration of Mn in roots, stems, and leaves was not significantly affected due to Mn-ZA application (Table 3). This is contrary to Pasković et al. (2012), where the concentration of Mn in leaves increased 200 days after applying Mn-ZA, and which was probably due to a different sampling period and different speed of Mn-ZA uptake. However, Mn total quantity in roots was significantly higher for Mn-ZA treatment (675 µg root DW) compared to control treatment (392 µg root DW) or application of Zn-ZA (428 µg root DW).

The root concentration of Mn in this study was higher than that reported by Chatzistathis, Therios, and Alifragis (2009) (31–100 mg/kg root DW) but far below the concentration which may lead to Mn toxicity (Chatzistathis et al. 2012).

The total root system quantity of Mn in this study was lower than quoted by Chatzistathis, Therios, and Alifragis (2009) but similar to that referred by Chatzistathis et al. (2012), suggesting that the total quantity of Mn in roots probably depends on numerous factors such as cultivar, soil, and plant age.

In all the treatments, the majority of Mn was retained in the root system (44–55%), with the rest distributed among the leaves (40–49%) and stems (5–6%) (Table 3). The assumption is that the root retention of Mn is attributed to the formation of metal complexes (Millaleo et al. 2010), while the relatively high distribution of Mn in leaves may possibly depend on low Mn phloem mobility (Mengel and Kirkby 2001).

The average concentration of Mn in dry olive leaf matter in this study was higher than the values recorded in the olive orchard with the same variety and subject to similar soil chemical characteristics (Pasković et al. 2013b). The assumption is that it may be due to the age of the plant since this work involved using non-fruiting plants, as well as the prolonged annual vegetative cycle in the growth chamber to which the plants were exposed. It may also be due to the soil texture (Table 1) or possible reduction conditions where the availability of Mn to plants is increased (Mengel and Kirkby 2001). Therefore, despite the high pH of the soil and expected reduced availability of Mn (Mulder and Gerretsen 1952), the obtained results are in line with the optimum concentration of Mn in olives (Connor and Fereres 2005; Freeman, Uriu, and Hartmann 2005). The total manganese quantity in the leaves (409–484 µg leaf DW) (Table3) was not affected by the applied treatments.

Table 3. The concentration and the total quantity of Mn in root, stem, and leaves dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

	Mn concentration (mg/kg DW)			Mn quantity (µg/plant)			
Treatment	Root	Stem	Leaf	Root		Stem	Leaf
Control	203 ± 25[z]	9 ± 1	51 ± 5	392 ± 21	b[y]	47 ± 2	409 ± 37
Zn-ZA	207 ± 27	11 ± 2	55 ± 6	428 ± 19	b	61 ± 8	479 ± 45
Mn-ZA	297 ± 14	11 ± 1	57 ± 1	675 ± 74	a	62 ± 5	484 ± 12
F	5.410	0.693	0.323	11.435		1.572	1.116
P	0.073	0.552	0.741	0.022		0.316	0.412

[z]Mean values ± SE, n = 3.
[y]Mean values followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test.

The concentration (9–11 mg/kg stem DW) and total quantity (47–62 µg stem DW) of Mn in the olive stems (Table 3) did not differ significantly between the treatments and were lower than those noted by Chatzistathis, Therios, and Alifragis (2009).

The increase of Zn and Mn in root samples (Tables 2 and 3) due to contamination with soil particles could be excluded because the root washing and preparation for analysis were performed according to standard procedures (Perica 1996). In addition, the absence of linear Fe increase in roots (Table 4) could also exclude possible sample contamination with soil particles.

The iron concentration and total quantity in roots were significantly higher in control treatment compared to Zn-Za and Mn-ZA treatments (Table 4). Regarding the reduced concentration and quantity of Fe in all zeolite treatments, some antagonism between Fe-Zn and Fe-Mn was observed (Kabata-Pendias 2011; Rajaie et al. 2009). Nonetheless, in the control treatment, Fe concentration in roots was already at the lower limit with respect to the data published by Chatzistathis, Therios, and Alifragis (2009) and which is typical for calcareous soils where Fe is less bioavailable (Imas 2000; Mengel and Kirkby 2001). In this experiment, around 80% of the total quantity of Fe in plants (Table 4) was concentrated in olive roots. In the olive, the root system seemed to perform a buffering role and controlled Fe levels in the upper parts of the olive (Chatzistathis, Therios, and Alifragis 2009). Nonetheless, for comparison purposes, there is insufficient data in the literature on root content from plants fertilized with modified synthetic zeolites.

Leaf Fe concentration (35–49 mg/kg leaf DW) (Table 4) did not indicate any difference among the treatments, with all the results below 50 mg/kg leaf (DW) and which may be defined as a relative deficiency in the olive according to Therios (2009). Nevertheless, there were no signs of iron deficiency symptoms in studied plants.

Data obtained on the total quantity of Fe in olive stems remained unclear, with a significant difference between Zn-ZA (65 µg stem DW) and the control treatment (55 µg stem DW) (Table 4). It is consistent with Pittman (2008) who reported that the transport of metals in plants is generally poorly understood.

In this study, the P concentration in roots and stems and its total quantity were not significantly affected by fertilization treatments, whereas the P concentration and total quantity in leaves were higher in Mn-ZA treatment compared to the control treatment or Zn-ZA treatment (Table 5). In general, the obtained P concentration in the dry leaf matter (0.7–0.9 g/kg leaf DW) was higher than the minimum value specified by Bouat (1968, cited in Lasram and Tnani 1992) or the deficiency threshold level of 0.5 g/kg noted by Connor and Fereres (2005) and Freeman, Uriu, and Hartmann (2005). Therios (2009) determined a lack of P in leaves with values lower than 0.7 g/kg leaf DW and a relative lack of phosphorus ranging from 0.7 to 0.9 g/kg leaf DW, which corresponds to the values of P obtained in this research. However, the plants in this study did not develop any visible symptoms of P deficiency.

A significantly higher total K content in stems as well as concentration in leaves as well as its total content (Table 6) was also linked to the use of Mn and Zn zeolites. This is in consistent with the

Table 4. The concentration and the total quantity of Fe in root, stem, and leaves dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

	Fe concentration (mg/kg DW)				Fe quantity (µg/plant)				
Treatment	Root		Stem	Leaf	Root		Stem		Leaf
Control	1067 ± 86[z]	a[y]	10 ± 1	35 ± 2	2084 ± 109	a	55 ± 1	b	283 ± 14
Zn-ZA	744 ± 32	b	11 ± 0	49 ± 10	1567 ± 88	b	65 ± 2	a	425 ± 79
Mn-ZA	580 ± 15	b	10 ± 0	35 ± 1	1316 ± 124	b	60 ± 1	ab	294 ± 1
F	40.431		0.533	2.092	23.027		9.890		1.361
P	0.022		0.624	0.239	0.006		0.028		0.354

[z]Mean values ± SE, n = 3.
[y]Mean values followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test.

Table 5. The concentration and the total quantity of P in root, stem, and leaves dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

	P concentration (g/kg DW)				P quantity (mg/plant)			
Treatment	Root	Stem	Leaf		Root	Stem	Leaf	
Control	0.8 ± 0[z]	0.7 ± 0	0.8 ± 0	b[y]	1.6 ± 0.1	3.5 ± 0.2	6.1 ± 0.4	b
Zn-ZA	0.8 ± 0	0.6 ± 0	0.7 ± 0	b	1.8 ± 0.2	3.6 ± 0.2	6.1 ± 0.3	b
Mn-ZA	0.8 ± 0	0.7 ± 0	0.9 ± 0	a	1.8 ± 0.1	4.2 ± 0.2	7.9 ± 0.6	a
F	1.000	2.800	26.000		0.587	2.978	10.477	
P	0.444	0.177	0.005		0.598	0.161	0.026	

[z]Mean values ± SE, n = 3.
[y]Mean values followed by different letters are significantly different at $P \le 0.05$ according to Tukey's test.

Table 6. The concentration and the total quantity of K in root, stem, and leaves dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

	K concentration (g/kg DW)				K quantity (mg/plant)				
Treatment	Root	Stem	Leaf		Root	Stem		Leaf	
Control	17.5 ± 0.2[z]	15.2 ± 0.9	16.6 ± 0.7	b[y]	34.6 ± 3.5	79.8 ± 3.2	c	132.3 ± 2.8	b
Zn-ZA	18.8 ± 0.6	18.4 ± 0.4	19.3 ± 0.6	a	40.2 ± 4.9	105.0 ± 2.6	a	167.9 ± 7.0	a
Mn-ZA	17.9 ± 0.4	16.3 ± 0.5	19.7 ± 0.4	a	40.5 ± 2.5	94.6 ± 2.9	b	167.5 ± 6.8	a
F	2.023	4.726	21.571		0.997	75.78		50.414	
P	0.247	0.088	0.007		0.445	<0.001		0.002	

[z]Mean values ± SE, n = 3.
[y]Mean values followed by different letters are significantly different at $P \le 0.05$ according to Tukey's test.

results of Ming and Allen (2001) who reported an increased efficiency of P and/or K mineral fertilizers coinciding with the use of zeolites.

In general, the studied K concentration levels found in the plants (16.6–19.7 g/kg leaf DW) (Table 6) are greater than those found in most of the literature references (Chatzistathis, Therios, and Alifragis 2009; Chatzistathis et al. 2010; El-Fouly et al. 2007; Fernandez-Escobar, Moreno, and Garcia-Creus 1999; Miljković 2006; Perica 1996; Rodrigues et al. 2012; Soyergin, Genc, and Fidan 2002; Tekaya et al. 2016; Toplu, Uygor, and Yildiz 2009; Ulger et al. 2004), but in agreement with results of Hartmann and Brown (1953) and Perica, Goreta, and Selak (2008) for his greenhouse study. The observed differences can be explained due to the age of the plants, cultivation methods, and soil and cultivar characteristics.

In terms of the other macronutrients, Ca and Mg concentrations in leaves were in the range reported by Chatzistathis, Therios, and Alifragis (2009), Connor and Fereres (2005), Freeman, Uriu, and Hartmann (2005), and Therios (2009). There was no significant difference among the treatments, regardless of plant tissue or values of concentration and total quantity (Ca: 5.1–18.3 g/kg DW, TQ 26.7–86.1 mg DW; Mg: 0.6–6.6 g/kg DW, TQ 3.3–14.4 mg DW) (Tables 7 and 8).

Table 7. The concentration and the total quantity of Ca in root, stem, and leaves dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

	Ca concentration (g/kg DW)			Ca quantity (mg/plant)		
Treatment	Root	Stem	Leaf	Root	Stem	leaf
Control	18.3 ± 0.3[z,y]	5.1 ± 0.2	10.5 ± 1.2	36.1 ± 2.9	26.7 ± 1.6	83.7 ± 7.8
Zn-ZA	19.4 ± 0.6	5.7 ± 0.4	9.9 ± 1.4	41.3 ± 5.1	32.2 ± 1.3	86.1 ± 11.2
Mn-ZA	18.6 ± 1.8	5.3 ± 0.5	9.1 ± 0.4	42.2 ± 5.7	30.7 ± 2.9	77.0 ± 3.0
F	0.209	0.438	0.323	0.369	1.367	0.242
P	0.820	0.673	0.741	0.713	0.353	0.796

[z]Mean values ± SE, n = 3.
[y]Mean values followed by different letters are significantly different at $P \le 0.05$ according to Tukey's test.

Table 8. The concentration and the total quantity of Mg in root, stem, and leaves dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

	Mg concentration (g/kg DW)			Mg quantity (mg/plant)		
Treatment	Root	Stem	Leaf	Root	Stem	Leaf
Control	6.6 ± 0.4^{z,y}	0.6 ± 0.1	1.5 ± 0.3	12.8 ± 0.2	3.3 ± 0.3	12.2 ± 2.2
Zn-ZA	4.8 ± 0.5	0.7 ± 0.1	1.5 ± 0.0	10.2 ± 1.6	3.8 ± 0.3	13.3 ± 0.2
Mn-ZA	4.9 ± 0.1	0.6 ± 0.1	1.7 ± 0.1	11.0 ± 1.1	3.5 ± 0.3	14.4 ± 0.1
F	5.140	0.200	0.329	1.054	0.474	0.809
P	0.060	0.826	0.737	0.429	0.654	0.507

^{z}Mean values ± SE, n = 3.
^{y}Mean values followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test.

Applying Zn-ZA or Mn-ZA to substrates in which olive transplants were grown did not affect the shoot length or dry weight of the roots, stems, and leaves (Table 9). However, zeolite fertilizers had a visible significant impact on root morphology (Table 10). Root length (3105.2 cm) was significantly reduced, whereas root volume (8.0 cm^3) and root diameter (0.57 mm) increased significantly due to Zn-ZA treatment compared to the control treatment (3708.6 cm; 5.8 cm^3; 0.45 mm) (Table 10).

A significant ($P \leq 0.05$) strong positive correlation ($r = 0.98$) between the root concentration of Zn and root diameter as well as a significant ($P \leq 0.05$) strong negative correlation ($r = -0.78$) for the root length and root concentration of Zn indicates potentially toxic effects (data not shown). Woolhouse (1983) stated that in many species, Zn toxicity is associated with the inhibition of root elongation and which can be connected to the elevated root concentrations of Zn in Zn-ZA treatments (the average value in this study is twice that of the control treatment). Li et al. (2009) reported that plant exposure to Zn may have a significant positive effect on average root diameters compared to untreated plants, which is also comparable to the results in this study.

Root length was significantly higher in Mn-ZA treatment (4364.9 cm) compared to all other treatments (Table 10). It can be linked to a generally positive zeolite impact on the physical properties of soils (water-air relationship) (Ciocinta et al. 2012; Ming and Allen 2001) or the impact of Mn on auxin metabolism (Zahan 2008) and root growth (Marschner 2003). Nonetheless, the

Table 9. Average shoot length and root, stem, and leaf total dry weight of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

Treatment	Shoot length (cm)	Root DW (g/plant)	Stem DW (g/plant)	Leaf DW (g/plant)
Control	77 ± 11^{z,y}	1.98 ± 0.18	5.32 ± 0.51	8.00 ± 0.15
Zn-ZA	92 ± 5	2.12 ± 0.20	5.70 ± 0.19	8.70 ± 0.15
Mn-ZA	91 ± 5	2.27 ± 0.18	5.79 ± 0.01	8.50 ± 0.20
F	0.962	0.735	0.832	3.513
P	0.456	0.535	0.499	0.132

^{z}Mean values ± SE, n = 3.
^{y}Mean values followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test.

Table 10. Effect of fertilization treatments on root morphology of olive plants grown without (control treatment) or with zeolite (Zn-ZA or Mn-ZA) soil application.

Treatment	Lenght (cm/plant)		Surface area (cm^2/plant)	Volume (cm^3/plant)		Diameter (mm/plant)	
Control	3708.6 ± 50.7^z	b^y	519.3 ± 21.4	5.8 ± 0.4	b	0.4 ± 0	b
Zn-ZA	3105.2 ± 47.5	c	556.5 ± 11.8	8.0 ± 0.3	a	0.6 ± 0	a
Mn-ZA	4364.9 ± 178.9	a	623.1 ± 23.5	7.1 ± 0.3	ab	0.5 ± 0	b
F	28.263		5.295	8.969		67.64	
P	0.004		0.075	0.033		0.001	

^{z}Mean values ± SE, n = 3.
^{y}Mean values followed by different letters are significantly different at $P \leq 0.05$ according to Tukey's test.

study did not identify a significant correlation (P = 0.06, r = 0.64, data not shown) between root concentration of Mn and root length. Marschner (2003) reported that Mn deficient plants have a smaller percentage of soluble carbohydrates in the roots, which is probably a key factor for reduced root growth. Moreover, according to Zahan (2008), the role of Mn in root growth is linked to auxin metabolism as well as phenol and lignin synthesis, given that Mn-deficient plants seem to have smaller amounts of these components.

Conclusions

The conclusion of this research is that applying zinc-enriched Zeolite A resulted in elevated levels of Zn in all measured plant tissues. This may very well be useful when applied to Zn-deficient soils. Moreover, Zn- and Mn-loaded zeolites had a positive impact on the characteristics of the olive root; Zn-ZA had a positive influence on root length, while Mn-ZA indicated a positive impact on the root volume and diameter. All these results are useful in determining nutrients for olive trees and the olive industry, in general.

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