Acta Bot. Croat. 72 (2), 295–310, 2013

CODEN: ABCRA 25
ISSN 0365–0588
eISSN 1847-8476
DOI: 10.2478/v10184-012-0021-9

Interactions between leaf macronutrients, micronutrients and soil properties in pistachio (*Pistacia vera* L.) orchards

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Abstract – The interactions between: (i) leaf dry matter macronutrietns, micronutrients and soil chemical properties, (ii) leaf macro- and micronutrients, (iii) soil macro- and micronutrients and (iv) soil chemical properties, and soil micro- and macronutrients in 50 pistachio orchards were investigated in leaves and soils by means of regression analysis. Most of the soils were deficient in plant-available P, Zn, Mn, Fe, and B, while they were excessively supplied with Cu. Leaf analysis showed that most of the trees were sufficient in K, Mg, Mn and B, but deficient in N, P and Fe, and excessive in Zn and Cu. It was found that almost all the significant elemental interactions occurring in pistachio leaves or soils were synergistic, contributing considerable quantities of available nutrients and, therefore, improving the nutrient status of pistachio trees, and the level of soil fertility. On the other hand, the interactions between K and Mg in leaves, and between soil pH and leaf N or soil Fe, Mn and B, were antagonistic. It is suggested that these results must be taken into account during fertilization of pistachio trees, in order to avoid nutritional disorders and to promote plant growth, productivity and nut quality.

Keywords: *Pistacia vera*, plant nutrition, leaf, macronutrients, micronutrients, soil

Introduction

In Greece, pistachio (*Pistacia vera* L.) trees are cultivated on about 5,000 ha and produce 9,000 tons of fruit (FAO 2008). It is an economically important species that produces nuts of high nutritional value. The proper nutrition of pistachio trees is a basic prerequisite for the production of nuts of excellent quality and high commercial value. The high produc-

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tivity of pistachio trees makes necessary, among other factors, knowledge of: a) the interactions between leaf dry matter (d.m.) nutrient content, and chemical soil characteristics and b) the interrelations among nutrient elements in leaves.

The interactions between leaf macro- and micronutrients, also between leaf nutrients and the soil properties, as well as between soil macro- and micronutrients, and between nutrients in soil and soil properties have been studied sporadically and in a very general way (BRADY and WEIL 2002). According to relatively recent work, there is a direct relationship between the plant nutrient content and soil properties (KIZILGOZ et al. 2001). Usually, the interactions between soil characteristics and nutrient elements contribute negatively to the available level of nutrients in pistachio, with serious consequences to nut yield and quality. The low pH is correlated positively with the availability of most micronutrients (e.g. Zn, Mn, Fe) and P (OLSEN 1972). A high $CaCO_3$ concentration in combination with a highly alkaline soil accentuates plant growth and yields, notwithstanding any reduction in nutrient availability (MENGEL and KIRKBY 1987). Organic matter in soil is related positively to an increase of nutrient availability due either to the favorable effect on decomposition of the rocks and minerals or to the increase of cation exchange capacity (CEC) (TISDALE et al. 1993, KOUKOULAKIS et al. 2000). Moreover, clay positively affects the increase of available cations (Ca^{++}, Mg^{++}, K^{+}) (BRADY and WEIL 2002).

The interactions between nutrients have been studied to a certain extent in several crops, but not in pistachios. There is a particular shortage of relevant data concerning the elemental contribution of these interactions in pistachio cultivation, at least under the conditions of Greece.. According to DIBB and THOMPSON (1985), the nutrient concentrations in plants depend on the rate of their absorption, translocation and accumulation, and during these processes the elemental interactions within the plants may play an important role (KALAVROUZIOTIS and KOUKOULAKIS 2009a).

The aim of this work was to investigate the elemental contribution of the interactions between nutrients in leaves, and in soils, as well as between soil properties and nutrients, and their effect on soil fertility and on the nutrient status of pistachio trees.

Materials and methods

Planting material and sampling procedure

Fifty productive pistachio (*Pistacia vera* cv. Aegenes) orchards were selected in various locations of the Fthiotida district (south Greece). The trees were grafted on the rootstock 'Tsikoudia' (*Pistacia terebinthus* cv. Tsikoudia), trained as a vase and they were mostly planted at a spacing of 6.5–7.0 m between and within rows. Standard commercial cultural practices were followed during the survey. Representative soil samples from a depth of 0 to 0.30 m were collected from 20 cores in each orchard. The sampling points (four cores per tree) were located 2 m from the trunk, along and between tree rows. At the end of July, ten mature leaves per tree were collected from the middle node of non-bearing 1-year-old shoots and from all around the periphery of the canopy.

Soil analyses

Each sample was air-dried, crushed, sieved through a 2-mm mesh sieve and tested for pH, free $CaCO_3$ and organic matter (OM). The basic soil characteristics were determined according to the accepted methods (JACKSON 1958). The clay content of the soil samples was determined using the Bouyoucos method (BOUJOUCOS 1962).

Most soils of the orchards studied (92%) had a high clay content, which ranged between 44 and 74%. The remaining 8% of the soils contained clay 30–38%, and they are characterized as sand-clay-loamy (SCL) and clay-loamy (CL) soils. As regards pH, 82% of the studied soils may be characterized as moderately alkaline, with 74% of them presenting pH values between 7.5 and 8.0. Interestingly, 79% of the studied orchards presented the optimum range of pH values (7.5–8.5) for pistachio tree growth. Seventy four percent of the soils had a high $CaCO_3$ content (above 4%), while the organic matter content was low (below 1%) for 54% of the studied soils and medium (1–2%) for 38% of the studied soils. Moreover, soil electrical conductivity was low and ranged between 0.06 and 0.70 mS cm^{-1} with a mean value of 0.40 mS cm^{-1}.

The available soil nutrients were extracted by the following methods: P by Olsen's procedure, exchangeable K and Mg by ammonium acetate, B by hot water, and the micronutrients (Mn, Zn, Fe and Cu) by DTPA (Diethylenetriamine pentaacetic acid). Analytical determination of the elements K, Mg, Fe, Mn, Zn and Cu in the soil samples was performed by atomic absorption spectroscopy (Perkin-Elmer 2340) using standard methods (CHAPMAN and PRATT 1961). Phosphorus was analyzed by the vanado-molybdo-phosphate yellow complex (CHAPMAN and PRATT 1961). Finally, B was determined by the Azomethine-H method (WOLF 1971).

Leaf tissue analyses

The leaf samples were washed twice with distilled water, air-dried at 85 °C for 48h (till constant weight was obtained) and ground in a mill to pass through a thirty-mesh screen. Tissue B extraction was made by dry ashing of a 0.5 g sample in a muffle furnace at 500 °C for 6 h. The ash was dissolved in 10 mL of 0.1 N hydrochloric acid (HCl) and B was determined colorimetrically (420 nm) by the Azomethine-H method (WOLF 1971). The analytical determination of N was performed by the Kjeldahl method (CHAPMAN and PRATT 1961). Phosphorus, K, Mg, Fe, Mn, Zn and Cu analyses were conducted by dry ashing of 0.5 g of dried tissue for 6h at 550 °C. Subsequently, the ash was dissolved in 3 mL of 6N HCl and the solution was diluted with deionised water to 50 mL final volume. Phosphorus concentration was determined by the vanado-molybdo-phosphate yellow complex method, while K, Mg, Fe, Mn, Zn and Cu were determined by atomic absorption spectroscopy (Perkin-Elmer 2340) using standard methods (CHAPMAN and PRATT 1961).

Statistical analysis

In each orchard, thirty uniform size pistachio trees were selected and they were separated in five blocks. Each replicate block consisted of six trees. Regression models were developed for each interaction found among soil properties, i.e. pH, $CaCO_3$, clay, organic matter and leaf N, P, K, Mg, Zn, Fe, Mn, B and Cu concentrations, as well as among leaf and soil nutrients. The estimators in the models were tested by t-test and the overall regression model by F-test at a level of significance of 0.05 and 0.001. Moreover, regression coefficients of the models were determined. All the regression models were obtained by using the statistical package of SPSS version 17.

In order to quantify the percent elemental contribution (PEC) of an interaction, the procedure developed by KALAVROUZIOTIS et al. (2010), was modified with respect to the calculation of PEC. More specifically, the quantification procedure and the modification mentioned are explained below in four steps.

1) Regression analysis is run between the concentrations of elements of soil or of plant dry matter, as given by the available analytical experimental data, and the statistically significant regression equations are chosen.
2) The interactions corresponding to the statistically significant regression equations, are classified on the basis of the same dependent variable, i.e. x_0*y, x_1*y, x_2*y etc., where x and y represent a different element, for example if x_0 is P, x_1 is Zn, x_2 is Mn, and y Fe, then the interactions will be P-Fe, Zn-Fe, and Mn-Fe. As can be seen, the dependent variable is written on the right to avoid confusion. Obviously, each one of the elements studied can be a dependent or independent variable according to which one is to be contributed. The number of interactions with the same dependent variable is not constant, but it varies according to the interactive capacity of the elements that participate in the interactions, and their respective concentration levels. In calculating the PEC, the mean value of the calculated dependent variables is found by dividing their total sum by the number of interactions involved.
3) The statistically significant equations are solved for the maximum (x_{max}) and the minimum (x_{min}) values of the independent variable, as given by the analytical data. Thus, the calculated maximum and minimum values are the dependent variables y_{maxcl} and y_{mincl}, respectively, are found, and the difference between them is determined.
4) Also, the maximum analytical value of the dependent variable (y_{maxan}) and minimum values (y_{minan}) are taken respectively from the analytical experimental data, and the PEC is calculated by means of the relation 1:

$$PEC = (y_{maxcl} - y_{mincl}) \times 100/(y_{maxan} - y_{minan}) \tag{1}$$

where y_{maxcl} represents the calculated maximum value of the dependent variable in mg kg^{-1}, y_{mincl} represents the calculated minimum value of the dependent variable in mg kg^{-1}, y_{maxan} represents the maximum value of the dependent variable obtained from the set of the existing analytical data of soil or plant tissue analysis (in mg kg^{-1}), and y_{minan} represents the minimum value of the dependent variable, obtained from the set of the existing analytical data of soil or plant tissue analysis (in mg kg^{-1}). It is noted that the relation 1 can be used for the calculation of PEC for interactions occurring either in the soil or in the plant tissues.

It should be mentioned at this point that the mathematical relation for the calculation of PEC, given in the publication of KALAVROUZIOTIS et al. (2010), was the following:

$$PEC= (y_{maxcl}- y_{mincl}) \times 100/E_{mc} \tag{2}$$

where y_{maxcl}, y_{mincl} represent the maximum and minimum calculated values of the dependent variable in mg kg^{-1}, and E_{mc} represents the mean total plant dry matter or soil content of the element representing the dependent variable. As it can be seen, relation 2 did not take into account the observed actual difference ($y_{maxan} - y_{minan}$), but only the difference between the calculated values of the dependent variables, and the mean dry matter concentration (E_{ec}), of the dependent variable, giving not very satisfactory results. Therefore, after a detailed consideration, we modified the calculation of PEC and replaced the above relation 2 with the relation 1, as mentioned above.

Results

Interactions between soil properties and leaf nutrient concentration

Regression equations of the statistically significant interrelations between leaf nutrient concentration and soil properties (pH, organic matter (OM), $CaCO_3$ (CC), and soil clay content (C)), are given in table 1. The interactions pH × N (Fig. 1c) and $CaCO_3$ × Cu (Fig. 1d) and C × Mn (Fig. 1e) are antagonistic, suggesting that the increase of pH or of $CaCO_3$ or of C decreases the leaf N, Cu and Mn concentrations, respectively. On the other hand, the interactions OM × Zn (Fig. 1a), and OM × B (Fig. 1b) are synergistic contributing to pistachio leaf Zn and B, respectively. The results of the interaction quantification procedure, as expressed by the percent elemental contribution (PEC), are shown in table 2. The negative figures, being the result of antagonistic interactions, reflect a decrease of the respective element in the soil or in the plant respectively. On the other hand, the positive figures show an increase of the nutrient level. It is noted that most of the figures are positive, a fact that emphasizes the synergistic nature of most of the interactions that occurred in the soil and in plants, underlining the importance of the elemental interactions.

Tab. 1. Interactions between pistachio leaf nutrients and soil properties.

No	Interactions	Regression equations	R	Sig.	Type
1	pH × N	$N = 0.275 \times (pH)^2 - 3.969 \times (pH) + 16.258$	0.439	0.007	A[a]
2	CC × Cu	$Cu = -0.048 \times (CC)^2 - 0.101 \times (CC) + 5.73$	0.356	0.041	A
3	OM × Zn	$Zn = -7.593 \times (OM)^2 + 21.67 \times (OM) + 8.037$	0.363	0.036	S[b]
4	OM × B	$B = -8.287 \times (OM)^2 + 18.054 \times (OM) + 78.905$	0.341	0.055	S
5	C × Mn	$Mn = 0.037 \times (C)^2 - 4.501 \times (C) + 162.202$	0.350	0.046	A

CC=$CaCO_3$, OM=Organic matter, C= Clay

[a] Antagonistic

[b] Synergistic

Interactions between leaf nutrients

Seventeen interactions (regression equations) among pistachio leaf macro- and micronutrients were statistically significant out of a total 81 interactions (9 × 9) (Tab. 3). From these 17 interactions 12 were synergistic (S), meaning that they contributed to leaves the respective quantities of the interacting elements, one was antagonistic (A), and four synergistic-antagonistic (S-A).

According to interactions between macro- and micronutrients occurring in pistachio leaves (Figs. 2 a–f), it is obvious that the elemental interactions among leaf nutrients are mainly described by quadratic, and secondarily by logarithmic regression equations. In addition, concerning the above interactions, the respective PEC values are presented in table 2.

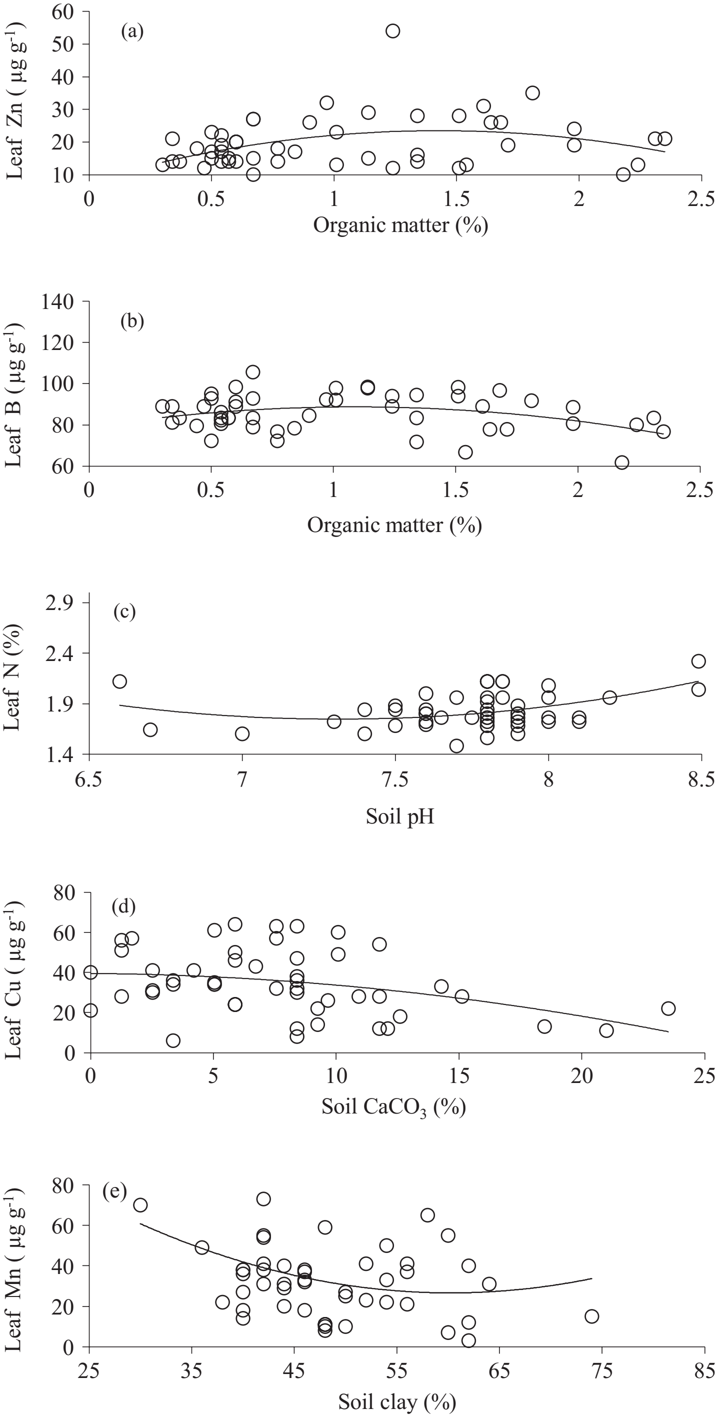

Fig. 1. Interactions of pistachio leaf Zn, B, N, Cu and Mn concentrations with soil organic matter, organic matter, pH, $CaCO_3$ and clay, respectively.

Tab. 2. Percent elemental contribution values by the interactions among i) soil properties and leaf nutrients, ii) leaf nutrients, iii) soil properties and soil nutrients, and iv) soil nutrients.

Elements	i	ii	iii	iv
N	–7.45	41.80	0.0	0.0
P	0.0	0.0	0.0	15.21
K	0.0	–14.33	29.20	47.09
Mg	0.0	12.90	–3.67	17.76
B	5.18	22.73	31.15	0.0
Fe	0.0	4.64	–31.97	13.83
Mn	–26.80	3.14	–18.61	2.10
Zn	13.53	20.07	4.56	13.59
Cu	–8.16	23.07	0.0	13.15

Tab. 3. Interactions among nutrients in pistachio leaves.

No	Interaction	Regression equations	R	Sig.	Type
1	K × N	$N = -0.37 \times (K)^2 + 1.046 \times (K) + 6.169$	0.350	0.046	S[a]
2	Mg × N	$N = 0.173 \times (Mg) + 1.717$	0.286	0.044	S
3	Mn × N	$N = 8.11 \times 10^{-5} \times (Mn)^2 + 0.009 \times (Mn) + 1.645$	0.371	0.031	(S-A[b])
4	Mg × K	$K = \ln(Mg) \times (-0.184) + 0.905$	0.278	0.050	A
5	N × Mg	$Mg = \ln(N) \times (0.863) + 0.125$	0.280	0.049	S
6	Zn × Fe	$Fe = 0.018 \times (Zn)^2 + 0.261 \times (Zn) + 50.438$	0.440	0.006	S
7	Mn × Fe	$Fe = 0.002 \times (Mn)^2 + 0.403 \times (Mn) + 46.988$	0.535	0.000	S
8	Mg × Zn	$Zn = -13.297 \times (Mg)^2 + 35.126 \times (Mg) + 3.827$	0.375	0.028	(S-A)
9	Fe × Zn	$Zn = 0.147 \times (Fe) + 10.373$	0.429	0.002	S
10	Mn × Zn	$Zn = 0.008 \times (Mn)^2 - 0.105 \times (Mn) + 18.822$	0.496	0.001	S
11	Cu × Zn	$Zn = -0.003 \times (Cu)^2 + 0.371 \times (Cu) + 10.781$	0.377	0.027	S
12	Fe × Mn	$Mn = -0.002 \times (Fe)^2 + 0.835 \times (Fe) - 8.556$	0.540	0.000	S
13	Zn × Mn	$Mn = 0.033 \times (Zn)^2 - 0.621 \times (Zn) + 31.975$	0.470	0.003	S
14	B × Mn	$Mn = -0.021 \times (B)^2 + 4.415 \times (B) - 186.661$	0.368	0.033	S
15	Mg × Cu	$Cu = -29.57 \times (Mg)^2 + 72.103 \times (Mg) + 3.038$	0.416	0.011	(S-A)
16	Zn × Cu	$Cu = -0.021 \times (Zn)^2 + 1.889 \times (Zn) + 6.997$	0.400	0.017	S
17	Mn × B	$B = -0.003 \times (Mn)^2 + 0.471 \times (Mn) + 75.14$	0.196	0.006	(S-A)

[a] Synergistic
[b] Antagonistic

Elemental contribution to soil by the interactions between soil nutrients, and soil properties

According to regression equations of the interactions between soil nutrients and soil properties (Tab. 4) the interactions OM × K, C × K, C × Mg, $CaCO_3$ × Zn and OM × Zn were synergistic while those of $CaCO_3$ × Fe, pH × Fe, $CaCO_3$ × Mn, pH × Mn, pH × B, $CaCO_3$ × B

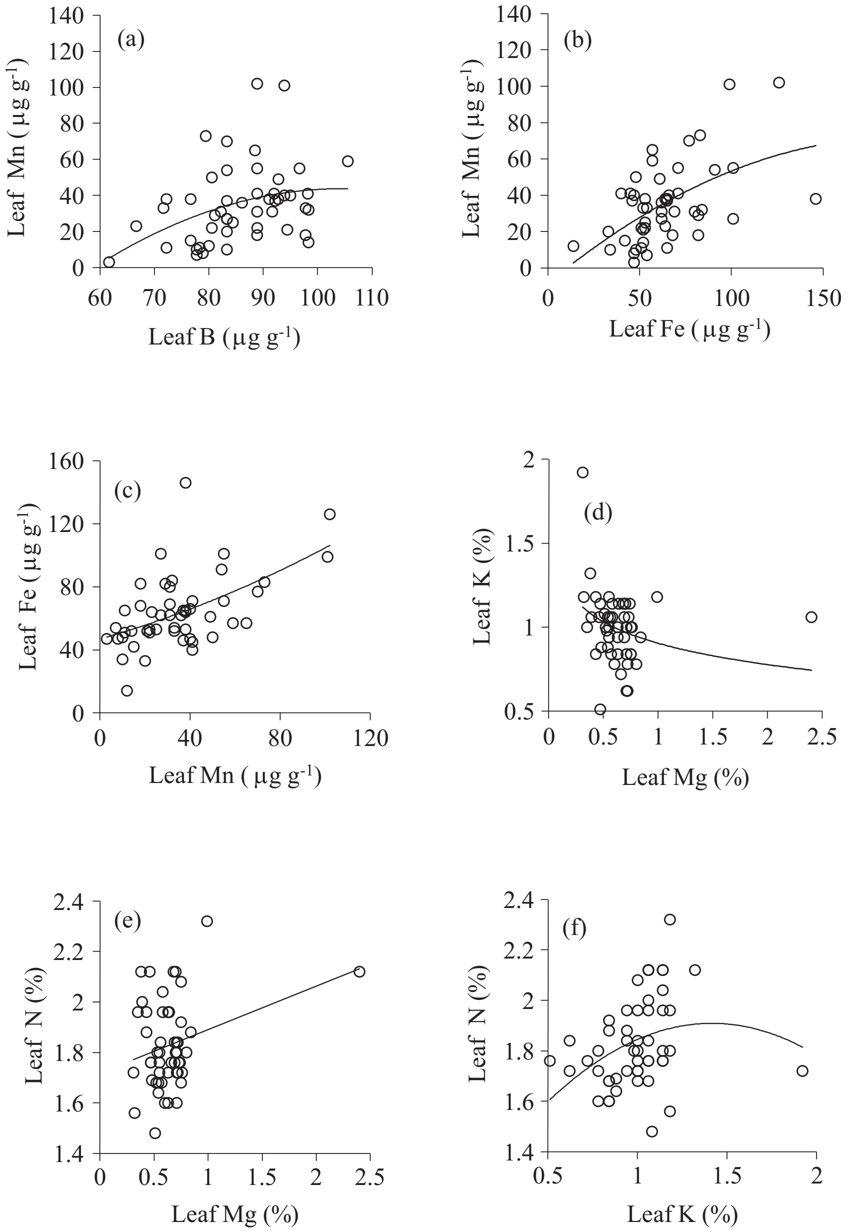

Fig. 2. Interactions among nutrients in pistachio leaves.

and C × B were antagonistic. Most of the interactions between soil properties and soil nutrients are described by quadratic regression equations.

In order to have a more concrete idea about the elemental contribution to soil, by the interactions mentioned in Table 4, a quantification procedure was applied and the results are shown in table 2.

Tab. 4. Interactions among soil properties and nutrients occurring in pistachio orchard soils.

No	Interactions	Regression equations	R	Sig.	Type
1	OM × K	$K = -23.505 \times (OM)^2 + 195.03 \times (OM) + 86.12$	0.549	0.000	S[a]
2	C × K	$K = \text{In}(C) \times 410.1 - 1325.823$	0.500	0.000	S
3	C × Mg	$Mg = 0.714(C)^2 - 61.268(C) + 1759.68$	0.432	0.007	S
4	CC × Fe	$Fe = 0.01 \times (CC)^2 - 0.365 \times (CC) + 4.977$	0.373	0.029	A[b]
5	pH × Fe	$Fe = 6.616 \times (pH)^2 - 95.376 \times (pH) + 371.1$	0.653	0.000	A
6	CC × Mn	$Mn = -0.079 \times (CC) + 2.219$	0.286	0.044	A
7	pH × Mn	$Mn = \ln(pH) \times (-9.86) + 21.802$	0.339	0.016	A
8	CC × Zn	$Zn = 0.023 \times (CC)^2 - 0.34 \times (CC) + 2.932$	0.359	0.039	S
9	OM × Zn	$Zn = 0.394 \times (OM)^2 + 2.04 \times (OM) - 0.219$	0.548	0.000	S
10	pH × B	$B = 0.066 \times (pH)^2 - 1.03 \times (pH) + 4.47$	0.447	0.005	A
11	CC × B	$B = -0.003 \times (CC) + 0.480$	0.398	0.004	A
12	C × B	$B = \ln(C) \times (-0.079) + 0.76$	0.342	0.015	A

C=Clay, CC=$CaCO_3$, OM =Organic matter
[a]Synergistic
[b]Antagonistic

Tab. 5. Interactions among soil nutrients occurring in pistachio orchards.

No	Interactions	Regression equations	R	Sig.	Type
1	Fe × P	$P = -0.204 \times (Fe)^2 + 3.579 \times (Fe) + 10.076$	0.363	0.036	S[a]
2	P × K	$K = -0.315 \times (P)^2 + 20.165 \times (P) + 33.757$	0.525	0.001	S
3	Cu × K	$K = \ln(Cu) \times 67.828 + 214.352$	0.657	0.000	S
4	P × Mg	$Mg = \ln(P) \times 114.341 + 212.76$	0.274	0.054	S
5	Fe × Mn	$Mn = 0.03 \times (Fe)^2 - 0.108 \times (Fe) + 1.511$	0.529	0.000	S
6	P × Fe	$Fe = \ln(P) \times 1.105 + 0.038$	0.303	0.033	S
7	P × Zn	$Zn = -0.009 \times (P)^2 + 0.434 \times (P) - 1.505$	0.386	0.023	(S-A[b])
8	K × Zn	$Zn = -1.81 \times 10^{-5} \times (K)^2 + 0.021 \times (K) - 1.255$	0.461	0.004	S
9	Cu × Zn	$Zn = -0.01 \times (Cu)^2 + 0.509(Cu) + 0.811$	0.481	0.002	S
10	K × Cu	$Cu = -0.0000472 \times (K)^2 + 0.043 \times (K) - 2.944$	0.465	0.003	S

[a] Synergistic
[b] Antagonistic

Elemental contribution to soil by the interactions between soil nutrients

Most of the statistically significant interactions between soil macro- and micronutrients (Tab. 5) were described by quadratic (Fig. 3 a, d, e, g, h) and some of them by logarithmic equations (Fig. 3 b, c, f). It can be seen that almost all of these interactions were synergistic with the exception of P × Zn (Fig. 3 d), which was 'synergistic-antagonistic' (S-A). Therefore, the elemental contribution of these interactions was positive, i.e. significant quantities

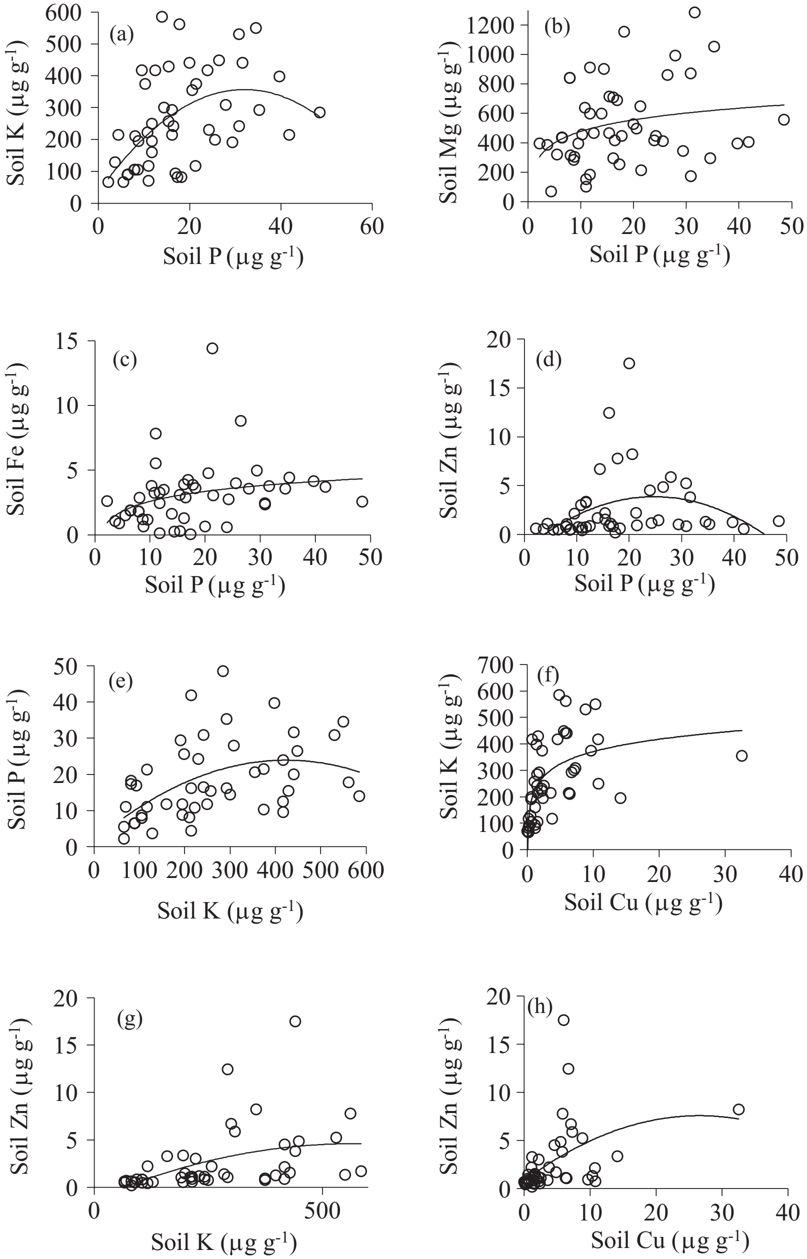

Fig. 3. Interactions among soil available nutrients

of macro- and micronutrients have been contributed to soil. More specifically, in terms of various nutrients, the PEC values are presented in table 2.

Discussion

The critical ranges of soil nutrients are the following (in μg g^{-1}): K 140–280, P 15–25, Ca 300–750, Mg 50–100, B 0.50–1.0, Fe 4–25, Mn 15–25, Zn 1.0–2.5 and Cu 0.9–1.5 (KOUKOULAKIS 1995). Regarding the previous values, most of the studied soils were deficient in P, Zn, Mn, Fe and B content (46%, 44%, 100%, 82% and 100%, respectively). On the other hand, the soils of 62% of the orchards were excessively supplied with available Cu. Interestingly, according to BASLAR et al. (1999) *Pistacia terebinthus* subsp. *palaestina,* a species related to the rootstock used in the present work, generally prefers neutral soils and thrives in the presence of variable $CaCO_3$ and P concentrations. Therefore, lime and P content in soil are not expected to be limiting factors for pistachio trees growth in the Fthiotida district.

On the other hand, the recommended critical values for pistachio nutritional status (CRANE and MARANTO 1988, MILLS and BENTON JONES 1996) are the following: N 2.5–2.9%, P 0.14–0.17%, K 1.0–2.0%, Mg 0.6–1.2%, B 50–230 μg g^{-1}, Fe 30–125 μg g^{-1}, Mn 30–80 μg g^{-1}, Zn 7–14 μg $g^{-1}$and Cu 3–4 μg g^{-1}. Based on the previous values, leaf analysis showed that K, Mg, Mn and B were sufficient in 60%, 52%, 54% and 100% of the studied orchards, respectively, while, N, P and Fe were deficient in 100%, 96% and 44% of the orchards, respectively. On the other hand, Zn and Cu were excessive in 68% and 100%, respectively, of the orchards under consideration.

Some soil properties seem to correlate significantly with the concentration of nutrients in the leaves. Such antagonistic interaction $CaCO_3 \times$ Cu (Fig. 1 d) has also been reported by KALAVROUZIOTIS et al. (2010) for this soil which was planted with *Brassica oleracea* var Gemmifera (Brussels sprouts). In another study with *Pistacia lentiscus* trees, $CaCO_3$ concentration in soil was found to be negatively correlated with leaf N and K concentrations (DOGAN et al. 2003). Also, the interaction OM × B (Fig. 1b) is in line with findings of STEVENSON and COLE (1999), who found that B is the only non metal which can combine with OM, primarily as organic complexes, with compounds that contain cis-hydroxyl groups such as saccharides, the plant-available B being released after mineralization of the organic matter. Similarly, in relation to Zn (Fig. 1a), it has been stated by HODGSON et al. (1966) that 28–90% of this element may be organically bound in the soil.

It has generally been found in the present work that the elemental interactions between leaf nutrients are mainly described by quadratic, and secondarily by logarithmic regression equations. Similar results have been reported by KALAVROUZIOTIS and KOUKOULAKIS (2009b) and KALAVROUZIOTIS et al. (2010) experimenting with Brussels sprout plants.

Similar interactions between leaf nutrients (Tab. 3) have also been found by other researchers. More specifically, experiments with broccoli (*Brassica oleracea* var Italica) plants resulted in the following synergistic interactions in leaves: N × K, Mg × N, Fe × Mn, Mn × B and B × Mn (KALAVROUZIOTIS et al. 2008, 2009). In accordance with the above findings, KALAVROUZIOTIS and KOUKOULAKIS (2009b) reported the synergistic interactions of N × K and Zn × Fe occurring in the leaves of Brussels sprout plants. On the other hand, HALDAR and MANDAL (1981) showed that the application of Zn decreased Fe concentration in shoots, but they mention that this decrease was not due to dilution effect or to reduced rate of translocation from roots to tops.

In relation to the interaction of Mn × Fe, contrary to our results, SRIVASTAVA and GUPTA (1996) state that the chlorosis of Fe, which is induced by increased levels of Mn, is related to the enzymatic disturbance due to the fact that Mn may compete with Fe for binding sites of the enzymes. Similarly, Mn may interfere with the translocation of Fe from roots to shoots.

The interaction Zn × Cu found in the present study to be synergistic was said by HEWITT (1983) to be antagonistic, each of the interacting elements affecting their respective concentration in the plant, competing for common carrier sites. Also, during metabolism they replace each other in some metalloenzymes. HALDAR and MANDAL (1981) found that high Zn concentration in soil accentuates Cu deficiency, each element competitively inhibiting the uptake of the other (GIORDANO et al. 1974). These workers also stated that the interaction Zn × Fe is mutually antagonistic, because excess Zn or Fe may cause reduction in the absorption of Fe or Zn, respectively. This mutual effect of Zn and Fe increases the possibility of their deficiency in the growing plants (KAUSAR et al. 1976).

A careful study of the results about PEC points out that the elemental synergistic interactions occurring within the pistachio leaves may contribute significant quantities of nutrients, while the antagonistic interactions can also deprive the plants of these nutrients, as in the case of K, which was found to be negative, suggesting a 14.33% decrease of K contribution to leaves (Tab. 2).

Moreover, the generally high PEC to soil found, suggests that the synergistic interactions mobilize from non-available sources significant quantities of nutrients, while the antagonistic interactions immobilize available nutrients to plants, unfavorably affecting soil fertility. Studying these results, the following may be concluded: the interactions between soil nutrients and soil properties play a significant role in supplying or decreasing the soil available plant nutrients, and therefore, they determine to a great extent the level of soil fertility.

Obviously, the interactions among soil nutrients favored significantly soil fertility, and hence, plant growth and nut yield. In detail, the synergistic and statistically significant interaction P × Mg (Fig. 3b) found in the soil of pistachio orchards has also been found to occur between soil P and leaf Mg (MERHAUT 2007). This interaction seems to be associated with the ionic balance, related to cation and anion uptake by plants as well as to the increased root growth, sometimes observed with increased P fertilization. The effect of P fertilization increasing Mg uptake has also been documented in rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), bean (*Phaseolus vulgaris* L.), and corn (*Zea mays* L.) (FAGERIA et al. 1995).

In relation to K, it has been suggested that the interaction of this element with other elements presents a great variability among plant species (DIBB and THOMPSON 1985). This interaction should be studied more scrupulously, considering that many other factors, such as the level of available Mg (very high in the present study), fertilization and age of plants, moisture and temperature in soil, could affect the above interaction (ARNON 1975). The macronutrient K is a very strong competitor, when it is present in high concentration. It particularly affects the uptake of Mg. However, in the present work, the interaction Mg × K was found to be antagonistic due to the high level of Mg in the leaves (2.4%); the mean K concentration in leaves was only 1.2%, close to the low limit of the critical range (1.0–2.0%) (CRANE and MARANTO 1988). It must be noted that, generally, the statistically significant interactions are usually biphasic, that is, the elements involved affect each other negatively or positively but at high concentrations. For example KOUKOULAKIS et al. (1988) observed that increasing the supply of K reduced leaf Mg concentration in tomato and cucumber, with an increase of K availability in the soil, due to K × Mg antagonism. However, increasing K concentrations of nutrient solutions in hydroponically grown tomatoes resulted in increased Mg concentration in fruits and seeds (MENGEL and KIRKBY 1987).

In the present work, it has been shown that in general, the increase of soil Cu concentration significantly increased K level in the soil, suggesting that the interaction CuxK is synergistic (Fig. 3f). Similar results have been reported by KALAVROUZIOTIS and KOUKOULAKIS (2009b) according to which the interaction Cu × K was synergistic in the roots, leaves and sprouts of Brussels sprouts. In contrast to the above findings, SONMEZ et al. (2007) reported that increasing Cu applications resulted in a decline of leaf and root K concentration in tomato seedlings. On the other hand, ALVA et al. (1999) found that the application of increased levels of Cu decreased the Zn content of leaves and roots of *Citrus* seedlings, grown in a sand substrate. However, in the present work, the interaction Cu x Zn was synergistic and statistically significant in both, soil and pistachio leaves (Tabs. 3, 5). The availability of nutrients, especially of micronutrients, in soil was strongly influenced by pH, $CaCO_3$ and organic matter (Tab. 4). It is known that Zn solubility increases at low pH values (LINDSAY 1991, BRADY and WEIL 2002). In particular, the optimum availability of Zn in soil is observed at a pH range 5–7. Indeed, we found that the interactions pH × N, $CaCO_3$ × Cu (Tab. 1), and $CaCO_3$ × Fe, pH × Fe, $CaCO_3$ × Mn, pH × Mn, pH × B, and $CaCO_3$ × B (Tab. 4) were antagonistic, as they were negatively interrelated. On the other hand, the interactions OM × B and OM × Zn (Tab. 1) as well as OM × K and OM × Zn (Tab. 4) were synergistic, suggesting the favorable effect of organic matter on the availability of soil plant nutrients. The fact that the interaction $CaCO_3$ × Zn was synergistic was unexpected, as most of the soils studied in this work were calcareous with high pH. A possible explanation could be the formation of $ZnCO_3$ due to the high Zn level in 13 cases of orchards out of a total of 50, varying from 3–17.5 mg kg^{-1} soil. The $ZnCO_3$ is a relatively soluble compound, which could even be used as fertilizer (TISDALE et al. 1993).

In conclusion, almost all the significant elemental interactions occurring in pistachio leaves or soils were synergistic. They contribute considerable quantities of available macro- and micronutrients and therefore improve the nutrient status of pistachio leaves, and the level of soil fertility. On the other hand, any antagonistic or synergistic interactions must be taken into account during fertilization of pistachio, because among other consequences, they may possibly lead to plant deficiencies. Soil analytical data showing the nutrient concentration levels, and their relationships, may be helpful in predicting possible antagonistic or synergistic interactions. Furthermore, the significant interactions between soil physical or chemical properties and nutrient content of the leaves or soils found in the present study, suggest that altering these properties (e.g. improving the organic matter or pH), could favorably modify the level of soil fertility, and therefore enhance plant growth, productivity and nut quality.

References

ALVA, A. K., HUANG, B., PRAKASH, O., PARAMASIVAM, S., 1999: Effects of copper rates and soil pH on growth and nutrient uptake by citrus seedlings. Journal of Plant Nutrition 22, 1687–1699.

ARNON, I., 1975: Mineral nutrition of maize. International Potash Institute, Bern, Switzerland.

BASLAR, S., DOGAN, Y., MERT, H. H., 1999: Studies on the ecology of *Pistacia terebinthus* L. subsp. *palaestina* (Boiss.) Engler in West Anatolia. Journal of the Faculty of Science, Ege University 22, 1–12.

BOUJOUCOS, G. J., 1962: Hydrometer method improved for making particle size analysis of soils. Journal of Agronomy 54, 464–465.

BRADY, N. C, WEIL, R. R., 2002: The nature and properties of soils. Prentice Hall, N.J., USA.

CHAPMAN, H. D., PRATT, P. F., 1961: Methods of analysis for soils, plants and waters. Division of Agricultural Sciences, University of California, Riverside, USA.

CRANE, J. C., MARANTO, J., 1988: Pistachio production. Cooperative Extension University of California, Division of Agricultural and Natural Resources. Publication 2279, Oakland, CA.

DIBB, D. W., THOMPSON, Jr. W. R., 1985: Interaction of potassium with other nutrients. In: MUNSON, R. D. (ed.), Potassium in agriculture, 515–533. SSSA, Madison, Wisconsin.

DOGAN, Y., BASLAR, S., AYDIN, H., MERT, H., 2003: A study of the soil-plant interactions of *Pistacia lentiscus* L. distributed in the western Anatolian part of Turkey. Acta Botanica Croatica 62, 73–88.

FAO (Food and Agriculture Organization), 2008. Retrieved September 5, 2012, from http://faostat.fao.org.

FAGERIA, N. K., ZIMMERMANN, F. J. P., BALIGAR, V. C., 1995: Lime and phosphorus interactions on growth and nutrient uptake by upland rice, wheat, common bean, and corn in an oxisol. Journal of Plant Nutrition 18, 2519–2532.

GIORDANO, P. M., NOGGLE, J. C., MORTVENDT, J. J., 1974: Zinc uptake by rice as affected by metabolic inhibitors and competing cations. Plant Soil 41, 637–646.

HALDAR, M., MANDAL, L. N., 1981: Effect of phosphorus and zinc on the growth and phosphorus, zinc, copper, iron and manganese nutrition of rice. Plant Soil 59, 415–425.

HEWITT, E. J., 1983: The essential and functional mineral elements. In: ROBINSON, J. B. D., BOULD, C., HEWITT, E. J., NEEDHAM, P. (eds.), Diagnosis of mineral disorders in plants, vol 1, 7–53. Her Majesty's Stationary Office, London, UK.

HODGSON, J. F., LINDSAY, W. L., TRIERWEILER, J. F., 1966: Micronutrient cation complexing in soil solution. II. Complexing of zinc and copper in displacing solution from calcareous soils. Soil Science Society of America Proceedings 30, 723–726.

JACKSON, M. L., 1958: Soil chemical analysis. Prentice Hall Inc., Englewood Cliffs, N.J., USA.

KALAVROUZIOTIS, I. K., KOUKOULAKIS, P. H., 2009a: Environmental implications of soil properties and essential nutrient interactions, under the effect of treated municipal wastewater. Water Air Soil Pollution 197, 267–276.

KALAVROUZIOTIS, I. K., KOUKOULAKIS, P. H., 2009b: Distribution of elemental interactions in Brussels sprout plants, under the treated municipal wastewater. Journal of Plant Interactions 4(3), 219–231.

KALAVROUZIOTIS, I. K., KOUKOULAKIS, P. H., ROBOLAS, P., PAPADOPOULOS, A. H., PANTAZIS, V., 2008: Macro- and micronutrient interactions in soil under the effect of *Brassica oleracea* var Italica, irrigated with treated municipal wastewater. Fresenius Environmental Bulletin 17, 1–15.

Kalavrouziotis, I. K., Koukoulakis, P. H., Sakellariou-Makrantonaki, M., Papanikolaou, C., 2009: Effects of treated municipal wastewater on the essential nutrients interactions in the plant of *Brassica oleracea* var Italica. Desalination 242, 297–312.

Kalavrouziotis, I. K., Koukoulakis, P. H., Mehra, A., 2010: Quantification of elemental interactions effects on Brussels sprouts under treated municipal wastewater. Desalination 254, 6–11.

Kausar, M. A., Chaudhary, F. M., Rashid, A., Latif, A., Alam, S. M., 1976: Micronutrient availability to cereals from calcareous soils. I. Comparative Zn and Cu deficiency and their mutual interaction in rice and wheat. Plant Soil 45, 397–410.

Kizilgoz, I., Kizilkaya, R., Acar, I., Kaptan, H., 2001: Nutrient contents of pistachio trees (*Pistacia vera* L.) growing in district of Sanliurfa and the relationship between their microelement deficiency and some soil properties. Cahiers Options Méditerranéennes 56, 47–52.

Koukoulakis, P., 1995: Principles of rational fertilization of crops (in Greek). Agriculture and Husbandry 9, 43–61.

Koukoulakis, P. H., Simonis, A. D., Bladenopoulou, S., 1988: Potassium-magnesium antagonism in tomato and cucumber, grown in plastic greenhouse. Proceedings of Athens Academy 63, 130–139.

Koukoulakis, P. C., Simonis, A. D., Gertsis, A., 2000: The organic matter in soils. The problem of Greek soils (in Greek). Stamoulis Publications, Athens.

Lindsay, W. L., 1991: Inorganic equilibria affecting micronutrients in soil. In: Mortdvedt, J. J., Giordano, M., Lindsay, W. C. (eds.), Micronutrients in agriculture, 90–111. SSSA, Madison, Wisconsin, USA.

Mengel, K., Kirkby, E. A., 1987: Principles of plant nutrition. International Potash Institute, Berne, Switzerland.

Merhaut, D. J., 2007: Magnesium. In: Barker, A. V., Pilbeam, D. J. (eds.), Handbook of plant nutrition, 146–181. CRC Press, Boca Raton, FL, USA.

Mills, H. A., Benton Jones Jr, J., 1996: Plant analysis handbook. MicroMacro Publishing Inc., Athens, USA.

Olsen, S. R., 1972: Micronutrient interactions. In: Mortdvedt, J. J., Giordano, M., Lindsay, W. C. (eds.), Micronutrients in agriculture, 243–264. SSSA. Madison, Wisconsin, USA.

Sonmez, S., Kaplan, M., Somnez, N. K., Kaya, H., Uz, I., 2007: Effect of both soil copper applications and foliar copper application frequencies on macronutrients contents of tomato plants. Asian Journal of Chemistry 19, 5372–5384.

Srivastava, P. C., Gupta, U. C., 1996: Trace elements in crop production. Science Publishers Inc., Lebanon, USA.

Stevenson, F. J., Cole, M. A., 1999: Cycles of soil. Carbon, nitrogen, phosphorus, sulfur, micronutrients. John Wiley and Sons Inc., New York, USA.

Tisdale, S. L., Nelson, W. L., Beaton, J. D., Havlin, J. L., 1993: Soil fertility and fertilizers. MacMillan Publishing Co, New York, USA.

WOLF, B., 1971: The determination of boron in soil extracts, plant materials, composts, manures, water and nutrient solutions. Communications in Soil Science and Plant Analysis 2, 363–374.