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MICRONUTRIENTS IN CALCAREOUS FOREST SOILS

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Abstract

The relations and the statistical comparisons of the available micronutrients Mn, Zn, Cu and Fe (DTPA extractable) were examined in alkaline soils of three forest ecosystems, i.e. pine, fir and maquis forest. Through a Principal Components Analysis (PCA) it was found that Mn and Zn had identical dependence on soil parameters. Both concentrations of these metals had negative relationship with pH, percentage of $CaCO_3$ and positive one with the concentrations of organic C, N and Cation Exchange Capacity (CEC) values. With the exception of $CaCO_3$, Fe had the same dependence. Copper had a negative relation with $CaCO_3$ and a positive one only with clay content and not with the organic carbon as would be expected. The Analysis of Variance (ANOVA) showed that the soil under fir had the highest concentrations of Mn probably due to the lowest pH values and $CaCO_3$ percentages. The soil in the maquis plot had the lowest concentrations of Zn and Fe because of the highest pH and lowest amount of organic C. Despite the lowest amount of organic C, the soil in the maquis plot had the highest concentration of Cu because of the nature of parent material.

Key words: $CaCO_3$, DTPA, fir, maquis, pH.

Introduction

The study of micronutrients was firstly carried out in agricultural soils. The general rules governing the behaviour of manganese (Mn) and iron (Fe) are the redox potential (i.e. oxidizing or reducing conditions) and pH (Porter et al. 2004; Colombo et al. 2014). Forest soils are usually aerobic and therefore the pH is the most important factor for these elements. Low pH values bring about dissolution of Fe and Mn oxides and enrichment of soil solution with Fe and Mn ready for plant uptake. The same dependency on pH exists with zinc (Zn). Its deficiency is limiting crop production in ±30 % of the world's soils (Sillanpää and Vlek 1985). Most of these soils are calcareous. Copper (Cu) also depends on soil pH although not as much as the previous mentioned metals (Sims 1986). It is the soil organic matter that Cu is strongly connected through chelation (McLaren et al. 1981). Soils with neutral to alkaline pH values may generally have lower Cu availability relative to acid soils, which can be explained by the presence of carbonates favouring Cu precipitation and adsorption (Bradl 2004). Forest plants have received less attention in this respect but there are some works on the subject. In northern Europe, the motivation, initially, was to ameliorate the acidification of forest soils through liming. Grønflaten and Steinnes (2005) used four extractants to predict metal uptake by understorey forest vegetation in Norway. In the same country, Grønflaten et al. (2005) determined the concentration of trace elements in soils and plants in Scots pine stand after liming. In other regions having an appreciable percentage of alkaline forest soils, the deficiency problem can be intense. In a review, Will (1990) quoted that pines growing in alkaline soils in New Zealand presented deficiencies (chlorotic symptoms) of Mn and Fe. Soil scientists have developed tests involving a single extractant to draw conclusions on the availability of micronutrients in soils. In agricultural calcareous soils, some thresholds concentrations have been established below which plant growth is retarded. Başar (2009) in Turkey, used 14 extractants to predict uptake of micronutrients and heavy metals in peaches grown on alkaline soils and Michopoulos et al. (2004) used 7 extractants to assess Mn uptake by fir in calcareous soils. It is a little risky to adopt threshold values for forest species. At most, these values can be an indication for micronutrient deficiencies, which have to be verified through fertilization trials.

The aims of this work were: 1) to identify the soil parameters affecting the content of the available micronutrients in calcareous forest soils in three ecosystems and 2) using this piece of information to compare the average concentrations in the selected ecosystems. The forests chosen were a pine and a fir forest in the mountain of Parnitha and maquis forest in the area of Corinth. When reforestations take place in calcareous soils, usually having low concentrations of available micronutrients, such information will be of avail.

Materials and Methods

Study sites and sampling

Mountain of Parnitha

Two sampling areas were located in the national park of the Mountain of Parnitha 40 km north of the city of Athens, capital of Greece. The Parnitha Mountain has a Mediterranean climate with frequent snowfalls in winter and pleasant temperatures in summer. The average temperature range is 8–13 ^{o}C and the yearly precipitation height was calculated 1231 mm (Michopoulos et al. 2007). The coordinates of the Parnitha Mountain are: latitude 38^{o}10′24" and longitude 23^{o}43′2.6".

The first sampling area was in the zone of Aleppo pine (*Pinus halepensis* (Mill.) and the second in the zone of Greek fir (*Abies cephalonica* Loudon).

Pine forests extend from the foot of the mountain, where they mix with shrubs (kermes oaks, strawberry trees, etc.), to 800 m a.s.l. In higher altitudes (800–1000 m), the Aleppo pine mixes with the Greek fir. Apart from the Aleppo pine the flora comprises kermes oak (*Quercus coccifera* L.), green olive trees (*Phylirea latifolia* L.), holm oak (*Quercus ilex* L.), strawberry trees (*Arbutus unedo* L.), junipers (*Juniperus* sp.), olives (*Olea europea* L.), garden sage (*Salvia officinalis* L.) and thorns (*Crataegus* sp.).

The fir zone extends from 900 to 1400 m a.s.l. The dominant plant species with ecological significance in that zone are: prickly juniper (*Juniperus oxycedrus* L.) wild roses (*Rosa canina* L.*, Rosa sempervirens* L.), barberry (*Berberis cretica* L.), hawthorns (*Crataegus heldreichii* Boiss., *Crataegus monogyna* Jack.). In addition, several trees species are included such as wild almonds (*Prunus webbii* Vierh.*, Prunus spinose* L.), wild pears

(*Pyrus amygdaliformis* Vill.) and naturally numerous herbs, like different kinds of daisies (*Bellis sylvestris* L., *Doronicum orientale* Hoffm.).

The soils of the sampling areas in both species zones are derived from hard limestone and tertiary deposits.

Soil samples were collected in a random way from a network of plots meant to monitor forest health in 2006. In total 40 sites of pine and 19 sites of the fir zone were selected for soil collection. In each collecting point, a composite sample was created by collecting soils in equal volumes from two orientations, north and east distancing 5 m each at a depth of 20 cm after the L layer was removed.

Maquis zone

The third sampling area is close to the city of Corinth (41 km) and 120 km southwest from the city of Athens. It is included in the *Quercetalia ilicis* vegetation zone, and *Quercion ilicis* and *Oleo-Ceratonion* subzones. The climate is typical Mediterranean with dry and warm summers and mild winters with a mean annual air temperature of 16.0 °C and mean annual rainfall of 560 mm (National Meteorological Service of Greece 2010). The altitude range is 360 to 500 m. The coordinates of the area are: latitude 37°48′22", longitude 22°40′19".

Maquis is an area covered dominantly by shrub vegetation of the Mediterranean region primarily of leathery, broad-leaved evergreen shrubs or small trees (Dallman 1998). The dominant species here are woody plants, i.e. mastic trees (*Pistacia lentiscus* L.), *Quercus coccifera, Arbutus unedo,* and *Olea* europaea. In addition, the herbaceous plants Greek cyclamen (*Cyclamen graecum* Link), and Bulbous dandelion (*Leontodon tuberosus* L.) are found.

Soil samples from this zone were collected in a systematic way at a depth of 20 cm (0–20 cm) after the L layer was removed. In a straight, line every 10 m a soil sample was collected. The first five samples made a composite sample and so on until there were 15 composite samples.

In terms of classification according to the WRB system (IUSS Working Group WRB 2015), the soils in the mountain of Parnitha are Regosols, whereas the maquis soil is a Luvisol.

Table 1 contains concise information of the ecosystems.

Table 1. Information on the forest ecosystems from which soil was collected.

Area	Altitude, m	Soil parent material	Rain height, mm	Main forest species
Parnitha	430–1000	Hard limestone, Tertiary deposits	1230	Aleppo pine
Parnitha	900–1400	Hard limestone, Tertiary deposites	1230	Greek fir
Korinth	360–500	Hard and soft limestone	560	Maquis

Soil analysis

All soil samples were air dried and passed through a 2 mm sieve prior to analysis. Subsamples of sieved soils were pulverized in a ball mill for the determination of organic C, calcium carbonate and Kjeldahl N.

The texture of soils was determined by the hydrometer method, while the $CaCO_3$

content was determined by a calcimeter.

The pH of soils (1:2.5 soil:water, ratio per weight) was measured by a glass electrode. Exchangeable cations (Ca, Mg, K and Na) were extracted with 1 M NH_4-acetate solution at a pH of 7. Cation exchange capacity (C.E.C.) of the samples was determined by the Na-acetate method (Bower et al. 1952).

Organic C was measured by wet oxidation with potassium dichromate ($K_2Cr_2O_7$), organic plus ammonium N was extracted with concentrated sulphuric acid (H_2SO_4), and its concentration was measured by the Kjeldahl distillation method (Jones 2001). Available trace elements (Fe, Zn, Mn and Cu) in soils were extracted with DTPA (Lindsay and Norvell 1978). In calcareous soils, the presence of calcium carbonate ($CaCO_3$) can result in higher concentrations of extracted micronutrients due to the dissolution of $CaCO_3$ particles where the metals are occluded. The use of DTPA depresses that dissolution and that is the reason it is suitable for calcareous soils (Mahashabde and Patel 2012, Al Jaloud et al. 2013, Chavda et al. 2018).

All results were expressed per dry weight of soil (105 °C).

Data handling and statistical analysis

The Statistical dependence of the available micronutrients were examined for some soil parameters in the mineral soil layers by means of the Principal Component Analysis (PCA). The PCA was conducted with the ordination software CANOCO (Ter Braak and Smilauer 2002). The parameters chosen were the pH, the percentages of $CaCO_3$, clay and organic C, the concentrations of N, the CEC value and the ratio of C/N. The first principal component is the linear combination of x-variables that has maximum variance (among all linear combinations). It accounts for as much variation in the data as possible. The second principal component is the linear combination of x-variables that accounts for as much of the remaining variation as possible, with the constraint that the correlation between the first and second component is 0 (Zar 1999). Values around 0.600 or higher are considered satisfactory as they explain at least 40 % of the parameters' variability (Comrey 1962).

The average values, the ranges and coefficients of variation (percentages of standard deviations over the means) were calculated for all soil parameters measured. The latter were compared with an analysis of variance (ANOVA). Before the ANOVA test a logarithmic transformation was applied to the data (apart from pH) to conform to normality requirement.

Results and Discussion

In Table 2, the component 1 represents the horizontal axis of Figure 1, whereas the component 2 the vertical one. In addition, the PCA axes and the vectors of the parameters are combined with the calculated values of the micronutrients. We can see that Mn, Zn and Fe have values higher than 0.600 in the component 1 column, whereas Cu in the component 2 column.

The PCA figure and Table 2 showed that the Mn and Zn had identical dependence on soil parameters. Both the concentrations of these metals had negative relationship with pH, $CaCO_3$ and positive ones with organic C, N and CEC. Similar behavior of these two metals, with regard to solubility ($CaCl_2$-extractability) was also found by Haynes and Swift (1985) in a greenhouse experiment. With the exception of $CaCO_3$, Fe had the same depend-

Table 2. Values of soil parameters derived from PCA analysis.

		Component 1		
Soil parameter	**Mn (0.768)**	**Zn (0.705)**	**Cu (0.25)**	**Fe (0.849)**
$CaCO_3$	-0.683	-0.557	-0.459	-0.452
pH	-0.849	-0.778	-0.743	-0.742
CEC	0.839	0.887	0.844	0.871
C	0.696	0.811	0.866	0.864
N	0.656	0.791	0.868	0.830
C/N	0.056	-0.009	-0.085	-0.024
Clay	0.141	-0.061	-0.243	-0.210-
		Component 2		
Soil parameter	**Mn (0.278)**	**Zn (0.315)**	**Cu (0.660)**	**Fe (0.222)**
$CaCO_3$	-0.291	0.410	-0.698	0.535
pH	-0.059	0.282	-0.294	0.381
CEC	-0.151	-0.081	0.127	-0.197
C	-0.444	0.164	-0.239	0.097
N	-0.686	0.505	-0.081	0.373
C/N	0.510	-0.617	-0.075	-0.530
Clay	0.861	-0.852	0.790	-0.866

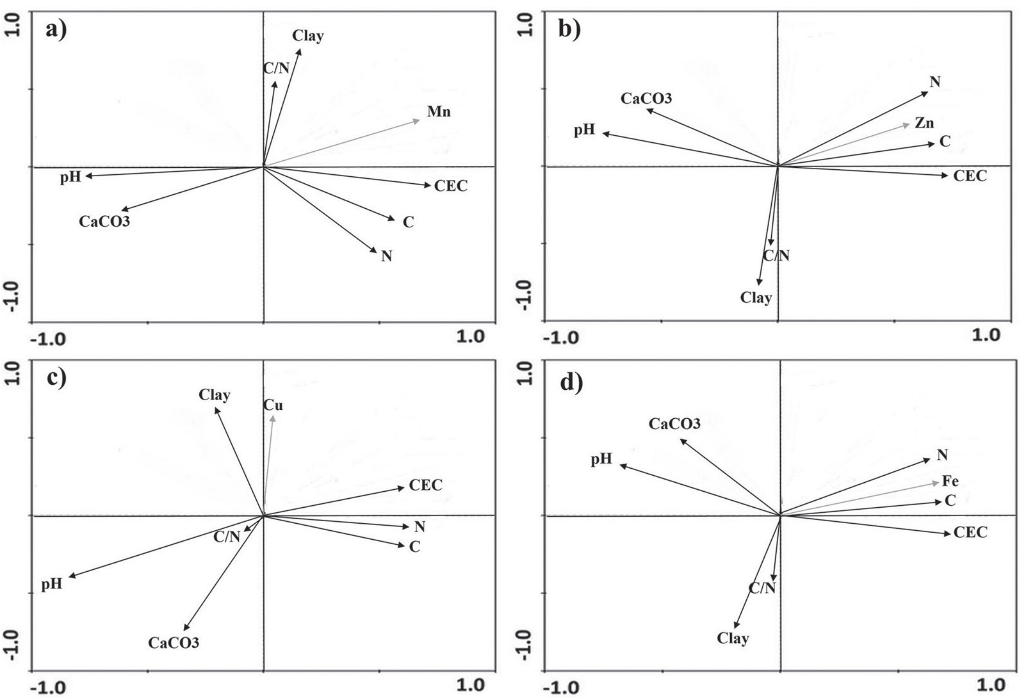

Fig. 1. Ordination diagrams for the relation of available micronutrients – Mn (a), Zn (b), Cu (c) and Fe (d) with the soil properties.

ence. Copper had a negative relation with the $CaCO_3$ and a positive relation only with clay and not with the organic matter as would be expected because of the strong bonds it usually forms with organic compounds. Not all researchers have found significant dependency of pH with DTPA extractable micronutrients. Mahmoudabadi et al. (2015) found negative relationships of the four micronutrients under consideration with $CaCO_3$ but not with pH in urban forest soils in Iran. Similarly, to this study they did not find significant relationship of Cu with organic matter. Copper was not found to correlate with pH. Likewise, Grønflaten and Steinnes (2005) did not find any effect on extractable (with NH_4NO_3) Cu after liming in a forest soil under Scots pine in Norway. In contrast, in agricultural soils in Spain Navarro-Pedreño et al. (2018) found that Cu, Mn, Fe (DTPA extractable) had a negative relationship with pH and Zn a positive one. The Organic C content of those soils was very low in comparison with the soils in this work and that fact was probably influential. It seems that different soils do not have the same behavior so relationships between available micronutrients and soil parameters should be examined separately for each soil type.

The deficiency limits for micronutrients extracted with DTPA have been defined for agricultural soils and have a range depending on the crop they refer to. So for Mn it is 3–3.5 mg·kg^{-1}, for Cu 0.2–1.0 mg·kg^{-1}, for Zn 0.5–0.54 mg·kg^{-1} and for Fe 4.5–4.8 mg·kg^{-1} (Lindsay and Norvell 1978, Nayyar et al. 1985, Sharma and Mukhopadhyay 2006). Table 3 shows the ranges of all soil parameters. With regard to the micronutrients, Cu seems to be the micronutrient in shortest supply. More specifically, the three ecosystems have some soil samples below the limit

Table 3. Averages, ranges and coefficients of variation (in parenthesis) of soil parameters.

Sites	C	N	C/N	Sand	Clay	Silt	CEC	$CaCO_3$	pH	Mn	Zn	Cu	Fe
Pine	7.36a	3.48a	22.7a	50.5a	23.9a	25.6a	47.5a	20.2a	7.66a	39.5a	7.33a	1.21a	67.6a
	3.9–15	1.6–8.9	11–43	29–69	15–44	16–33	18–96	2.5–45	6.9–8.1	17–77	1.0–58	0.7–2.8	30–183
	(33)	(45)	(29)	(18)	(28)	(13)	(37)	(57)	(3.5)	(36)	(126)	(32)	(54)
Fir	7.51a	4.39a	18.4b	55.7a	20.3a	24.0ab	60.8b	5.11b	7.37b	68.1b	9.09a	1.82b	82.8a
	1.17–15	0.7–11	12–31	32–85	5.4–39	9.4–31	29–116	0.2–44	6.7–7.7	21–126	0.6–72	0.9–4.0	20–163
	(43)	(51)	(30)	(24)	(48)	(200	(28)	(196)	(3.5)	(42)	(170)	(47)	(46)
Maquis	4.43b	3.58a	12.8c	54.1a	24.5a	21.4b	24.8c	13.9c	7.93c	43.8a	2.01b	3.02c	12.0b
	0.80–8.0	0.9–5.6	6.7–16	28–78	8.0–48	14–26	6.4–44	1.6–48	7.4–8.5	9.2–106	0.25–7.2	0.5–6.7	3.3–22
	(45)	(46)	(26)	(23)	(45)	(18)	(37)	(108)	(4.6)	(71)	(92)	(52)	(39)

Note: Values in the same column followed by different letters differ significantly for at least 0.05 probability level.

of 1.0 mg·kg^{-1} in Cu. The soil in the pine stand has nine cases, the maquis two and the fir one. In addition, in the maquis plot the soil has low concentrations (below the limits) of Zn (four cases) and of Fe one. It must be taken into account that these ranges refer to the bulk soil and not to the rhizosphere one. Plants exude a variety of organic compounds and inorganic ions (protons, phosphate, etc.) to change the chemistry and biology of the rhizosphere (Rengel 2015). In any case, when reforestation takes place these low concentrations should be considered carrying out, for example, an experiment with fertilization trials.

The parameter that had the lowest coefficient of variation was the pH (Table 3). This is due to the strong buffer capacity calcareous soils have to withstand pH changes. Interestingly the micronutrient with the highest coefficient of variation was Zn.

Although the focus is on the micronutrients, the ANOVA was applied to all soil parameters to draw conclusions on the differences among the three ecosystems. Significant differences were found among the parameters on which the micronutrient concentrations depend. It is obvious that the soil in the maquis plot has striking differences with the soils in the two high forests of Parnitha. The first difference is the low content of organic matter (Table 3). This is due to the fact that the decomposition of needles in the high forests in a mountainous environment is slower and organic matter accumulates in soils. In addition, the C/N ratio had the lowest values in the maquis stand, a sign of rapid decomposition of the litterfall. The magnitude of the CEC followed that of the organic C in the three stands. The percentage of $CaCO_3$ and the pH had the highest values in the maquis plot as the result of the nature of the parent material (soft limestone). The low amount of organic matter and the high pH contributed to the low concentrations of Zn and Fe in the maquis stand. The pH had the lowest value in the soil of the fir stand. Consequently, the soil had the highest concentration of available Mn and relatively high concentrations of Zn and Fe (Table 3). The highest concentration of Cu in the soil of the maquis stand is a little difficult to explain. The maquis plot had the least concentration of organic C. In addition, the clay content in soils with which Cu had a good relationship did not differ significantly among the three forest stands. Probably the explanation lies in the nature of the parent material of the soil in the maquis plot. Okoli et al. (2019) stressed the importance of parent material in soil fractionation in four different locations in Imo State, Southeastern Nigeria. They found significant differences with regard to Cu concentrations among almost all the different fractions in the various types of parent material.

Conclusion

The pH was a decisive factor for the mobility of Mn, Zn and Fe in the calcareous soils in this work. Despite this similarity, the Zn concentration had very high variability compared with the two other metals. The relationships of available Cu with soil parameters did not always follow the patterns mentioned in literature, especially with regard to the close relation of the metal with the organic matter. The parent material seems to be an additional important factor to affect the Cu content in soils. The Mn concentration was satisfactory in all stands but the Cu content was low especially in the pine stand and Zn in the maquis stand. Grown plants can overcome the low concentrations of micronutrients in soils with root exudation. In case of reforestation young plants may need some fertilization which can nevertheless be proved through trial.

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