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Original Article

Effect of Irrigation and Fertilization Levels on Mineral Composition of *Cannabis sativa* L. Leaves

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Abstract

A field experiment was conducted in central Greece to study, the effect of two irrigation (I1: 100% ETo, I2: 60% ETo) and N-fertilization levels (N1: 244, N2: 184 kg ha^{-1}), on the nutrients concentration of *Cannabis sativa* leaves (cv. 'Fibranova'). The N, K, Ca, Mg, P -concentration in the leaves was ranged by 2.8 to 3.51%, 1.8 to 2.57%, 1.96 to 2.17%, 0.86 to 0.88%, and 0.3 to 0.37% respectively, while by the micronutrients the iron showed the highest concentration that ranged by 129 to 139.8 mg kg^{-1}dw. The treatment I1F1, where the highest level of irrigation and N -fertilization was applied, compared to the other treatments, showed the highest dry biomass yield, however, in the leaves the highest concentrations of N, K, Mn and Cu were not observed. Moreover, it was found that the N, K, Ca, Mg, P and Fe removal only by one ton dry biomass of leaves was ranged by 28 to 35.12 kg, 18.01 to 25.65 kg, 19.6 to 21.7 kg, 8.34 to 8.75 kg, 3.01 to 3.70 kg and 0.129 to 0.140 kg, respectively. These results could contribute optimal fertilizer application and therefore to the reduction of production costs of the crop.

Keywords: cannabis; fertilization; irrigation; mineral composition; nutrient uptake

Introduction

Cannabis sativa is an annual herbaceous crop cultivated in a wide range environmental conditions for its fiber production, medicine, as fuel source, and as raw material for thermal insulation (Small and Marcus, 2002; Van der Werf and Turunen, 2008; Bertoli *et al.*, 2010; Mihoc *et al.*, 2012).

Although all hemp varieties contain psychoactive compounds as Δ9-tetrahydrocannabinol (THC), the fiber hemp varieties contain low concentration, and in the European Union, the majority of the countries, have defined a maximum THC concentration of 0.2 g 100g^{-1} dry weight for the fiber hemp cultivation (Small and Marcus, 2003).

During the last years hemp cultivation has increased significantly in Europe, because it has a positive environmental impact, can be used as an alternative rotation crop, can improve soil structure and preserving soil fertility(Bertoli *et al.*, 2010; Zatta *et al.*, 2012; Bouloc and Werf, 2013; Finnan and Styles, 2013).

Hemp crop yield and quality of hemp products is influenced significantly by hemp cultivars, agro-technical methods, soil properties, and climate. Therefore, is important the choice of the appropriate hemp variety in an area (Amaducci *et al.*, 2012; Cosentino *et al.*, 2012; Tang *et al.*, 2016).

Intake of the nutrient elements from the plant affects the biomass production and the amounts of available soil nutrients. However, there is little literature on the mineral composition of fibre hemp, in order to optimize the mineral fertilization, mainly when is cultivated under Mediterranean conditions (Hakala *et al.*, 2009; Angelini *et al.*, 2014).

The objective of the present study was the effect of irrigation and fertilization levels on the macronutrients and micronutrients of *Cannabis sativa* leaves (variety 'Fibranova'), in order to set up removal amounts from the leaves and to optimize mineral fertilization, under the Mediterranean conditions of Thessaly region, central Greece.

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Materials and Methods

Experimental

The experiment was conducted at the Experimental Farm of the Technological Educational Institute of Thessaly, Larissa (latitude 39°62′69″ N, longitude 22°38′14″ E). The seeding took place on April 2017 applying 45 kg ha^{-1} of seed cultivar 'Fibranova' in a row-distance of 12.5 cm. The crop density was 160 plants m^{-2}. Two levels of irrigation (I1=100% ETo and I2=60% ETo), and two levels of fertilization (F1=244 kg N, 60 kg P_2O_5 and 60 kg $K_2O$and F2=184 kg N, 60 kg $P_2O_5$and 60 kg K_2O per hectare) were applied. The evapotranspiration (ETo) in the area was500 mm. Thus, hemp cultivation consists of four experimental units, I1F1, I1F2, I2F1 and I2F2. The size on each experimental unit was 14 m^2 with four replications. The study area is characterized by a typical Mediterranean climate with cold winter, hot summer and low precipitation in spring and summer. In particular, during the growing period of hemp, the average temperature ranged to 22.9 °C, while the precipitation was 290 mm.

Methods of analyses

The Texture of soils was determined by the bouyoucos hydrometer method (Bouyoucos, 1962). Soil samples were analyzed using the following methods which are referred by (Page *et al.*, 1982).

Soil pH is determined in (1:5) soil/water extract; pH value is measured by using glass electrode pH meter**.** Electrical conductivity, (EC) measured in (1:5) soil/water extract; using conductivity meter. The calcium carbonate was determined by the method Bernard. Organic matter was analyzed by chemical oxidation with 1 mol L^{-1} $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol L^{-1} $FeSO_4$.

Both ammonium and nitrate nitrogen were extracted with 0.5 mol L^{-1} $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively**.** Exchangeable forms of potassium and sodium were extracted with 1 mol L^{-1} CH_3COONH_4 and measured by flame Photometer (Essex, UK)**.** Available P forms was extracted with 0.5 mol L^{-1} $NaHCO_3$ and measured colorimetrically. Available forms of Cu, Zn, Mn and Fe were extracted with DTPA (diethylenetriaminepentaacetic acid 0.005 mol L^{-1} + $CaCl_2$ 0.01 mol L^{-1}+triethanolamine 0.1mol L^{-1}) and measured by atomic absorption. The samples were analyzed by Atomic Absorption, Spectroscopy Varian Spectra AA 10 plus, Victoria, Australia, with the use of flame and air-acetylene mixture (Varian, 1989).

Dried samples of hemp were ground in a rotor mill to < 300 μm prior the analysis. The minerals in plant tissues were determined by dry combustion according to the methods described by (Jones and Case, 1990).Total nitrogen content in leaves was measured using the Kjeldhal method described by Jones (Jones, 1998).

Statistical analysis

Data analysis was made using the MINITAB (Ryan *et al.*, 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences (P=0.05) between treatments were found.

Results and Discussion

Soil physical-chemical characteristics

The soil of the experimental field before hemp's sowing at depth 0-30 cm was characterized as Sandy Clay Loam (SCL), bulk specific gravity of soil was 1.3 g cm^{-3}, the pH at (1:5) soil/water extract was 7.26, while the electrical conductivity measured in the same extract was 0.2 ms/cm, organic matter and $CaCO_3$ soil content was 1.44% and 2.42%, respectively. Moreover, the exchangeable Na content and the cation-exchange capacity (CEC) of soil was 103.5 mg kg^{-1}and 19.3 cmolkg^{-1}, respectively. The exchangeable Ca and Mg soil content was 1988 mg kg^{-1} and 397.5 mg kg^{-1}, respectively. Finally, the levels of available mineral nutrient elements N, P, K, Cu, Zn, Mn, and Fe before hemp sowing and after the crop harvest are presented in Table 1 and Fig. 1. According to other studies, the hemp crop requires slightly alkaline and well-drained soils, rich in nutrients and organic matter (Struik *et al.*, 2000).

The soil inorganic nitrogen content ($N\text{-}NH_4^+$ + $N\text{-}NO_3^-$) showed a decrease in all treatments at the end of the crop period (harvest) compared with the begin of the crop period, part of this decrease is likely due to plant nitrogen uptake and in leaching to lower soil layers. The lower inorganic nitrogen content at the end of the crop period compared with the begin of the crop period was observed in treatments I1F2 and I2F2, where the smallest nitrogen fertilizer dose was added, irrespective of the irrigation levels (Table 1 and Fig. 1).

The available phosphorus (Olsen) content of soil increased at the end of the crop period compared with the beginning of crop period in the treatment I2F1, where the lowest level of irrigation was applied. This increase to available phosphorus content is probably due to the lower phosphorus leaching or in lower uptake by the plant, and not only in the added phosphorus fertilizer. On the contrary, the available phosphorus content decreased at end of the crop period compared with the beginning of crop period for the treatment I1F2, where the higher level of irrigation was applied. This decrease to available phosphorus content is probably due to greater phosphorus leaching or in increased uptake by the plant. In the other treatments, the available soil phosphorus content did not show statistically significant differences at end of the crop period compared with the beginning of the crop period (Table 1 and Fig. 1).

The levels of the available forms K and Mn in the soil showed a decrease at end of the crop period compared with the beginning of the crop period for treatments I1F1 and I1F2, where the higher level of irrigation was applied. This decrease to available forms K and Mn is probably due to greater leaching of the mineral elements or in increased uptake by the plant. Finally, the levels of the available forms Cu, Zn and Fe of soil for all treatments did not show statistically significant differences at end of the crop period compared with the begin of the crop period (Table 1 and Fig. 1). The inorganic N, P_2O_5, and K_2O concentrations were transformed to nutrient contents presented in Table 2 and Fig. 2 using the following equation:

Nutrient content ($kg\ ha^{-1}$) = nutrient concentration ($kg\ 10^{-6}\ kg^{-1}$) × 1300 ($kg\ m^{-3}$) × 10^4($m^2\ ha^{-1}$) × 0.3(m)

Where:

1300 $kg\ m^{-3}$ is the soil bulk density value.

It is important to refer that the concentrations of available P and K forms, first were transformed in concentrations of P_2O_5, and K_2O.

Table 2 and Fig. 2, shows the balance of mineral nutrient elements in the soil at depth of 0-30 cm, during the cultivation period. Our results confirm the high demands of the cannabis in available forms of N and K during the growing period.

Table 1. Levels of nutrient elements in soil before hemp sowing and after the crop harvest at soil depth 0-30 cm

Treatments	Available forms			DTPA			
	N	P	K	Cu	Zn	Mn	Fe
	mg kg^{-1} soil						
Before hemp sowing							
	381.5±29.3^a	17.2±1.12^{bc}	555.8±23.4^a	0.93±0.07^a	0.70±0.05^a	4.92±0.37^a	0.37±0.03^a
After crop harvest							
I1F1	171.5±12.7^b	18.7±1.29^b	432.4±16.0^b	0.85±0.05^a	0.78±0.07^a	2.96±0.21^b	0.30±0.02^a
I1F2	54.5±4.54^d	13.9±0.84^d	462.6±16.1^b	0.92±0.06^a	0.64±0.05^a	3.11±0.20^b	0.32±0.02^a
I2F1	118.5±8.78^c	21.8±1.23^a	512.9±17.6^a	0.85±0.06^a	0.46±0.04^a	4.15±0.29^a	0.37±0.03^a
I2F2	66.5±5.45^d	15.5±1.17^{cd}	512.9±18.5^a	0.85±0.05^a	0.55±0.05^a	4.26±0.31^a	0.37±0.03^a

Data represent average means and SE deviation, n= (4); For each chemical property of soil, columns of the table with the same letter do not differ significantly according to the Tukey's test (P=0.05).I1=100% ETo; I2=60% ETo; F1=244 kg N, 60 kg P2O5 and 60 kg K2O per hectare; F2=184 kg N, 60 kg P2O5 and 60 kg K2O per hectare

Table 2. Balance of mineral nutrient elements in the soil at depth (0-30) cm

Treatments	Mineral-N	Available-P_2O_5	Available-K_2O
	$kgha^{-1}$		
Start of growing period			
	1488±114.46	153.6±10.04	2612.3±109.76
Added fertilization			
I1F1	244	60	60
I1F2	184	60	60
I2F1	244	60	60
I2F2	184	60	60
End of growing period			
I1F1	668.9±49.55	167.0±11.53	2032.3±75.02
I1F2	212.6±17.72	124.2±7.51	2174.2±75.49
I2F1	462.2±34.24	194.7±10.99	2410.6±82.87
I2F2	259.4±21.26	138.5±10.46	2410.6±86.74

Data represent average means and SE deviation, n= (4); I1=100% ETo; I2=60% ETo; F1=244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare; F2=184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare

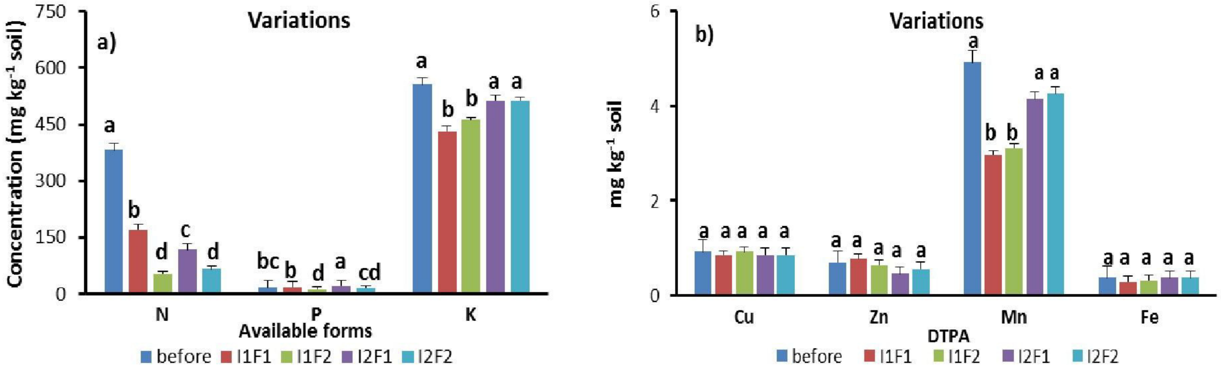

Fig. 1. Levels of nutrient elements in soil before hemp sowing and after the crop harvest at soil depth 0-30 cm; I1F1 (100% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I1F2 (100% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare, I2F1 (60% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I2F2 (60% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare); Data represent the average means ± standard deviation, n= (4); For each chemical property of soil, columns with the same letter do not differ significantly according to the Tukey's test (P = 0.05)

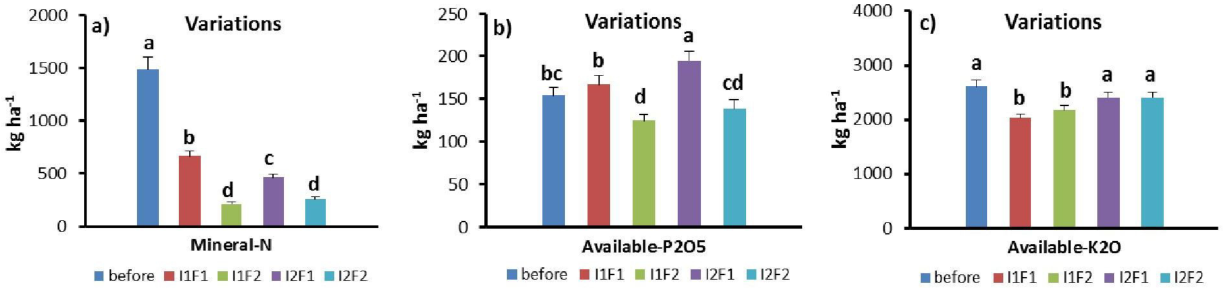

Fig. 2. Inorganic elements availability in the soil at depth (0-30) cm during growing period; Before, start of growing period; I1F1, I1F2, I2F1, and I2F2, end of growing period; Added irrigation and fertilization, I1F1 (100% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I1F2 (100% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare, I2F1 (60% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I2F2 (60% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare);Data represent the average means ± standard deviation, n= (4); For each chemical property of soil, columns with the same letter do not differ significantly according to the Tukey's test (P = 0.05)

Mineral composition of hemp leaves

The mineral composition of hemp leaves, grown under the environmental conditions of central Greece, is shown in Table 3 and Fig. 3. Among the applied treatments, I1F2 showed the highest nitrogen concentration in the hemp leaves. This is probably due to the lower inorganic nitrogen concentration in the soil for the treatment I1F2 at the end of the growing season (Table 2 and Fig. 2). Comparing the treatments where the highest level of irrigation (100% ETo) was applied with the two N-fertilization levels (I1F1 and I1F2), the treatment I1F2 showed the highest nitrogen concentration in the hemp leaves. This is probably due to lower biomass production and the existence of lower inorganic nitrogen concentration in the soil at the end of the growing season for the treatment I1F2 (Table 3 and Fig. 3). Comparing the treatments where the lowest level of irrigation (60% ETo) was applied, with the two N-fertilization levels (I2F1 and I2F2), I2F1 showed highest nitrogen concentration in the hemp leaves. This is probably due to the application of highest level fertilization of nitrogen in treatment I2F1 and the same amount biomass production observed between the two treatments.

Moreover, hemp leaves in the treatments where the highest irrigation level (100% ETo) was applied, compared to the treatments with the lowest applied irrigation level (60% ETo), showed highest potassium concentration. This is probably due to the existence of lower potassium concentration in the soil at the end of the growing season combined with the higher uptake of potassium from the hemp leaves, in the treatments where the highest level of irrigation was applied (Table 1 and 5; Fig. 1 and Fig. 6). Furthermore, no effect of the two irrigation levels was observed in Ca, Mg and P -concentrations in the hemp leaves (Table 3 and Fig. 3).

Therefore, the hemp cultivation under the environmental conditions of central Greece, for treatment I1F1 where was applied the highest irrigation and N-fertilization level, showed highest biomass production, although not observed highest N and K concentration in the leaves (Table 4 and Fig. 4).

The results showed that for the studied macronutrients, the hemp leaves had highest in N concentration, followed by K, Ca, Mg and P, in all treatments (Table 3 and Fig. 3). These results are similar to those reported by other authors for different hemp varieties in central and south Europe (Ivanyi, 2011; Angelini *et al.*, 2014). In particular, the results in the leaves of 'Fibranova' variety showed highest Mg and P concentration, compared with the leaves of same variety grown under the environmental conditions of central Italy, while showed similar in N, Ca and K concentration (Angelini *et al.*, 2014).

There is little information on the micronutrients concentrations at hemp leaves. In these results, regarding in Fe, Cu, Zn, Mn and Mo concentrations in hemp leaves, no effect of the irrigation levels was observed. The results showed highest Fe concentration, followed by Mn, Zn, Cu and Mo, in all treatments (Table 3 and Fig. 3). Moreover, these results showed lower copper and zinc content in the leaves compared to some varieties grown in Ethiopia and Pakistan (Ghani *et al.*, 2012; Zerihun *et al.*, 2015). Furthermore, the hemp leaves of the study, showed lower Cu and Fe concentration, similar in Zn and Mo, and highest Mn concentration, compared with the leaves of some varieties grown under the environmental conditions of central Italy, including of 'Fibranova' variety (Angelini *et al.*, 2014).

The accumulation of the mineral elements in the leaves may be related with factors as the mobility of the elements in the plant, the rate of uptake of the nutrients by the roots the availability of the elements in the soil solution, the climatic conditions, and the variety (Benner and Bazzaz, 1988).

According to previous studies (Mengel *et al.*, 2001), the accumulation tendencies of nutrients to the various plant organs, may indicate synergy or competition between the nutrients, and the various plants organs to have different allocation of nutrients.

Dry yield and nutrient uptake from the hemp leaves

The harvest was carried out at flowering stage, in July and August 2017. Plants from each treatment were hand cut at 6 cm from soil. The aerial part of the plant was separated in stem and leaves and allowed to dry into in oven at 59 °C for the air dry weight determination. The dry biomass hemp yield of, is shown in Table 4 and Fig. 4. The total dry hemp biomass for all treatments ranged from 12.28 to 15.41 ton ha^{-1}, while the treatment I1F1 showed the highest dry biomass yield.

Table 3. Mineral composition of hemp leaves at different cultivation practices

Mineral elements in hemp leaves	Treatments			
	I1F1	I1F2	I2F1	I2F2
N (g kg^{-1}dw)	28.360±1.25^{c}	35.118±1.59^{a}	31.356±1.40^{b}	27.998±1.24^{c}
P (g kg^{-1}dw)	3.326±0.17^{ab}	3.696±0.17^{a}	3.481±0.20^{ab}	3.006±0.16^{b}
K (g kg^{-1}dw)	21.826±0.81^{b}	25.647±0.95^{a}	18.006±0.62^{d}	19.654±0.69^{c}
Ca(g kg^{-1}dw)	21.7±0.78^{a}	21.2±0.82^{ab}	19.6±0.74^{bc}	20.2±0.79^{b}
Mg (g kg^{-1}dw)	8.75±0.40^{a}	8.84±0.39^{a}	8.55±0.41^{a}	8.72±0.39^{a}
Na (mg kg^{-1}dw)	159.6±7.16^{a}	171.8±8.81^{a}	159.6±8.19^{a}	159.6±8.19^{a}
Cu (mg kg^{-1}dw)	4.78±0.27^{b}	6.96±0.51^{a}	6.37±0.42^{a}	4.78±0.30^{b}
Zn (mg kg^{-1}dw)	27.9±2.08^{ab}	30.2±1.88^{a}	23.9±1.82^{b}	25.6±1.81^{b}
Mn(mg kg^{-1}dw)	42.6±3.56^{b}	56.2±4.23^{a}	44.6±3.66^{b}	50.4±3.85^{ab}
Fe (mg kg^{-1}dw)	129.0±8.60^{a}	129.0±9.63^{a}	129.0±9.56^{a}	139.8±10.92^{a}
Mo (mg kg^{-1}dw)	1.85±0.13^{a}	2.07±0.12^{a}	1.9±0.14^{a}	2.10±0.14^{a}

Data represent average means and SE deviation, n= (4); For each chemical property of leaves hemp, lines of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). I1=100% ETo; I2=60% ETo; F1=244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare; F2=184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare

Table 4. Dry yield (ton ha^{-1}), and mineral element uptakes (kg ha^{-1}) from the hemp leaves at different cultivation practices

Dry Yield	Treatments			
	I1F1	I1F2	I2F1	I2F2
Total dry yield	15.41±0.950^{a}	12.28±0.777^{b}	12.73±0.827^{b}	12.83±0.839^{b}
leaves dry yield	2.407±0.145^{a}	2.291±0.143^{a}	2.020±0.132^{b}	2.208±0.152^{ab}
leaves dry biomass % of total plant biomass	15.62	18.66	15.87	17.21
Mineral element uptakes				
N	68.26±2.98^{b}	80.46±3.64^{a}	63.34±2.83^{bc}	61.82±2.74^{c}
P	8.01±0.41^{ab}	8.47±0.39^{a}	7.03±0.41^{bc}	6.64±0.35^{c}
K	52.54±1.95^{a}	58.76±2.18^{a}	36.37±1.25^{c}	43.40±1.52^{b}
Ca	52.23±1.88^{a}	48.57±1.88^{ab}	39.59±1.50^{c}	44.60±1.75^{bc}
Mg	21.06±0.96^{a}	20.25±0.89^{ab}	17.27±0.83^{c}	19.25±0.86^{b}
Na	0.384±0.017^{a}	0.394±0.020^{a}	0.322±0.017^{b}	0.352±0.018^{ab}
Cu	0.012±0.001^{a}	0.016±0.001^{a}	0.015±0.001^{a}	0.011±0.001^{a}
Zn	0.067±0.005^{a}	0.069±0.004^{a}	0.048±0.004^{b}	0.057±0.004^{b}
Mn	0.103±0.009^{ab}	0.129±0.010^{a}	0.090±0.007^{b}	0.111±0.009^{ab}
Fe	0.311±0.021^{a}	0.296±0.022^{ab}	0.261±0.019^{b}	0.309±0.024^{a}
Mo	0.004±0.001^{a}	0.005±0.001^{a}	0.004±0.001^{a}	0.005±0.001^{a}

Data represent average means and SE deviation, n= (4); For each chemical property and each dry yield, lines of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). I1=100% ETo; I2=60% ETo; F1=244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare; F2=184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare

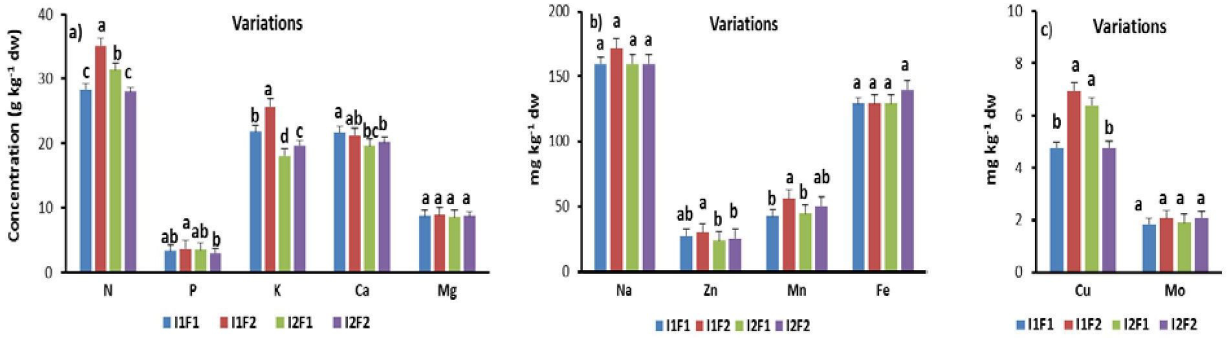

Fig. 3. Mineral composition of hemp leaves at different cultivation practices; I1F1 (100% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I1F2 (100% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare, I2F1 (60% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I2F2 (60% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare); Data represent the average means ± standard deviation, n= (4); For each chemical property of hemp leaves, columns with the same letter do not differ significantly according to the Tukey's test (P = 0.05)

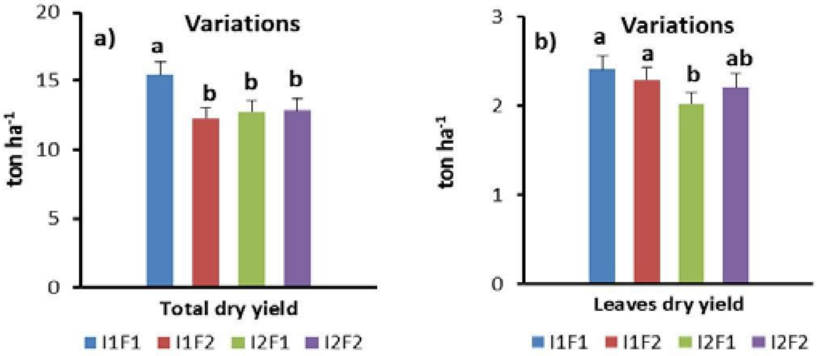

Fig. 4. Total dry biomass and leaves dry biomass of hemp at different cultivation practices; I1F1 (100% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I1F2 (100% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare, I2F1 (60% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I2F2 (60% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare); Data represent the average means ± standard deviation, n= (4); For dry yield of hemp leaves and total biomass, columns with the same letter do not differ significantly according to the Tukey's test (P = 0.05)

The dry biomass of leaves ranged from 2.020 to 2.407 ton ha^{-1}. The treatment I1F1 where the highest levels of irrigation and N-fertilization was applied, showed highest dry biomass yield of leaves, compared to treatment I2F1 where the lower level of irrigation was applied and the highest level of N-fertilization. Furthermore, the dry biomass of leaves in all treatments constitute by 15.62 to 18.66% of total plant biomass.

The mineral elements uptake from the hemp leaves, are shown in Table 4 and Fig. 5. Table 5 and Fig. 6, shows the mineral element uptake from hemp leaves for the production of one ton dry biomass of leaves.

Among the studied treatments, for the production of one ton dry biomass of leaves required the following amounts: The higher amount N (35.1 kg) for treatment I1F2 and the lower amount N (28.0 kg) for treatment I2F2, the higher amount P (3.7 kg) for treatment I1F2 and the lower amount P (3.01 kg) for treatment I2F2, the higher amount K (25.65 kg) for treatment I1F2 and the lower amount K (18.01 kg) for treatment I2F1, the higher amount Ca (21.70 kg) for treatment I1F1 and the lower amount Ca (19.60 kg) for treatment I2F1. Furthermore, magnesium uptake from the hemp leaves did not show statistically significant differences between treatments. The average amounts magnesium required to produce one ton dry biomass of hemp leaves is 8.59 kg. Generally, under the environmental conditions of central Greece, the irrigation of 100% ETo requires higher amounts of N, P, K and Ca, compared to the irrigation of 60% ETo, for the production of one ton dry biomass of leaves.

The results of this study showed high requirements of the hemp leaves in nutrients. Similar results for high requirements of the hemp leaves in nutrients were obtained in literature (Bócsa and Karus, 1998; Angelini *et al.*, 2014). Therefore, for production of one ton dry biomass of hemp leaves required highest uptake of N, followed by Ca, K, Mg and P in all treatments.

The micronutrients are essential in small amounts in the hemp leaves. The results showed that no effect of the two irrigation levels was observed in Cu, Mn, Fe and Mo concentrations in the hemp leaves (Table 5 and Fig. 6).The average amounts micronutrients required to produce one ton dry biomass of leaves are 0.294 kg Fe, 0.108 kg Mn, 0.013 kg Cu and 0.0045 kg Mo. Furthermore, for the production of one ton dry biomass of leaves, higher amount Zn (0.068 kg) required for the irrigation level of 100% ETo and lower amount Zn (0.052 kg) for the irrigation level of 60% ETo (Table 5 and Fig. 6).

According to other studies, leaves contribute significantly to the overall uptake of the plants in N, Mg, Ca, P, Zn and Mn by 66 %, 59%, 58%, 53%, 50%, and 42%, respectively. The contribution of leaves to the overall uptake of the plants for the elements K, Cu, and Mo it's about 33% (Angelini *et al.*, 2014).

Table 5. Mineral element uptakes (kg) from the hemp leaves to produce one ton dry biomass of leaves at different cultivation practices

Mineral element uptakes (kg)	Treatments			
	I1F1	I1F2	I2F1	I2F2
N	28.36 ± 1.24^{bc}	35.12 ± 1.59^{a}	31.36 ± 1.40^{b}	28.00 ± 1.24^{c}
P	3.33 ± 0.17^{ab}	3.70 ± 0.17^{a}	3.48 ± 0.20^{a}	3.01 ± 0.16^{b}
K	21.83 ± 0.81^{b}	25.65 ± 0.95^{a}	18.01 ± 0.62^{c}	19.66 ± 0.69^{c}
Ca	21.70 ± 0.78^{a}	21.20 ± 0.82^{ab}	19.60 ± 0.74^{c}	20.20 ± 0.79^{bc}
Mg	8.75 ± 0.40^{a}	8.34 ± 0.37^{a}	8.55 ± 0.41^{a}	8.72 ± 0.40^{a}
Na	0.159 ± 0.007^{a}	0.172 ± 0.009^{a}	0.159 ± 0.008^{a}	0.159 ± 0.008^{a}
Cu	0.005 ± 0.001^{a}	0.007 ± 0.001^{a}	0.007 ± 0.001^{a}	0.005 ± 0.001^{a}
Zn	0.028 ± 0.002^{a}	0.030 ± 0.002^{a}	0.024 ± 0.002^{b}	0.026 ± 0.002^{ab}
Mn	0.043 ± 0.004^{a}	0.056 ± 0.004^{a}	0.045 ± 0.004^{a}	0.050 ± 0.004^{a}
Fe	0.129 ± 0.009^{a}	0.129 ± 0.010^{a}	0.129 ± 0.009^{a}	0.140 ± 0.011^{a}
Mo	0.002 ± 0.0003^{a}	0.002 ± 0.0003^{a}	0.002 ± 0.0003^{a}	0.002 ± 0.0003^{a}

Data represent average means and SE deviation, n= (4); For each chemical property and each dry yield, lines of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). I1=100% ETo; I2=60% ETo; F1=244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare; F2=184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare

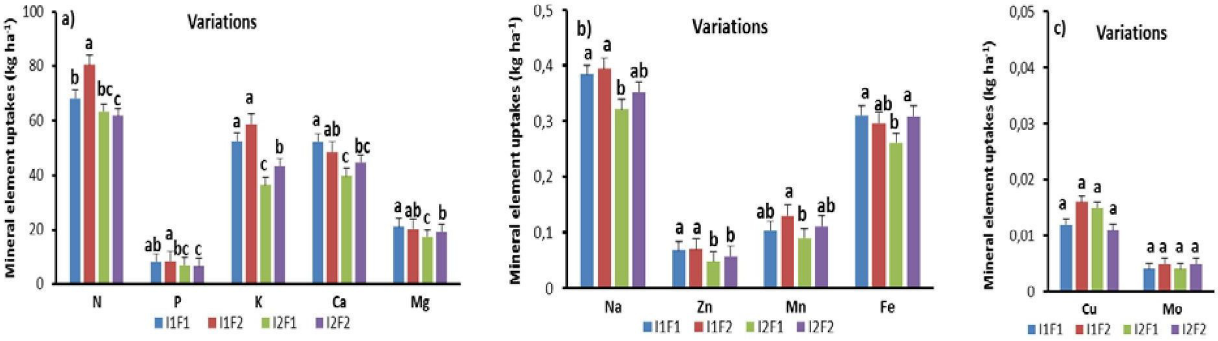

Fig. 5. Mineral element uptakes from the hemp leaves at different cultivation practices; I1F1 (100% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I1F2 (100% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare, I2F1 (60% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I2F2 (60% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare); Data represent the average means ± standard deviation, n= (4); For each chemical property of hemp leaves, columns with the same letter do not differ significantly according to the Tukey's test (P = 0.05)

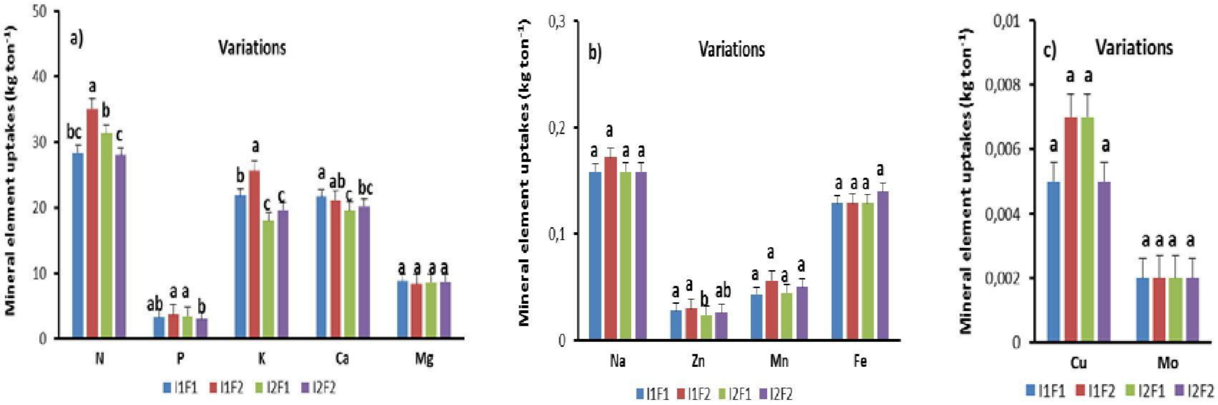

Fig. 6. Mineral element uptakes (kg) from the hemp leaves to produce one ton dry biomass of leaves at different cultivation practices; I1F1 (100% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I1F2 (100% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I2F1 (60% ETo and 244 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare), I2F2 (60% ETo and 184 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare); Data represent the average means ± standard deviation, n= (4); For each chemical property of hemp leaves, columns with the same letter do not differ significantly according to the Tukey's test (P = 0.05)

Conclusions

These results confirm the effect of the two irrigation and N-fertilization levels on the macro- and micro-nutrient concentration of hemp leaves, under the environmental conditions of central Greece. The application of the highest level of N-fertilization, showed highest nitrogen concentration in hemp leaves at the treatment where the lowest irrigation level (60% ETo) was used compared to the highest irrigation level (100% ETo). On the other hand, the application of the lowest N-fertilization level, showed highest nitrogen concentration in hemp leaves at the treatment with the highest irrigation level compared to the lowest irrigation level. The application of the highest irrigation level compared to the lowest irrigation level showed highest potassium concentration in hemp leaves.

Irrespective of the two irrigation and N-fertilization applied levels, the macronutrient required by the leaves of hemp in higher amounts it was the N followed by K, Ca, Mg and P, while from the micronutrients in higher amounts required the Fe. This is probably due to the mobility of the elements in the plant, to the synergy or competition between the nutrients and the fluctuation in irrigation which could affect the availability of nutrients.

The results could contribute optimal fertilizer application and therefore to the reduction of production costs, to achieve more sustainable cultivation.

Conflict of Interest

The authors declare that there are no conflicts of interest related to this article.

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