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# Spatial distribution of nutrients and morpho-physiological indicators of salinity tolerance among five olive cultivars - The use of relative nutrient concentration as an efficient tolerance index

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## ABSTRACT

Olive is the most important fruit tree species grown in Mediterranean basin, where many times it grows under saline conditions, due to irrigation with low quality water. Olive response to salinity is cultivar dependent and the evaluation of native genotypes is an important tool in finding tolerant genetic material. For this reason, five Greek olive cultivars, i.e. "Koroneiki", "Gaidourelia", "Lefkolia Serron", "N-K Gigas" and "Throumbolia", were evaluated regarding their tolerance to sodium chloride salinity (0, 50, 100 and 200 mM). All cultivars exhibited a significant reduction of growth, while the most severe symptoms were found in "Gaidourelia" while "Lefkolia Serron" presented the least symptoms. The latter exhibited the lowest sodium concentration in the leaves and the highest in the roots. Both potassium to sodium and calcium to sodium ratios were high in "Gaidourelia" leaves and stems, suggesting that the use of nutrient concentration for comparison of olive tolerance among cultivars could lead to erroneous results. The best indicator proved to be the determination of nutrient relative concentration compared to corresponding cultivar's control, as "Gaidourelia" exhibited the highest sodium relative concentration as well as the lowest nutrient ratios, revealing its inability to restrain sodium in the root level.

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growth; leaf sclerophylly indexes; sodium chloride; soil

## Introduction

Olive (*Olea europaea* L.) is the iconic and most important fruit-tree species of the Mediterranean basin, adequately adapted to the pedoclimatic conditions of the area. Under these conditions, characterizing the Mediterranean type climate, olive trees are frequently exposed to harsh conditions, such as temperature extremes in summer months and high irradiance levels as well as long dry periods (Cimato et al. 2010; Goreta et al. 2007; Melgar et al. 2009; Wiesman, Itzhak, and Ben Dom 2004). Moreover, in most coastal areas where olive tree is cultivated, the good quality water is primarily directed for municipal use, restricting thus its use for irrigation (Methenni et al.

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Color versions of one or more of the figures in the article can be found online at [www.tandfonline.com/lpla](http://www.tandfonline.com/lpla).

2018). As irrigation increases crop production and to some extent improves oil quantity and quality, the need for irrigating olive groves is increasing. Since the only available water for irrigation in many olive growing areas is of poor quality, the trees often face stressful conditions, due to high salt accumulation in the soil (Ben-Gal et al. 2017). Since rainfall is naturally low and insufficient to deplete salts accumulated in the soil, the only way to make the cultivation profitable is the selection and use of salt tolerant cultivars.

Olive is considered to be moderately tolerant to salinity (Maas and Hoffman 1977), but the magnitude of its tolerance relies to the genetic material (Tattini and Traversi 2009; Roussos et al. 2017). According to previous studies, olive tolerance to salinity mostly depends on the effective exclusion of sodium (Na) at root level, preventing it to reach actively growing tissues such as young leaves and growing shoots (Cimato et al. 2010; Melgar et al. 2008). Irrigation with saline water causes growth and productivity reductions which are mostly related to Na accumulation in the leaves rather than chloride (Cl) (Tattini, Bertoni, and Caselli 1992).

Generally plants grown under saline conditions apart from increased accumulation of Na and Cl exhibit also reduced concentrations of potassium (K), calcium (Ca) and magnesium (Mg) and non consistent imbalances of micronutrient concentrations (Chartzoulakis et al. 2006; Wiesman, Itzhak, and Ben Dom 2004; Assimakopoulou et al. 2017). The high Na and Cl concentration in soil solution may inhibit nutrient uptake either by direct competition at absorption sites (Na vs K and Cl vs nitrates) or by increased osmotic potential at root level, preventing thus the unhindered mass flow of nutrients to the root. Salt tolerant species or cultivars differ from salt sensitive ones by either preventing the high salt accumulation in the growing tissues, limiting thus toxic ions at root level (exclusion mechanisms) or by effectively compartmentalizing the toxic ions into the vacuoles, preventing further damage (Tattini and Traversi 2009; Chartzoulakis et al. 2006; Gucci and Tattini 1997). Most olive cultivars characterized as salt tolerant develop the salt exclusion strategy, preventing the toxic ions from reaching the aerial part of the tree (Tattini, Lombardini, and Gucci 1997). The effectiveness of this mechanism though, is greatly dependent on the salinity level, as in most cultivars it is quite effective under low salt concentration (50 mM) while at higher salinity levels Na cannot be restricted at the root level, reaching thus the aerial part, causing toxicity symptoms (Chartzoulakis et al. 2002; Kchaou et al. 2010). The exact salt level where the exclusion mechanism fails is not strictly defined, as it is cultivar dependent (Assimakopoulou et al. 2017; Tattini and Traversi 2009).

The aim of the present research was to study the salinity tolerance of five indigenous olive cultivars (most of them never studied before), on the basis of plant growth, leaf sclerophylly characteristics and nutrient concentration, in order to further elucidate the role of toxic ions exclusion and nutrient imbalances caused by salinity.

## Materials and methods

### Plant material

One year old self-rooted, uniform in size, olive trees of the five Greek cultivars: “Koroneiki”, “Gaidourelia”, “Lefkolia Serron” (from here on called “Lefkolia”), “N-K Gigas” (“Kostelenos G.D. nurseries” selection) and “Throumbolia” were used, grown in 3.5 L pots with the substrate used in the nursery (“Kostelenos G.D. nurseries”). The substrate was characterized as clay loam, with pH value 8.08, total  $\text{CaCO}_3$  15.75%, and CEC 29.57 meq  $100\text{ g}^{-1}$ . The plants were grown outdoors during the summer months July to August, in the orchard of Agricultural University of Athens (latitude,  $37^\circ 58' \text{ N}$ , longitude,  $23^\circ 32' \text{ E}$ , and altitude 30 m).

The plants were irrigated with tap water three times a week in order to keep the substrate moist before the onset of salt stress. Four treatments were applied, i.e. the control (using tap water with an electrical conductivity of  $0.307\text{ mS cm}^{-1}$ ), addition of sodium chloride to irrigated water at concentration of 50 mM ( $\text{EC} = 5.68\text{ mS cm}^{-1}$ ), 100 mM ( $\text{EC} = 10.9\text{ mS cm}^{-1}$ ) and

200 mM ( $EC = 20.4 \text{ mS cm}^{-1}$ ). During the first days of salt imposition, plants were irrigated with the NaCl solution in concentration increments of 25 mM per irrigation event, in order to reach the desired salinity concentration gradually, avoiding salt shock. Thereafter the plants were irrigated thrice a week with the desired salt solution (approximately 500 ml per plant) for a total of 40 days, when one of the cultivars exhibited severe leaf drop.

### **Growth measurements**

In order to assess the effects of the different salinity level on the growth of each cultivar, the height of each plant was measured at the onset and end of salinity trial along with the diameter of the trunk at pre-marked height. Furthermore, the effects of the osmotic impact of salinity on the plants was evaluated by measuring the following leaf sclerophylly characteristics: relative water content (RWC), leaf tissue density (LTD), succulence (SUC), water content at saturation (WCS), water saturation deficit (WSD) and specific leaf area (SLA) according to Denaxa et al. (2012).

At the end of the trial the symptoms of salinity stress were evaluated based on a four grade scale as follows: 0- no symptoms at all, 1- symptoms at approximately 25% of plant canopy, 3- symptoms at 25–50% of plant canopy and 4 symptoms at more than 50% of plant canopy.

### **Soil and plant tissue samplings**

Forty days after salt imposition, at the end of the trial, plants were uprooted and the substrate of each pot was separately sampled. The substrate was air-dried and ground to 2 mm prior to analysis. Particle size was assessed using the hydrometer method, with a 2-h reading for clay content. Electrical conductivity and soil pH was measured in a 1:1 soil: distilled water (w/v) suspension. Organic matter was determined using the Walkley-Black wet digestion method and total N titrimetrically after distillation of  $\text{NH}_3$  by Kjeldahl digestion. Exchangeable cations and Cation Exchange Capacity (CEC) were determined using ammonium acetate extraction. Soil available P was determined according to Olsen (1954). Available metal contents were extracted from the soils by shaking 10 g samples for 2 h with 20 ml 0.005 diethylenetriamine-pentaacetic acid (DTPA) adjusted to pH = 7.3.

The plants were destructively harvested and separated into leaves, shoots and roots and transferred to the laboratory. All plant parts were washed with distilled water and thereafter oven dried at  $70^\circ\text{C}$  till constant weight, the dry weight recorded in order to assess the impacts of salinity on dry mass accumulation and used for mineral nutrient analysis. The dried samples were ground to fine powder and dry-ashed in a furnace for 6 h at  $500^\circ\text{C}$ . The concentration of P was determined by vanado-molybdo-phosphate yellow color method, Cl by titration with 0.1 N silver nitrate, K and Na by flame emission spectroscopy whereas Ca, Mg, Fe, Mn and Zn was determined by atomic absorption spectrometry (Varian SpectraAA, 240 FS) in the dry digest. The ratios K/Na and Ca/Na of the leaves, roots, stems and entire plant were also calculated.

### **Statistical analysis**

The trial followed the completely randomized design with three replications (one plant per replication) per treatment. The effects of salt treatments on soil properties and on tree growth within each cultivar (intra-cultivar comparison), were evaluated by ANOVA and significant differences were determined with Tukey HSD test at  $p = 0.05$ .

Nutrient concentration in the various plant parts, as well as plant growth was also expressed as relative values (%) to the corresponding control of each cultivar for inter-cultivar comparison. Data derived were analyzed as a Two-Way Anova, with the factors being the cultivar and the salt

**Table 1.** Effects of salinity on soil physico-chemical properties.

Salinity level	pH	EC (mS cm <sup>-1</sup> )	Organic matter (%)	CaCO <sub>3</sub> (%)	CEC (meq 100g <sup>-1</sup> )
0	8.08 a	3.74 c	5.2 a	15.8 a	29.6 a
50	7.87 b	18.40 b	5.5 a	15.2 a	29.6 a
100	7.87 b	22.10 b	5.0 a	16.3 a	28.9 a
200	7.84 b	36.40 a	5.0 a	14.0 a	30.1 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

concentration in the irrigation water. Differences between treatments were determined with Tukey HSD multiple range test at  $\alpha = 0.05$ .

Principal component analysis (PCA) after varimax rotation was performed in order to summarize the effect of each salinity level on all the measured variables, in a reduced number of factors and group the tested cultivars based on the measured variables.

## Results

### *Effects of salinity on soil properties*

Salinity did not have an effect on soil organic matter, which was quite high, on the concentration of calcium carbonate and cation exchange capacity (Table 1). On the other hand, salinity reduced soil pH, even by the first level of 50 mM while it significantly increased electrical conductivity. The more the salt in the medium the higher was the electrical conductivity.

Nitrogen concentration did not alter by the presence of salt and the same stood also for P, K, Ca, Fe, Zn and Cu concentrations (Table 2). Sodium and Cl increased with increased salt concentration in irrigation water, while Mg concentration decreased. Manganese concentration on the other hand increased with increased salinity level.

### *Effect of salinity on plant growth and scerophylly indexes*

There were significant interactions between salinity level and cultivar, in almost all indexes calculated (Table 3). The highest RWC was recorded in «Lefkolia» under 50 mM salt concentration while the lowest in «Gaidourelia» under 200 mM. LTD was highest in «Throumbolia» leaves under 200 mM and lowest in the same cultivar under 50 mM salt. The highest SUC was observed in «Throumbolia» under both 50 and 100 mM while under 200 mM it presented the lowest. WSD was the highest in «Gaidourelia» grown under 200 mM salt concentration and lowest in «Koroneiki» (200 mM), «Lefkolia» (50 and 200 mM) and «N-K Gigas» (0 mM). SLA was found to be highest in «Gaidourelia» grown under 50 mM and lowest in the same cultivar under 100 mM.

In most of the cultivars tested, salinity resulted in a significant decrease of leaf dry weight, and this was mostly evident in «Gaidourelia», as the higher the salt stress the lower was the leaf dry weight (Table 4). «Lefkolia» did not exhibit any significant reduction of the leaf dry weight, while none of the cultivars presented any significant change due to salinity regarding the shoot or root dry weight. The total dry weight of the plant was significantly reduced by salinity in «N-K Gigas» and «Koroneiki». There was not any significant change in the canopy to root ration in any of the cultivars under salt stress.

When the dry weight changes were calculated as percentage of each cultivar respective control, salinity found to have a significant impact on total relative dry weights (Table 5). The total relative dry weight was significantly reduced under the highest salt concentration. «Throumbolia» presented the highest relative leaf dry weight under 50 mM of salt concentration and the lowest under the highest (200 mM) salt concentration.

**Table 2.** Effects of salinity on soil mineral nutrient concentration.

Salinity level(mM)	N(%)	P	K	Na	Mg	Ca	Cl	Fe	Mn	Zn	Cu
		mg kg <sup>-1</sup>	meq 100 g <sup>-1</sup>			mg kg <sup>-1</sup>		mg kg <sup>-1</sup>			
0	0.10 a	20.1 a	0.53 a	0.6 d	2.8 a	36.0 a	423 d	2.7 a	1.5 c	4.5 a	1.3 a
50	0.11 a	18.6 a	0.55 a	15.4 c	2.6 ab	34.7 a	4230 c	2.9 a	2.3 a	6.1 a	1.4 a
100	0.07 a	22.8 a	0.56 a	24.7 b	2.3 bc	34.2 a	6620 b	3.2 a	2.4 a	5.9 a	1.7 a
200	0.08 a	23.1 a	0.52 a	38.5 a	2.0 c	33.4 a	10800 a	3.1 a	2.7 a	7.0 a	1.6 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

**Table 3.** Effects of salinity on leaf sclerophylly indexes (RWC, relative water content (%), LTD, leaf tissue density (g kg<sup>-1</sup>), SUC, succulence (mg H<sub>2</sub>O mm<sup>-2</sup>), WCS, water content at saturation (g H<sub>2</sub>O g<sup>-1</sup> DW), WSD, water saturation deficit (%), SLA, specific leaf area (mm<sup>2</sup> mg<sup>-1</sup>)).

Parameters	Leaf characteristics (sclerophylly indexes)					
	RWC	LTD	SUC	WCS	WSD	SLA
<b>Cultivar</b>						
Gaidourelia	80.6 a	551 a	0.016 b	0.18 a	19.4 a	49.3 ab
Koroneiki	87.6 a	518 ab	0.019 ab	0.13 a	12.5 a	52.2 a
Lefkolia	89.4 a	478 ab	0.020 ab	0.14 a	10.6 a	52.9 a
N-K Gigas	89.0 a	450 b	0.025 a	0.17 a	11. a	50.6 ab
Throumbolia	85.7 a	502 ab	0.024 a	0.16 a	14.2 a	46.4 b
<b>Salinity level (NaCl mM)</b>						
0	89.5 a	495 ab	0.021 ab	0.12 a	10.5 a	46.7 b
50	88.5 a	444 b	0.024 a	0.18 a	11.5 a	56.0 a
100	84.6 a	501 ab	0.021 ab	0.18 a	15.4 a	50.0 b
200	83.2 a	558 a	0.018 b	0.14 a	16.8 a	48.4 b
<b>Cultivar x Salinity Level</b>						
Gaidourelia x 0	88.4 ab	489 abc	0.021 abc	0.14 a	11.6 ab	46.0 d-g
x 50	85.1 ab	483 abc	0.020 abc	0.19 a	14.9 ab	67.4 a
x 100	82.9 ab	599 abc	0.013 bc	0.14 a	17.0 ab	41.6 g
x 200	65.9 b	633 ab	0.013 bc	0.25 a	34.1 a	52.1 fg
Koroneiki x 0	87.2 ab	495 abc	0.027 abc	0.14 a	12.8 ab	42.2 fg
x 50	90.8 ab	441 bc	0.020 abc	0.13 a	9.2 ab	59.2 ab
x 100	79.5 ab	577 abc	0.013 bc	0.17 a	20.5 ab	54.0 bcd
x 200	93.1 a	561 abc	0.017 abc	0.06 a	6.9 b	53.3 b-e
Lefkolia x 0	86.1 ab	484 abc	0.020 abc	0.18 a	13.9 ab	53.1 b-f
x 50	95.7 a	486 abc	0.020 abc	0.05 a	4.3 b	50.1 b-g
x 100	83.0 ab	477 abc	0.023 abc	0.24 a	17.0 ab	50.4 b-g
x 200	92.9 a	464 bc	0.020 abc	0.09 a	7.1 b	58.1 abc
N-K Gigas x 0	94.1 a	505 abc	0.020 abc	0.06 a	5.9 b	45.0 d-g
x 50	84.4 ab	421 c	0.027 abc	0.27 a	15.5 ab	55.1 bcd
x 100	87.8 ab	427 bc	0.023 abc	0.18 a	12.2 ab	59.4 ab
x 200	89.6 ab	449 bc	0.030 ab	0.15 a	10.4 ab	42.7 efg
Throumbolia x 0	91.8 ab	506 abc	0.020 abc	0.08 a	8.2 ab	47.3 c-g
x 50	86.6 ab	391 c	0.033 a	0.26 a	13.4 ab	48.1 c-g
x 100	89.8 ab	428 bc	0.033 a	0.15 a	10.2 ab	44.5 d-g
x 200	74.8 ab	685 a	0.010 c	0.15 a	25.2 ab	45.9 d-g

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

The most severe symptoms, with excessive leaf drop were found under 200 mM in “Gaidourelia” (with a mean symptom index of 3.33) (Figure 1). “Lefkolia” presented the least symptoms, even under 200 mM exhibiting a mean index of 1.0, while the rest of the cultivars presented intermediate values.

### Effect of salinity on nutrient concentration in leaves, stems, root and entire plant

The genotype had a significant effect on Na, Cl and Mg concentration in leaves as seen in Table 6. Sodium was low in “Gaidourelia” and “Lefkolia” compared to the other cultivars, while

**Table 4.** Effects of salinity on the growth of olive trees based on dry weight changes (g).

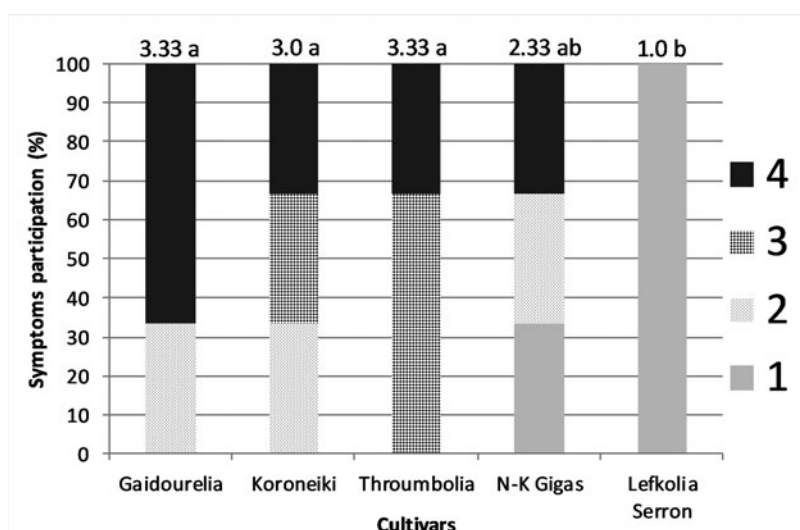
Cultivar	Salinity level(NaCl mM)	Effect of salinity on the dry weight of olive trees				
		Leaf DW	Shoot DW	Root DW	Total DW	Canopy/Root
Gaidourelia	0	29.8 a	33.2 a	6.4 a	69.4 a	11.8 a
	50	23.9 ab	31.7 a	5.5 a	61.1 a	10.1 a
	100	16.2 ab	32.3 a	3.9 a	52.4 a	12.7 a
	200	13.0 b	31.2 a	4.5 a	48.8 a	9.9 a
Koroneiki	0	17.8 a	33.0 a	4.3 a	55.2 a	12.6 a
	50	15.8 a	27.9 a	4.4 a	48.1 ab	10.2 a
	100	9.8 b	28.0 a	3.6 a	41.5 ab	11.9 a
	200	7.8 b	27.5 a	3.2 a	38.5 b	11.2 a
Lefkolia	0	22.7 a	38.4 a	3.0 a	64.1 a	21.1 a
	50	18.8 a	28.5 a	3.7 a	51.0 ab	12.9 a
	100	15.7 a	25.7 a	2.8 a	44.2 b	15.7 a
	200	18.2 a	26.6 a	2.7 a	47.5 ab	17.2 a
N-K Gigas	0	25.0 a	26.2 a	2.7 a	53.9 a	19.4 a
	50	18.3 ab	23.6 a	2.5 a	44.4 ab	18.4 a
	100	20.0 ab	21.7 a	3.6 a	45.2 ab	11.6 a
	200	10.3 b	20.5 a	2.1 a	33.0 b	15.0 a
Throumbolia	0	24.4 ab	34.1 a	4.1 a	62.6 a	14.9 a
	50	29.63 a	32.4 a	3.6 a	65.6 a	20.0 a
	100	14.84 ab	29.5 a	4.4 a	48.7 a	9.9 a
	200	8.40 b	34.6 a	4.2 a	47.2 a	10.3 a

Means within the same column and for the same cultivar followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

**Table 5.** Effects of salinity on the relative dry weight changes of olive trees.

Parameters	Relative growth based on dry weight changes (%)			
	Leaf relative DW	Shoot relative DW	Root relative DW	Total relative DW
<b>Cultivar</b>				
Gaidourelia	59.4 a	95.7 a	72.4 a	78.0 a
Koroneiki	62.6 a	84.2 a	86.0 a	75.8 a
Lefkolia	77.2 a	70.2 a	101.6 a	77.4 a
N-K Gigas	64.8 a	83.7 a	100.1 a	74.1 a
Throumbolia	70.6 a	90.0 a	95.1 a	82.7 a
<b>Salinity level (NaCl mM)</b>				
50	89.3 a	87.9 a	97.4 a	88.4 a
100	63.8 b	83.7 a	95.3 a	76.3 ab
200	47.7 b	82.6 a	80.4 a	68.1 b
<b>Cultivar x Salinity Level</b>				
Gaidourelia				
x 50	80.4 ab	95.6 a	85.6 a	88.1 a
x 100	54.2 b	97.4 a	61.4 a	75.5 a
x 200	43.7 b	94.2 a	70.2 a	70.3 a
Koroneiki				
x 50	88.8 ab	84.5 a	100.4 a	87.1 a
x 100	55.1 b	85.0 a	83.1 a	75.2 a
x 200	43.9 b	83.3 a	74.6 a	69.9 a
Lefkolia				
x 50	82.9 ab	74.3 a	121.6 a	79.6 a
x 100	68.9 ab	67.1 a	91.8 a	68.9 a
x 200	79.8 ab	69.2 a	91.3 a	74.0 a
N-K Gigas				
x 50	73.1 ab	90.2 a	90.9 a	82.4 a
x 100	79.9 ab	82.7 a	131.6 a	83.9 a
x 200	41.1 b	78.3 a	77.7 a	61.1 a
Throumbolia				
x 50	121.2 a	95.1 a	88.5 a	104.8 a
x 100	60.7 b	86.6 a	108.5 a	77.9 a
x 200	29.8 b	88.3 a	88.2 a	65.4 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .



**Figure 1.** Symptoms participation percentage on leaves, according to the four-grade scale, under 200 mM sodium chloride concentration. Numbers above each column indicate the mean sensitivity index based on the four-grade scale. Means followed by the same letter do not differ significantly according to Tukey HSD test at  $\alpha = 0.05$ .

**Table 6.** Effects of salinity on macronutrient concentration and ratios in olive leaves.

Parameters	Nutrients in Leaves (g kg <sup>-1</sup> D.W.)						Nutrient ratios	
	Na	Cl	P	K	Ca	Mg	K/Na	Ca/Na
<b>Cultivar</b>								
Gaidourelia	5.5 b	21.8 a	0.9 b	9.7 ab	13.5 a	1.0 b	8.2 a	9.8 a
Koroneiki	9.9 a	12.7 bc	0.6 cd	10.0 a	14.4 a	1.5 a	3.4 b	4.1 c
Lefkolia	4.7 b	15.5 b	1.1 a	10.9 a	10.4 bc	1.0 b	3.8 b	3.9 c
N-K Gigas	8.5 a	9.9 c	0.6 d	8.6 b	9.3 c	1.1 b	6.3 ab	5.9 bc
Throumbolia	9.7 a	15.6 b	0.8 c	9.9 ab	11.0 b	1.2 b	8.6 a	8.3 ab
<b>Salinity level (NaCl mM)</b>								
0	0.5 c	2.0 c	0.8 a	8.7 b	9.1 b	0.9 b	20.5 a	21.1 a
50	7.6 b	15.3 b	0.8 a	9.8 ab	12.6 a	1.3 a	1.5 b	1.9 b
100	11.1 a	21.4 a	0.8 a	10.0 a	12.7 a	1.3 a	1.1 b	1.3 b
200	11.3 a	21.5 a	0.7 a	10.6 a	12.5 a	1.2 a	1.1 b	1.3 b
<b>Cultivar x Salinity Level</b>								
Gaidourelia x 0	0.3 a	4.1 a	0.9 b-f	8.0 bcd	9.1 e-h	0.7 a	28.4 a	32.5 a
x 50	5.3 a	24.1 a	1.0 bcd	9.4 a-d	13.4 a-e	1.0 a	1.8 c	2.6 d
x 100	8.0 a	30.0 a	0.9 b-e	10.9 abc	15.5 abc	1.1 a	1.4 c	2.0 d
x 200	8.4 a	28.7 a	0.9 b-f	10.4 abc	16.0 a	1.3 a	1.3 c	1.9 d
Koroneiki x 0	1.0 a	0.9 a	0.7 c-g	10.6 abc	11.9 a-g	1.2 a	11.4 b	12.8 c
x 50	10.3 a	11.5 a	0.6 d-g	9.7 a-d	15.6 ab	1.7 a	1.0 c	1.6 d
x 100	14.5 a	20.4 a	0.6 c-g	10.8 abc	15.7 a	1.8 a	0.7 c	1.1 d
x 200	13.8 a	17.9 a	0.6 c-g	8.8 a-d	14.3 a-d	1.4 a	0.6 c	1.0 d
Lefkolia x 0	0.3 a	2.7 a	1.4 a	9.1 a-d	9.0 fgh	0.7 a	12.2 b	12.5 c
x 50	4.0 a	13.0 a	1.2 ab	10.6 abc	11.3 c-h	1.1 a	1.1 c	1.0 d
x 100	6.3 a	22.5 a	1.0 abc	11.5 ab	9.5 e-h	1.1 a	0.5 c	0.9 d
x 200	8.2 a	23.8 a	0.9 b-e	12.3 a	11.8 a-g	1.1 a	1.2 c	1.2 d
N-K Gigas x 0	0.6 a	1.1 a	0.5 g	7.5 cd	8.0 gh	0.9 a	22.7 a	20.4 b
x 50	9.4 a	12.3 a	0.7 c-g	10.1 abc	9.5 e-h	1.2 a	1.0 c	1.4 d
x 100	12.5 a	12.9 a	0.5 fg	6.1 d	10.7 d-h	1.3 a	0.8 c	0.9 d
x 200	11.2 a	13.3 a	0.6 efg	10.4 abc	9.2 e-h	1.1 a	0.7 c	0.8 d
Throumbolia x 0	0.4 a	1.1 a	0.7 c-g	8.3 bcd	7.4 h	0.8 a	27.9 a	27.2 ab
x 50	9.3 a	15.8 a	0.7 c-g	9.2 a-d	13.1 a-f	1.4 a	2.6 c	2.8 d
x 100	14.1 a	21.4 a	0.9 b-f	10.9 abc	12.2 a-g	1.2 a	2.2 c	1.7 d
x 200	15.1 a	24.0 a	0.7 c-g	11.1 abc	11.4 b-h	1.2 a	1.5 c	1.4 d

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

**Table 7.** Effects of salinity on micronutrient concentration in olive leaves.

Parameters	Nutrients in Leaves (mg kg <sup>-1</sup> D.W.)			
	Fe	Mn	Zn	Cu
<b>Cultivar</b>				
Gaidourelia	61.56 b	37.40 ab	26.25 a	8.50 a
Koroneiki	83.40 ab	43.80 a	14.50 b	4.94 b
Lefkolia	84.56 ab	44.25 a	25.00 a	8.33 a
N-K Gigas	103.21 a	30.70 b	15.12 b	4.71 b
Throumbolia	67.62 b	37.03 ab	19.40 b	5.82 b
<b>Salinity level (NaCl mM)</b>				
0	82.70 a	39.45 a	20.56 a	6.06 a
50	88.35 a	40.54 a	20.89 a	6.65 a
100	72.76 a	37.82 a	20.15 a	6.42 a
200	76.48 a	36.73 a	18.60 a	6.71 a
<b>Cultivar x Salinity Level</b>				
Gaidourelia x 0	65.20 a	39.40 a	31.67 a	8.16 a
x 50	64.03 a	36.63 a	23.33 a	8.33 a
x 100	45.66 a	35.73 a	23.33 a	8.66 a
x 200	71.36 a	37.83 a	26.67 a	8.83 a
Koroneiki x 0	81.60 a	44.96 a	15.36 a	4.36 a
x 50	86.06 a	46.86 a	17.46 a	5.63 a
x 100	86.10 a	43.43 a	13.87 a	5.06 a
x 200	79.86 a	39.96 a	11.30 a	4.70 a
Lefkolia x 0	81.13 a	42.76 a	23.33 a	7.67 a
x 50	105.50 a	46.63 a	30.00 a	8.67 a
x 100	76.56 a	42.76 a	26.67 a	8.33 a
x 200	78.06 a	44.86 a	20.00 a	8.67 a
N-K Gigas x 0	109.56 a	32.96 a	16.56 a	4.60 a
x 50	105.16 a	29.60 a	13.50 a	4.70 a
x 100	98.46 a	30.93 a	16.70 a	4.36 a
x 200	99.67 a	29.30 a	13.73 a	5.20 a
Throumbolia x 0	76.03 a	37.16 a	15.90 a	5.50 a
x 50	81.00 a	43.00 a	20.16 a	5.93 a
x 100	60.00 a	36.26 a	20.20 a	5.70 a
x 200	53.46 a	31.70 a	21.33 a	6.16 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

“Lefkolia” exhibited high P and K. The lowest Cl concentration was determined in “N-K Gigas” while Mg was found to be highest in “Koroneiki”.

The higher the salinity level the higher was the concentration of Na and Cl, while salinity resulted in increased concentrations of Mg.

There were not any significant interactions between cultivar and salinity level concerning the concentration of Na, Cl and Mg. The highest P concentration was determined in “Lefkolia” under control conditions while the lowest in “N-K Gigas” under the same conditions. High Ca was detected in leaves of both “Gaidourelia” under Tr200 and “Koroneiki” under Tr100. Under Tr0 both ratios (K/Na and Ca/Na) were highest in “Gaidourelia”.

Genotype had a significant effect on micronutrient concentration too (Table 7). “Lefkolia” presented high concentration of Mn, Zn and Cu while the highest concentration of Fe was detected in “N-K Gigas”. Salinity did not affect micronutrient concentrations while there were not any interactions between salinity and cultivar too.

Macronutrient concentration (P, K, Ca and Mg) in stems was also influenced by genotype, as “Throumbolia” exhibited high concentration of Ca and Mg and “Lefkolia” of P and K (Table 8). There was not any significant interaction concerning the concentrations of P, K, Ca and Mg in stems, while the highest Na and Cl concentration was detected in “Throumbolia” under Tr200.

“Gaidourelia” under Tr0 exhibited the highest Mn concentration and “Lefkolia” the lowest, irrespective of the salt level (Table 9). “Throumbolia” under control conditions presented the highest Zn concentration, while under Tr200 the highest Cu one. Zinc was highest in “Throumbolia” under control conditions.

**Table 8.** Effect of salinity on macronutrient concentration and their ratio in olive stems.

Parameters	Nutrients in Stem (g kg <sup>-1</sup> D.W.)						Nutrient ratios	
	Na	Cl	P	K	Ca	Mg	K/Na	Ca/Na
<b>Cultivar</b>								
Gaidourelia	5.56 a	5.88 ab	0.64 bc	6.31 bc	7.21 bc	0.70 b	4.87 a	5.65 a
Koroneiki	5.06 a	3.82 d	0.56 c	5.01 c	6.81 c	0.50 c	2.50 ab	3.59 b
Lefkolia	6.05 a	5.01 bc	0.89 a	8.34 a	7.07 c	0.66 b	1.78 b	2.33 b
N-K Gigas	6.56 a	4.55 cd	0.51 c	6.12 bc	8.21 b	0.94 a	2.91 ab	3.67 b
Throumbolia	6.42 a	6.32 a	0.79 ab	7.32 ab	9.44 a	1.03 a	3.73 ab	2.70 b
<b>Salinity level (NaCl mM)</b>								
0	1.06 d	1.02 d	0.57 b	6.78 a	7.73 a	0.72 a	9.07 a	10.56 a
50	5.28 c	4.81 c	0.77 a	6.80 a	7.73 a	0.77 a	1.99 b	1.91 b
100	7.70 b	6.31 b	0.69 ab	6.14 a	8.05 a	0.83 a	0.86 b	1.10 b
200	9.67 a	8.33 a	0.69 ab	6.77 a	7.48 a	0.74 a	0.72 b	0.79 b
<b>Cultivar x Salinity Level</b>								
Gaidourelia x 0	0.43 g	1.13 e	0.56 a	7.08 a	8.22 a	0.74 a	16.68 a	19.35 a
x 50	5.08 c-g	5.76 cd	0.63 a	5.99 a	7.09 a	0.68 a	1.25 c	1.49 cd
x 100	6.59 b-f	6.64 c	0.63 a	6.13 a	6.63 a	0.64 a	0.94 c	1.03 d
x 200	10.15 ab	10.02 ab	0.76 a	6.05 a	6.92 a	0.73 a	0.62 c	0.71 d
Koroneiki x 0	0.62 g	0.87 e	0.55 a	4.63 a	6.63 a	0.43 a	7.57 bc	10.93 b
x 50	4.45 d-g	3.50 de	0.63 a	3.38 a	6.01 a	0.42 a	0.76 c	1.35 cd
x 100	6.18 b-f	4.76 cd	0.53 a	5.63 a	7.40 a	0.55 a	0.96 c	1.29 d
x 200	9.01 a-d	6.13 cd	0.56 a	6.39 a	7.21 a	0.61 a	0.71 c	0.80 d
Lefkolia x 0	2.06 fg	1.00 e	0.72 a	9.05 a	6.71 a	0.64 a	4.82 bc	6.33 bc
x 50	3.95 efg	4.63 cd	1.07 a	9.83 a	7.18 a	0.72 a	0.93 c	1.06 d
x 100	9.00 a-d	7.14 bc	0.89 a	7.08 a	7.45 a	0.75 a	0.52 c	1.03 d
x 200	9.19 abc	7.26 bc	0.89 a	7.42 a	6.94 a	0.55 a	0.86 c	0.91 d
N-K Gigas x 0	1.38 g	1.00 e	0.34 a	5.78 a	8.26 a	0.91 a	8.67 b	10.67 b
x 50	7.88 a-e	5.38 cd	0.72 a	7.30 a	8.16 a	0.93 a	1.46 bc	2.03 cd
x 100	8.88 a-d	6.13 cd	0.37 a	4.61 a	9.14 a	1.09 a	0.93 c	1.24 d
x 200	8.10 a-e	5.70 cd	0.61 a	6.80 a	7.28 a	0.83 a	0.60 c	0.76 d
Throumbolia x 0	0.84 g	1.13 e	0.69 a	7.35 a	8.83 a	0.89 a	7.63 bc	5.50 cd
x 50	5.07 c-g	4.76 cd	0.78 a	7.50 a	10.23 a	1.09 a	5.55 bc	3.60 cd
x 100	7.88 a-e	6.89 c	1.04 a	7.24 a	9.66 a	1.13 a	0.93 c	0.92 d
x 200	11.88 a	12.53 a	0.66 a	7.20 a	9.05 a	0.99 a	0.82 c	0.76 d

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

“Lefkolia” along with “Koroneiki” exhibited the lowest Ca concentration in the roots (Table 10). Na concentration was found to be higher in “Lefkolia” under Tr200, while Cl highest concentration was determined in “Gaidourelia” under the same salt level. “Koroneiki” and “Lefkolia” under control conditions exhibited the highest K concentration while the highest Ca/Na ration was determined in “Gaidourelia” under Tr0.

Manganese concentration in the roots was highest in “Gaidourelia” (Table 11). Salinity did not affect significantly Mn and Cu concentration, while the highest Fe concentration was detected in “Gaidourelia” under Tr50 and the highest Zn concentration in “Throumbolia” under Tr100.

### ***Effect of salinity on the relative macronutrient content in leaves, stems, root and entire plant compared to the corresponding control***

The highest relative Na concentration was observed in “Throumbolia” under Tr50 (almost 3000% higher than the corresponding control) (Table 12). Higher increase of Cl in the leaves compared to control was observed under Tr50, especially in “Throumbolia”. Salinity resulted in decreased P concentration in the leaves, especially under Tr200, while the same stood also for K. K/Na ratio was found highest in “Lefkolia” under control conditions and lowest in “Throumbolia” under Tr100 and 200. Ca/Na ratio was highest in “Koroneiki” under control conditions and lowest in “Throumbolia” under Tr200.

**Table 9.** Effect of salinity on micronutrient concentration in olive stems.

Parameters	Nutrients in Stem (mg kg <sup>-1</sup> D.W.)			
	Fe	Mn	Zn	Cu
<b>Cultivar</b>				
Gaidourelia	19.50 a	42.71 a	11.37 c	2.83 c
Koroneiki	28.22 a	20.31 b	10.21 c	3.22 c
Lefkolia	31.21 a	8.67 b	10.02 c	3.12 c
N-K Gigas	29.37 a	20.97 b	21.32 b	4.25 b
Throumbolia	35.58 a	20.21 b	29.62 a	6.43 a
<b>Salinity level (NaCl mM)</b>				
0	27.00 a	26.95 a	17.47 a	3.90 a
50	34.08 a	24.16 ab	14.34 a	3.70 a
100	28.44 a	24.88 ab	16.13 a	3.83 a
200	25.58 a	14.30 b	18.10 a	4.46 a
<b>Cultivar x Salinity Level</b>				
Gaidourelia x 0	27.38 a	60.90 a	13.75 c-g	2.82 ef
x 50	21.66 a	53.60 ab	11.47 d-g	3.48 c-f
x 100	15.61 a	51.30 abc	7.99 g	2.50 f
x 200	13.35 a	5.05 d	12.28 c-g	2.50 f
Koroneiki x 0	35.29 a	23.59 bcd	15.91 b-g	3.52 c-f
x 50	16.63 a	16.43 d	7.63 g	2.90 ef
x 100	38.70 a	21.68 bcd	9.52 fg	3.24 def
x 200	22.29 a	19.52 bcd	7.77 g	3.24 def
Lefkolia x 0	25.17 a	8.74 d	9.16 g	3.85 b-f
x 50	37.16 a	9.43 d	11.41 d-g	2.86 ef
x 100	22.06 a	7.52 d	10.07 efg	3.30 def
x 200	40.47 a	8.99 d	9.45 fg	2.47 f
N-K Gigas x 0	19.80 a	21.66 bcd	14.02 c-g	3.19 def
x 50	47.63 a	20.48 bcd	12.45 c-g	4.31 b-f
x 100	29.20 a	22.67 bcd	26.15 a-f	4.18 b-f
x 200	20.86 a	19.06 cd	32.68 ab	5.33 bcd
Throumbolia x 0	27.39 a	19.85 bcd	34.49 a	6.11 b
x 50	47.35 a	20.85 bcd	28.75 abc	4.97 b-e
x 100	36.64 a	21.26 bcd	26.90 a-e	5.90 bc
x 200	30.96 a	18.88 cd	28.35 a-d	8.75 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

The highest accumulation of Na was found in “Gaidourelia” under Tr200 (Table 13). Similarly, Cl was significantly increased under saline conditions in “Gaidourelia” and “Throumbolia” stems, especially under Tr200. Chloride increase reached almost 1000% compared to the corresponding control in “Throumbolia” under Tr200. All other macronutrients were equally reduced by salinity, while both ratios were found to be higher in “Lefkolia” under Tr50.

The lowest increase of Na in the roots was observed in “Gaidourelia” and under Tr50 (Table 14). Chloride accumulated significantly in the roots of “N-K Gigas” and “Throumbolia” as the salinity level increased above Tr50. “Throumbolia” exhibited a significant increase in P and Ca concentration in the roots compared to its control, while “Gaidourelia” a significant decrease. Phosphorus accumulated in the roots under Tr100, while it was reduced under both Tr50 and Tr200. Magnesium concentration was reduced in the roots, and was more pronounced in “Gaidourelia” where the reduction reached almost 50% compared to its control. The highest ratios were observed in “Throumbolia” roots and under Tr50 compared to other salinity levels.

When the entire plant was taken into account, “Gaidourelia” presented a significant increase of Na and “Throumbolia” a significant increase in Cl compared to other cultivars (Table 15). The higher the salt concentration the lower was the K and Mg concentration in the plant. The lowest ratios were observed in “Gaidourelia”, especially under Tr200 and the highest in “Lefkolia” under Tr50.

**Table 10.** Effect of salinity on macronutrient concentration and their ratio in olive roots.

Parameters	Nutrients in Roots (g kg <sup>-1</sup> D.W.)						Nutrient ratios	
	Na	Cl	P	K	Ca	Mg	K/Na	Ca/Na
<b>Cultivar</b>								
Gaidourelia	1.16 c	13.22 a	0.08 b	0.42 d	14.91 a	2.44 bc	0.54 b	16.68 a
Koroneiki	10.43 b	5.07 d	0.67 a	5.44 a	11.69 b	2.41 bc	0.81 a	1.49 b
Lefkolia	12.43 a	9.96 b	0.79 a	4.15 b	11.42 b	2.18 c	0.29 c	1.71 b
N-K Gigas	11.56 ab	6.87 c	0.65 a	2.08 c	14.94 a	2.75 b	0.36 bc	1.54 b
Throumbolia	11.21 b	5.57 cd	0.71 a	2.91 c	14.44 a	3.34 a	0.53 b	1.19 b
<b>Salinity level (NaCl mM)</b>								
0	3.73 d	3.26 d	0.54 a	4.66 a	14.24 a	2.86 a	1.32 a	9.38 a
50	9.78 c	7.66 c	0.59 a	2.73 b	13.54 a	2.71 a	0.29 b	3.64 b
100	11.44 b	10.00 b	0.66 a	2.44 b	12.93 a	2.55 ab	0.21 b	2.67 bc
200	12.48 a	11.63 a	0.52 a	2.17 b	13.20 a	2.37 b	0.20 b	2.39 c
<b>Cultivar x Salinity Level</b>								
Gaidourelia x 0	0.49 g	4.85 gh	0.080 b	0.67 ef	16.93 a	2.76 b-e	1.38 b	34.84 a
x 50	1.12 fg	12.30 cd	0.078 b	0.32 f	15.43 a	2.40 b-e	0.28 de	13.78 b
x 100	1.42 efg	17.15 ab	0.080 b	0.39 f	13.76 a	2.26 cde	0.27 de	9.67 c
x 200	1.60 efg	18.57 a	0.076 b	0.33 f	13.53 a	2.33 cde	0.21 de	8.43 c
Koroneiki x 0	3.76 def	2.50 h	0.79 a	7.56 a	11.74 a	2.44 b-e	2.04 a	3.13 d
x 50	10.07 c	4.88 gh	0.50 ab	5.99 ab	11.19 a	2.21 de	0.59 cd	1.11 d
x 100	13.27 b	7.01 fg	0.71 a	4.11 bc	10.36 a	2.39 b-e	0.31 de	0.78 d
x 200	14.64 ab	5.88 fgh	0.67 a	4.10 bc	13.46 a	2.60 b-e	0.28 de	0.92 d
Lefkolia x 0	4.93 d	4.97 gh	0.83 a	7.23 a	12.20 a	2.33 cde	0.82 c	3.67 d
x 50	13.06 bc	9.34 def	0.83 a	3.46 cd	11.03 a	2.23 de	0.09 e	1.11 d
x 100	14.69 ab	11.95 cde	0.83 a	2.26 c-f	11.76 a	2.20 de	0.10 e	0.98 d
x 200	17.06 a	13.60 bc	0.68 a	3.67 cd	10.70 a	1.97 e	0.15 de	1.07 d
N-K Gigas x 0	4.50 de	2.12 h	0.57 ab	3.59 cd	16.43 a	3.32 abc	0.88 c	2.77 d
x 50	12.28 bc	5.01 gh	0.72 a	1.15 ef	13.46 a	2.91 a-e	0.22 de	1.36 d
x 100	15.20 ab	8.39 d-g	0.82 a	1.53 def	14.56 a	2.85 a-e	0.20 de	1.13 d
x 200	14.27 ab	11.95 cde	0.48 ab	2.05 c-f	15.33 a	1.93 e	0.14 de	0.88 d
Throumbolia x 0	4.98 d	1.88 h	0.46 ab	4.27 bc	13.91 a	3.45 ab	1.49 b	2.49 d
x 50	12.36 bc	6.76 fg	0.82 a	2.75 cde	16.63 a	3.84 a	0.27 de	0.84 d
x 100	12.67 bc	5.51 fgh	0.87 a	2.56 c-f	14.22 a	3.06 a-d	0.15 de	0.80 d
x 200	14.83 ab	8.14 efg	0.70 a	2.05 c-f	13.00 a	3.04 a-d	0.21 de	0.63 d

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

### Classification of cultivars based on PCA analysis

Principal component analysis was carried out to minimize the number of variables that influence each factor in order to facilitate the interpretation of the results. Fifteen components were extracted with eigenvalues higher than 1, explaining 92.38% of the variability in the original data. The first two factors were selected (explaining together 35.92% of the total variance) and the scatterplot produced is presented in Figure 2.

Component one comprised of relative concentration in leaves of P, K, Ca, Mg, Fe, Mn, Zn and Cu, of relative concentration in whole plant of P, K, Ca, Mg, Fe and Mn, while component 2 comprised of the ratio Ca/Na in the entire plant compared to the respective control and of Na and P concentration in the root. “Gaidourelia” was located separately from the other cultivars in the negative side of component 2, characterized thus by low Ca/Na ratio and Na and P concentration in the root. The exact opposite stood for “Lefkolia”, which was clearly located in the positive side of component 2. The other three cultivars were not clearly located into one side of each component.

### Discussion

Irrigation with saline water resulted, as expected, in increased electrical conductivity and Na and Cl concentrations in the soil, according to the literature (Moreno et al. 2001; Roussos et al. 2013). Soil pH was reduced even by the first salinity level. According to Al-Busaidi and Cookson (2003)

**Table 11.** Effect of salinity on micronutrient concentration in olive roots.

Parameters	Nutrients in Roots (mg kg <sup>-1</sup> D.W.)			
	Fe	Mn	Zn	Cu
<b>Cultivar</b>				
Gaidourelia	1054.8 a	71.37 a	56.08 b	15.90 a
Koroneiki	1158.4 a	57.08 b	99.20 b	15.22 a
Lefkolia	484.9 b	53.84 b	40.00 b	15.20 a
N-K Gigas	1098.2 a	65.86 ab	60.73 b	15.57 a
Throumbolia	1062.7 a	61.30 ab	161.02 a	16.73 a
<b>Salinity level (NaCl mM)</b>				
0	913.2 ab	59.64 a	60.45 a	14.89 a
50	1131.3 a	66.11 a	96.53 a	16.16 a
100	896.4 b	61.10 a	88.86 a	16.64 a
200	946.3 ab	60.71 a	87.78 a	15.20 a
<b>Cultivar x Salinity Level</b>				
Gaidourelia x 0	817.2 b-e	70.00 a	82.63 bc	16.67 a
x 50	1690.6 a	79.70 a	66.23 bc	15.80 a
x 100	765.0 b-e	66.36 a	32.00 c	15.60 a
x 200	946.6 b-e	69.43 a	43.46 bc	15.53 a
Koroneiki x 0	1003.5 a-e	50.70 a	39.53 bc	11.80 a
x 50	1077.3 a-e	52.06 a	93.73 abc	17.33 a
x 100	1126.9 a-e	53.40 a	75.63 bc	14.10 a
x 200	1425.8 ab	72.16 a	187.90 abc	17.67 a
Lefkolia x 0	457.1 de	44.86 a	43.16 bc	15.67 a
x 50	494.2 cde	58.03 a	46.00 bc	15.23 a
x 100	581.9 cde	64.50 a	34.63 bc	15.83 a
x 200	406.6 e	47.97 a	36.23 bc	14.06 a
N-K Gigas x 0	1136.4 a-e	70.36 a	37.70 bc	14.13 a
x 50	1189.5 a-d	67.63 a	82.06 bc	15.06 a
x 100	989.5 a-e	66.83 a	54.33 bc	20.95 a
x 200	1077.5 a-e	58.63 a	68.83 bc	12.13 a
Throumbolia x 0	1151.9 a-d	62.30 a	99.23 abc	16.20 a
x 50	1204.7 abc	73.13 a	194.63 ab	17.40 a
x 100	1018.8 a-e	54.43 a	247.73 a	16.73 a
x 200	875.3 b-e	55.36 a	102.50 abc	16.60 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

as long as calcium dominates the cation exchange complex rather than sodium, as in the present trial, the soil pH is buffered and it is unlikely to rise above 8.5. The increasing concentration of Na in the soil though, displaces Ca into the soil solution, where in well aerated soils as the one used here, it precipitates as  $\text{CaCO}_3$ , liberating  $\text{H}^+$ , reducing thus the pH of the soil (Al-Busaidi and Cookson 2003). Manganese concentration was also increased by salinity, which has also been observed in alkaline soils irrigated with high salinity water, according to Dahiya and Singh (1976). Magnesium concentration instead decreased with increasing salinity level. As the Na in the soil increases, it can replace Mg present on the exchange sites of soil, causing it to precipitate as insoluble magnesium carbonate, reducing thus the exchangeable Mg concentration in the soil (Al-Busaidi and Cookson 2003; Lowell 1964).

Olive is considered to be a moderately salt tolerant species, with great differences among cultivars, concerning their tolerance (Boussadia, Zaabar, and Braham 2017). In the present trial “Gaidourelia” was the most sensitive cultivar to salinity, losing most of its leaves under high salt concentration, while “Lefkolia” was the most tolerant one, showing minor symptoms of toxicity. The other cultivars tested presented moderately tolerant behavior. Since survival in evergreen sclerophylls seems to be a more reliable indicator of salt tolerance than growth (Munns 2002), “Gaidourelia” can be safely ranked as salt sensitive while “Lefkolia” as salt tolerant. This was further confirmed by the PCA which clearly showed the differences between the two cultivars.

Salinity has been found to induce a reduction of olive tree growth (Goreta et al. 2007; Perica, Goreta, and Selak 2008; Therios and Misopolinos 1988; Chartzoulakis et al. 2002; Rahemi et al. 2017), as has been also found in the present experiment, which was most evident above 50 mM,

**Table 12.** Effect of salinity on macronutrient content and their ratio in leaves.

Parameters	Nutrient content in leaves (% of corresponding control)						Nutrient Ratio	
	Na	Cl	P	K	Ca	Mg	K/Na	Ca/Na
<b>Cultivar</b>								
Gaidourelia	1468.35 ab	376.79 b	61.82 a	95.36 a	94.42 a	87.50 a	5.17 b	6.54 b
Koroneiki	788.55 b	1126.84 a	58.14 a	86.27 a	79.86 a	85.17 a	6.82 a	9.47 a
Lefkolia	1425.78 ab	559.36 b	58.55 a	74.91 a	92.57 a	113.71 a	7.13 a	7.08 b
N-K Gigas	1140.67 b	724.74 b	78.35 a	73.27 a	80.53 a	83.77 a	6.81 a	7.23 b
Throumbolia	2112.33 a	1155.34 a	78.51 a	57.38 a	120.66 a	122.71 a	3.67 c	4.93 c
<b>Salinity level (NaCl mM)</b>								
50	1503.99 a	902.55 a	93.61 a	100.98 a	128.23 a	136.05 a	7.44 a	8.96 a
100	1502.64 a	857.22 a	63.75 b	71.35 b	88.53 b	91.75 b	5.09 b	6.26 b
200	1154.78 a	606.07b	43.85 b	59.98 b	64.07 b	67.92 b	5.24 b	5.93 b
<b>Cultivar x Salinity Level</b>								
Gaidourelia x 50	1521.56 ab	454.17 cd	86.52 a	94.78 a	116.52 b	108.43 b	6.26 b-e	7.72 b-e
x 100	1580.97 ab	379.19 cd	55.49 a	72.94 a	91.62 b	75.21 b	4.90 def	6.02 c-g
x 200	1302.51 b	297.02 d	43.46 a	57.01 a	75.12 b	78.85 b	4.34 ef	5.88 c-g
Koroneiki x 50	935.82 b	1167.60 abc	78.97 a	80.31a	116.19 b	122.16 ab	8.31 abc	11.82 a
x 100	821.54 b	1292.95 ab	54.20 a	55.00 a	73.11 b	82.33 b	6.56 b-e	8.52 bc
x 200	608.31 b	919.98 a-d	41.24 a	36.83 a	50.28 b	51.01 b	5.60 c-f	8.07 bcd
Lefkolia x 50	1000.44 b	402.06 cd	71.67 a	95.71 a	103.59 b	129.60 ab	9.49 a	10.36 ab
x 100	1344.17 b	578.81 bcd	50.39 a	84.83 a	71.32 b	98.06 b	6.52 b-e	5.59 d-g
x 200	1932.72 ab	697.22 bcd	53.58 a	105.55 a	102.82 b	113.47 b	5.38 def	5.28 efg
N-K Gigas x 50	1088.35 b	794.35 bcd	101.49 a	95.68 a	87.89 b	95.62 b	8.81 ab	8.10 bcd
x 100	1557.33 ab	890.94 a-d	83.85 a	64.18 a	108.68 b	107.67 b	4.04 ef	6.91 c-f
x 200	776.31 b	488.92 bcd	49.71 a	59.94 a	45.03 b	48.01 b	7.59 a-d	6.68 c-f
Throumbolia x 50	2973.76 a	1694.57 a	129.41 a	138.45 a	216.96 a	224.44 a	4.34 ef	6.79 c-f
x 100	2209.21 ab	1144.22 abc	74.85 a	79.80 a	97.92 b	95.47 b	3.42 f	4.26 fg
x 200	1154.03 b	627.23 bcd	31.27 a	40.57 a	47.09 b	48.24b	3.25 f	3.72 g

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

**Table 13.** Effect of salinity on macronutrient content and their ratio in stems.

Parameters	Nutrient content in stems (% of corresponding control)						Nutrient Ratio	
	Na	Cl	P	K	Ca	Mg	K/Na	Ca/Na
<b>Cultivar</b>								
Gaidourelia	1596.76 a	624.70 a	114.88 a	82.83 a	79.85 a	89.43 a	5.61 b	5.57 b
Koroneiki	882.47 b	459.07 b	85.03 a	92.96 a	86.09 a	103.39 a	10.70 ab	10.49 b
Lefkolia	268.71 c	431.37 b	93.33 a	63.02 a	75.17 a	74.17 a	31.91 a	32.04 a
N-K Gigas	512.88 bc	474.40 b	141.26 a	89.44 a	83.09 a	87.84 a	15.97 ab	15.81 ab
Throumbolia	857.10 b	628.51 a	106.11 a	92.93 a	98.75 a	104.79 a	11.50 ab	12.56 b
<b>Salinity level (NaCl mM)</b>								
50	592.28 b	407.49 b	122.49 a	86.55 a	87.12 a	92.73 a	25.28 a	24.27 a
100	790.10 b	505.90 b	98.06 a	79.95 a	87.86 a	96.69 a	10.39 a	12.35 ab
200	1088.37 a	657.44 a	103.82 a	86.20 a	78.80 a	86.35 a	9.74 a	9.27 b
<b>Cultivar x Salinity Level</b>								
Gaidourelia x 50	1095.60 bcd	479.46 bc	107.20 a	115.48 a	108.86 a	116.26 a	7.47 b	7.69 b
x 100	1466.62 ab	563.20 abc	110.83 a	110.83 a	100.26 a	113.51 a	5.64 b	5.35 b
x 200	2228.07 a	831.43 ab	126.62 a	102.60 a	94.29 a	110.28 a	3.73 b	3.68 b
Koroneiki x 50	601.93 b-e	337.06 c	95.85 a	100.07 a	91.08 a	109.35 a	10.06 b	12.33 b
x 100	860.86 b-e	468.91 bc	78.25 a	92.77 a	89.00 a	99.23 a	12.65 b	11.81 b
x 200	1184.60 bcd	571.24 abc	80.98 a	92.66 a	87.79 a	93.42 a	9.40 b	7.31 b
Lefkolia x 50	162.16 e	338.44 c	110.40 a	85.95 a	87.13 a	93.32 a	72.81 a	65.57 a
x 100	306.68 de	460.13 bc	82.47 a	85.82 a	81.80 a	91.52 a	12.19 b	16.72 b
x 200	337.28 cde	495.54 abc	87.13 a	81.56 a	79.73 a	88.96 a	10.72 b	13.84 b
N-K Gigas x 50	527.30 cde	480.40 bc	189.64 a	81.12 a	79.15 a	86.01 a	19.22 b	16.77 b
x 100	543.75 cde	504.15 abc	89.69 a	79.52 a	78.59 a	84.12 a	10.77 b	16.26 b
x 200	467.60 cde	438.65 bc	144.43 a	64.84 a	76.19 a	83.65 a	17.91 b	14.40 b
Throumbolia x 50	574.39 cde	402.09 bc	109.35 a	60.79 a	74.51 a	78.60 a	16.82 b	19.00 b
x 100	772.60 b-e	533.09 abc	129.06 a	55.81 a	71.27 a	70.86 a	10.72 b	11.60 b
x 200	1224.31 bc	950.36 a	79.93 a	53.73 a	69.20 a	59.78 a	6.97 b	7.09 b

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

**Table 14.** Effect of salinity on macronutrient content and their ratio in roots.

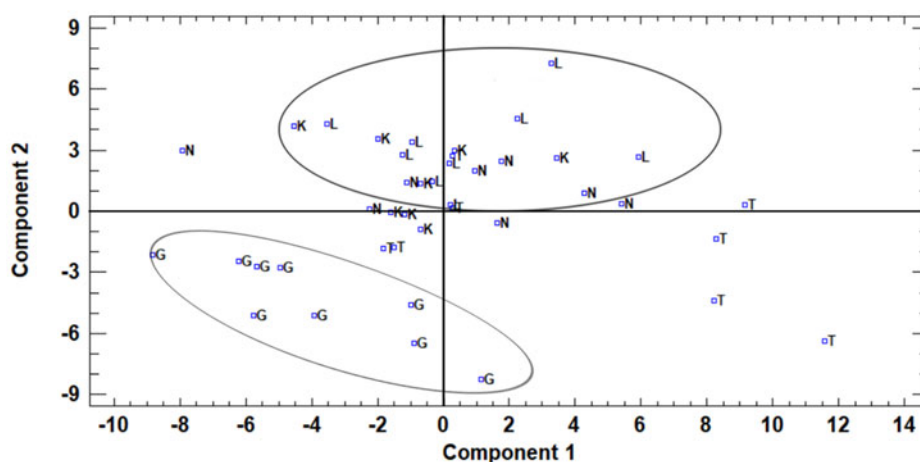
Parameters	Nutrient content in roots (% of corresponding control)						Nutrient Ratio	
	Na	Cl	P	K	Ca	Mg	K/Na	Ca/Na
<b>Cultivar</b>								
Gaidourelia	161.13 b	187.08 c	52.42 c	28.53 a	44.15 b	43.32 b	18.54 abc	30.50 b
Koroneiki	290.05 a	194.16 c	59.99 bc	54.89 a	86.66 ab	86.47 a	19.27 abc	30.06 b
Lefkolia	303.41 a	236.64 bc	97.20 bc	45.39 a	94.07 a	93.37 a	14.09 bc	30.49 b
N-K Gigas	305.71 a	390.45 a	108.41 b	42.15 a	87.96 a	80.26 a	13.84 c	28.66 b
Throumbolia	255.11 ab	338.03 ab	167.74 a	58.61 a	107.61 a	94.90 a	21.25 a	40.54 a
<b>Salinity level (NaCl mM)</b>								
50	224.34 b	204.15 b	91.68 ab	48.72 a	85.68 a	83.94 a	20.81 a	37.68 a
100	293.88 a	302.51 a	121.18 a	47.38 a	89.45 a	86.43 a	16.01 ab	30.50 b
200	271.02 ab	301.16 a	78.60 b	41.64 a	77.14 a	68.63 a	15.38 b	27.97 b
<b>Cultivar x Salinity Level</b>								
Gaidourelia x 50	64.95 c	69.76 c	27.10 a	13.73 a	25.36 a	23.60 a	20.59 a	39.54 a
x 100	182.77 bc	219.95 bc	61.66 a	35.97 a	50.20 a	48.66 a	19.95 a	27.77 a
x 200	235.68 abc	271.53 abc	68.50 a	35.89 a	56.89 a	57.70 a	15.08 a	24.18 a
Koroneiki x 50	276.10 abc	189.03 bc	45.42 a	80.82 a	97.47 a	94.38 a	29.07 a	35.57 a
x 100	297.54 ab	222.06 bc	71.42 a	43.31 a	74.45 a	82.48 a	15.00 a	25.09 a
x 200	296.51 ab	171.38 bc	63.14 a	40.54 a	88.08 a	82.55 a	13.75 a	29.52 a
Lefkolia x 50	319.85 ab	227.44 bc	121.16 a	58.25 a	110.83 a	116.97 a	17.76 a	33.93 a
x 100	273.00 abc	226.02 bc	93.78 a	30.81 a	89.54 a	86.37 a	10.15 a	32.29 a
x 200	317.36 ab	256.47 abc	76.66 a	47.12 a	81.85 a	76.78 a	14.37 a	25.24 a
N-K Gigas x 50	239.37 abc	213.18 bc	90.35 a	29.30 a	75.67 a	80.79 a	11.54 a	30.15 a
x 100	434.43 a	519.93 a	174.84 a	56.22 a	116.43 a	113.99 a	12.19 a	26.65 a
x 200	243.32 abc	438.25 ab	60.04 a	40.92 a	71.77 a	46.01 a	17.79 a	29.19 a
Throumbolia x 50	221.44 abc	321.33 abc	174.36 a	61.50 a	119.09 a	103.96 a	25.09 a	49.19 a
x 100	281.67 ab	324.59 abc	204.19 a	70.60 a	116.65 a	100.63 a	22.76 a	40.69 a
x 200	262.21 abc	368.16 ab	124.67 a	43.75 a	87.09 a	80.10 a	15.91 a	31.74 a

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.05$ .

**Table 15.** Effect of salinity on relative macronutrient content and their ratio in the entire olive tree.

Parameters	Nutrient content in entire plant (% of corresponding control)						Nutrient Ratio	
	Na	Cl	P	K	Ca	Mg	K/Na	Ca/Na
<b>Cultivar</b>								
Gaidourelia	1382.79 a	394.44 c	78.35 a	78.35 a	80.11 b	75.78 b	5.93 c	6.07 c
Koroneiki	677.62 b	593.21 b	71.76 a	71.76 a	83.41 ab	91.03 ab	10.67 bc	12.58 b
Lefkolia	366.06 c	473.54 bc	73.96 a	73.96 a	83.68 ab	90.57 ab	19.03 a	20.91 a
N-K Gigas	629.43 bc	583.21 b	79.47 a	79.47 a	82.47 ab	84.93 ab	12.32 b	12.93 b
Throumbolia	852.18 b	795.81 a	88.79 a	88.79 a	107.02 a	108.05 a	10.14 bc	12.22 b
<b>Salinity level (NaCl mM)</b>								
50	686.01 a	555.12 a	92.02 a	92.02 a	101.68 a	104.61 a	15.82 a	17.33 a
100	805.44 a	591.75 a	73.41 a	73.41 ab	87.82 ab	91.51 a	9.70 b	11.72 b
200	853.39 a	557.26 a	69.97 a	69.97 b	72.51 b	74.10 b	9.34 b	9.77 b
<b>Cultivar x Salinity Level</b>								
Gaidourelia x 50	1111.03 a	398.33 a	87.60 a	87.60 a	87.10 b	77.22 a	7.94 b	7.94 bcd
x 100	1350.04 a	389.38 a	78.89 a	78.89 a	79.63 b	71.80 a	5.69 b	5.75 cd
x 200	1687.30 a	395.60 a	68.58 a	68.58 a	73.59 b	78.33 a	4.17 b	4.52 d
Koroneiki x 50	614.16 a	540.34 a	72.35 a	72.35 a	95.99 ab	104.24 a	11.81 b	15.52 bc
x 100	682.26 a	650.52 a	73.51 a	73.51 a	82.92 b	90.91 a	11.04 b	12.47 bcd
x 200	736.42 a	588.76 a	69.42 a	69.42 a	71.31 b	77.94 a	9.17 b	9.74 bcd
Lefkolia x 50	253.61 a	357.54 a	84.53 a	84.53 a	91.78 b	104.55 a	30.44 a	31.92 a
x 100	383.74 a	492.21 a	64.02 a	64.02 a	74.31 b	86.43 a	13.89 b	15.99 bc
x 200	460.82 a	570.86 a	73.33 a	73.33 a	84.96 b	80.73 a	12.76 b	14.81 bcd
N-K Gigas x 50	611.37 a	601.40 a	100.48 a	100.48 a	87.22 b	92.31 a	15.71 b	14.29 bcd
x 100	775.94 a	686.15 a	64.25 a	64.25 a	101.13 ab	105.11 a	7.87 b	12.85 bcd
x 200	500.99 a	462.07 a	73.68 a	73.68 a	59.06 b	57.38 a	13.38 b	11.64 bcd
Throumbolia x 50	839.88 a	877.97 a	115.17 a	115.17 a	146.31 a	144.73 a	13.18 b	16.97 b
x 100	835.22 a	740.46 a	86.36 a	86.36 a	101.12 ab	103.32 a	10.00 b	11.54 bcd
x 200	881.44 a	768.99 a	64.84 a	64.84 a	73.64 b	76.10 a	7.23 b	8.15 bcd

Means within the same column followed by the same letter do not differ significantly based on Tukey HSD at  $\alpha = 0.0$ .



**Figure 2.** Principal component analysis results of the effect of salinity stress on the measured variables. The ellipses indicate where “Gaidourelia” and “Lefkolia” are located in the scatterplot. Abbreviations: G, “Gaidourelia”, L, “Lefkolia”, K, “Koroneiki”, T, “Throumbolia” and N, “N-K Gigas”.

irrespective of the cultivar. It also induced a slight decrease of RWC, which was most evident in the sensitive “Gaidourelia” under 200 mM reaching 66%. Similar high RWC has been reported by Goreta et al. (2007) and Gucci, Lombardini, and Tattini (1997), who found that salinity imposed a reduction of RWC but the values reported were always higher than 84% even under 200 mM NaCl. Since there were not any significant effects on WCS and WSD, one can assume that the adverse effects observed should not be the result of severe osmotic stress.

Salinity has a direct effect on macronutrient concentration in various plant parts, as has been reported in the literature (Cimato et al. 2010; Perica, Goreta, and Selak 2008) while there were no such intense and consistent effects on micronutrient concentration in accordance with the literature (Bartolini, Mazuelos, and Troncoso 1991). Irrigation with saline water resulted in increased concentration of both Na and Cl in all parts of the tree (leaves, stems and roots) and a significant decrease in the ratios K/Na and Ca/Na as has been found in other cultivars too (Chartzoulakis et al. 2002; Melgar et al. 2008; Soda et al. 2017; Vigo, Therios, and Bosabalidis 2005; Baccari, Chelli-Chaabouni, and Chaari-Rkhis 2018). According to Kchaou et al. (2010) the significant decrease of K/Na and Ca/Na ratios indicates the imbalance in nutrient absorption and the down regulation of the exclusion mechanism, which prevents the accumulation of toxic ions in the aerial parts.

The most sensitive cultivar, based on the symptoms, “Gaidourelia”, exhibited the highest concentration of Cl in the leaves but not the highest Na one, which was found in “Koroneiki”. According to several researchers (Flowers and Yeo 1995; Cimato et al. 2010) the “physiological tolerance” i.e. the control of salt entry and allocation, and the “low-Na strategy” are better descriptors of salt tolerance in olive. In the case of “Gaidourelia” though, this is not entirely true, since it did not exhibit the highest concentration of Na in its leaves nor in its stems. Thus it seems that the concentration of Na is not a universal, uni-cultivar indicator. Furthermore, the most interesting thing was the fact that “Gaidourelia” presented one of the highest K/Na ratios in its leaves, which according to Al-Absi, Qrunfleh, and Abu-Sharar (2002) is an indicator for choosing a tolerant cultivar, which clearly is not the case here. Another interesting finding was that Cl was found in high concentration in the leaves of the sensitive “Gaidourelia”, indicating that there could be a relation between the sensitivity of this cultivar and Cl accumulation. The ability of an olive cultivar to withstand high salt concentration at the root level is also dependent on the ability to exclude Cl ions from the aerial parts (Chartzoulakis 2005; Kchaou et al. 2010). Thus, it is possible that “Gaidourelia” is sensitive to high Cl accumulation. According to

Cassaniti, Cherubino, and Flowers (2009) some of the ornamental species they tested accumulated more Cl than Na in their leaves and growth reduction was correlated to the concentration of Cl found, as was noticed in the case of “Gaidourelia”. Furthermore, according to Kchaou et al. (2010) the fallen olive leaves presented high amounts of both Cl and Na and the leaf fall was attributed to the sensitivity of toxic ion accumulation. “Gaidourelia” probably presents a higher sensitivity to accumulated Cl than other cultivars, which together with a moderate (compared to other cultivars) concentration of Na leads to leaf fall.

When the concentration of the ions was expressed as relative to the corresponding control of each cultivar, the effects of salinity became clearer. In stems, the most sensitive cultivar “Gaidourelia” exhibited the highest relative Na content while at the same time it presented the lowest K/Na ratio. On the contrary the most tolerant cultivar “Lefkolia” showed the highest K/Na and Ca/Na ratios, which according to Gucci and Tattini (1997) is a trait of salt tolerant cultivars. The most obvious differences though were found at the entire plant level. As excess Na displaces Ca from the binding sites at plasma membrane this leads to a consequent loss of K/Na selectivity (Cramer, Läuchli, and Polito 1985) which was clearly the case here, as the relative Ca concentration in the roots of “Gaidourelia” was significantly reduced under salinity (44% of the control compared to at least 87% in the other cultivars). The fact that “Gaidourelia” exhibited also the highest relative Na concentration on an entire plant level among cultivars, indicates that the relative concentration could be a more useful indicator than just the concentration of this nutrient on dry mass basis, when a comparison is being made among cultivars. Furthermore it was obvious that “Gaidourelia” restrained very low amounts of Na in the roots, indicating that the “low-Na strategy” does not function in this cultivar, and this may be the reason of its susceptibility, along with the low relative water content observed in its leaves under 200 mM (salt induced osmotic stress). According to Tattini (1994), Tabatabaei (2006) and Chartzoulakis (2005) the resistance mechanism of a salt-tolerant olive cultivar is based on the effectiveness of sodium exclusion by roots and the ability to maintain a high K/Na ratio, something that was not the case in “Gaidourelia”. On the contrary, “Lefkolia” retained high amounts of Na in the roots while maintaining low levels in the stems. The data presented here indicate that when working with genotypes with different or even unknown salinity tolerance, an effective alternative indicator would be the relative content of ions and especially the ratios at the whole plant level, as the concentration of nutrients in the leaves could give partly inconclusive evidence regarding cultivar’s tolerance to salinity.

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