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# Salt stress-mineral nutrient relations in olive (*Olea europaea* L.) plant

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## Abstract

In order to investigate the effect of salt stress on mineral nutrients, one year-old olive (*Olea europaea* L. cv. Gemlik) seedlings were exposed to increasing levels of NaCl salinity (4 dS $m^{-1}$, 8 dS $m^{-1}$ and 12 dS $m^{-1}$, respectively) in pot culture and Na, K, Ca, Mg, N, P, Cl, Fe, Mn, Zn concentrations, ratios of K/Na and (K+Ca+Mg)/Na of the plants were ascertained. Sodium and Cl concentrations of plant parts increased with the salinity and the level in the aerial parts of the plants were lower than that of root. Salinity led to a general decrease in K concentrations in the all organs with the exception of subsoil trunk. Calcium concentrations of the plant parts decreased significantly by salinity with the exception of roots and subsoil trunk. Salinity affected Mg concentrations only in trunk and leaves. Treatments significantly decreased the ratios of K/Na and (K+Ca+Mg)/Na of all the plant organs. Compared to control application the highest salinity level (12 dS $m^{-1}$) decreased the N concentrations of all the plant organs statistically except roots. Similarly salinity increased the concentrations of P in all plant parts except trunks compared to control treatment. Concentrations of all the micronutrients detected in the study were found lower in aerial parts than the roots.

**Keywords**: Olive cultivar, salinity, nutrient contents, nutrient ratios.



## Introduction

Salinization is the process that leads to an excessive increase of water-soluble salts in the soil. A soil is considered saline if the electrical conductivity of its saturation extract (ECe) is above 4 dS $m^{-1}$ (US Salinity Laboratory Staff, 1954). However, the threshold value above which deleterious effects occur can vary depending on several factors including soil water regime, climate and plant type (Maas, 1986). Olive (*Olea europaea* L.) plant is considered as moderately salt tolerant (Maas, 1986) and is grown preferentially in semiarid areas where irrigation is required to produce maximum yield. Gemlik is one of the most important olive cultivar of Northern Regions of Turkey (Canözer, 1991). However the cultivar has been preferred intensively by the growers of the Southern and the Southeastern parts of Turkey in the last decade. Gemlik cultivar is selected for growing by the growers for its high rooting capacity. Since these regions have more arid climate than that of North, resources of high quality water for irrigation are limited and the use of lower quality water resources such as saline water and reclaimed sewage effluent for irrigation is implemented. Utilization of such water resources accelerate the salinisation of the soil and generally decreased crop production (Lauchli and Epstein, 1990; Maas, 1990). Previous findings demonstrated that genotypic response of olive cultivars to salt stress is variable (Tattini et al. 1992; Demiral, 2005).

An increase in soil salinity commonly results in a reduction of plant water uptake. Passive nutrient uptake of the plants is related to water uptake, and any decrease in water availability causes a reduction in uptake of nutrients such as $NO_3^-$, K, Zn and Ca. Additionally any imbalance in composition of saline soil solution results in uptake of some ions, such as Cl, Na or Mg, in excessive amounts. An increase in the concentration of these

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ions either has a toxic effect directly to the plants or promotes imbalance in plant nutrient metabolism (Ghafoor et al. 2004). All these processes result in lower crop yields. However, toxic effect of an ion can be controlled depending on the cationic or anionic balance especially the K/Na and (K+Ca+Mg)/Na ratios. Salt tolerant varieties of higher plant species have wider K/Na ratio than that of salt sensitive ones (Chauhan et al. 1980). The main objectives of this experiment were to investigate short-term effects of NaCl-induced salinity on chemical composition of Gemlik olive cultivar.

## Material and Methods

### Plant material and salinity treatments

Gemlik olive (*Olea europaea* L.) cultivar was used as test plant in the experiment. The experiment was carried out in a net-house at the University of Adnan Menderes, Aydın, Turkey from March 15 to June 15, 2007. One year-old homogeneous self-rooted plants produced through mist propagation system were planted in 18 L containers with soil/coarse sand mixture (1/1.5, w/w). Soil analysis made before planting (Westerman, 1990) showed the following soil characteristics: very low in available K (0.21 me 100 $g^{-1}$) (Pizer, 1967); low in total N (0.07 %), available P (4.69 mg $kg^{-1}$), Mg (0.05 me 100 $g^{-1}$) and Zn (0.60 mg $kg^{-1}$) (Loue, 1968; Lindsay and Norwell, 1978; Olsen and Sommers, 1982) medium in available Ca (3.05 me 100 $g^{-1}$), Fe (7.44 mg $kg^{-1}$), Mn (60.60 mg $kg^{-1}$) and Cu (0.68 mg $kg^{-1}$) (Loue, 1968, Lindsay and Norwell, 1978). Texture; sandy loam (SL) (sand 60.50%, silt 23.70% and clay 15.80%); alkaline (pH 7.99); poor in organic matter (1.01%) with high $CaCO_3$ (24.64%); and slightly-salty (251 µmhos $cm^{-1}$) (Soil Survey Staff, 1951; Kellogg, 1952; Thun et al. 1955; Evliya,1964).

Treatments comprised of salinized half-strength Hoagland's solution with 3 different NaCl levels (2560 mg $L^{-1}$, 5120 mg $L^{-1}$ and 7680 mg $L^{-1}$ of NaCl, which is equal to 4 dS $m^{-1}$, 8 dS $m^{-1}$ and 12 dS $m^{-1}$, respectively). The salinity levels were adjusted by the addition of appropriate amounts of NaCl to half-strength Hoagland's solution (Hoagland and Arnon, 1950). The electrical conductivity (EC) of the solutions was measured with a hand-held EC meter (WTW-Cond 330i, Germany). Half-strength Hoagland's solution was used as control treatment. The experiment was laid out in completely randomized design. Each treatment was replicated 12 times with 1 plant per container. Seedlings (approx. 30 cm in length) were grown for a month by using half-strength Hoagland's solution then saline solutions were applied to the plants. Salinity of the solutions was increased gradually reaching to the final application levels by the end of the second month in order to prevent possible shock effect of salinity on the experimental plants. The experiment was terminated 4 months after planting.

In order to keep the salinity under control in the root area; (1) the plants were irrigated with an amount of the solutions accounting for a leaching factor of 20-25%, (2) electrical conductivity (EC) was measured throughout the entire irrigation period in the leachate obtained from the containers, (3) when the EC of the leachate exceeded the EC of the application doses about 20% the growing media was washed with fresh water and the EC was dropped to the normal levels. Additionally the containers were covered with aluminum foil to prevent evaporation that causes accumulation of salt at the surface.

### Sampling and chemical analyses

For chemical analyses, all the plants were gently removed from the substrate and were divided into root, subsoil trunk, trunk and leaves. Roots were then sampled by hand from growing media and were washed with deionised water. All samples were then weighed and dried in a forced-air oven (Memmert UM 500, Schwabach, Germany) at 70 °C for 72 h. Dried samples were then prepared for analysis by grinding in a stainless steel mill (IKA A 11 Basic, Staufen, Germany). The ground samples were wet digested in a mixture of $HNO_3/HClO_4$ (4/1, v/v) solution (Westerman, 1990). Na, K and Ca concentrations were determined using flame photometry (Jenway PFP7, Staffordshire, UK); Mg, Fe, Zn and Mn concentrations by an atomic absorption spectrophotometer (Varian SpetrAA 220FS, Mulgrave, Australia), and P by the vanadomolybdophosphoric method (Westerman, 1990). Total N concentrations of dried samples were analyzed by Kjeldahl digestion method (Westerman, 1990). Cl was extracted from 0.1 g of the ground sample with 10 mL of deionized water by shaking the mixture for 2 h. Chloride concentrations of the extracts were measured by a chloridemeter (Jenway PCLM3, Staffordshire, UK) and results were expressed as % Cl in the DM (Brown and Jackson, 1955).

### Statistical analyses

Analysis of variance (ANOVA) was performed for the experimental data using MSTAT statistical software (Little and Hills, 1978). Mean separation was performed with the Least Significant Difference (LSD) test at $P \leq 0.05$.

## Results

Compared to control treatment, Na and Cl concentrations of plant parts increased progressively and significantly with salinity except leaves (Table 1). Elevated salinity led to a general decrease in K concentrations of the all plant organs studied, except subsoil trunks. Among the different plant parts, the highest concentration of K was found in leaves (Table 2). Salinity treatments generally led to a significant decrease in the Ca concentrations of the plant trunks and in leaves (Table 2). Salinity affected Mg concentrations only in trunk and leaves. A fluctation was observed in the Mg concentrations of aerial parts of the plants (Table 2).

Table 1. Effect of salinity on Na and Cl concentrations in different tissues of Gemlik olive cultivar

| Salinity Treatment (dS $m^{-1}$) | Root | Subsoil Trunk | Trunk | Leaf |
|---|---|---|---|---|
| | | Na (%) | | |
| Control | 0.20 c | 0.05 d | 0.13 d | 0.03 c |
| 4 | 0.53 b | 0.17 c | 0.28 c | 0.13 b |
| 8 | 0.58 b | 0.24 b | 0.42 b | 0.33 a |
| 12 | 1.07 a | 0.31 a | 0.58 a | 0.38 a |
| | | Cl (%) | | |
| Control | 0.38 c | 0.12 d | 0.15 d | 0.19 c |
| 4 | 0.93 b | 0.21 c | 0.26 c | 0.34 b |
| 8 | 0.98 b | 0.30 b | 0.44 b | 0.56 a |
| 12 | 1.40 a | 0.56 a | 0.68 a | 0.55 a |

Means with the same letter are not significantly different using LSD at $P \leq 0.05$

Table 2. Effect of salinity on K, Ca and Mg concentrations in different tissues of Gemlik olive cultivar

| Salinity Treatment (dS $m^{-1}$) | Root | Subsoil Trunk | Trunk | Leaf |
|---|---|---|---|---|
| | | K (%) | | |
| Control | 1.97 a | 0.39 ns | 1.71 a | 2.53 a |
| 4 | 0.70 b | 0.40 | 1.39 b | 2.32 ab |
| 8 | 0.35 c | 0.35 | 0.57 c | 2.08 b |
| 12 | 0.35 c | 0.34 | 0.55 c | 2.04 b |
| | | Ca (%) | | |
| Control | 0.92 ns | 0.91ns | 1.35 a | 1.11 a |
| 4 | 0.84 | 0.92 | 1.16 b | 1.03 a |
| 8 | 0.85 | 0.87 | 1.22 ab | 0.74 b |
| 12 | 0.84 | 0.75 | 0.88 c | 0.70 b |
| | | Mg (%) | | |
| Control | 0.16 ns | 0.06 ns | 0.11 a | 0.10 b |
| 4.00 | 0.16 | 0.06 | 0.09 b | 0.12 a |
| 8.00 | 0.17 | 0.06 | 0.11 b | 0.11 ab |
| 12.00 | 0.17 | 0.07 | 0.12 a | 0.12 a |

Means with the same letter are not significantly different using LSD at $P \leq 0.05$; ns: nonsignificant.

Salinity decreased the ratios of K/Na and (K+Ca+Mg)/Na of the plant parts significantly. The ratios of K/Na and (K+Ca+Mg)/Na were found higher in leaves and lower in roots and subsoil trunks when compared to other plant parts (Table 3). Salinity led to a significant decrease in N concentration of the plant parts, except the roots (Table 4). Compared to control treatment, salinity significantly increased the concentrations of P in all plant parts, except in the trunk. Among different plant parts, the highest concentration of P was determined in leaves (Table 4).

Concentrations of all the micronutrients detected in the study were found lower in aerial parts of the plants compared to the roots (Table 5). Salinity treatments significantly affected the concentrations of Fe in roots and trunks of the plants. The treatments increased the root Fe concentrations in 8 dS $m^{-1}$ salinity level. Compared to control treatment, salinity led to a decrease in Fe concentration of trunk. Salinity affected significantly the concentration of Zn in all plant parts, except leaf. In general, root and trunk Zn concentrations decreased, and subsoil trunk Zn concentrations increased with salinity (Table 5). Compared to control treatment, salinity led to an increase in the concentrations of Mn in roots and subsoil trunk, a decrease in leaf and variable effect on trunk of the plants (Table 5).

Table 3. Effect of salinity on K/Na and (K+Ca+Mg)/Na ratios in different tissues of Gemlik olive cultivar

| Salinity Treatment (dS $m^{-1}$) | Root | Subsoil Trunk | Trunk | Leaf |
|---|---|---|---|---|
| | | K/Na | | |
| Control | 9.97 a | 8.18 a | 13.45 a | 122.80 a |
| 4 | 1.33 b | 2.49 b | 4.92 b | 20.61 b |
| 8 | 0.62 c | 1.58 c | 1.48 c | 7.45 b |
| 12 | 0.36 c | 1.18 c | 0.99 c | 5.81 b |
| | | (K+Ca+Mg)/Na | | |
| Control | 15.48 a | 28.40 a | 24.98 a | 182.14 a |
| 4 | 3.26 b | 8.61 b | 9.45 b | 31.51 b |
| 8 | 2.40 c | 5.72 c | 4.86 c | 10.57 b |
| 12 | 1.38 d | 3.89 c | 2.85 c | 8.09 b |

Means with the same letter are not significantly different using LSD at P ≤ 0.05;

Table 4. Effect of salinity on N and P concentrations in different tissues of Gemlik olive cultivar

| Salinity Treatment (dS $m^{-1}$) | Root | Subsoil Trunk | Trunk | Leaf |
|---|---|---|---|---|
| | | N (%) | | |
| Control | 1.63 ns | 0.53 a | 0.87 a | 2.31 a |
| 4 | 1.63 | 0.54 a | 0.77 b | 2.31 a |
| 8 | 1.65 | 0.51 ab | 0.74 b | 2.29 a |
| 12 | 1.62 | 0.47 b | 0.70 b | 2.21 b |
| | | P (%) | | |
| Control | 0.04 c | 0.03 c | 0.05 ns | 0.12 b |
| 4 | 0.08 b | 0.05 a | 0.06 | 0.14 a |
| 8 | 0.10 ab | 0.04 b | 0.05 | 0.13 ab |
| 12 | 0.12 a | 0.05 ab | 0.06 | 0.13 ab |

Means with the same letter are not significantly different using LSD at P ≤ 0.05; ns: nonsignificant.

Table 5. Effect of salinity on Fe, Zn and Mn concentrations in different tissues of Gemlik olive cultivar

| Salinity Treatment (dS $m^{-1}$) | Root | Subsoil Trunk | Trunk | Leaf |
|---|---|---|---|---|
| | | Fe (mg $kg^{-1}$) | | |
| Control | 362.4 b | 129.9 ns | 174.5 a | 128.5 ns |
| 4 | 367.2 b | 148.0 | 106.1 c | 126.7 |
| 8 | 475.6 a | 152.3 | 125.7 bc | 131.6 |
| 12 | 394.0 b | 151.3 | 127.7 b | 129.5 |
| | | Zn (mg $kg^{-1}$) | | |
| Control | 70.1 a | 20.6 c | 53.6 a | 45.0 ns |
| 4 | 67.5 a | 29.3 b | 49.8 ab | 48.3 |
| 8 | 62.4 a | 36.7 ab | 39.0 bc | 51.2 |
| 12 | 46.5 b | 40.6 a | 28.5 c | 53.6 |
| | | Mn (mg $kg^{-1}$) | | |
| Control | 42.5 b | 10.1 b | 13.0 a | 26.5 a |
| 4 | 47.7 ab | 11.3 ab | 12.1 a | 23.7 ab |
| 8 | 45.5 ab | 10.9 ab | 10.0 b | 20.2 c |
| 12 | 56.0 a | 14.1 a | 12.4 a | 23.2 bc |

Means with the same letter are not significantly different using LSD at P ≤ 0.05;ns: nonsignificant.

## Discussion

The observation dealing with lower Na and Cl concentrations in aerial parts of the plants than that of roots may indicate that the cultivar is able to protect above ground meristematic tissues efficiently from the accumulations of Na and Cl. As reported by Demiral (2005), salt tolerant olive cultivar Barnea regulated the transport of salts from roots to aerial parts more effectively than salt sensitive olive cultivar Leccino. This ability was regarded as an essential phyto-physiological mechanism for salinity tolerance in plants (Munns, 2002) and was related to the potential growth of the plants (Ghafoor et al., 2004). Therefore, it may be speculated that Gemlik cultivar regulated the transport of to the leaves in order to maintain minimum total

leaf area for photosynthesis and the production of carbohydrates crucial for the sustainable plant growth under salinity.

Probably, high K concentration of leaves represents an adaptation to salinity of the cultivar. With high K concentration in leaves, the cultivar prevented osmotically Na transport from the roots to the aerial parts of the plants (Jacoby, 1999). According to Demiral (2005), salt tolerant olive cultivar Barnea accumulated higher concentrations of K in its tissues than salt sensitive olive cultivar Leccino. On the other hand, salinity decreased concentrations of K of roots. Compared to control treatment, K concentration of the roots were 64% lower in the lowest salinity level (4.0 dS $m^{-1}$), and 81% lower in the 8.0 dS $m^{-1}$ and 12.0 dS $m^{-1}$ salinity levels. Sodium influx into root cells are partly achieved by K influx transporters (Epstein et al., 1963). Therefore, it is suggested that this reduction might be related to the antagonistic effect of Na on K influx in the plants.

Not only the concentrations of Na and K but also the ratio of K/Na can be used as a parameter giving clues about the physiological response of the plants to salt stress (de Lacerda et al., 2005). Therefore, it may be speculated that higher K/Na and (K+Ca+Mg)/Na ratios of the plant leaves can be accepted as key indicators reflecting the levels of adaptation of the cultivar to salt stress. Most likely, these findings indicate that salinity affected more efficiently K, Ca and Mg concentrations of the aerial parts than the roots. According to Ghafoor et al. (2004), passive nutrient uptake is relevant to water intake, and any decrease in water availability reduces the uptake of plant nutrients. Additionally, an imbalance in the composition of saline soil solution can cause an excessive or insufficient uptake of some ions. Calcium is supposed to be directly involved in Na exclusion and retention mechanisms regulating Na transport (Melgar et al., 2006). The results of this study agree with the aforementioned reports. In our study, all the plants survived until the end of the experiment and restricted the concentrations of Na and Cl in the aerial parts in all salinity treatments (Table 1). Therefore, the low Na and Cl and, high K and Ca concentrations of the leaves may be an evidence of the salinity tolerance ability in Gemlik olive cultivar. The low Mg concentrations of the plant parts might be one of the consequences of the competition with Ca and K. As reported by Marschner (1995), Ca is strongly competitive with Mg and the binding sites on the root plasma membrane appear to have less affinity for Mg than for Ca.

Among different plant parts, the highest concentration of N was found in the leaves. The concentration of N in the trunk was more affected than the other plant parts by salinity. Compared to the control treatment, the highest salinity treatment (12.0 dS $m^{-1}$) decreased N concentration by 11.3%, 19.5% and 4.3% in subsoil trunk, trunk and leaves, respectively. These results agree with previous findings (Peuke et al., 1996, Rubinigg et al., 2003). According to Rubinigg et al. (2003), a decreased rate of N translocation to the trunks could be the consequence of a general lower rate of solute flow in xylem. The authors stated that this phenomenon is a result of a reduced transpiration rate in trunk, inhibition of the xylem loading rate for $NO_3^-$ or amino acids, a lower requirement for $NO_3^-$ in the trunk, or a decrease $NO_3^-$ influx in *Plantago maritima* L. growth in saline conditions.

As reported by Köhler and Raschke (2000) anion channels with similar permeability for both Cl and $NO_3^-$ play a significant role in the xylem loading of these ions. Therefore, it may be speculated that in the presence of high Cl concentrations, the translocation of $NO_3^-$-N from the root to the trunk decreased at the site of entrance into xylem via competition for the same channel. Our results seem to confirm this hypothesis. The accumulation of N in Gemlik olive was negatively and significantly correlated with the concentrations of Cl (r= - 0.736** for roots, r= - 0.945** for subsoil trunk, r= - 0.908**for trunk and r= – 0. 692* for leaves; ** significant at $p \leq 0.01$, * significant at $p \leq 0.05$). According to previous reports, the competition between $NO_3^-$ and $Cl^-$ ions uptakes across root plasma membrane are performed by various transport systems (Cerezo et al., 1997) and the internal N demand of plant was one of the properties regulating the activity of these transport systems (Forde and Clarkson, 1999). According to the authors the high N concentrations of the plant roots under salinity may have attributed to the following factors; first, the inhibition of $NO_3^-$-N translocation from the roots to the trunk; and second, reduced transpiration rate of the plants as a result of salinity stress.

Some researchers reported that salinity induces inhibition of the high affinity mechanism in plants (Martinez and Lauchli, 1994). According to Chabra et al. (1976) salinity decreased P uptake because of the possible competition between P and Cl absorption. However, the results of some other studies indicated that salinity may increase P uptake through the low affinity system in plants under high external P concentrations

(Martinez et al. 1996). Plants have two different P uptake systems: either with a high affinity (uptake of P at low P concentrations) or low affinity (uptake of P at higher P concentrations) (Furihata et al. 1992). The low affinity system is considered constitutive (Dunlop et al. 1997) and its activity is connected with the existence of multiple transporters of P in the plasma membrane and tonoplast (Schachtman et al. 1998). The transporters are regulated by the external P concentration (Leggewie et al., 1997) and high cytosol pH (Martinez and Lauchli, 1994). In our study, the nutrient solution used in the experiment had sufficient amount of P (15.5 mg $L^{-1}$or 0.5 mmol P) for growing plants. Additionally, plant analysis showed that test plants accumulated sufficient amounts of P in their leaves (Jones et al., 1991). Therefore, we suggest that a Cl based limitation in uptake of P did not occur in the Gemlik cultivar.

It might be speculated that an increase in salinity and/or in salinity-induced increase in Fe and Mn concentrations led to a significant decrease in the Zn concentration of experimental plants' roots. However, as reported by Grattan and Grieve (1999), the relationships between salinity and micronutrients are complex and differences can be attributed to plant type and tissue, salinity level and composition, micronutrient concentration in the medium, growing conditions and the duration of study. Some of the possible reasons above-mentioned are beyond the scope of this study. Whatever the exact reason of the micronutrients alteration in olive plants under salinity is, we may accept that micronutrient concentrations of root of Gemlik cultivar are higher than that of micronutrient concentrations of plant parts above ground under salinity.

## Conclusion

The result showed that compared to other plant parts K and Ca concentrations of the leaves were in highest concentrations under salinity. Therefore the concentrations of K and Ca, and the ratios of K/Na and (K+Ca+Mg)/Na of the leaves were regarded as an indication of adaptation to salinity in olive. In general salinity decreased N concentrations and increased P concentrations of the plant parts. Regarding the micronutrients, salinity increased root Fe and Mn concentrations, and decreased root Zn concentrations compared to control treatment. The findings of this investigation may be significant to explain the behaviour of *Olea europaea* L. under salt stress, in particular to a deeper understanding on how salinity interferes with concentration of mineral nutrients in plants.

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