Physiological Responses of Tomato Plants and Soil Microbial Activity in Salt Affected Greenhouse Soil

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Crop productivity decreases globally as a result of salinization. However, salinity impact on greenhouse-grown crops is much higher than on field-grown crops due to the overall concentrations of nutrients in greenhouse soils. Therefore, this study was performed to determine the short-term changes in growth, photosynthesis, and metabolites of tomato plants grown in greenhouse under heavily input of fertilizers evaluated by microbial activity and chemical properties of soils. The soils (< 3, 3.01~6, 6.01~10 and >10.01 dS m^{-1}) from farmer's greenhouse fields having different fertilization practices were used. Results showed that the salt-accumulated soil affected adversely the growth of tomato plants. Tomato plants were seldom to complete their growth against > 10.0 d Sm^{-1} level of EC. The assimilation rate of CO_2 from the upper fully expanded leaves of tomato plants is reduced under increasing soil EC levels at 14 days, however; it was the highest in moderate or high EC-subjected (3.0 ~ 10.0 dS m^{-1}) at 28 days. In our experiment, soluble sugars and starch were sensitive markers for salt stress and thus might assume the status of crops against various salt conditions. Taken together, tomato plants found to have tolerance against moderate soil EC stress. Various EC levels (<3.0 ~ 10.0 dS m^{-1}) led to a slight decrease in organic matter (OM) contents in soils at 28 days. Salinity stress led to higher microbial activity in soils, followed by a decomposition of OM in soils as indicated by the changes in soil chemical properties.

Key words: Electric conductivity, Organic acids, Soluble sugars, Microbial activity, Tomato

Introduction

Salinity causes significant reductions in growth and yield of many crop plants throughout the world due to soil degradation (Abd El-Azeem et al. 2012; Jung et al. 2011; Ok et al. 2007). However, salinity impact on greenhouse-grown crops differs from that on field-grown crops due to the overall much higher concentrations of nutrients in greenhouse soils. This high ion levels are maintained in the soil to improve quality of the product and to control crop growth under poor light (Sonneveld 2000). Salinity can cause water stress through increasing the osmotic pressure of the soil solution, resulting in much higher concentration of an internal ion and thus leading to plant growth reduction (Greenway and Munns 1980). In particular, water stress by salinity causes deleterious effects on dry matter partitioning, cell extension,

cell division, and leaf photosynthesis and transpiration (Greenway and Munns 1980; Maas and Hoffman, 1977). Additionally, water stress accumulates sucrose as soluble sugars and starch as carbohydrate in the leaves (Balibrea et al. 2000; Gao et al. 1998; Yin et al. 2010).

Since 1990s, many problems such as the frequent occurrence of soil borne diseases, salt accumulation, degradation of soil quality and decrease in soil productivity have been reported in vegetable greenhouse-soils under heavy application of fertilizers with a constant increased greenhouse industry (Chen et al. 2004; Tong and Chen 1991; Xu et al., 2004). More attention has been raised to improve greenhouse vegetable production and minimize salinity adverse effects, however, few studies in relation to the responses of plant and soil to human activities have been done in greenhouse soils compared to the artificially adjusted soils with NaCl. With a consideration of keeping an expansion of greenhouse industry and an input of mineral sources, it might be more productive to understand

how well plants can perform their own growth features under the conditions of various EC levels.

Therefore, the objective of the present study was to determine the short-term changes in growth, photosynthesis, and metabolites of tomato plants grown in greenhouse soils encountered by heavy input of fertilizers. In addition, the temporal changes in microbial activity and chemical properties of soils were investigated to evaluate soil quality.

Materials and Methods

Plant Materials and Soil Preparation This study was conducted in a greenhouse at the National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA) in 2011. Soil was collected from farmer's greenhouse fields having different fertilization practices and their chemical properties were analyzed (Table 1). The EC of the collected soils was firstly measured by using an electro-conductivity meter after the extraction of soil solution (1:5, w/v), and divided into four EC levels ($< 3, 3.01\sim6, 6.01\sim10$ and >10.01 dS m^{-1}). The three weeks-old tomato (*Lycopersicon esculentum* cv. Seonmyoung) seedlings were transplanted with 6 replications into different EC-leveled soils (pot volume: 25 cm diameter × 40 cm height) after mixing soil thoroughly. Plants were constantly exposed for 28 days with average day temperature of $28 \pm 2°C$ and night temperature of $18 \pm 2°C$. Mid-day photosynthetic photon flux density was

$800-1,100$ μmol m^{-2} s^{-1}. The 200 mL of water was supplied everyday without any application of chemical fertilizer throughout the experiment. Plants and soils were harvested between 10:00 and 14:00 at 14 and 28 days after transplanting, and used for further analysis.

CO_2 Assimilation Measurements Photosynthetic activities in response to different EC levels were measured with a CO_2 analyzer (ADC LCpro$^+$, ADC BioScientific Ltd, UK) operating in a differential mode. Mid-fully expanded tomato leaves from each treatment were enclosed in the square (6.25 cm^2) chamber to monitor CO_2 assimilation (*A*), transpiration rate (*E*) and stomatal conductance (g_s). Photosynthesis measurement with PPFD ($800-1,100$ μmol m^{-2} s^{-1}) and atmosphere CO_2 (380 ± 10 μmol m^{-2}) were performed between 10:00 and 14:00.

Metabolites Determination Leaf samples (1g FW) were subjected to 10 ml of 0.5 N HCl (v/v) and heated at 80°C for 10 min with shaking. The extracts were centrifuged ($12,000 \times g$, 10 min, 4°C) and passed through 0.45 μm membrane filter. Organic acids (citrate, malate and succinate) were determined using HPLC system (Agilent LC 1100, USA). Separation of organic acids was performed for 30 min with an Aminex 87H column (Temp., 40 °C) under 0.01 N H_2SO_4 as a mobile phase. The injection volume of each sample was 20 μL and flow rate was maintained 0.5 mL min^{-1}. Absorbance was detected with an UV detector for organic acids

Table 1. Soil chemical properties under various EC levels and different soil EC-exposed tomato plants at 14 and 28 days.

	EC level	pH	E.C	OM	T-N	NO_3^--N	NH_4^+-N	Av.P_2O_5	K	Ca	Mg	Na	B
	(dS/m)	(1:5)	(dS m^{-1})	(g kg^{-1})	(%)	-------- mg kg^{-1} --------			------------- mg/kg -------------				
Collected	<3.00	6.8	1.6	33.4	0.13	10	6	1,442	1.92	7.8	2.8	0.30	2.25
	3.01~6.00	6.8	4.4	41.2	0.18	66	6	1,275	2.74	8.6	4.1	0.70	4.27
	6.01~10.00	6.7	7.5	52.8	0.23	47	27	1,829	3.81	10.9	5.6	1.17	7.83
	>10.01	6.4	16.5	53.1	0.27	201	29	1,652	4.08	13.6	7.4	1.88	6.63
14 days	<3.00	6.8	1.6	33.6	0.13	20	12	1,412	1.93	9.7	2.9	0.50	1.97
	3.01~6.00	6.8	6.1	40.2	0.19	111	12	1,397	2.77	15.3	4.7	1.14	4.59
	6.01~10.00	6.5	9.5	48.3	0.23	136	10	1,654	3.88	15.5	6.6	1.67	8.18
	>10.01	6.4	18.2	51.1	0.27	293	11	1,666	4.90	16.8	9.2	2.77	8.04
28 days	<3.00	6.9	2.1	31.3	0.12	13	6	1,458	1.62	7.8	2.6	0.47	2.24
	3.01~6.00	7.0	4.8	37.9	0.18	55	22	1,476	2.27	11.3	4.1	0.98	5.07
	6.01~10.00	6.6	8.4	44.3	0.20	118	9	1,865	3.40	12.6	6.0	1.40	8.85
	>10.01	6.4	16.6	49.4	0.23	234	9	1,602	4.30	13.1	8.0	2.32	8.57

using a wavelength of 210 nm. Quantification was based on standard curves with a range from 0.1−10 mmol μL^{-1} of each standard of organic acids. Acid-soluble carbohydrates were determined using HPLC system (Dionex Ultimate 3000, USA) composed of an auto-sampler and detector (Shodex RI-101, Japan). Separation of carbohydrates was carried out with a Waters sugar-pak (Temp. 75°C) under distilled-deionized water as a mobile phase. The injection volume of each sample was 20 μL and flow rate was maintained 0.5 mL min^{-1}. Quantification was based on the standard curves of each carbohydrates with a range from 50 ~ 10,000 mg L^{-1}.

Mineral Analysis of Plant and Soil The extraction and measurement of macro nutrients were determined according to Walinga method (1989). Three randomly selected-plants per treatment were divided into leaves, stem, and roots and dried in an oven at 70°C for 2 days to determine dry weights and elemental concentrations. Chemical analyses of leaves, stem, and roots were carried out on dry weight basis with three replications. The absorbance of N and P was measured at 660 and 880 nm, respectively, using UV-spectrophotometer, while K, Ca, Mg, and Na were measured with ICP-OES (INTEGRA XMP, GBC, Australia).

Soil samples from each treatment were collected from the rhizosphere at 14 and 28 days after transplanting (DAT) in order to examine the changes in inorganic N and total C of soil. Inorganic N was extracted with 2 M KCl (1:5, w/v) from fresh soil samples (Kopp and McKee, 1978; Keeney and Nelson, 1982) and was colorimetically determined by flow injection analyzer (BRAN LUBBE, Germany). Total soil carbon contents were determined using a CN elemental analyzer (Variomax CN, ELEMENTAR, Germany). The selected chemical properties of soils used in the experiment were determined by the Korean standard methods (RDA, 1988).

Soil Microbial Activity After fumigation and extraction of soil, the released fumigation extractable carbon (FEC) and nitrogen (FEN) were measured using a method modified from Vance et al. (1987). The fumigation time was 24 hr and a 1 : 4 ratio of soil to extractant was used. All extracts were stored at - 20 °C until analysis. Before analysis, samples were thawed and kept at room temperature for 30 min. Samples were diluted by 5-folds prior to the determination of

extractable C by a TOC analyzer (TOC-5050, Shimadzu, Japan) to prevent precipitation of K_2SO_4 from the extraction medium onto the catalyst pellets. Extractable N was determined as ninhydrin-reactive N, which includes NH_4-N, amines, amino acids, peptides and proteins (Cater, 1991).

Statistical Analysis Statistical analysis was performed with SAS software package (version 9.1). Data were subjected to one-way ANOVA. If the ANOVA yielded a significant F value ($p < 0.05$), the differences among treatments were compared using a least significant difference (LSD

Results and Discussion

Plant Growth and Photosynthetic Activities The salt-accumulated soil affected adversely the growth of tomato (Fig. 1) and the difference in growth was observed almost immediately after treatment. The growth rates were the highest in soil EC level below 3.0 dS m^{-1}and followed by 3.0 ~ 6.0, 6.0 ~ 10.0 and above 10.0 dS m^{-1}. However, it was observed that tomato in 3.0 ~ 6.0 dS m^{-1} of EC level reached the similar growth rates compared to the below 3.0 dSm^{-1} at 28 days (Fig. 1). This result suggests that tomato plants can resist moderate or high EC soils (3.0 ~ 10.0 dS m^{-1}) and recover their growth at 28 days after early slightly
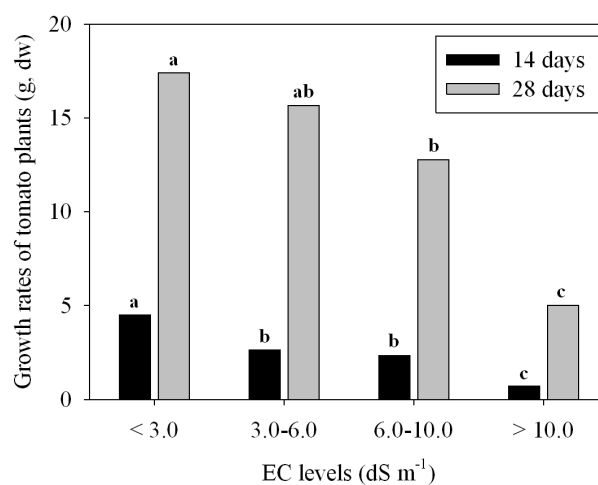

Fig. 1. Temporal changes in the growth of tomato plants under various soil EC levels. Three week-old tomato seedlings were transplanted and harvested to measure shoot dry weight at 14 and 24 days after treatment (n = 6). Bars with the same letters are not significantly different at P< 0.05.

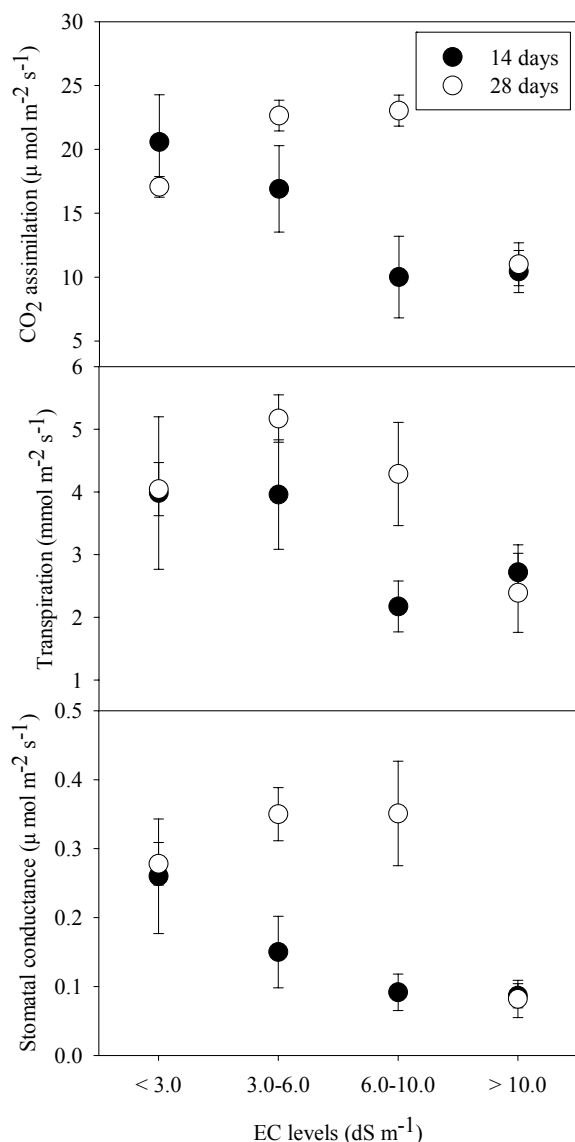**Fig. 2. Temporal changes in CO₂ assimilation rates (*A*), transpiration rates (*E*), stomatal conductance (*gₛ*) under various soil EC levels. The rate of photosynthesis was measured with Mid-fully expanded tomato leaves between 10:00 and 14:00. Error bars represent the standard deviation of the mean (n = 6).**

retarded growth at 14 days, while plants were seldom to complete their growth against severe level of EC (> 10.0 dS m⁻¹). Many previous studies are in accordance with our present study as they reported decreased growth rates in tomato plants under salt stress (Dumbroff and Cooper, 1974; El-Shourbagy and Ahmed, 1975; Cruz and Cuartero, 1990, Bolarin et al., 1991; Perez-Alfocea et al., 1993).

The measured assimilation rate of CO₂ from the upper fully expanded leaves of tomato plants is reduced under increasing soil EC levels at 14 days (Fig. 2), and the

range found to be 10.44 ± 1.65 (> 10.0 dS m⁻¹) ~ 20.58 ± 3.71 μmol m⁻² s⁻¹ (< 3.0 dS m⁻¹). In contrast, assimilation rates of CO₂ at 28 days were the highest in moderate or high EC-subjected (3.0 ~ 10.0 dS m⁻¹) tomato (an average of 22.84 ± 1.20 μmol m⁻² s⁻¹), which meant 34 % greater than that in below 3.0 dS m⁻¹ of EC level (Fig. 2). However, there was no change in assimilation rate of CO₂ under severe EC level (> 10.0 dS m⁻¹) during the experiment. Likewise, transpiration and stomatal conductance had similar behavior with CO₂ assimilation in all treatments (Fig. 2). It is noteworthy from our study that tomato plants found to have tolerance against moderate soil EC stress. The rate of photosynthesis is reduced under salt stress in many NaCl-treated salt stress studies (Seemann and Critchley, 1985; Yeo et al., 1991; Alarcon et al., 1994; Hocking and Steer, 1994). In the present study, the used EC-leveled soils were derived from excessive supply of mineral nutrients, and thus it is assumed that tomato plants are able to withstand high EC levels which can sufficiently cause severe damage evenly at seedling stages.

Concentration of Carbohydrates and Organic Acids
Water soluble carbohydrates, soluble sugars and starch of leaves, stems and roots of tomato were measured at 28 days under different EC treatments (Fig. 3). The contents soluble sugars and starch showed the highest values in below 3.0 dS m⁻¹ of EC, which represented 56.3 mg g⁻¹ in leaves, 91.9 mg g⁻¹ in stems and 58.4 mg g⁻¹ in roots for soluble sugars and 114.9 mg g⁻¹ in leaves, 65.4 mg g⁻¹ in stems and 55.5 mg g⁻¹ in roots for starch. Whereas, no significant differences were observed regarding contents of sugars and starch in above 3.0 dS m⁻¹ of EC levels. Interestingly, the concentrations of soluble sugars (55.1 mg g⁻¹, roots) and starch (97.4 mg g⁻¹, leaves) were substantially increased in above 10.0 dS m⁻¹ of EC compared to that in moderate or high EC regime (3.0 ~ 10.0 dS m⁻¹). In addition, a difference in the concentrations of soluble sugars and starch were substantial in leaves and roots while they remained rarely in stems. In our experiment, soluble sugars and starch were sensitive markers for salt stress and thus might assume the status of crops against various salt conditions. There are contradictory results about the effect of salt stress on sugar accumulation. For instance, some studies have reported the increased sugar content (Sacher and Staples, 1985; Balibrea et al., 2000), while others have
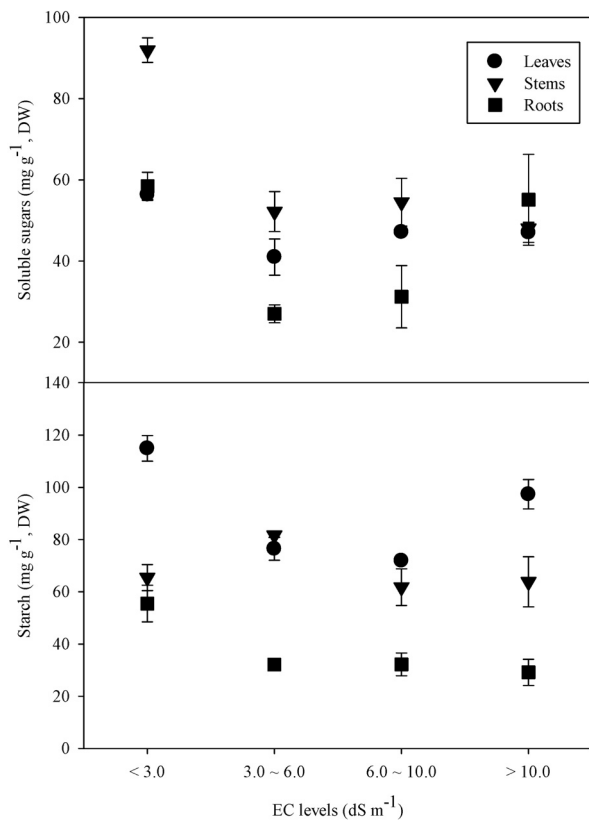
Fig. 3. The concentration of soluble sugars and starch in leaves, stems and roots of different soil EC-exposed tomato plants at 28 days after treatments. Error bars represent the standard deviation of the mean (n = 6).
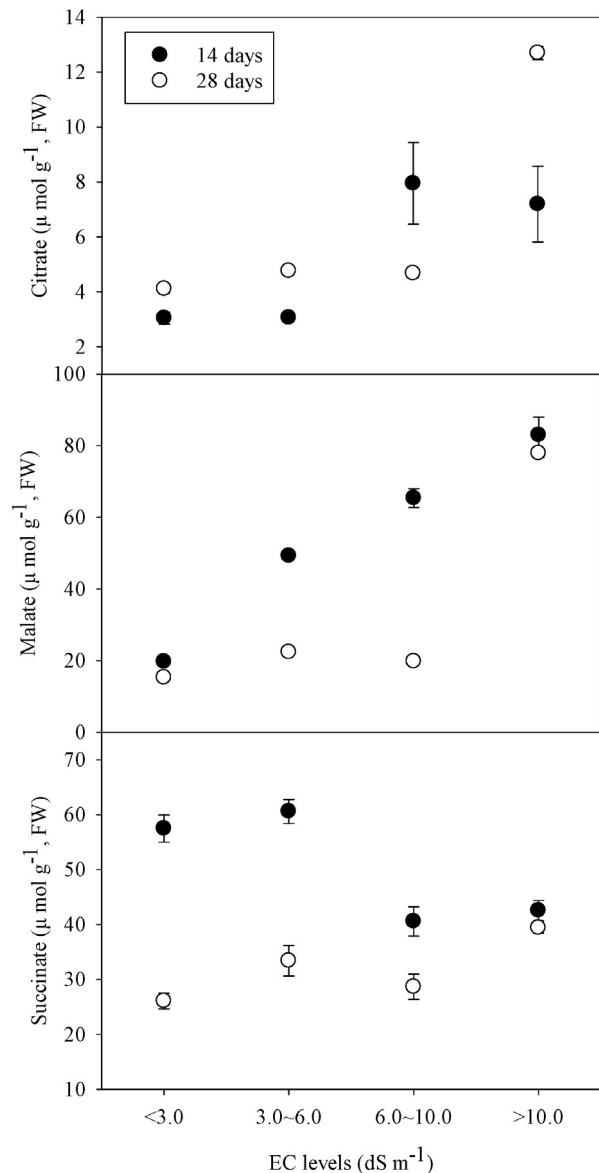

Fig. 4. Temporal changes in the concentration of organic acids, citrate, malate and succinate, in the leaves of different soil EC-exposed tomato plants. Error bars represent the standard deviation of the mean (n = 6).

found a constant or decreased sugar content (Hanson and Hitz, 1982; Morgan, 1992) during stress conditions. Overall, it is obvious that soluble sugars and starch showed a slight decrease in high nutrients-induced salt stress. This result might be assumed that a decline in CO_2 assimilation in below 3.0 dS m^{-1} led to an accumulation of soluble sugars and starch through the acceleration of soluble sugar and starch catabolism, opposite to its enhancement in moderate salt stress (3.0 ~ 10.0 dS m^{-1}).

Increasing EC levels induced significant accumulation of citrate (R^2 = 0.733) and malate (R^2 = 0.995), in the leaves of tomato plants at 14 days (Fig. 4), while the concentration of succinate (R^2 = 0.674) decreased constantly. Interestingly, all organic acids which measured at 28 days did not represented distinct difference up to 10.0 dSm^{-1} of EC level; however, citrate and malate in high salt regime (> 10.0 dS m^{-1}) were substantially accumulated in tomato leaves. Organic acids are rapidly accumulated to regulate cellular osmotic potential against salinity (Perez-Alfocea et al., 1993), and malate

increases substantially as a function of salinity stress (Martinez et al., 1994). In the present study, organic acids accumulation was closely correlated with the concentration of soluble carbohydrates, while a negative correlation between plant growth and photosynthesis was observed.

Plant Mineral Uptake Increasing EC levels influenced significantly the uptake of major elements of tomato plants (Fig. 5). In particular, the contents of N, Ca, Mg and Na on the basis of dry weight in organs were closely correlated with soil EC while P and K were not. The N
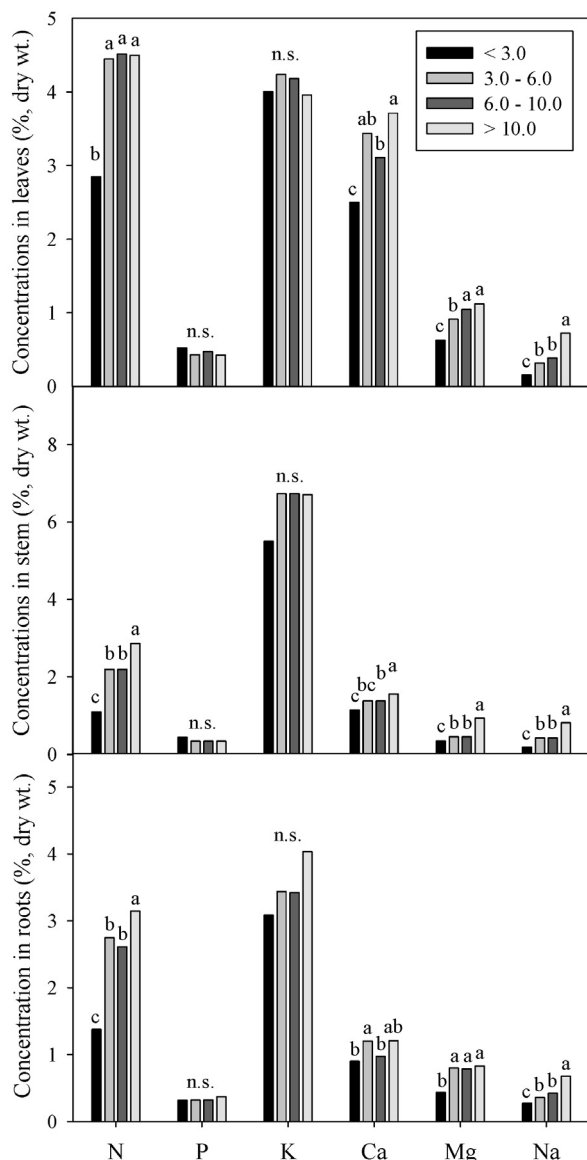
Fig. 5. The concentration of macro elements in leaves, stems and roots of different soil EC-exposed tomato plants at 28 days after treatments (n = 6). Bars with the same letters are not significantly different at *P*< 0.05.

uptake of leaves, stems and roots at above 3.0 dS m^{-1} of EC levels was 58 to 120 % higher than the control (< 3.0 dS m^{-1}). Contents of Ca, Mg and Na were higher than that in the control by 20 to 37 %, 64 to 84 %, and 79 to 212 %, respectively. In addition, macro elements were mostly accumulated in leaves with the exception of stems where possessed K the highest. Mineral absorption by plants is mainly correlated positively with the application rates of fertilizers at the optimal range. Numerous studies revealed that high salinity has suppressed the uptake of mineral elements such as the reduction in the accumulation of N (Al-Rawahy et al., 1992; Feigin et al., 1991; Khan et al., 1990), P (Sharpley

et al., 1992), K (Janzen and Chang, 1987; Sharma, 1996), Ca (Janzen and Chang, 1987; Puntamkar et al., 1988; Rengasamy, 1987) and Mg (Hansen and Munns, 1988; Awada et al., 1995), owing to the competitiveness between ions. Contrary to these previous studies, positive increases in terms of soil ionic compositions with soil EC levels (Table 1) might be suggested that element-excessive soils had little or no effect on limiting the absorption of individual element.

Soil Chemical properties It is clear from Table 1 that increasing EC levels led to a significant increase in concentrations of inorganic N (In-N), NO$_3$-N, Na and B in soils at 14 and 28 days compared to the control. In addition, Ca, Mg and K concentrations were increased in moderate or high EC soils (3.0 ~ 10.0 dS m^{-1}). Only soil EC has increased in moderate or high EC soils (3.0 ~ 10.0 dS m^{-1}) compared to the control. Various EC levels (<3.0 ~ 10.0 dS m^{-1}) led to a slight decrease in organic matter contents in soils at 28 days and this finding might be assumed that salinity stress affected the microbial biomass, owing to the acceleration of OM decomposition (Table 1).

Microbial Biomass As shown in Fig. 6, all EC levels led to a significant increase of microbial biomass carbon (MBC) and nitrogen (MBN) in soils at 14 and 28 days compared to the control with the exception of MBC in moderate salt stress (3.0 ~ 6.0 dS m^{-1}). In particular, MBC under various EC leveled soils increased by an average of 62.1% and 36% at 14 and 28 days, respectively, while a 57.1% and 180.4% increase in MBN was observed at 14 and 28 days, respectively, compared to the control (Fig. 6). Our findings prove that salinity stress led to higher microbial activity in soils compared to the control, followed by a decomposition of OM in soils as indicated by the changes in soil chemical properties in Table (1).

Take together, response to salinity stress can be determined by plant growth and its physiological mechanisms of salt tolerance (Vasques et al., 2006). For example, plant can tolerate salt stress by exclusion and accumulation of Na$^+$ or K$^+$ (Bell and O'Leary, 2003; Peng et al., 2004; Vasques et al., 2006). It is clear from our findings (Fig. 5) that K$^+$ is the main cation leading to osmotic adjustment in leaves, stems and roots of different soil EC-exposed tomato plants at 28 days as reported in studies by Matoh et al. (1988), Lissner et al.
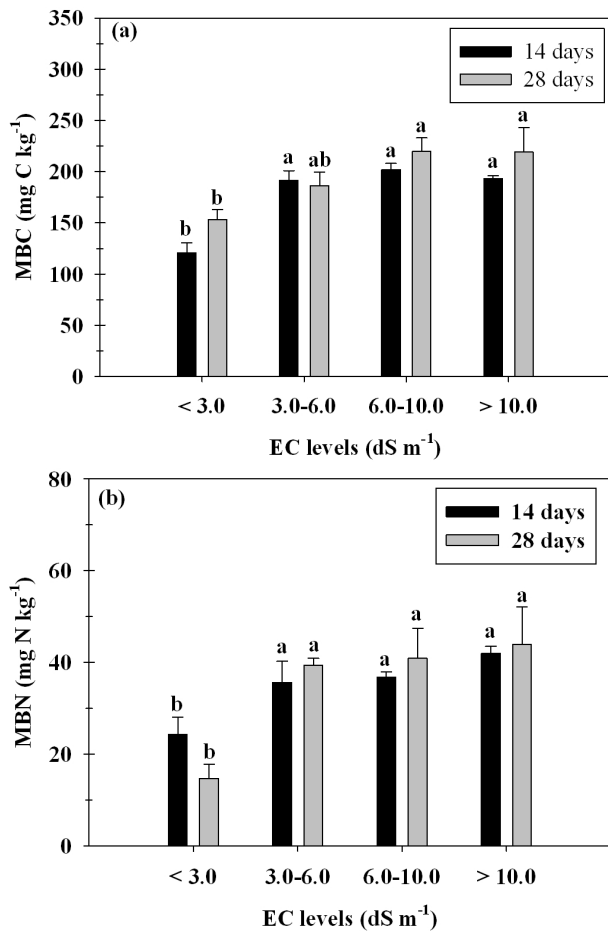
Fig. 6. Temporal changes in microbial biomass carbon (MBC, a) and nitrogen (MBN, b) in different soil EC-exposed tomato plants (n = 3). Bars with the same letters are not significantly different at _P_< 0.05.

(1999) and Vasquez et al. (2005). Saltinity influenced plant metabolism, resulting in decreased crop growth and yield through osmotic inhibition of the available water uptake by roots or the specific ion affects (Abd El-Azeem et al. 2012; Yang et al. 2009).

Acknowledgement

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