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Differential effect of nitrogen forms on physiological parameters and micronutrient concentration in maize (*Zea mays* L.)

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Abstract

Nitrogen is an essential macro nutrient for the plant growth. Plants can absorb nitrogen in cationic (NH_4^+) and anionic (NO_3^-) forms that can influence the physiological parameters, absorption of micronutrients and can cause the nutritional imbalance in plants. This study investigated the effects of urea, ammonium nitrate, ammonium sulphate and calcium nitrate on growth, physiological parameters and absorption of micronutrients by maize. Nitrogen forms significantly affected shoot and root dry weights, leaf stomatal conductance, transpiration rate, photosynthetic rate and concentrations of zinc, manganese and copper in maize shoots and roots. Urea fed plants had maximum shoot dry weight (56.76 g pot^{-1}) and root dry weight (5.50 g pot^{-1}) while nitrate fed plants had minimum shoot (51.74 g pot^{-1})and root dry weights (4.42 g pot^{-1}). Ammonium fed plants had significantly ($P < 0.05$) higher stomatal conductance (0.21 mmol m^{-2}s^{-1}), transpiration rate (5.58 mmol m^{-2}s^{-1}) and photosynthetic rate (20.95 mg CO_2 m^{-2}s^{-1}) compared to the plants fed with other forms of nitrogen. Ammonium fed plants had significantly ($P < 0.05$) higher concentrations of zinc (53.79 mg kg^{-1}), manganese (51.72 mg kg^{-1}) and copper (7.19 mg kg^{-1}) in shoots and similar trend was observed in roots. It was concluded that urea fed plants produced maximum shoot and root dry matter and ammonium fed plants contained maximum concentration of micronutrients in shoots and roots.

Keywords: ammonium; micronutrient; nitrate; stomatal conductance; transpiration rate; maize.

Introduction

Nitrogen is an essential macro nutrient for the plant growth. Plants can absorb nitrogen (N) in cationic (NH_4^+) and anionic (NO_3^-) forms which can influence the pH changes in rooting medium (Imas et al., 1997; Savvas et al., 2006). Nitrogen forms can influence the growth and yield of plants grown in soil based and non-soil growth media (Forde and Clarkson, 1999). Preferred absorption of ammonium (NH_4^+) by plants is electrochemically neutralized by release of protons (H^+) resulting in lowering of pH in rooting zone (Bolan et al., 1991). Contrarily, preferred absorption of nitrate (NO_3^-) by the plants triggered the release of equivalent influx of H^+ or extrusion of anions (OH^-) resulting in increasing the pH of root zone (Barber, 1984; Savvas et al., 2006). Changes in the pH of rooting zone could greatly influence the uptake of micronutrients and thus causing the nutritional imbalance in plants (Savvas et al., 2006). Several researchers have investigated differential effect of NO_3^- and NH_4^+ on plant growth and yield but comparison of urea with these two forms is not very common in literature (Chaillou and Lamaze, 1997; Britto and Kronzucker, 2002). Some researchers have reported that urea is less efficient than other forms of N for plant growth and yield (Gerendas and Sattelmacher, 1997; Tan et al., 2000). It has also been observed that different N forms influence the transpiration rate of alfalfa and stomatal conductance of white clover (Høgh-Jensen and Schjoerring, 1997; Lugert et al., 2001). Nitrogen forms supplied to the plants greatly influenced absorption of cations and anions by altering the root zone pH (Marschner, 2003; Kosegarten et al., 1997). Effect of N forms absorbed by the plants on the root zone pH and acquisition of nutrients from the soil is important particularly in relation to absorption of micronutrients (Marschner, 2003). Ammonium is assimilated by the roots near to the absorption sites to avoid its accumulation in cell vacuoles (Epstein and Bloom, 2005). However, NO_3^- can be stored by plants in their tissues without any toxic effects and thus translocate to the shoots (Goyal and Huffaker, 1984; Andrews, 1986). Forms of N supplied to the plants greatly influence chemical composition of plants and thus provides excellent tool to control relative uptake of different ions (Sonneveld, 2002; Kotsiras et al., 2002). Plants supplied with NH_4^+ absorbed more metals from rooting medium compared to those plants fed with NO_3^- (Smoleń and Sady, 2009). Keeping in view the role of different N forms in plant growth, absorption of nutrients and physiological parameters, this study was planned to investigate effect of N forms *viz.* urea, NH_4^+ and

Table 1. Physico-chemical properties of experimental soil.

Soil Parameter	Unit	Value
Textural Class		Loam
pH_s		7.65
EC_e	$dS\ m^{-1}$	1.75
SAR	$(mmol\ L^{-1})^{1/2}$	1.69
Saturation percentage	%	28.40
Organic Matter	%	0.65

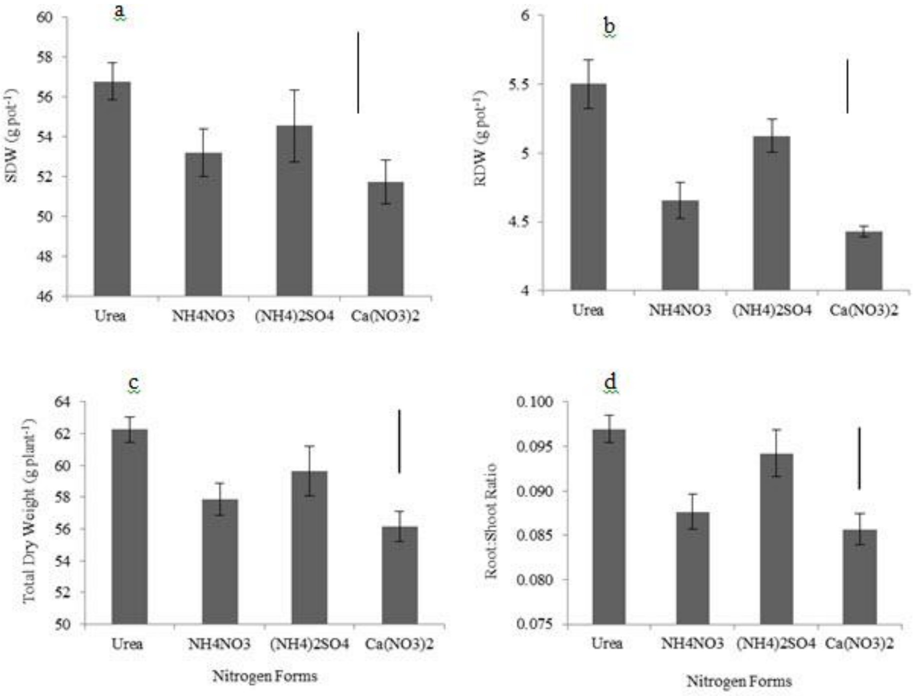

Fig 1. Shoot dry weight (a) root dry weight (b), total dry weight (c) and root shoot ratio (d) of maize grown with different forms of N, LSD at P < 0.05 is represented by line bar in each figure.

NO_3^- on growth, physiological parameters and micronutrient concentration in maize plants.

Results

Soil pH

Nitrogen forms significantly ($P < 0.05$) affected the soil pH in post-experiment soil (Fig. 2d). The maximum soil pH was recorded in the pots where NO_3^- was applied as N source while minimum soil pH was record where only NH_4^+ was applied.

Shoot and root dry weights

Nitrogen forms significantly ($P < 0.05$) affected shoot dry weight (SDW) of maize (Fig. 1a). Maximum SDW (56.76 g pot^{-1}) was recorded with the application of urea while minimum SDW (51.74 g pot^{-1}) was produced by maize with the application of NO_3-N{$Ca(NO_3)_2$}. The SDW values with urea, combination of NH_4^+ and NO_3^- and with sole NH_4^+ form of N {$(NH_4)_2SO_4$) were statistically at par with each other. Only SDW with urea was significantly higher (10 %) than SDW with NO_3^- only. Similarly, root dry weight (RDW) was significantly ($P < 0.001$) affected by different N forms applied (Fig 1b). Maize produced maximum RDW (5.50 g pot^{-1}) with the application of urea and minimum RDW (4.42 g pot^{-1}) with the application of NO_3^-. Root dry weight was statistically similar between the plants fed with urea and NH_4^+. Distribution of dry matter between maize shoots and roots were significantly ($P < 0.05$) affected by N forms supplied to the plants (Fig 1d). Dry matter was preferably distributed towards roots in the plants fed with urea and NH_4^+ compared with those plants fed with both NH_4^+-NO_3^- and NO_3^-.

Physiological parameters

Stomatal conductance

Stomatal conductance is significantly ($P < 0.05$) affected by N forms supplied to the plants (Fig. 2a). Stomatal conductance was maximum (0.21 mmol $m^{-2}s^{-1}$) in the plants fed with NH_4^+ and minimum (0.13 mmol $m^{-2}s^{-1}$) with the plants fed with NO_3^-. Stomatal conductance was statistically similar in the plants fed with urea and both forms of N (NH_4^+-NO_3^-). The differences in stomatal conductance of the plants fed with NO_3^- and both forms of N (NH_4^+-NO_3^-) were also statistically similar. However, stomatal conductance of the plants fed with only NH_4^+ was statistically higher than the plants fed with all other forms of N.

Transpiration rate

Transpiration rate was significantly ($P < 0.05$) affected by N forms supplied to the maize plants (Fig 2b). The plants fed with NH_4^+ had maximum transpiration rate (5.58 mmol $m^{-2}s^{-1}$) while those fed with both form of N (NH_4^+-NO_3^-) had minimum transpiration rate (3.04 mmol $m^{-2}s^{-1}$). With the exception of plants supplied with NH_4^+ only, all other plants had statistically similar transpiration rates.

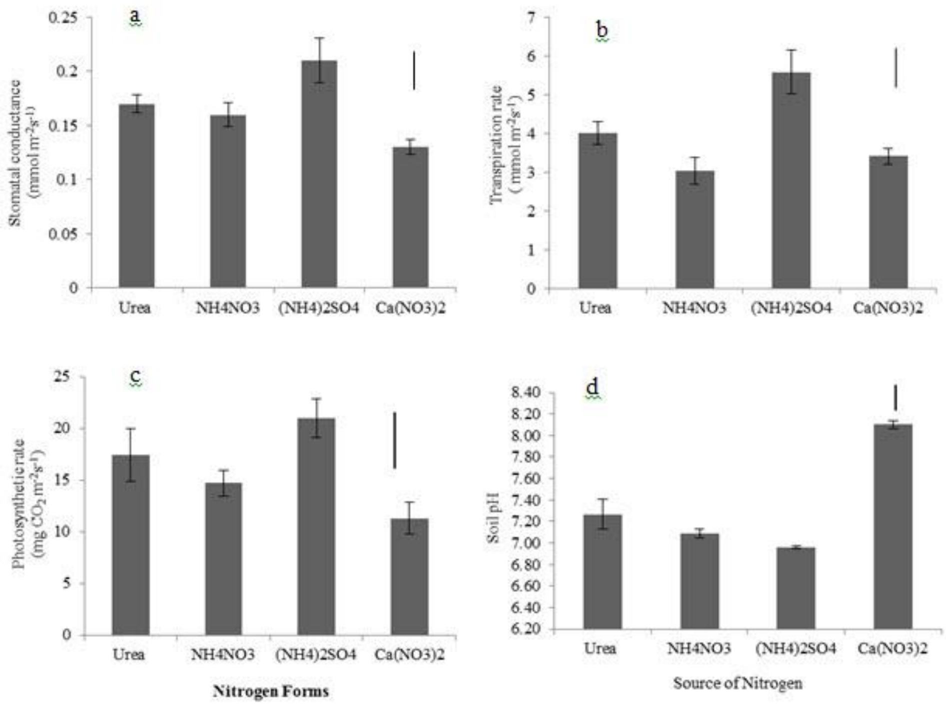

Fig 2. Stomatal conductance (a), Transpiration rate (b) and Photosynthetic rate (c) of maize plants grown with different forms of N and soil pH (d), LSD at $P < 0.05$ is represented by line bar in each figure.

Photosynthetic rate

Nitrogen forms supplied to the maize plants significantly ($P < 0.05$) affected photosynthetic rate (Fig. 2c). Photosynthetic rate was maximum (20.95 mg CO_2 $m^{-2}s^{-1}$) in the plants fed with NH_4^+, followed by the plants supplied with urea (17.45 mg CO_2 $m^{-2}s^{-1}$) and both N forms (NH_4^+-NO_3^-) (14.67 mg CO_2 $m^{-2}s^{-1}$) and it was minimum (11.30 mg CO_2 $m^{-2}s^{-1}$) in plants grown with NO_3^-. Photosynthetic rate was statistically different in the plants fed with NO_3^- and NH_4^+ only while the plants fed with urea had statistically similar photosynthetic rate with those plants fed with both forms of N (NH_4^+-NO_3^-) and NH_4^+. Nitrate and NH_4^+-NO_3^- fed plants had statistically similar photosynthetic rate.

Ionic composition

Zinc (Zn)

Nitrogen forms significantly ($P < 0.05$) affected Zn concentration in maize shoot (Fig. 3a). Plants supplied with NH_4^+ had maximum Zn concentration (53.79 mg kg^{-1}) and NO_3^- fed plants contained minimum Zn concentration (45.71 mg kg^{-1}) in shoots. Zinc in shoots of plants fed with urea, NH_4^+-NO_3^- and NO_3^- were statistically at par with each other. . Zinc concentration in roots of maize was significantly ($P < 0.001$) affected by N forms supplied to the plants (Fig. 3b). Zinc concentration in roots of maize plants fed with NH_4^+ was maximum (44.32 mg kg^{-1}) followed by the plants fed with NH_4^+-NO_3^-.,Urea fed plants had minimum Zn concentration (30.94 mg kg^{-1}) which was statistically similar to Zn concentration (35.73 mg kg^{-1}) in root of NO_3^- fed plants. Total Zn uptake by maize plants was significantly ($P < 0.05$) affected by N forms supplied to the plants and its distribution between roots and shoots was also significantly ($P < 0.05$) affected (Fig. 3c & d). Zinc was preferably distributed towards roots of plants fed with both forms of N (NH_4^+-NO_3^-) and NH_4^+ compared to the roots of plants fed with urea and NO_3^-.

Manganese (Mn)

Nitrogen forms significantly ($P < 0.001$) affected Mn concentration in maize shoots but non-significantly in roots (Fig. 4a). Maximum Mn concentration (51.72 mg kg^{-1}) was recorded in the shoots of maize plants fed with NH_4^+ followed by NO_3^- fed plants but concentration in shoots of these plants was statistically at par with each other. However, concentrations in the shoots of these plants were statistically higher than plants fed with urea and NH_4^+-NO_3^-. Manganese concentration was minimum (38.93 mg kg^{-1}) in the shoots of plants supplied with NH_4^+-NO_3^-. Although, differences in Mn concentration in roots of plants were not significant but the plants supplied with NH_4^+ had higher Mn (60.54 mg kg^{-1}) in roots compared to other plants. Total Mn uptake by plants and distribution between roots and shoots was affected significantly ($P < 0.05$) by N forms supplied to the plants (Fig. 4c & d). Plants fed with NH_4^+ had maximum Mn uptake (3.12 µg $plant^{-1}$) and those plants fed with both forms of N (NH_4^+-NO_3^-) had minimum Mn uptake (2.32 µg $plant^{-1}$). Distribution of Mn towards roots was maximum in the plants fed with both forms of N (NH_4^+-NO_3^-) and it was minimum in the roots of plants supplied with NO_3^-.

Copper (Cu)

Nitrogen forms supplied to the maize plants significantly ($P < 0.05$) affected Cu concentration in maize shoots (Fig. 5a). Maximum Cu concentration (7.19 mg kg^{-1}) was recorded in shoots of maize fed with NH_4^+ and it was minimum (4.66 mg kg^{-1}) in the shoots of plants fed with both forms of N (NH_4^+-NO_3^-). Shoots of NH_4^+ and NO_3^- fed plants had statistically similar Cu concentration and NO_3^- fed plants had statistically similar Cu concentration with that of urea fed plants.

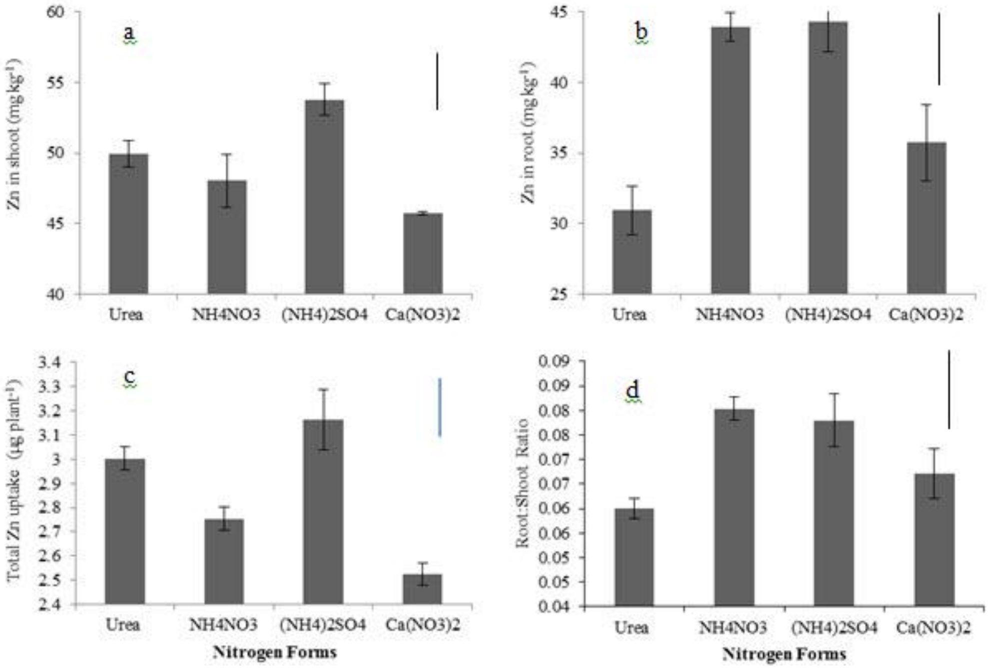

Fig 3. Concentration of Zn in maize shoots (a) and roots (b), Total Zn uptake (d) and root:shoot ratio of Zn (d) in maize grown with difference forms of N, LSD at $P < 0.05$ is represented by line bar in each figure.

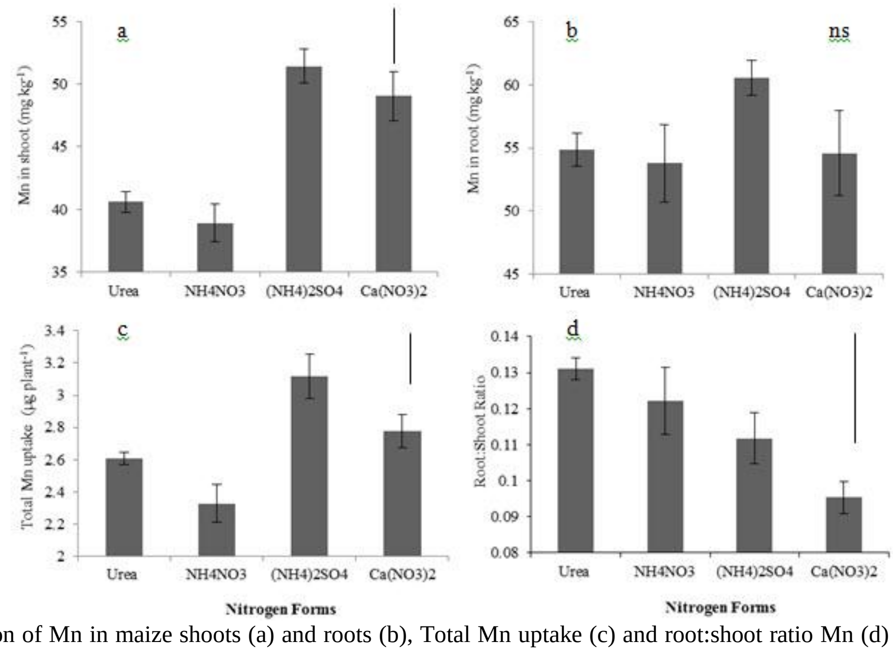

Fig 4. Concentration of Mn in maize shoots (a) and roots (b), Total Mn uptake (c) and root:shoot ratio Mn (d) in maize grown with difference forms of N, LSD at $P < 0.05$ is represented by line bar in each figure.

Contrary to the Cu concentration in shoots, Cu in roots of maize was non-significantly affected by N forms. Ammonium supplied plants contained the maximum (15.13 mg kg^{-1}) and those supplied with NH_4^+-NO_3^- had the minimum Cu (12 mg kg^{-1}) in the roots. Total Cu uptake by maize plants was significantly ($P < 0.05$) affected by N forms supplied to the plants, however, N forms did not have significant effect on distribution of Cu between roots and shoots (Fig. 5 c & d)

Discussion

Results of the present study showed the superiority of NH_4^+ form of N in dry matter production as compared to the other sources, more particularly the NO_3^- form. Similar results were also reported by Kraus and Staurt (2002) who concluded that NH_4^+ fed cotoneaster plants had higher dry matter compared to other plants fed with NO_3^- alone or combination of NO_3^- and NH_4^+. Similarly, Ruan et al. (2007) recorded increase in dry weights of tea plants with the application of NH_4^+ compared with other forms of N. This could be due to decreased absorption of NO_3^--N compared with other forms of N, and inefficient assimilation owing to low nitrate reductase activity (Lavoie et al., 1992; Poonnachit and Darnell, 2004). However, in contradiction to these results, Zhou et al. (2011) recorded higher dry matter production of cucumber plant fed with NO_3^--N compared with that of NH_4^+ fed plants but non-significant differences for dry matter

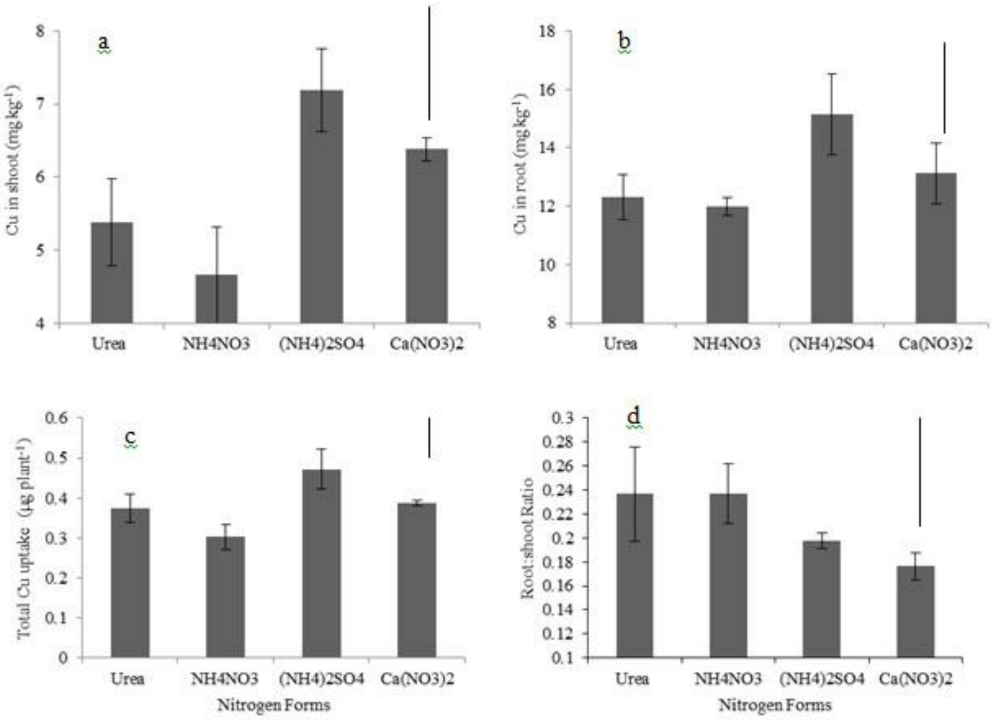

Fig 5. Concentration of Cu in maize shoots (a) and roots (b), Total Cu uptake (c) and root:shoot ratio of Cu (d) in maize grown with difference forms of N, LSD at $P < 0.05$ is represented by line bar in each figure.

of rice due to different forms of N. Stomatal conductance is higher in the plants fed with NH_4^+ than those fed with other forms of N which was in agreement with the results reported by Zhou et al. (2011) who recorded higher stomatal conductance in rice plants fed with NH_4^+ compared with other forms of N. The NH_4^+ fed plants had maximum transpiration rate while those fed with both form of N (NH_4^+-NO_3^-) had minimum transpiration rate. These results are in agreement with the results reported by Khan et al. (1994) and Lugert et al. (2001) who recorded increase in transpiration of alfalfa and tomato, respectively due to the application of NH_4^+. Nitrogen is an important pre-requisite for photosynthesis which determines plant productivity (Li, 2007). The NH_4^+ -fed plants had maximum photosynthesis compared with those fed with other forms of N and these results are in agreement with the reports of other workers (Guo et al., 2008; Xin et al., 2011). It has been reported that N forms remarkably affected photosynthesis by influencing chlorophyll contents, photosynthetic rate, activity of Rubisco and the photorespiration (Xin et al., 2011). The different effect of N forms on photosynthesis is associated with stomatal conductance (Guo et al., 2008). Generally, it had been shown that the plants fed with NH_4^+ had higher stomatal conductance and transpiration rate (Hogh-Jensen and Schjoerring, 1997; Guo et al., 2002). These findings indicate that ammonium causes a higher RuBP regeneration rate than nitrate (Raab and Terry, 1994). Form of N supplied to the plants greatly influenced the absorption of other ions particularly micronutrients like Fe, Cu, Mn and Zn, in consonance with reports of other workers (Kotsiras et al., 2002; Borgognone et al., 2012). In the present study, plants fed with NH_4^+ had higher concentration of micronutrients compared to the plants fed with other forms of N. Ammonium supply to the plants resulted in acidification of rhizosphere and enhanced acquisition by plants (Thomson et al., 1993). These results were in agreement with the results reported by (Bar-Tal et al., 2001) for pepper and by Borgognone et al., 2012 for tomato plants.

Material and methods

Plant material

Seeds of maize were collected from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Healthy seeds of uniform size were treated with fungicide for protection of the seedlings against disease. The treated seeds were soaked in moist cloth to enhance germination. The germinating seeds were sown in the soil filled in the glazed pots.

Collection and preparation of soil

The experiment was conducted in rain protected wire house at the Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan. Bulk soil sample was collected from Farm area. Soil sample was analysed for determination of soil physico-chemical properties by using methods described in United States Salinity Laboratory Staff Hand Book 60 (Richards, 1954). Different soil physical and chemical properties are given in Table 1. Soil was air dried ground to pass through 2 mm sieve. Soil was filled into pots at 9 kg per pot and N was applied to the pots at rate of 6 mM per pot using urea, ammonium nitrate (NH_4NO_3), ammonium sulphate {$(NH_4)_2SO_4$}and calcium nitrate {$Ca(NO_3)_2$}. Nitrogen was applied in splits with half N at sowing and remaining N in two equal splits. The four treatments were arranged in Completely Randomized Design (CRD) with four replications. Phosphorus and potassium was applied to the pots through single super phosphate and sulphate of potash, respectively at the time of sowing. The plants were irrigated throughout growth period with canal water. Infra Red Gas Analyzer (IRGA) was used to measure transpiration rate, stomatal conductance and photosynthetic rate before harvesting of 75 days old plants. Hydrometer method (Bouyoucos, 1962) was used for particle size analysis of soil. A pH meter (model 671P) was used in determination of soil pH. Instrument was first calibrated with standard buffer solutions of pH 4.00 and 9.00. The pH was measured by placing electrode of pH meter in the paste. Soil saturated paste was extracted by applying a positive pressure with the help of air pump. Extract was preserved in air tight plastic bottles for further analysis. Electrical conductivity of saturated soil extract (EC_e) was measured by using a conductivity meter by calibrating it 0.01 *N* KCl solution. Soil organic matter (OM) was determined with following Walkly-Black method (Nelson and Sommers, 1982). After harvesting, plants were washed with tap water followed by diluted HCl and distilled water to remove aerial deposition. Washed plant samples were oven dried at 70°C till the constant weight and ground. A 0.5 gram of ground shoot and root samples were digested with diacid (HNO_3:$HClO_4$) with 3:1 ratio. After digestion, samples were cooled and volume of digests was made 25 ml with distilled water and stored in air tight bottles for the determination of Fe, Cu, Mn and Zn with Atomic Absorption Spectrophotometer (Solar S-series, Thermoelectron) after its calibration with respective standard solutions.

Data Quality

The data quality control and assurance was achieved by including blanks with every batch of the samples. Three replications of all the samples and reagent blanks were used to ensure accuracy and precision in the analysis. The working standard solutions of each metal were prepared using standard stock solutions certified by the supplier. Five working standard solutions and blanks were used to calibrate the AAS. Each metal was determined using AAS by optimizing instrumental conditions for maximum sensitivity as directed in manufacture's manual.

Statistical analysis

The data obtained were subjected to statistical analysis using software statistix 8.1 and means were separated from each other following LSD test.

Conclusion

Maize plants responded differentially to different of forms of N supplied. Urea fed plants produced maximum shoot and root dry matter. However, NH_4^+ fed plants absorbed higher concentrations of metal micronutrients that could disturb the ionic balance of plants. It could be concluded that NH_4^+ form of N should not be applied to those plants which are grown in metal contaminated soils.

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