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Growth, heavy metal status and yield of salt-stressed wheat (*Triticum aestivum* L.) plants as affected by the integrated application of bio-, organic and inorganic nitrogen-fertilizers

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Summary

Efforts have been made to use the integrated application of bio-, organic and inorganic nitrogen (N)-fertilizers to decrease waste accumulation, and to minimize nutrient losses and yield contamination with heavy metals for human nutrition and health. Therefore, a field experiment was conducted to assess the effect of integrated applications of organic manures, bio-fertilizer and/or mineral-N fertilizers on growth, yield, some chemical constituents and shoot and yielded grain heavy metal contents of wheat (*Triticum aestivum* L. cv. Sakha 93) plants grown under salinity stress (ECe = 7.84 dS m^{-1}). Results showed that, the treatment comprised of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (bio-fertilizer; 4 Kg ha^{-1}) + cattle manure (10 t ha^{-1}) was found to be most effective, producing the best status of growth characteristics, osmoprotectants concentrations, essential nutrient contents, shoot heavy metal concentrations, and grain yield and its content of heavy metals compared to the all other treatments. The treatment comprised of Cerealine (4 Kg ha^{-1}) + cattle manure (20 t ha^{-1}) was occupied the second order. We can recommend to use the integrated treatment of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (bio-fertilizer; 4 Kg ha^{-1}) + cattle manure (10 t ha^{-1}) effectively in saline soils to improve wheat growth and yield with minimum contents of heavy metals for human health and nutrition.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crops used for human nutrition in most countries worldwide. It is a moderately salt-tolerant crop (Maas and Hoffman, 1977), and is often grown on newly-reclaimed saline soils in Egypt. On the other hand, the cultivation of wheat will be restricted or even prevented in such soils if their salinity reached about 10 dS m^{-1} due to the severe reduction in wheat growth and productivity.

Salinity stress is one of the major problems of agriculture in arid and semiarid regions. Salt stress affects plant physiology due to the osmotic and ionic stresses at both the cellular level and whole plant. It generates a physiological drought by affecting the plant water relations (Munns, 2002). The accumulation of toxic salts in the leaf apoplasm leads to dehydration, loss of turgor and the death of cells and tissues. Photosynthesis is one of the most severely affected processes during salinity stress, mediating by decreased levels of chlorophyll (Rady and Mohamed, 2015) and the inhibition of various enzymes, including Rubisco (Soussi et al., 1998). In addition, lipid peroxidation and the antioxidant system of the plant have been reported to be stimulated by salt stress (Rady et al., 2013; Semida and Rady, 2014). All these, and other altered processes lead to poor plant growth and productivity.

Saline soils have low fertility and a poor structure with a high bioavailability of heavy metals (Acosta et al., 2011) to be available for plant roots. Heavy metal bioavailability can be minimized *via* biological immobilization and stabilization methods using a range of organic fertilizers (OFs), such as cattle manure (CM) and poultry manure (PM). The beneficial effects of OFs, which are sourced locally, are an increase soil quality, total C and N content of the soil and soil-waterholding capacity, alteration of soil pH, elevation of soil humus and soil aggregation, and water infiltration properties (Domagala-Swiatkiewicza and Gastol, 2013; Yagioka et al., 2014; Abdou and Mohamed, 2014; Bobul'ska et al., 2015), and a reduction in the bioavailability of heavy metals (Angelova et al., 2013). Although some studies have reported that organic farming tends to reduce plant growth and yield (Jansen et al., 2015), an important one of the OFs, CM application was found to increase the growth and yield of many crops without any undesirable impacts on the environment (Suthar, 2009; Rady, 2011). Poultry manure (PM) has long been recognized as perhaps the most desirable of these OFs because of its high nitrogen content. The application of OFs and other organic amendments to farmland is an economical and environmentally sustainable mechanism for increasing crop production and reducing bioavailability of heavy metals (Younis et al., 2015). Cerealine (Bio-fert) is a commercial product of biofertilizer contains *Azospirillum sp*. For decreasing high doses of chemical fertilizer, the integrative effect of Bio-fert, OFs and chemical fertilizers is a promising tool to increase wheat productivity, get cleaner product with low undesirable high doses of heavy metals and other pollutants (Sushila et al., 2000).

Different strategies are being employed for attaining optimum growth and productivity under saline conditions. One of them is the proper management of nitrogen (N) fertilizer (Rady, 2012). To alleviate deleterious salinity effects such as a reduction in growth and yield, and an increase of bioavailability and plant absorption of heavy metals, different N-forms are being used. In view of these reports, it was postulated that the integrated application of bio-, organic- and mineral-N fertilizer could alleviate the adverse effects of salt stress on the growth and productivity of wheat. Thus, the major objective of this study was to examine the extent to which N-forms could ameliorate the effects of salt stress on growth, ion accumulation, heavy metal uptake, and yield of wheat crop.

Materials and methods

Plant material, treatments and growth conditions

A field experiment was conducted at the Experimental Farm of the Faculty of Agriculture, Fayoum University, Egypt. The main characteristics of the experimental soil, organic manures and irrigation water (Southeast Fayoum; 29° 17´N; 30° 53´E) used in this study were determined according to Jackson (1973) and Wilde et al. (1985) and are shown in Tab. 1.

Healthy seeds of wheat (*Triticum aestivum* L. cv. Sakha 93) were obtained from The Crop Research Institute, The Agricultural Research Center, Giza, Egypt, and were sown on 15 November 2009 and final harvest was done on 20 May 2010. Seeds were washed with distilled

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Tab. 1: Physical and chemical properties of experimental soil, organic manures and irrigation water.

Soil analysis		Organic manure analysis		
Property	Values	Property	Cattle	Poultry
Sand%	76.8	$1m^3$ wt. (kg)	738	498
Silt%	18.1	Moisture%	70.0	66.7
Clay%	5.1	pH	7.50	6.34
Texture	Sandy loam	EC (dSm^{-1})	5.76	5.21
*pH	7.68	*O.C.%	34.0	43.6
EC ($dS\ m^{-1}$)	7.84	*O.M.%	47.6	69.8
Total N%	0.63	C/N ratio	23:1	22:1
Total P%	0.36	Total N%	1.47	1.99
Total K%	1.25	Total P%	0.50	0.78
$CaCO_3$%	11.1	Total K%	1.32	1.52
Fe (ppm)	16.9	CaCO3%	2.86	3.26
Mn (ppm)	8.4	Fe (ppm)	946	1928
Zn (ppm)	7.2	Mn (ppm)	429	876
Cu (ppm)	6.4	Zn (ppm)	146	230
Pb (ppm)	12.8	Cu (ppm)	68.2	98.2
Cd (ppm)	13.6	Pb (ppm)	12.2	31.2
Ni (ppm)	11.9	Cd (ppm)	5.91	18.0
Cr (ppm)	9.04	Ni (ppm)	7.13	16.9
Se (ppm)	6.17	Cr (ppm)	5.81	17.3
Co (ppm)	4.96	Se (ppm)	4.95	13.0
		Co (ppm)	5.01	11.9

*pH (1:1 suspension), O.C.% = Organic carbon%, O.M.% = Organic matter%.

water, sterilized in 1% (v/v) sodium hypochlorite for approx. 2 min, washed thoroughly again with distilled water, and left to dry at room temperature (25 ºC) for approx. 1 h before sowing. Fourteen treatments in a randomized complete block design were used with three replications, generating 42 plots. The area of each plot was 10.5 m^2 (3 m × 3.5 m). The distance between plots was 1 m from all sides to avoid treatments overlapping. The treatments imposed in the present investigation were detailed in Tab. 2.

The experiment was designed to examine the influence of N-form (organic, bio- and/or inorganic) fertilizer on growth, heavy metal uptake, nutrients concentration and yield of wheat plants grown at salinity with EC = 7.84 dS m^{-1}. PM and CM were added to soil before sowing, during soil preparation. They spread on the soil surface, and then mixed into the soil-surface layer. Ammonium nitrate and urea quantities according to treatments were added before sowing. A quantity of 360 kg ha^{-1} of single super phosphate (15.5% P_2O_5) was added before sowing as recommended dose. Cerealine is a commercial product of biofertilizer contains *Azospirillum* sp. produced by General Organization of Agriculture, Egypt, and was added before sowing at rate of 4 Kg ha^{-1}. All other agricultural practices for wheat production were conducted according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation.

Plant sampling, and growth and yield measurements

At fourteen weeks after sowing, plants were sampled to study the growth characteristics, and to perform the chemical analyses in leaves or shoots. At harvest, yield parameters were estimated. The following growth and yield parameters were measured: Plant height (cm), number (No.) of leaves $plant^{-1}$, leaf area $plant^{-1}$ (cm^2), No. of tillers $plant^{-1}$, shoot dry weight (DW) $plant^{-1}$ (g), 1000-grain weight (g) and grain yield ha^{-1} (ton).

Chemical analyses

At fourteen weeks after sowing, the fifth leaf or shoot of four selected plants were collected from each experimental unit for chemical determinations. The concentrations of chlorophylls and carote-

Tab. 2: Components of the 14 treatments used in the present experiment

Treatment	Nitrogen (N) source	Amount of N source ha^{-1}	Amount of N ha^{-1}
T_1 (Control)*	Not found	Not found	Not found
T_2 (Recom.)**	Ammonium nitrate (NH_4NO_3)	475 kg (33.5% N)	160 kg
T_3 (Recom.)**	Urea (NH_2-CO-NH_2)	350 kg (46% N)	160 kg
T_4	Poultry manure (PM_1)	10 t	199 kg
T_5	Cattle manure (CM_1)	20 t	294 kg
T_6	Bio-N (Cerealine)	4 Kg	………
T_7	½NH_4NO_3 + PM_2	237.5 kg (33.5% N) + 5 t	80 kg + 99.5 kg***
T_8	½NH_4NO_3 + CM_2	237.5 kg (33.5% N) + 10 t	80 kg + 147 kg***
T_9	½NH_4NO_3 + Cerealine	237.5 kg (33.5% N) + 4 kg	80 kg + ………
T_{10}	½NH_2-CO-NH_2 + PM_2	175 kg (46% N) + 5 t	80 kg + 99.5 kg
T_{11}	½NH_2-CO-NH_2 + CM_2	175 kg (46% N) + 10 t	80 kg + 147 kg
T_{12}	½NH_2-CO-NH_2 + Cerealine	175 kg (46% N) + 4 kg	80 kg + ……..
T_{13}	Cerealine + CM_1	4 kg + 20 t	……. + 294 kg
T_{14}	⅓NH_4NO_3 + Cerealine + CM_2	158.3 kg + 4 kg + 10 t	55 kg + …... + 147 kg

* The control treatment was not received any N-fertilizer.
** The recommended dose of N for wheat from either ammonium nitrate or urea as the Egyptian Ministry of Agriculture and Land Reclamation.
*** Calculated from the Tab. 1 (Analyses of cattle and poultry manures).

noids of the fresh upper fifth leaf were extracted by acetone 80%, and were then measured colorimetrically using the method of ARNON (1949). The concentration of free proline was determined colorimetrically by using acid ninhydrin reagent with the sulfosalicylic acid (3%)-extract as outlined by BATES et al. (1973). The concentration of total free amino acids was determined according to MUTING and KAISER (1963). The concentration of total soluble sugars was determined in ethanolic extract with freshly-prepared anthrone reagent [150 mg anthrone + 100 ml of 72% (v/v) sulphuric acid] according to IRIGOYEN et al. (1992). The contents of nutrients were determined in digested samples according to JACKSON (1973). The N content was measured using the semi-micro Kjeldahl method (BREMNER, 1965). The P content was determined colorimetrically by the molybdenum blue method (CHAPMAN and PRATT, 1961). The contents of K and Na were assessed using flame photometry (JACKSON, 1973). The Ca content, and the all tested heavy metals (Pb, Cd, Ni, Cr, Se, Co, Zn and Cu) contents were determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (CHAPMAN and PRATT, 1961).

Statistical analysis
The data were subjected to the analysis of variance (ANOVA) appropriate to the randomized complete block design according to the procedure outlined by GOMEZ and GOMEZ (1984). The significant differences among treatments were compared using the Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Results

Influences of N-fertilizer form on plant height, No. of leaves plant^{-1}, leaf area plant^{-1}, No. of tillers plant^{-1}, shoot DW plant^{-1} of wheat grown under saline soil conditions are shown in Tab. 3. The treatment comprised of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (4 Kg ha^{-1}) + cattle manure (CM_2; 10 t ha^{-1}) was found to be most effective, producing the highest values of the all mentioned growth characteristics compared to the all other treatments. The treatment comprised of Cerealine (4 Kg ha^{-1}) + cattle manure (CM_1; 20 t ha^{-1}) was occupied the second order. The treatment of the recommended mineral-N dose of ammonium nitrate or urea was in a parallel line with each of the treatments of ½Urea (80 kg N ha^{-1}) + PM_2 (5 t ha^{-1}), ½Urea (80 kg N ha^{-1}) + CM_2 (10 t ha^{-1}) and ½Urea (80 kg N ha^{-1}) + Cerealine (4 Kg ha^{-1}) in the third order compared to the other treatments, including the control.

Fig. 1 shows the influences of N-fertilizer forms on the concentrations of total chlorophyll, total carotenoids, free proline, total soluble sugars and free amino acids in wheat leaves under saline soil conditions. The treatments comprised of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (4 Kg ha^{-1}) + cattle manure (CM_2; 10 t ha^{-1}) and Cerealine (4 Kg ha^{-1}) + cattle manure (CM_1; 20 t ha^{-1}) were found to be most effective, producing the highest values of the all aforementioned parameters, occupying the first and the second orders, respectively compared to the all other treatments.

Tab. 4 shows the effects of N-fertilizer forms on nutrient contents (nitrogen; N, phosphorus; P, potassium; K, calcium; Ca and sodium; Na), and K/Na and Ca/Na ratios in shoots of wheat plants grown under saline soil conditions. The treatment comprised of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (4 Kg ha^{-1}) + cattle manure (CM_2; 10 t ha^{-1}) was found to be most effective, producing the highest values of the contents of N, P, K and Ca and the ratios of K/Na and Ca/Na, and the lowest values of Na contents compared to the all other treatments. The treatments comprised of Cerealine (4 Kg ha^{-1}) + cattle manure (CM_1; 20 t ha^{-1}) and ½NH_4NO_3 (80 kg N ha^{-1}) + cattle manure (CM_2; 10 t ha^{-1}) were occupied the second and third orders, respectively compared to the other treatments, including the control.

Tab. 3: Effect of N-fertilizer form applications on plant height, number (No.) of leaves plant^{-1}, leaf area plant^{-1}, and shoot dry weight (DW) plant^{-1} of wheat grown under saline soil conditions

Treatments	Plant height (cm)	No. of leaves plant^{-1}	Leaf area plant^{-1} (cm^2)	Shoot DW plant^{-1} (g)
T_1	58.4e*	5.11e	20.1g	6.1e
T_2	78.4c	6.89c	37.8cde	11.5c
T_3	78.4c	6.79c	36.7cde	11.3c
T_4	76.9cd	6.68c	35.3de	11.1c
T_5	77.1cd	6.61c	34.9e	11.0c
T_6	70.7d	5.98d	30.3f	9.9d
T_7	80.1c	6.95c	37.9cd	11.6c
T_8	78.4c	6.78c	36.9cde	11.3c
T_9	81.6bc	7.09bc	38.8c	11.8bc
T_{10}	77.8c	6.66c	35.5de	11.1c
T_{11}	80.1c	6.73c	35.7de	11.2c
T_{12}	80.0c	6.96c	37.8cde	11.6c
T_{13}	87.8b	7.63b	41.8b	12.7b
T_{14}	96.3a	8.37a	46.6a	14.0a

*Values are means (n = 9), and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.
T_1: Control (without any N-fertilizer); **T_2**: Recommended N as ammonium nitrate (160 kg N = 476 kg NH_4NO_3 ha^{-1} with 33.5% N); **T_3**: Recommended N as Urea (160 kg N = 348 kg ha^{-1} urea [$(NH_2)_2CO$] with 46% N); **T_4**: Poultry manure (PM_1 = 10 t ha^{-1}); **T_5**: Cattle manure (CM_1 = 20 t ha^{-1}); **T_6**: Bio-N (Cerealine at rate of 4 Kg ha^{-1}); **T_7**: ½NH_4NO_3+PM_2 (PM_2 = 5 t ha^{-1}); **T_8**: ½NH_4NO_3+CM_2 (CM_2 = 10 t ha^{-1}); **T_9**: ½NH_4NO_3+Cerealine; **T_{10}**: ½Urea+PM_2; **T_{11}**: ½Urea+CM_2; **T_{12}**: ½Urea+Cerealine; **T_{13}**: Cerealine+CM_1; **T_{14}**: ⅓NH_4NO_3+Cerealine+CM_2.

The effects of N-fertilizer forms on the concentrations of heavy metals (lead; Pb, cadmium; Cd, nickel; Ni, chromium; Cr, selenium; Se, cobalt; Co, zinc; Zn and copper; Cu) in shoots and yielded grains of wheat plants grown under saline soil conditions are shown in Tab. 5 and 7. The treatments comprised of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (4 Kg ha^{-1}) + cattle manure (CM_2; 10 t ha^{-1}) and Cerealine (4 Kg ha^{-1}) + cattle manure (CM_1; 20 t ha^{-1}) were found to be most effective, producing the lowest values of the concentrations of all heavy metals, except some fluctuations compared to the all other treatments. On the other hand, the treatment of ½Urea (80 kg N ha^{-1}) + poultry manure (PM_2; 5 t ha^{-1}) was found to generate the highest concentration values of all heavy metals, followed by all treatments with urea either solely or combined with poultry manure, or cattle manure, and even with Cerealine.

N-fertilizer form effects on the yield parameters (1000-grain weight and grain yield ha^{-1}) of wheat plants grown under saline soil conditions are shown in Tab. 6. The treatments comprised of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (4 Kg ha^{-1}) + cattle manure (CM_2; 10 t ha^{-1}) and Cerealine (4 Kg ha^{-1}) + cattle manure (CM_1; 20 t ha^{-1}) were found to be most effective, producing the highest values of the 1000-grain weight and grain yield ha^{-1}, occupying the first and the second orders, respectively compared to the all other treatments.

Discussion

The salinity of soil (EC_e = 7.84 dS m^{-1}; Tab. 1) may affect the plant metal uptake through the toxic effects of the Na^+ and Cl^- ions. The

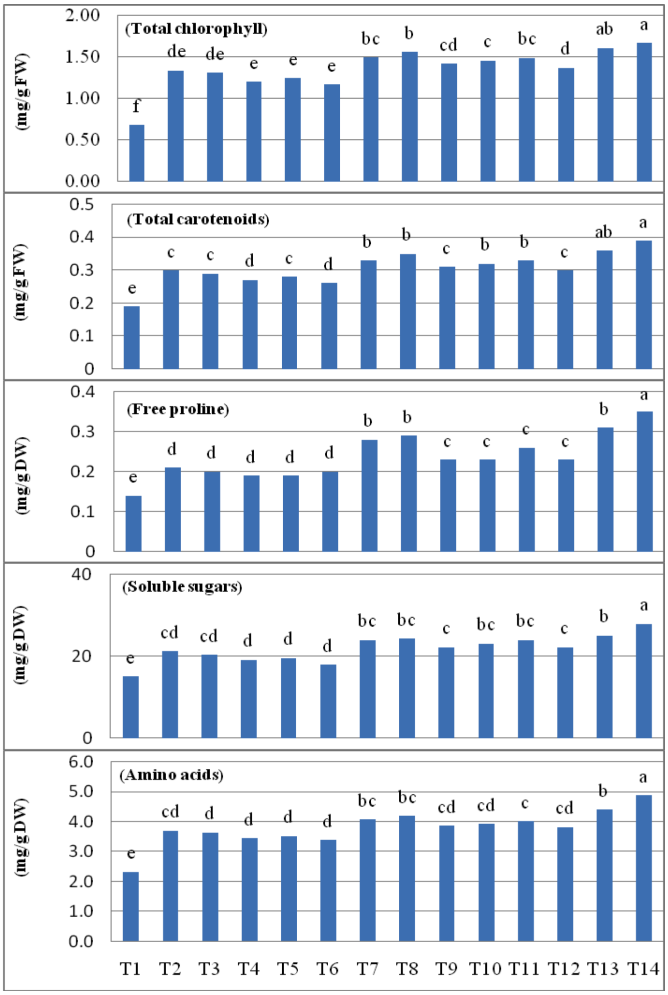

Fig. 1: Effect of N-fertilizer form applications on the leaf concentrations of total chlorophyll, total carotenoids, free proline, total soluble sugars, and amino acids in wheat plants grown under saline soil conditions. *Mean values are presented in form of vertical bars. Bars with a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.
T_1: Control (without any N-fertilizer); **T_2**: Recommended N as ammonium nitrate (160 kg N = 476 kg NH_4NO_3 ha^{-1} with 33.5% N); **T_3**: Recommended N as Urea (160 kg N = 348 kg ha^{-1} urea [$(NH_2)_2CO$] with 46% N); **T_4**: Poultry manure (PM_1 = 10 t ha^{-1}); **T_5**: Cattle manure (CM_1 = 20 t ha^{-1}); **T_6**: Bio-N (Cerealine at rate of 4 Kg ha^{-1}); **T_7**: ½NH_4NO_3+PM_2 (PM_2 = 5 t ha^{-1}); **T_8**: ½NH_4NO_3+CM_2 (CM_2 = 10 t ha^{-1}); **T_9**: ½NH_4NO_3+Cerealine; **T_{10}**: ½Urea+PM_2; **T_{11}**: ½Urea+CM_2; **T_{12}**: ½Urea+Cerealine; **T_{13}**: Cerealine+CM_1; **T_{14}**: ⅓NH_4NO_3+Cerealine+CM_2.

Na^+ ions may release heavy metals from the sediment to the soil solution, thereby increasing the heavy metal accumulation in the plant organs (Tab. 5 and 7). In arid regions including Egypt, soils and irrigation water are characterized by high salinity levels that may aggravate the problem of heavy metal pollutions. In addition, high levels of chloride ions-containing irrigation water might increase soil heavy metal mobilization through the formation of metal-chloride complexes (McLean and Bledsoe, 1992; Ghallab and Usman, 2007). They also reported that the consequences of soil salinity can increase heavy metal solubility and availability, depending on the composition of soil solution and its dominant inorganic ligands. The soil applications of organic manures have been shown to improve soil fertility, and to reduce salt stress and the bioavailability of heavy metals to plant roots (Weggler et al., 2004; Ok et al., 2011). It has been also reported that the application of organic residues to heavy metal-contaminated soils increased soil organic matter content, improved the chemical and biological properties, and decreased the heavy metal bioavailability to plant roots (Ok et al., 2011). Moreover, the application of organic manures to heavy metal-contaminated soil reduced the metal toxicity and consequently increased soil microbial activities (Usman et al., 2013). In the present study, unlike the poultry manure the addition of cattle manure (CM) as a full alternative or a partial alternative to mineral fertilizers [i.e., the treatment comprised of ⅓NH_4NO_3 (55 kg N ha^{-1}) + Cerealine (4 Kg ha^{-1}) + cattle manure (CM_2; 10 t ha^{-1}) and the treatment comprised of Cerealine (4 Kg ha^{-1}) + cattle manure (CM_1; 20 t ha^{-1})] was significantly increased wheat growth characteristics (Tab. 3), nutritional status (Tab. 4), photosynthetic pigments and osmoprotectants (Fig. 1) and grain yield (Tab. 6), and significantly decreased the concentrations of heavy metals in plant shoots and yielded grains (Tab. 5 and 7). The most interesting finding is that these treatments were found to produce higher grain yield than average grain yield productivity of Egypt (6.452 t ha^{-1}) in 2009, compared to 7.85 and 6.66 t ha^{-1} of these two treatments, respectively.

Our results showed that the addition of CM caused a significant increase in shoot mineral nutrients (Tab. 4). This finding may be attributed to the release of nutrients with the decomposition of this organic manure (Wang et al., 2014). Results showed also that soil salinity caused increases in the bioavailability of heavy metals, as indicated by a significant increase in the wheat shoot and grain heavy metal concentrations in the control and the treatments contained inorganic N fertilizers and/or poultry manure (Tab. 5 and 7). This was observed in the treatment comprised of urea (inorganic N) + poultry manure (T_{10}) which resulted in the highest heavy metal concentrations in wheat plants. This increase in plant heavy metal concentration may be due to poultry manure application that has shown to increase the soil concentration of heavy metals (Hanč et al., 2008; Arroyo et al., 2014). In addition, the frequent use of chemical fertilizers such as urea and pesticides in common agricultural fields can also cause an increase in the concentration of heavy metals in soil and water (Hadi et al., 2014) and, consequently, in plants grown on this soil. However, the addition of CM significantly decreased shoot and grain heavy metal concentrations of wheat plants. These results are in accordance with those of Ahmad et al. (2011), noting that farm manure amendment significantly decreased Cd and Pb content in wheat grains. The lower heavy metal availability in the farmyard amended soils could be mainly related to the heavy metal immobilization through sorption, chelation, and sequestration by the solid and soluble organic matter. Indeed, soil heavy metal availability can be decreased by adding the organic materials, particularly CM through heavy metal transformation from more readily available forms to less available forms such as fractions associated with organic materials, carbonates, or heavy metal oxides, causing a reduction in heavy metal uptake by plants (Ok et al., 2011; Bolan et al., 2003). Narwal and Singh (1998) observed that the Cd, Cu, Ni and Zn in soils decreased with increasing the addition of farmyard manure and peat that correspond with our results by soil application with CM (Tab. 5 and 7). Soil organic matter may contribute to the adsorption and immobilization of heavy metals, restricting its solubility and bioavailability. Therefore, organic manures such as CM may have an important role in improving the quality and minimizing the metal toxicity of contaminated soils under heavy metals and salinity stress (Usman, 2015). It has been speculated that the application of organic manure to metal-contaminated soil may create a good environmental condition for mitigating the toxicity induced by high heavy metal concentrations (Usman et al., 2013).

The application of CM in combination with a partial inorganic fertilization and/or bio-fertilization (Cerealine) found to significantly increase wheat growth characteristics (plant height, number of

Tab. 4: Effect of N-fertilizer form applications on the contents of various elements [nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and sodium (Na)] and the ratios of Ca:Na and K:Na in shoots of wheat plants grown under saline soil conditions

Treatments	N (%)	P (%)	K (%)	Ca (%)	Na (%)	Ca/Na ratio	K/Na ratio
T_1	1.85h*	0.24i	1.53h	0.13h	0.30a	0.43h	5.1g
T_2	2.29defg	0.42gh	2.20efg	0.23e	0.22c	1.05de	10.0def
T_3	2.26efg	0.42gh	2.16fg	0.21f	0.24b	0.88fg	9.0ef
T_4	2.24fg	0.40h	2.12g	0.19g	0.24b	0.79g	8.8f
T_5	2.24fg	0.42gh	2.14g	0.21f	0.22c	0.95ef	9.7def
T_6	2.22g	0.40h	2.11g	0.19g	0.24b	0.79g	8.8f
T_7	2.42cd	0.51c	2.41bc	0.25cd	0.18e	1.39c	13.4c
T_8	2.46c	0.51c	2.40bc	0.26c	0.18e	1.44c	13.3c
T_9	2.35cdefg	0.46ef	2.29cdef	0.24de	0.22c	1.09d	10.4d
T_{10}	2.38cdef	0.48de	2.33cde	0.25cd	0.22c	1.14d	10.6d
T_{11}	2.39cde	0.50cd	2.36bcd	0.25cd	0.18e	1.39c	13.1c
T_{12}	2.30defg	0.44fg	2.22defg	0.24de	0.22c	1.09d	10.1de
T_{13}	2.68b	0.55b	2.49b	0.28b	0.14f	2.00b	17.8b
T_{14}	2.91a	0.60a	2.71a	0.31a	0.10g	3.10a	27.1a

*Values are means (n = 9), and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.
T_1: Control (without any N-fertilizer); **T_2**: Recommended N as ammonium nitrate (160 kg N = 476 kg NH_4NO_3 ha^{-1} with 33.5% N); **T_3**: Recommended N as Urea (160 kg N = 348 kg ha^{-1} urea [$(NH_2)_2CO$] with 46% N); **T_4**: Poultry manure (PM_1 = 10 t ha^{-1}); **T_5**: Cattle manure (CM_1 = 20 t ha^{-1}); **T_6**: Bio-N (Cerealine at rate of 4 Kg ha^{-1}); **T_7**: ½NH_4NO_3+PM_2 (PM_2 = 5 t ha^{-1}); **T_8**: ½NH_4NO_3+CM_2 (CM_2 = 10 t ha^{-1}); **T_9**: ½NH_4NO_3+Cerealine; **T_{10}**: ½Urea+PM_2; **T_{11}**: ½Urea+CM_2; **T_{12}**: ½Urea+Cerealine; **T_{13}**: Cerealine+CM_1; **T_{14}**: ⅓NH_4NO_3+Cerealine+CM_2.

Tab. 5: Effect of N-fertilizer form applications on the concentrations of heavy metals [lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), selenium (Se), cobalt (Co), zinc (Zn), and copper (Cu)] in shoots of wheat plants grown under saline soil conditions

Treatments	Pb	Cd	Ni	Cr (mg kg^{-1} DW)	Se	Co	Zn	Cu
T_1	2.85g*	4.16f	2.51g	3.27gh	1.26bcd	2.25f	138i	55.2ij
T_2	3.86e	5.61d	3.40e	4.41e	1.20de	3.06d	182f	71.6f
T_3	6.24c	8.96b	5.49c	7.12c	1.25bcde	4.94b	229bc	92.1bc
T_4	7.38b	9.80a	6.56b	8.39b	1.12e	5.95a	238b	94.8ab
T_5	2.64gh	3.82fg	2.33gh	3.01h	1.41a	2.11fg	132i	52.6jk
T_6	2.85g	4.13f	2.50g	3.45g	1.25bcde	2.24f	151h	60.3hi
T_7	5.75d	8.13c	5.00d	6.70d	1.20de	4.55c	212de	84.3de
T_8	3.48f	5.05e	3.05f	4.00ef	1.37abc	2.75e	164g	65.1gh
T_9	3.45f	4.99e	3.10f	3.94f	1.26bcd	2.73e	165g	66.2g
T_{10}	7.83a	9.98a	6.82a	9.12a	1.18de	6.03a	256a	98.7a
T_{11}	5.70d	8.24c	4.97d	6.58d	1.30abc	4.47c	202e	80.4e
T_{12}	5.88d	8.53c	5.16d	6.67d	1.24cde	4.66c	218cd	87.0cd
T_{13}	2.52h	3.68g	2.19h	2.86h	1.30abc	1.96g	117j	47.4k
T_{14}	2.02i	3.21h	1.81i	2.33i	1.38ab	1.59h	101k	40.8l

*Values are means (n = 9), and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.
T_1: Control (without any N-fertilizer); **T_2**: Recommended N as ammonium nitrate (160 kg N = 476 kg NH_4NO_3 ha^{-1} with 33.5% N); **T_3**: Recommended N as Urea (160 kg N = 348 kg ha^{-1} urea [$(NH_2)_2CO$] with 46% N); **T_4**: Poultry manure (PM_1 = 10 t ha^{-1}); **T_5**: Cattle manure (CM_1 = 20 t ha^{-1}); **T_6**: Bio-N (Cerealine at rate of 4 Kg ha^{-1}); **T_7**: ½NH_4NO_3+PM_2 (PM_2 = 5 t ha^{-1}); **T_8**: ½NH_4NO_3+CM_2 (CM_2 = 10 t ha^{-1}); **T_9**: ½NH_4NO_3+Cerealine; **T_{10}**: ½Urea+PM_2; **T_{11}**: ½Urea+CM_2; **T_{12}**: ½Urea+Cerealine; **T_{13}**: Cerealine+CM_1; **T_{14}**: ⅓NH_4NO_3+Cerealine+CM_2.

Tab. 6: Effect of N-fertilizer form applications on the 1000-grain weight and grain yield of wheat plants grown under saline soil conditions

Treatments	1000-grain weight (g)	Grain yield ha^{-1} (ton)
T_1	29.5d*	3.18g
T_2	43.9bc	5.40cdef
T_3	43.6bc	5.32cdef
T_4	42.7bc	5.26def
T_5	42.4bc	5.23ef
T_6	41.0c	5.11f
T_7	44.5bc	5.68cde
T_8	44.3bc	5.55cdef
T_9	44.9bc	5.80c
T_{10}	44.2bc	5.63cde
T_{11}	44.1bc	5.53cdef
T_{12}	44.4bc	5.74cd
T_{13}	45.3b	6.66b
T_{14}	49.9a	7.85a

*Values are means (n = 9), and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

T_1: Control (without any N-fertilizer); **T_2**: Recommended N as ammonium nitrate (160 kg N = 476 kg NH_4NO_3 ha^{-1} with 33.5% N); **T_3**: Recommended N as Urea (160 kg N = 348 kg ha^{-1} urea $[(NH_2)_2CO]$ with 46% N); **T_4**: Poultry manure (PM_1 = 10 t ha^{-1}); **T_5**: Cattle manure (CM_1 = 20 t ha^{-1}); **T_6**: Bio-N (Cerealine at rate of 4 Kg ha^{-1}); **T_7**: ½NH_4NO_3+PM_2 (PM_2 = 5 t ha^{-1}); **T_8**: ½NH_4NO_3+CM_2 (CM_2 = 10 t ha^{-1}); **T_9**: ½NH_4NO_3+Cerealine; **T_{10}**: ½Urea+PM_2; **T_{11}**: ½Urea+CM_2; **T_{12}**: ½Urea+Cerealine; **T_{13}**: Cerealine+CM_1; **T_{14}**: ⅓NH_4NO_3+Cerealine+CM_2.

leaves $plant^{-1}$, leaf area $plant^{-1}$ and shoot dry weight $plant^{-1}$; Tab. 3), positively reflecting in yield parameters. This finding agrees with earlier reports of Baiyeri and Tenkouano (2007) and Ndukwe et al. (2011) that animal manure is a valuable source of crop nutrients and organic matter, which can improve the soil biophysical conditions making the soil more productive and sustainable for plant growth. Growth and yield improvements in plants grown in soil amended with a combination of bio-, organic and inorganic fertilizers might be due to nutrient availability, particularly N, P and P ions (Maman and Mason, 2013) that agreed with our results obtained under salt stress (Tab. 4). Organic manures not only provide nutrients by making them more available for plants but also add to the most important constituent of the soil humus, which provides excellent substrate for plant growth. This could be attributed to the fact that the nutrients in the organic fertilizers were released gradually through the process of mineralization (Mehedi et al., 2012), maintaining optimal soil levels over prolonged periods of time. Some of the organic substances released during the mineralization may act as chelates, which help in the absorption of essential ions and other micro-nutrients (Lobo et al., 2012). They added that, organic materials could have formed complex (or chelate), preventing the precipitation of P, reduced the P-sorption capacity of the soil, enhanced P availability, improved P-recovery or resulted in better utilization by plants. Organic materials add carbon into the soil, provides substrate for microbial growth, and subsequent microbial activity. The turnover resulting from the decomposition of organic materials improves C and N mineralization rates, and enzyme activities, which affect nutrient cycling and availability to the plants (Smith et al., 1993). In addition, Vanlauwe et al. (2002) reported that the combination of organic and inorganic fertilizers result into synergy and improved conservation and synchronization of nutrient release and crop demand, leading to increased fertilizer efficiency and higher growth and yields. The combined treatment of CM (10 t ha^{-1}) + ⅓NH_4NO_3

Tab. 7: Effect of N-fertilizer form applications on the concentrations of heavy metals [lead (Pb), cadmium (Cd), nickel (Ni), chromium (Cr), selenium (Se), cobalt (Co), zinc (Zn), and copper (Cu)] in harvested grains of wheat plants grown under saline soil conditions

Treatments	Pb	Cd	Ni	Cr	Se	Co	Zn	Cu
				(mg kg^{-1} DW)				
T_1	1.31de*	1.40de	1.89fgh	1.18e	0.93c	0.38g	141efg	56.3efg
T_2	1.36de	1.48d	1.99def	1.24de	0.87cd	0.42f	150ef	59.8e
T_3	4.26c	2.34c	4.63c	2.58c	0.71e	0.58c	194c	77.5c
T_4	6.12b	2.99b	6.02b	3.16b	0.64f	0.79b	212b	84.6b
T_5	1.20e	1.26e	1.75hi	1.04f	1.00b	0.33h	128h	51.4g
T_6	1.30de	1.38de	1.85fghi	1.18e	0.92c	0.38g	140fg	56.1efg
T_7	1.42d	1.56d	2.09d	1.30d	0.81d	0.47d	168d	67.2d
T_8	1.30de	1.39de	1.84ghi	1.19de	0.92c	0.38g	141efg	56.0efg
T_9	1.33de	1.43de	1.92efg	1.20de	0.90c	0.40fg	144ef	57.6e
T_{10}	7.10a	3.52a	7.31a	3.48a	0.58f	0.91a	244a	94.9a
T_{11}	1.36de	1.49d	1.98defg	1.24de	0.87cd	0.43ef	152e	60.7e
T_{12}	1.40d	1.55d	2.07de	1.29de	0.82d	0.46de	170d	68.1d
T_{13}	1.20e	1.24e	1.72i	1.04f	1.02b	0.32h	131gh	52.1fg
T_{14}	1.01f	0.93f	1.53j	0.91g	1.11a	0.27i	114i	46.2h

*Values are means (n = 9), and mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

T_1: Control (without any N-fertilizer); **T_2**: Recommended N as ammonium nitrate (160 kg N = 476 kg NH_4NO_3 ha^{-1} with 33.5% N); **T_3**: Recommended N as Urea (160 kg N = 348 kg ha^{-1} urea $[(NH_2)_2CO]$ with 46% N); **T_4**: Poultry manure (PM_1 = 10 t ha^{-1}); **T_5**: Cattle manure (CM_1 = 20 t ha^{-1}); **T_6**: Bio-N (Cerealine at rate of 4 Kg ha^{-1}); **T_7**: ½NH_4NO_3+PM_2 (PM_2 = 5 t ha^{-1}); **T_8**: ½NH_4NO_3+CM_2 (CM_2 = 10 t ha^{-1}); **T_9**: ½NH_4NO_3+Cerealine; **T_{10}**: ½Urea+PM_2; **T_{11}**: ½Urea+CM_2; **T_{12}**: ½Urea+Cerealine; **T_{13}**: Cerealine+CM_1; **T_{14}**: ⅓NH_4NO_3+Cerealine+CM_2.

+ Cerealine was found to significantly increase wheat growth and productivity and significantly decrease plant and grains heavy metals concentrations. These results show that it is important to choose the suitable N source and its suitable amount to sustain the agricultural productivity, by minimizing the concentrations of heavy metals in the edible parts for human nutrition and health.

Conclusions

The higher growth and yield, and the lower plant and grain heavy metal contents obtained as a result of soil application with CM in addition to bio- and inorganic fertilizers are an indication that integrated use of CM, bio- and mineral fertilizers for salt-affected soil is advantageous over the use of others. Integration of CM as organic fertilizer with bio- and inorganic fertilizers could therefore be recommended as a best option in increasing fertilizer use efficiency and provision of a more balanced supply of essential nutrients and a lower bioavailability of heavy metals under salt stress to obtain economical wheat grain yield with minimum heavy metal content for human nutrition and health.

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