

## THE IMPACT OF CATTLE MANURE ON THE CONTENT OF MAJOR MINERALS AND NITROGEN UPTAKE FROM <sup>15</sup>N ISOTOPE-LABELED AMMONIUM SULPHATE FERTILIZER IN MAIZE (*ZEA MAYS L.*) PLANTS

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### Abstract

Jordan Valley is considered as one of the main intensified irrigated agricultural areas in Jordan with high use of fertilizers especially of nitrogen source which lead to increased ground water pollution with nitrate. A field trial was conducted at the National Agricultural Research Center in Jordan Valley, North West Amman. Six soil treatments with organic cattle manure at the following rates were applied in summer 2014 and 2015: 0 (control), 8, 12, 16 and 20 tons ha<sup>-1</sup>. Treatments were arranged in a completely randomized block design using four replicates. Nitrogen fertilizer from ammonium sulphate, as source of nitrogen, labeled with 1% <sup>15</sup>N isotope was added at a rate of 75 kg ha<sup>-1</sup>. The objective of this study was to evaluate the effect of different rates of organic cattle manure on nitrogen uptake from ammonium sulphate fertilizer by grains of maize (*Zea mays L.*) plants using <sup>15</sup>N isotope-tracer technology. It was found that nitrogen use efficiency was decreased as the amount of applied cattle manure increased owing to the high competition of organic versus mineral nitrogen. Whereas cattle manure positively affected the uptake of Zn, Fe and Cu by maize grains, causing increased mineral uptake instances, the uptake of K, P and Ca was not or slightly affected by cattle manure at various levels. The results of the present study could be beneficial for the development of an efficient fertilization schedule that would satisfy maize plant requirement of major elements, in particular N without its accumulation in the soil and the subsequent hazards to environment.

**Key words:** Fertilizer use efficiency, Isotope technology, Maize, Manure, Nitrogen.

### Introduction

Jordan valley accounts for 2.25% of Jordan's total area, and is considered as the food basket of the country. This is because of its unique climate and fertile soil which enables the production of high quality products all over the year (Anon., 2014). Maize crop is important in terms of its total production that is estimated around 800 million tons per year, and is widely distributed cereal after wheat and rice (Nafziger, 2010). Most maize grain produced is used as animal feed; and as raw material for many industrial uses, including bio-fuel production (Helander *et al.*, 2015; Barrière *et al.*, 2016). Maize is rich in carbohydrate, and good in protein and crude fiber content (Qamar *et al.*, 2017). It is a good source of starch, minerals, vitamins and unsaturated fatty acids, particularly linolenic acid (2%) (Ullah *et al.*, 2010; Qamar *et al.*, 2017). The use of bio- or organic-fertilizers from different sources has proven beneficial to enhance the productivity of many crops including maize plants (Amin, 2010; Solomon *et al.*, 2012; Hassan & Bano, 2016; Wang *et al.*, 2017). The first part of this research has addressed the effect of cattle manure at rates of 0, 4, 8, 12, 16 and 20 tons ha<sup>-1</sup> on maize growth and yield (Alhrouf *et al.*, 2017). Cattle manure at 20 tons ha<sup>-1</sup> resulted in the highest productivity and growth performance of maize plants (Alhrouf *et al.*, 2017).

Nitrogen use efficiency (NUE) is low in many crops, leading to serious environmental problems as a result of increasing N in environment (Singh *et al.*, 2001; Selvaraj *et al.*, 2017). Thus farmers, who use fertilizer of both organic and non-organic sources, could schedule the quantities of mineral-nitrogen fertilizer applied to plants grown under organic amended soil for higher growth, but at environmentally safe amounts. In addition, the increased demand for adequate and nutritious food products has drawn more attention to organic foods. Sustainable farming has emerged globally as billions of people in developing countries are faced with malnutrition due to inadequate supplies of essential micronutrients in staple cereals which they routinely consume (Arzani & Ashraf, 2017). The enrichment of staple food crops with mineral elements provided a temporary solution for combating micronutrient malfunction. A balance must, therefore, be established between yield costs using new farming practices and a sustainable high-quality crop production (Arzani & Ashraf, 2017). The objectives of the present study were a) monitoring nitrogen accumulation in seeds obtained from ammonium sulphate fertilizer as influenced by different levels of organic cattle manure using mineral fertilizer <sup>15</sup>N isotope-tracer technology, and b) evaluating the effects of cattle manure on the content of major minerals in seeds of maize.

## Materials and Methods

**Location:** The experiment was conducted during summer of 2014 and 2015 at Jordan Valley north-west of Amman in Dair Alla Agricultural Research station. Soil samples were analyzed for chemical properties before experiment started (EC, 1.32 ds/m; pH, 7.7; N 0.07%; P 18.5 ppm; K 420 ppm).

**Site preparation and fertilizer treatments:** Prior to sowing, in March of the two growing seasons, well prepared seed bed was attained by plowing and disking the soil. Then organic cattle manure was mixed with surface soil layer before planting at the following rates comprising the treatments of the study: T1: no manure (control); T2: 4 tons ha<sup>-1</sup>; T3: 8 tons ha<sup>-1</sup>; T4: 12 tons ha<sup>-1</sup>; T5: 16 tons ha<sup>-1</sup>; and T6: 20 tons ha<sup>-1</sup>. The percent composition of N, P and K in the organic manure was 1.8%, 1.3% and 1.5%, respectively. Plants were planted in the mid of April in rows that were 0.7 m apart with intra-row spacing of 0.2 m. The quantity of nitrogen fertilizer from ammonium sulphate, as source of nitrogen, labeled with 1% <sup>15</sup>N isotope was added at rate 75 kg N ha<sup>-1</sup> as recommended for each level of the organic manure mentioned. The treatments were arranged in a completely randomized block design using four replicates. Observations were made on 9 m<sup>2</sup> (3 x 3m) plots. Nitrogen fertilizer applied in the form of Ammonium sulfate was applied at four doses. The first dose was applied after germination, and the other three doses were applied at 20 days interval thereafter.

**Harvesting, plant sampling and data analysis:** Mature corn plants were harvested in the mid of July for each season. Plant parts were oven dried for 24 hours at 60°C. Kjeldahl method was adopted for the determination of total N content in seeds (Bremner & Mulvaney, 1982). Total nitrogen uptake was represented by total nitrogen content in the dried seeds. Seeds were analyzed for <sup>15</sup>N content using an emission spectrophotometer. Fertilizer N utilization percent (NUE %) was calculated as N traced from <sup>15</sup>N isotope-labeled fertilizer divided by the total amount of fertilizer used (Anon., 2001). The content of Cu, Zn, K, Fe, P, and Ca (mg kg<sup>-1</sup>) in seeds was determined following standard procedures (Walinga *et al.*, 1995). The experiments for both production seasons were pooled because they relatively gave similar results. Pooled data from the two growing seasons 2014-2015 were subjected to statistical analyses using SAS program (Anon., 2006) and differences among means were compared using the least significant difference test at 0.05 probability level.

## Results and Discussion

**Nitrogen uptake by plants:** Total nitrogen uptake by maize plants was increased significantly with the increase in cattle manure rate (Table 1, Fig. 1). Increasing the rate of cattle manure up to 12 t ha<sup>-1</sup> resulted in a steady increase in total nitrogen from

109.6 kg ha<sup>-1</sup> in non-treated plants to 145.3 kg ha<sup>-1</sup> in plants treated with 12 t ha<sup>-1</sup> of cattle manure. Further increase in manure from 12 to 16 t ha<sup>-1</sup> resulted in total nitrogen uptake at a higher rate than did the stepwise increase of manure from 0 to 12 t ha<sup>-1</sup>. Total nitrogen uptake showed a plateau for plants treated with manure from 16 to 20 t ha<sup>-1</sup>, and was 176.1, 180.4 kg ha<sup>-1</sup>, respectively (Fig. 1). These results supported the increase in maize plant growth and yield indices in response to the stepwise increase of cattle manure from 0 to 20 t ha<sup>-1</sup> as reported in the first part of this investigation (Alhrouf *et al.*, 2017).

**Nitrogen use efficiency:** Nitrogen fertilizer applied to maize plants from <sup>15</sup>N isotope-labeled ammonium sulphate fertilizer was significantly influenced by the different levels of organic manure (Table 1, Fig. 2). Nitrogen use efficiency showed a significant decrease with the stepwise increase of cattle manure from 0 to 16 t ha<sup>-1</sup>, and was significantly the least in plants receiving manure at 16 or 20 t ha<sup>-1</sup>, 20.0 and 20.8%, respectively. The decrease in mineral fertilizer efficiency with the increase in rate of organic fertilizer indicated that beneficial effects of N came mostly from organic sources and that increasing mineral N source will be unjustified in terms of cost and its hazardous accumulation in soil.

**Nutrient concentration:** Application of ammonium sulphate mineral fertilizer combined with the different levels of cattle manure to maize plants resulted in variable responses with regard to seed mineral content of Zn, Fe, Cu, K, P and Ca (Tables 1 and 2, Fig. 3). The results showed that fertilization, although not for all the stepwise changes in manure levels, positively influenced Fe, Zn and Cu content of seeds (Tables 1 and 2, Fig. 3). In contrast, the content of K, P and Ca in maize seeds was not (Ca and K) to slightly influenced (P) by the combined application of manure and mineral fertilizer, where they exhibited somewhat a plateau pattern of change in response to manure level (Table 2). Application of manure at 8 to 20 t ha<sup>-1</sup> resulted in a significant increase in Fe content of seeds, with a maximum of 35.38 mg kg<sup>-1</sup> in seeds of plants treated with 20 t ha<sup>-1</sup> organic manure in comparison to untreated- and 4.0 t ha<sup>-1</sup> - treated plants (Fig. 3). The content of Zn, on the other hand, was marginally influenced by fertilization where its content ranged from 34.73 to 36.03 mg kg<sup>-1</sup> in seeds of plants treated with 4 to 20 t ha<sup>-1</sup> of cattle manure than in untreated plants (32.73 mg kg<sup>-1</sup>) (Fig. 3). The content of Cu showed a progressive increase with manure fertilization up to 16 t ha<sup>-1</sup>, then significantly recorded the highest content in seeds of plants treated with 20 t ha<sup>-1</sup> (4.72 mg kg<sup>-1</sup>) (Table 2). However, as a result of the small standard errors, statistical analyses showed significance in P content of seeds with manure level change as compared to K (Tables 1 and 2). Overall, the highest content of K (0.72 mg kg<sup>-1</sup>), P (0.58 mg kg<sup>-1</sup>) and Ca (0.27 mg kg<sup>-1</sup>) were obtained in maize plants treated with cattle manure at 8, 20 and 16 t ha<sup>-1</sup>, respectively (Table 2).

**Table 1. Analysis of variance for effects of <sup>15</sup>N isotope-labelled ammonium sulphate fertilizer applied at 75 kg N ha<sup>-1</sup> for each level of the organic manure on content of total nitrogen and major minerals in maize seeds as well as on nitrogen use efficiency by seeds.**

Source of variation	Degrees of freedom (df)	F values							
		Nitrogen use efficiency	N	Fe	Zn	Cu	K	P	Ca
Treatments	5	9.3**	8.8**	14.2**	2.8*	9.0**	0.8 <sup>ns</sup>	4.5**	1.1 <sup>ns</sup>
Blocks	3	4.4	2.9	0.6	0.8	0.7	1.7	2.5	1.0

Significant differences between means were expressed as \**p*<0.05 and \*\**p*<0.01, ns: Treatments are not significant at 5%

**Table 2. Effect of <sup>15</sup>N isotope-labelled ammonium sulphate fertilizer added at 75 kg N ha<sup>-1</sup> for each level of organic cattle manure on Cu, K, P and Ca content in seeds of maize. The data are presented as the mean ± standard deviation.**

Manure (ton ha <sup>-1</sup> )	Cu	K	P	Ca
	mg kg <sup>-1</sup>			
0	2.32±0.25c	0.60 ± 0.14	0.43±0.03b	0.23±0.04
4	3.27±0.40b	0.60 ± 0.32	0.42±0.11b	0.24±0.03
8	3.79±0.59b	0.72 ± 0.20	0.46±0.12b	0.24±0.01
12	4.02±0.84ab	0.66 ± 0.13	0.37±0.03b	0.23±0.02
16	3.98±0.31ab	0.47 ± 0.01	0.40±0.02b	0.27±0.02
20	4.72±0.55a	0.55 ± 0.23	0.58±0.10a	0.25±0.02

Means followed by the same letter are not significantly different at *p*<0.05. The effect of the combined use of cattle manure and ammonium sulphate fertilizer was not significant for K and Ca as shown in table 1.

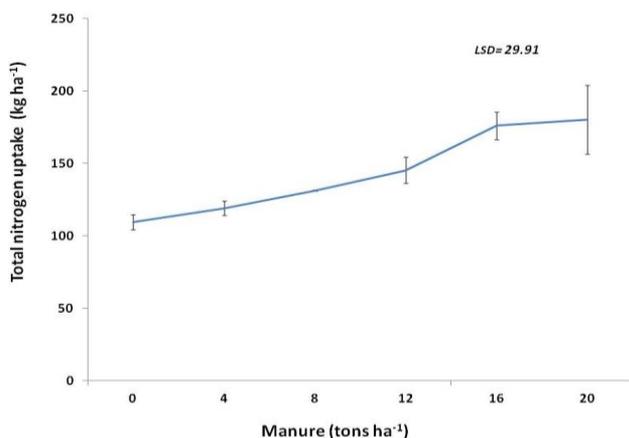


Fig. 1. Effect of cattle manure application on total nitrogen (N) content of maize seeds amended with 1% <sup>15</sup>N isotope-labelled ammonium sulphate fertilizer added at rate 75 kg N ha<sup>-1</sup> for each level of organic manure tested. The data are presented as the mean ± standard error.

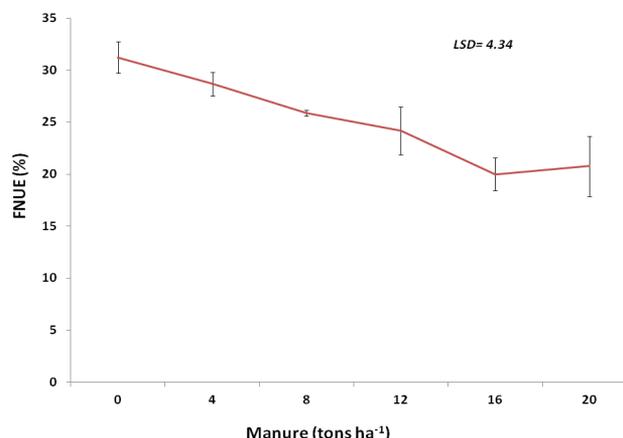


Fig. 2. Nitrogen utilization efficiency from <sup>15</sup>N isotope-labelled ammonium sulphate fertilizer by maize plants treated with different levels of cattle organic manure. The data are presented as the mean ± standard error.

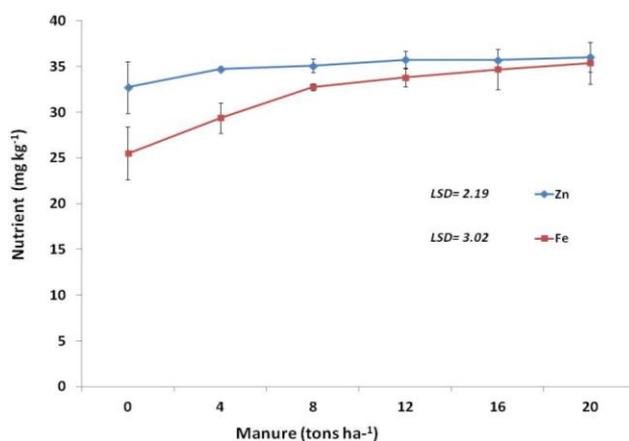


Fig. 3. Effect of ammonium sulphate fertilizer added at rate 75 kg N ha<sup>-1</sup> for each level of organic cattle manure tested on Zn and Fe content in seeds of maize. The data are presented as the mean ± standard deviation.

Manure provides essential nutrients to soil (Baiyeri & Tenkouano, 2008) and as illustrated in the present study resulted in an increased availability of certain minerals to plants. Martínez *et al.*, (2017) showed that apparent recovery of nitrogen in maize plants supplemented with cattle manure under the Mediterranean conditions during the first two seasons of growth resulted from mineralization of organic nitrogen. They also reported that loss of nitrogen from organic source is less likely than that from mineral fertilizers. Aerobically composted cattle manure was found useful in preventing N deficiency when used with some mineral N in early season in a maize field in sub-Saharan Africa (Nyamangara, 2007). The use of poultry litter and no-till management system enhanced soil fertility represented by an increase in N, P, K, Ca and Mg of soil collected from continuous soybean and corn systems (Watts *et al.*, 2010). Phosphorus and K in the present study were considered initially high in the

study site as indicated by Dinkins and Jones, (2013) and, therefore, exhibited small magnitude of change in response to different levels of the organic manure (Table 2). In spite of that, P content of seeds was significantly influenced by cattle manure amendment (Table 2). Significant effects of cattle manure in the present study were also observed on the seed content of Fe and Cu (Fig. 3, Table 2). The effect of manure on availability of macronutrients as N and P as well on micronutrients as Cu and Fe is well documented (Eghball *et al.*, 2002; Parsons *et al.*, 2007; Lentz & Ippolito, 2012). Application of manure fertilizer was reported to enhance P availability in P-fixing soils (Gichangi & Mnkeni, 2009) and in acidic soils (Hua *et al.*, 2008), by the formation of complexes, chelate, with Fe and Al in soil solution, therefore avoiding the precipitation of phosphate, or shielding P ions from sorption sites (Guppy *et al.*, 2005; Agbenin & Igbokwe, 2006; Gichangi & Mnkeni, 2009). Another role manure could play is to solubilize P from insoluble Ca, Fe, and Al phosphates (Khan *et al.*, 2009; Abbasi *et al.*, 2015). Generally, mineralization is an important mechanism for the increase of nutrient availability from manure and is influenced by soil properties, manure composition, temperature and soil microbial activities (Eghball *et al.*, 2002; Ferguson *et al.*, 2005; Abbasi *et al.*, 2015). Thus, as nutrient availability from manure fertilizers is actually influenced by number of factors, this could explain the beneficial effects of cattle manure on N, Fe, Zn and P but not on K or Ca as observed in the present study. Our findings are in accordance with that of Eghball *et al.*, (2002) where the interaction of the above mentioned factors on mineralization of organic manure could not be accurately predicted and thus approximation of nutrient mineralization following application of manure dung is only possible.

Although there is generally a need to integrate organic manure with inorganic fertilizers in order to achieve better crop yields, many researchers have also suggested that the interaction between organic matter and inorganic fertilizers may lead to an increase or decrease in soil nutrients depending on nutrients and plant material (Clemente & Bernal, 2006; Agbede *et al.*, 2008; Muhammad & Khattak, 2009).

## Conclusion

Using  $^{15}\text{N}$  isotope as a tracer proved to be an efficient method for detecting plant utilization of N- mineral-derived fertilizer as indicated by the results of the present study. Total nitrogen uptake by plants and FNUE% showed a contrasting response to increased manure fertilizer level. While total nitrogen uptake increased from 109.6 kg ha<sup>-1</sup> in non-treated plants to 176.1 kg ha<sup>-1</sup> in plants amended with 16 t ha<sup>-1</sup> cattle manure combined with 1%  $^{15}\text{N}$  isotope labeled ammonium sulphate at 75 kg N ha<sup>-1</sup>, a corresponding decline was evident in FNUE% from 31.2% to 20%. However, total nitrogen uptake and FNUE% showed a plateau for plants treated with manure from 16 to 20 t ha<sup>-1</sup>. Approximation of mineral nutrient after combined fertilizers amendment in maize seeds indicated variable degree of responses. While manure

fertilizer amendment positively affected seed content of Fe, Cu, and Zn, it had non to slight effects on seed mineral content of K, Ca and P. It could be concluded that organic derived N most likely showed increased availability to maize plants with the increase in manure up to 16 t ha<sup>-1</sup>. Meanwhile, mineral- derived N showed a progressive decline in N utilization efficiency, from 31.2% without manure amendment, to 20% with manure applied at 16 t ha<sup>-1</sup> and remained unchanged with increasing manure to 20 t ha<sup>-1</sup>. Therefore, it seems of a loss and hazardous to apply N-derive mineral fertilizer with cattle manure at 20 t ha<sup>-1</sup>.

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