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IN VITRO ANTIOXIDANT ACTIVITY, MACRONUTRIENTS AND HEAVY METALS IN LEAVES OF MAIZE (*ZEA MAYS* L.) PLANTS GROWN AT DIFFERENT LEVELS OF CATTLE MANURE AMENDED SOIL IN JORDAN VALLEY

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Abstract

A field experiment was conducted at the National Center for Agricultural Research and Extension in the Jordan Valley, during summer 2015 to evaluate the effect of cattle manure on antioxidant activity of maize leaves. The study also measured the effect of cattle manure on the bioavailability of heavy metals in maize leaves at harvest. Six treatments were compared, no cattle manure (T1, control), and 4 tons ha^{-1} (T2), 8 tons ha^{-1} (T3), 12 tons ha^{-1} (T4), 16 tons ha^{-1} (T5), and 20 tons ha^{-1} (T6) cattle manure. ANOVA followed by LSD test were used to compare the treatments at the 5% significance level. Antioxidant activity using DPPH and ABTS were measured for the six leaves extracts representing six treatments of organic manure. A wet digestion method was used to extract the heavy metals from the dry leaf powder samples using atomic absorption spectroscopy. Antioxidant activity for leaves increased significantly with increasing cattle manure amount applied to soil according to DPPH and ABTS radical scavenging activity. The highest antioxidant activity was reached at the highest amount of cattle manure while the control had the lowest. Concentrations of Ni, Cu, Mn, Zn, Cr and Fe contents in maize leaves of plants grown in soil amended with cattle manure were higher compared to plants grown in no-amended soil. Cattle manure amended soil increased Ca, K and P concentrations in the seeds and leaves of maize. The cattle manure was able to encourage a raise on mineral buildup in aerial parts of tested plants.

Key words: Antioxidants, Free radical scavenging potential, Micronutrients, Heavy metals, Cattle manure, Jordan valley.

Introduction

Maize (*Zea mays* L.) is the third important cereal crops after wheat (*Triticum aestivum*) and rice (*Oryza sativa*) (Haddad *et al*., 2016b) which was widely distributed (Kaul *et al*., 2011) and domesticated in Mexico about_7000 years ago from a wild grass before a Native Americans converted it into an improved source of food. The major components of maize are starch (72%), protein (10%), and fat (4%), giving it an energy density of 365 kcal per 100 g; however, it has lower protein content than rice (*Oryza sativa*) and wheat (*Triticum aestivum*) (Nuss & Tanumihardjo, 2010). Maize crop is grown for both grain and forage and is considered as gorgeous as conserved forage because of its ability of increasing the feed consumption of livestock and producing higher yields of high protein milk than other conserved feeds (Crowley, 1998).

The main challenge for maize production is to improve yield in a way that is sustainable every time. Increasing maize yield necessitates highly fertilized soils and those soils should be preserved through incorporated plant nutrient management system (Bationo & Koala, 1998). The use of inorganic fertilizers only has not proven beneficial in intensive agriculture because it exacerbates soil degradation. The long-period extreme application of inorganic fertilizers destroys beneficial soil microflora, contaminates water aquifer and changes soil natural system (Ahmadian *et al*., 2011)

The useful effect of combined application of both organic and inorganic dressings by farmers has been reported to increase yield and conserve soil productivity (Chukwu *et al.*, 2012). The application of organic fertilizers such as of poultry manure, cattle manure, and household wastes has been shown to increase the efficiency of mineral fertilizers by providing secondary and micro-nutrients that are not present in chemical fertilizers (Rayar, 2000; Bajeli *et al*., 2016; Alhrout *et al*., 2017), also improve the biological characteristics of the soil, as well as crop nutrition, production, and quality (Asroh, 2010; Tiamiyu *et al*., 2012; Eivazi *et al*., 2013) and ameliorate of *soil* physicochemical properties of soil resulted in yield increase (Alagöz & Yilmaz, 2009). On the other hand, toxic substances could be accumulated as a result of recycling of organic materials, particularly biosolids (e.g., animal manures). So, it should be applied with care to reduce the accumulation of toxic substances and contamination of the environment (Rezig *et al*., 2013).

In recent years, more attention has been paid to yield increase associated with organic applications with little attentions on crop nutritional composition. The impact of cattle manure application on antioxidant activities of plants has received very little consideration by scientists. Antioxidants are substances that can prevent the oxidation of lipids or other molecules by constraining the beginning of oxidative chain reactions or otherwise scavenging the free radicals generated during oxidative damage (Velioglu *et al.*, 1998). Other researchers describe an antioxidant as a material that when existing at low concentration compared with those of an oxidizable substrate, significantly prevents oxidation of that substrate (Halliwell & Gutteridge, 1999). Numerous studies indicated that the majority of the antioxidant activity may be attributed to anthocyanin, flavones, flavonoids, isoflavones, catechin and other phenolics (Kahkonen *et al*., 1999) that were found in the tissues of plants (Loliger, 1991; Abu-Romman *et al*., 2015; Haddad *et. al*., 2016a, Haddad *et al*., 2016b, Jaradat *et al*., 2018). Crops which have high antioxidant activity can serve as an excellent dietary source to improve health (Narwal *et al*., 2015; Al-Qudah *et al*., 2016). Increasing attention has been paid to antioxidant compounds derived from plants in recent years due to their valuable role in reducing various disorders such as heart disease, immune system decline, ageing, cardiovascular disease, brain dysfunction, and cancer (Pooja & Sunita, 2014). Increasing antioxidant levels in the plants is hardly *safer* than synthetic *antioxidants* added to foods because legislations are doubted to have some toxic effects and as potential carcinogens (Madhavi & Salunkhe, 1996). Thus, antioxidants are of interest to both health professionals and food scientists. The impact of cattle manure application on antioxidant activities of plants has received very little consideration by scientists. Therefore, It was hypothesized that maize crop, fertilized with cattle manure treatments, can provide superior antioxidant levels and mineral content. The aim of this field experiment was to investigate the antioxidant activity and rate of mineral contamination of maize leaf extracts having different manure treatments.

Materials and Methods

Experimental site and soil analysis: A field study was conducted at the National Agriculture Research Center (NARC) for in the Jordan Valley, northwest of Amman, Jordan, during summer season (April-July) of the year 2015. Top soil (0-15 cm) and organic manure were collected from various points in the plots, air dried, and ground and sieved with 2 mm mesh at the NARC before planting. The electric conductivity- EC (mmho/cm) of the soil paste was 1.32, the pH was 7.7, and the concentrations of K(mg/kg), P (mg/kg) and N (%) were 420, 18.5 and 0.07 respectively. All the analysis was executed according to Estefan *et al*., (2013) in which potassium was analyzed by Olsen method using spectrophotometer at 882 nm, Phosphorus analyzed by flame photometer at 767-nm, whereas nitrogen was determined by Kjeldahl method. The EC (mmho/cm) for organic manure was less than 1 and K(%), P(%), N(%) were 1.5, 1.3, 1.8 respectively as reported earlier (Alhrout *et al*., 2017). The soil analysis after organic manure addition was done (Table 1).

Table 1. The analysis of soil after organic manure addition.

EC mmho/cm	PH	K mg/kg	P mg/kg	N %	Treatment
1.15	7.6	420	23.7	0.06	T1
1.20	7.5	560	24.2	0.07	T2
1.24	7.7	520	26.9	0.08	T3
1.28	7.6	629	28.9	0.10	T4
1.30	7.6	672	32.9	0.11	T5
1.36	7.7	720	33.3	0.12	T6

Treatments, experimental design and plot size: An experimental area of 144 m^2 was divided into four replicates which was divided into six plots each with 6 m^2 (3 m×2 m). *Zea mays* seeds were planted at a spacing of 0.7 m between rows and 0.2 m within rows. The experiment was placed in a randomized complete block design (RCBD) with six treatments designated as: T1, T2, T3, T4, T5, and T6 referring to the control (No cattle manure), 4 t ha^{-1} cattle manure, 8 t ha^{-1} cattle manure, 12 t ha^{-1} cattle manure, 16 t ha^{-1} cattle manure, and 20 t ha^{-1} cattle manure, respectively.

Antioxidant activity

DPPH free radical scavenging assay: Antioxidant activity of plant extracts was measured in terms of hydrogen donation or radical scavenging activity by using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), which produce a violet solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to no color, which has been used to evaluate the antioxidant activity of plant extracts. DPPH containing an odd electron gives a strong absorption at 517 nm in visible spectrophotometer. As this electron becomes paired off in the presence of antioxidant compounds, the absorption fades and the resulting decolorization is stoichiometric with respect to the number of electrons taken up (Blois, 1958; Abdille *et al*., 2005). As designated by Blois (1958), 50 µl of different concentrations of plant extracts and the standard material ‘rutin’, were taken in test tubes separately, and 2.5 ml of 0.1 mM of DPPH methanolic solution was added to these tubes and shaken vigorously. The tubes were allowed to stand for 20 minutes at 27°C. The control was prepared as indicated above using methanol solvent (instead of plant extract) for the correction. The absorbance changes of the samples were measured at 517 nm by spectrophotometer. Activity of the radical scavenging was expressed as the percentage of inhibition and was calculated using the following formula:

$$\text{\% Radical scavenging activity} = \frac{\text{(OD (Control) - OD (sample)}}{\text{OD (Control)}} \times 100$$

Antioxidant capacity was expressed as rutin equivalent antioxidant capacity (TEAC) in milligrams of rutin per gram of sample dry matter using standard curve with different concentrations of rutin. All determinations were carried out at least three times.

ABTS free radical scavenging assay: Free radical scavenging ability was also determined by the use of 2, 2'-azinobis(3- ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assay. Generation of radical cation ($ABTS^{\bullet}$) involves the production of the blue/ green $ABTS^{\bullet}$ chromophore directly, through the reaction between potassium persulfate and ABTS. At wavelength of 734 nm this has maximum absorption. Antioxidants addition to the pre-formed radical cation will reduce it to ABTS, and the extent of reduction depends on the antioxidant activity, the duration of the reaction and the concentration of the antioxidant. Thus the extent of decolorization as inhibition percentage of the $ABTS^{\bullet}$ radical cation is determined as a function of concentration and calculated relative to the standard ‘reactivity of rutin’, under the same conditions (Re *et al*., 1999).

Seven mM of ABTS was prepared in water according to Re, *et al*., (1999). (ABTS•) was produced by reacting 2.45 mM potassium persulfate (final concentration) with ABTS stock solution and allowing the mixture to stand at room temperature before use for 16 h in the dark. Generated ABTS• solution was then diluted with ethanol to 0.70 (±0.02) absorbance at 734 nm. After addition of 2.0 mL of diluted ABTS$^{\bullet}$ solution (A734 nm = 0.700 ± 0.020) to 20 µL of different concentration of plant extract or rutin (standard) or solvent (control). The absorbance reading was taken exactly after six minutes. All determinations were carried out at least three times. The percentage inhibition of absorbance at 734 nm, and TEAC (mg/g) were determined according to standard curve of ABTS scavenging by rutin.

Determination of metals in leaf samples

Digestion procedure: A wet digestion method was used to extract the heavy metals (Jaradat *et al.*, 2016) from the dry leaf powder samples. For each sample, exactly (1, 0000) g was accurately measured using OHAUS analytical balance with high accuracy (0.0001) and transferred to the precleared Teflon digestion vessels. Then 10 ml of high purity nitric acid HNO_3 (65%) of AR grade was added to each vessel, followed by addition of 2 ml of high purity analytical reagent grade perchloric acid , $HClO_4$ (70)%. Then the vessels were capped and transfer to an oven at temperature of 70°C overnight. After the digestion complete, the vessel were cooled, and disassembled under the fume hood then the sample extracts were then quantitatively transferred to standard polyethylene volumetric flask and diluted to a final volume of 50 ml using deionized water. The diluted solution was filtrated using 0.7 µm online syringe micro filter. Finally, the cleared solutions after filtration were stored in 50 ml polyethylene bottle and stored at 4 °C in the refrigerator until analysis time.

Analytical Instrumentation and Calibration: Six metals (Ni, Cu, Mn, Zn, Cr and Fe) were analyzed quantitatively based on calibration curves for series of standard solutions ranging between 0.01 to 10 mg/kg (0.01, 0.05, 0.08, 0.1, 0.3, 0.5, 0.8, 1.00, 3.00, 5.00 and 10 mg/kg) using Atomic absorption spectroscopy (AAS) 6200 PerkinElmer, Canada-USA. The working standard were prepared by a serial dilution of the stock standard solution (1000 mg/kg for each metal) purchased from Merck (Germany). The calibration curves for these elements were built up by the instruments with best regression lines with correlation coefficient (r^2>0.998). Measurements were done in triplicate and the average value for each concentration was used to build up the calibration curve.

A total of six digested sample solutions and one blank solution were analyzed for their contents of six metals (Ni, Cu, Mn, Zn, Cr, Fe). Again, measurements were done in triplicate and the average value for each heavy metal was calculated.

Statistical analysis

The data were analyzed using analysis of variance (ANOVA) followed by Fisher's Least Significant Difference (LSD) test to compare the plots. All statistical analyses were conducted in (SAS, 2006).

Results

Soil analysis after organic manure addition: Soil samples for the different organic manure treatments were analyzed for N, P, K and E.C. and listed in Table 1. It is obvious from the table that the increase of the addition of organic manure will increase the N, P, K and soil electric conductivity.

Determination of the antioxidant activity in maize leaves extracts: Antioxidant activity assays can be categorized into two types based on the reactions involved; (1) Hydrogen atom transfer (HAT) and (2) Electron transfer (ET) (Huang *et al*., 2005). The latter was implemented in our investigations using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay. ET-based assays involve two reacting species an oxidant (also known as a probe which is an electron acceptor) and an antioxidant (electron donor). Fig. 1 shows the chemistry behind ABTS and DPPH assays.

Antioxidant activity is expressed as percent DPPH and ABTS radical scavenging activity with higher values indicating greater activity. The activity of antioxidant was significantly ($p \leq 0.05$) affected by cattle manure (Table 2). Antioxidant activity of leaves was increased significantly with increasing of cattle manure applied to soil (Table 3) by 6.08 %, 6.11%, 6.23%, 6.46% and 6.46%, respectively at 4, 8, 12, 16, and 20 ton ha^{-1} compared to control (untreated soil) according to DPPH radical scavenging activity and significantly increased by 6.4%, 6.77%, 6.89%, 8.61% and 10.70%, respectively at 4, 8, 12, 16, and 20 ton ha^{-1} compared to control (untreated soil) according to ABTS radical scavenging activity. The highest antioxidant activity was achieved at the highest amount of cattle manure with 90.6 % and 90 %, while the control had the lowest leaves antioxidant activity with 85.1% and 81.3% according to DPPH and ABTS radical scavenging activity, respectively.

Table 3. The antioxidant activity of maize leaves extract by two *In vitro* methods, DPPH and ABTS radical scavenging activity.

Treatment	*DPPH (% Radical scavenging)	*ABTS (% Radical scavenging)
T1	85.1 b	81.3 b
T2	90.2 a	86.5 a
T3	90.3 a	86.8 a
T4	90.4 a	86.9 a
T5	90.6 a	88.3 a
T6	90.6 a	90.0 a

*Means within column followed by the same letters are not significant at LSD p<0.05

Table 2. Analyses of variance for antioxidant activity in leaves, selected heavy metal contents in leaves and selected mineral contents in seeds and leaves of maize (*Zea mays* L.) plants grown at different levels of cattle manure amended soil in Jordan Valley.

Source of variation	Antioxidant activity in leaves		Heavy metals in leaves						Mineral contents in seed			Mineral contents in straw		
	DPPH	ABTS	Ni	Cu	Mn	Zn	Cr	Fe	Ca	K	P	Ca	K	P
Treatment	14.25 *	25.39 **	0.002 **	0.006 **	0.173 **	0.034 **	0.001 **	4.43 **	0.003 **	0.093 *	0.065 *	0.055 *	3.96 *	0.043 *
Replication	1.05 ns	5.98 ns	0.0002 **	0.005 **	0.0006 ns	0.009 ns	0.0002 ns	0.04 *	0.001 ns	0.007 ns	0.005 ns	0.007 *	0.014 ns	0.29 ns
Error	3.38	4.17	0.00002	0.0002	0.005	0.005	0.00002	0.003	0.004	0.008	0.003	0.001	0.042	0.014
C.V (%)	2.05	2.35	1.18	7.31	3.59	3.4	3.06	0.92	8.72	13.3	9.45	5.22	6.88	13.01

*, ** Indicate significant difference at 5%, 1% probability, respectively while NS indicate no significant differences between treatments

Determination of selected heavy metal contents in maize leaves: Concentrations of Ni, Cu, Mn, Zn, Cr and Fe accumulated in leaves of maize plants growing in the controlled soils T1 and in five treated soils (T2- T6) after three months (Table 4). It is obvious from analysis of variance that the addition of cattle manure at different rates to soil significantly affects the accumulation of heavy metals in leaves of maize (Table 2).There were significant increases in the Ni, Cu, Mn, Zn, Cr and Fe contents of leaves in plants grown in organic fertilizer-treated soil, with the T6 treatment leading to 21.9%, 64.29%, 34.7%, 26.9%, 42.15% and 74.43% increase in Ni, Cu, Mn, Zn, Cr and Fe, respectively, compared with T1.

Determination of selected macronutrient contents in seeds and leaves of maize: Significant effects of the rate of cattle manure (p<0.01) on concentration of selected macronutrient contents in both seeds and leaves of maize were observed (Table 2). Compared with the control, cattle manure amended soil noticeably concentrations of Ca, K and P were increased in the seeds and leaves of maize. The cattle manure was able to encourage a raise on mineral buildup in aerial parts of tested plants. There were increases of 42.11% in Ca, 91.9% in K and 78.98% in P concentrations in the seeds of maize grown in cattle manure treated soil (20 ton ha^{-1}) compared to those grown in control soil. The concentrations of minerals in the leaves of maize were increased by 47.38 % in Ca, 186.35% in K and 157.3% in P in cattle manure treated soil (20 ton ha^{-1}), over the control (Table 5).

Correlation coefficient, multiple correlation and partial regression analysis: Correlation coefficient, multiple correlation and Partial regression analysis were calculated (Tables 6, 7, and 8).

Discussion

Organic manure provides major elements and trace elements that are necessary for the plant, which facilitate plant growth (Han *et al.*, 2016); on the other hand, the movement of heavy metals from soil to the plant and their consequent accumulation in plant organs increase the concentration they receive as major environmental pollutants. The results for the grain and leaves heavy metal content proposes significant alterations in the nutrient grade of maize plants in many cases, where the increase or decrease in plant nutrient contents are resulted from different levels of cattle manure amended. The significant increase of Ni, Cu, Mn, Zn, Cr and Fe in leaves of maize designated that the heavy metals in organic manure are further accessible to plants. Alterations in the response of plants to organic manure application have been described by numerous authors. Zhao *et al*., (2014) demonstrated that application of cattle manure increased the contents of heavy metals in the stem and seed of corn. Other studies has found similar trend for chicken manures (Mandisi *et al.*, 2016). Similarly, it was stated earlier that organic manure application increases, Mg, Fe, Mn and Zn content of annual ryegrass (Yolcu *et al*., 2011a) and Fe and Zn content of Italian ryegrass (Yolcu *et al.*, 2011b) and Hungarian vetch (Yolcu, 2014).

The use of cattle manure in land farming improves soil fertility. Its application as soil amendments could be a good source of macronutrient for various crops with short growth cycles such as maize. Cattle manure at various rates increased concentrations of selected macronutrients in seeds and leaves of maize. This agreed with the results of Fonge *et al*., 2016 who found that leaf macronutrients concentration in *Amaranthus cruentus* L. and *Vernonia hymenolepis* was increased after organic manure addition, demonstrating that this reaction could be predictable in various crops. Numerous other studies have confirmed a positive effect of organic fertilizer-treated soil on plant leaves. Animal manure has been known as an excellent fertilizer to soil and contains most of plant macro and micronutrients (Subramanian & Gupta, 2006). These effects are consistent with those revealed by studies of Ouda & Mahadeen (2008) and Adekiya & Agbede (2009) for vegetable plants in which nutrient content increased with increasing organic fertilizer rate. Similarly, it was stated previously that organic manure increased P and K contents of tall wheatgrass (Butler & Muir, 2006) and Italian ryegrass (Yolcu *et al*., 2011b); K and Ca content of annual ryegrass (Yolcu *et al*., 2011a) .Organic manure releases nutrients slowly and is not leached easily, producing a long remaining effect in the soil, which, when accompanied with easily absorbed macronutrients, enhance progress in enlargement (Kristaponyte, 2005). The rise in macronutrients uptake with cattle manure amended treatments in this study was a consequence of microbial activity which improved the rate of nutrient mineralization (Rathod *et al*., 2013) resulted the increase in the availability of these nutrients in the soil.

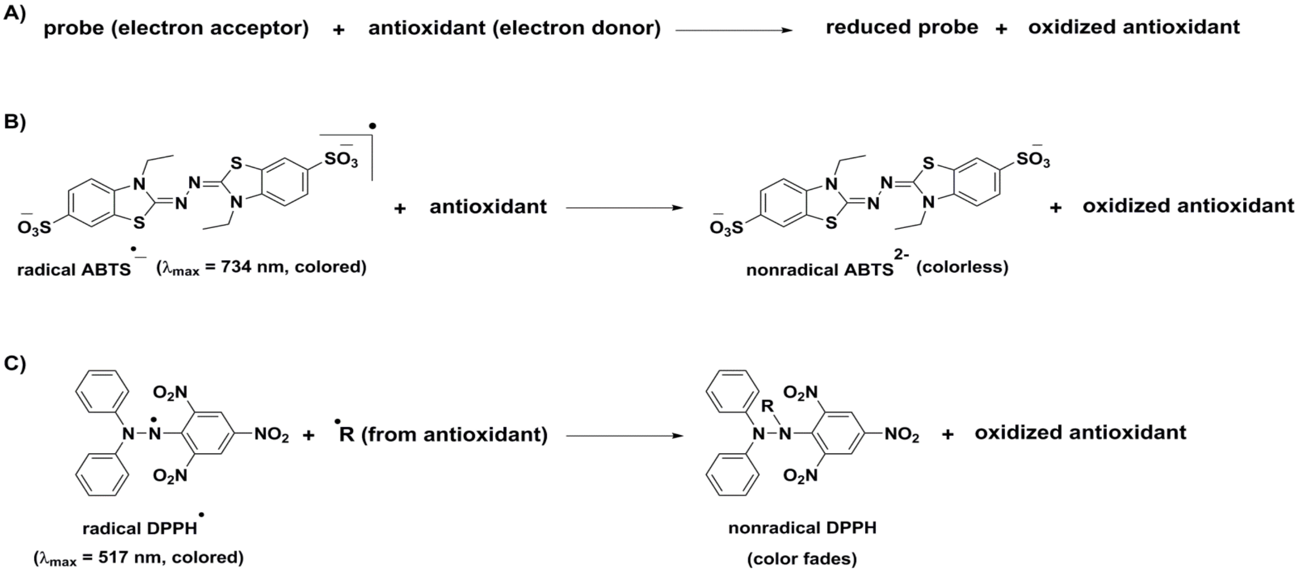

Fig. 1. Chemistry behind ET-based assays including ABTS and DPPH assays.

Table 4. Concentration g/kg of selected heavy metal contents in leaves of maize (*Zea mays* L.) plants grown at different levels of cattle manure amended soil in Jordan Valley.

Treatments	Ni	Cu	Mn	Zn	Cr	Fe
T1	3.2 f	15.4 c	16.5 c	11.3 b	1.3 d	42.6 f
T2	3.3 e	16.3 c	17.3 cb	13.1 a	1.3 d	55.1 e
T3	3.5 d	16.2 c	18.3 b	13.3 a	1.5 c	60.4 d
T4	3.6 c	20.7 b	18.4 b	13.8 a	1.7 b	68.1 c
T5	3.7 b	25.4 a	21.9 a	14.0 a	1.7 a	73.1 b
T6	3.8 a	25.3 a	22.3 a	14.4 a	1.8 a	74.3 a

*Means within column followed by the same letters are not significant at LSD 5%

Table 5. Concentration g/kg of selected mineral contents in seeds and leaves of maize (*Zea mays* L.) plants grown at different levels of cattle manure amended soil in Jordan Valley.

	Seeds			Straw		
T	Ca	K	P	Ca	K	P
T1	1.9 c	4.7 d	4.3 d	6.5 d	14.8 e	1.8 d
T2	2.4 b	5.7 dc	4.9 cd	7.4 c	22.2 d	2.2 d
T3	2.5 ab	6.9 bc	5.2 c	7.6 c	32.2 c	2.3 d
T4	2.6 ab	7.4 b	6.0 b	8.7 b	33.1 bc	2.9 c
T5	2.7 ab	7.8 ab	6.7 b	9.2 ab	36.0 b	3.7 b
T6	2.7 a	9.0 a	7.8 a	9.6 a	42.4 a	4.6 a

*Means within column followed by the same letters are not significant at LSD 5%

Table 6. Correlation coefficients between antioxidant activity of corn leaves (using ABTS radical scavenging activity method) and selected heavy metal contents and minerals in leaves and seeds of maize (*Zea mays* L.) plants.

Leaves	Ni	0.67 **
	Cu	-0.79 **
	Mn	0.64**
	Zn	0.83 **
	Cr	0.68 **
	Fe	0.76 **
Seeds	ca	0.67 **
	k	0.52 *
	p	0.83 **
Leaves	ca	0.73 **
	k	0.77 **
	p	0.69 **

Table 7. Multiple correlation analysis between radical scavenging activity of leaves using ABTS method and each of leaves heavy metals (a) and mineral contents in seeds (b) and mineral content in leaves (c).

	a	b	c
Multiple R	0.86	0.89	0.79
R Square	0.74	0.78	0.62
Adjusted R Square	0.60	0.74	0.54
Standard Error	2.06	1.67	2.22

Table 8. Partial regression analysis of radical scavenging activity using ABTS method with heavy metals in leaves (a) mineral contents in seeds (b) and mineral contents in leaves (c).

		Partial regression coefficient
a	Ni	-42.54
	Cu	38.96
	Mn	2.95
	Zn	20.59
	Cr	-82.14
	Fe	3.33
b	Ca	45.65 *
	K	-8.48
	P	23.10 *
c	Ca	6.58
	K	1.71
	P	3.46

*: Significance at probability level ($p<0.05$)

Antioxidant capacity has been widely used for different plants extracts (Fallaha *et al.*, 2018). The results of this study suggest that the application of cattle manure can improve antioxidant capacity of the maize leaves extracts. Increased antioxidant activity due to cattle manure addition was in agreement with earlier studies. Fauriel *et al.,* (2007); Hallmann and Rembialkowska (2007) reported that practices of organic agriculture, compared to conventional practices, improved the content of antioxidants of peaches and tomato plants. The antioxidant capacity of basil and *Labisia pumila* was improved by addition of poultry manure compared to chemical fertilizers (Ibrahim *et al.*, (2013); Pandey *et al.,* (2016). Similarly, Hassan *et al.,* (2012) described that organic manure enhanced activity of antioxidants in *Cosmos caudatus*. The improvement in leaves antioxidant activity with the application of organic manure could be attributed to different effects including indorsing effect of cattle manure regimes on pathways of secondary metabolites of plants (Pandey *et al.*, 2015), availability of macro and micronutrients in cattle manure for longer periods of time which are involved in some of the chemical reactions responsible for antioxidant activity in cells (Pandey *et al.*, 2015; Pandey & Patra (2015) as well as to larger bioactive compounds contents than those created by traditional cultivation (Barański *et al.*, 2014).

Table 6 displays the coefficients of correlation between antioxidant activity of maize leaves (using ABTS radical scavenging activity method) and selected heavy metal contents and minerals in leaves and seeds of maize (*Zea mays* L.) plants. Zn, Fe, Cr, Ni and Mn had a positive influence on antioxidant activity in leaves. Only Cu had a negative effect on antioxidant activity in leaves. Moreover, increasing concentration of Ni, Mn, Zn, Cr, and Fe in leaves as a result of cattle manure lead to increase antioxidant activity. The antioxidant activity of corn leaves had a significant positive correlation with Ca, K and P concentration in seeds and leaves of corn.

In order to determine the effect of heavy metals in leaves and mineral contents in seeds and leaves on antioxidant activity, multiple correlation analyses were carried out (Table 7). The multiple correlation coefficients is 0.86, 0.89, 0.79 of antioxidant activity using ABTS method on the basis of heavy metals in leaves, mineral contents in seeds and mineral content in leaves, respectively. This indicates that the correlation among the independent and dependent variables is positive. The coefficient of determination, R^2, is 74 %, 78% and 62%. This means that the variation in the dependent variable (antioxidant activity) is explained by the independent variables (heavy metals in leaves, mineral contents in seeds and leaves, respectively).

The significance of partial regression coefficients was also tested. Partial regression analysis of radical scavenging activity related to heavy metals in leaves, mineral contents in seeds and leaves are given in Table 8. Radical scavenging activity showed a significant partial regression coefficient with Ca and P in seeds.

Conclusion

In conclusion, this study indicates that introducing of cattle manure to the soil can result in an increased antioxidant activity of leaves as well as elevate the concentration of Ca, K and P in both seeds and leaves of maize, even at the lowest level. The benefits obtained from cattle manure and availability with low prices throughout the year makes cattle manure as an ideal option to fertilize plants in Jordan valley. Further studies are needed to reduce the harmful effect of heavy metals in leaves of plants grown in soil amended with cattle manure.

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