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Efficient Eco-Friendly Organic Wastes Mixed with Growth Promoting Bacteria to Remediate and Increase Fertility of Saline Sodic Soil in Egypt

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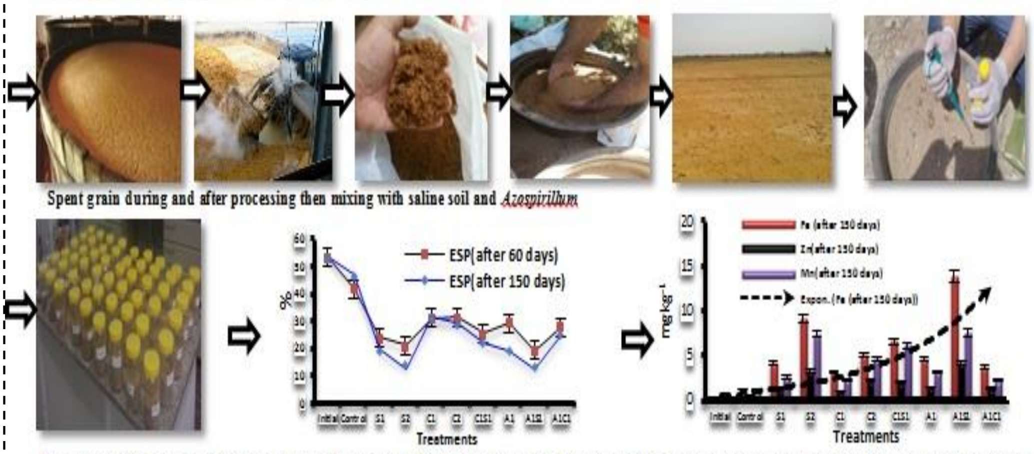
HIGHLIGHTS:

1- Spent grain is a costless by-product which can be used for saline-sodic soil reclamation.
2- The combined application of *Azospirillum* with spent grain decreased the ESP in soil.
3- SOC, pH, Dehydrogenase, and Total acidity were the main factors affecting ESP and then influencing, CEC and Ex-Na$^+$.
4- Variable-rate compost application was more expensive than the spent grain and *Azospirillum*.

GRAPHICAL ABSTRACT:



Azospirillum inoculation with spent grain decreased ESP and enhanced soil fertility without cost, compared to any other reclaimed material.

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ABSTRACT

In Egypt, the total area of agricultural land is 3.36 million acres, which is 3.8 % of the entire territory of the country. One of the main obstacles to agricultural production in Egypt is soil salinization and degradation. Therefore, saline-sodic soil reclamation in arid regions is highly relevant. This study aimed to use *Azospirillum* inoculation with eco-friendly organic wastes for free remediation of saline-sodic soils. In this work nine treatments included two levels of spent grain (S1 and S2), two levels of compost (C1 and C2), a mix of both sources (C1S1), one level of *Azospirillum* (A1), a mix of both sources with *Azospirillum* (A1S1 and A1C1) and an untreated control. The treatments were previously incubated with soil at field capacity for five months under laboratory conditions at 28° C. The most relevant chemical and biological parameters were analysed every month for five months. Results indicate that *Azospirillum* inoculation with spent grain increased soil organic carbon (SOC), dehydrogenase and urease enzymes, micro-nutrients (Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} and B^{+}), and macro-nutrients (N, P and K); while decreased exchangeable sodium percentage (ESP), pH and EC, by 75 %, 12%, and 43 % respectively, compared to initial conditions. The significant variation was observed in chemical and biological properties among all treatments in the order of S2>A1S1>A1>C2>C1S1>A1C1>C1>control. In conclusion, the addition of *Azospirillum* with spent grain is highly recommended for amelioration of the saline-sodic soil and is more effective compared with compost to remediate saline-sodic soils.

Keywords: Soil incubation, *Azospirillum,* amelioration, dehydrogenase, spent grain, saline-sodic

INTRODUCTION

One of the main obstacles to agricultural production in Egypt is soil salinization, which lead to land degradation (Rashad *et al*., 2010). Soil salinization and degradation subsequent to salinization is a key environmental impediment that severely influences agricultural productivity and sustainability in arid and semi-arid regions (Qadir *et al*., 2008). Globally salt-affected soils account for approximately 46 million hectares (20%) of agricultural land (Ahmad *et al*., 2013). More than 60 % of salt-affected soils classified as saline-sodic soils, the saline-sodic soils possess EC >4 dSm^{-1}, pH >8.2 and exchangeable sodium percentage (ESP) >15 % (Rath and Rousk, 2015). The remediation of saline-sodic soils requires removal of Na^{+} ions from the exchange areas and leaching of the substituted Na^{+} out of the root zone in percolating water (Sastre-Conde *et al.*, 2015; Zhang *et al*., 2019). The chemical amendments as desulfurization gypsum open a source of Ca^{2+} to substitute the exchangeable sodium from the cation exchange areas (Luo *et al*., 2018), which plays a very important role in the ameliorant of saline-sodic soils. The worldwide expansion of saline-sodic soils is increasing due to

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mismanagement and using low quality of irrigation water. The soil called saline-sodic due to its high ESP, cultivation of saline-sodic soils faces many challenges, such as poor soil structure, low soil stability aggregates, soil crusting, low hydraulic conductivity, low water infiltration, low water holding capacity, bulk density is high, alkalinity, high Na^+ concentrations in soil solution, low soil fertility (Dodd *et al*., 2013; Rath and Rousk, 2015), low biological activity, nutrient deficiencies, microbial community and soil fertility (Zhang *et al*., 2019). Effects of salinity, including low agricultural productivity, low economic returns and soil erosion (Hu and Schmidhalter, 2005). The salt effects are the results of complex interactions between the physiological, biochemical and morphological processes, including plant growth, nutrients, seed germination, and water absorption (Shrivastava and Kumar, 2015; Zhang *et al*., 2019). Soil salinity imposes ionic toxicity, osmotic pressure and nutrients (N, Ca, K, P, Fe, and Zn) deficiency and oxidative stress on plants, thus limiting the absorption of water from the soil. Soil salinity significantly reduces the absorption of phosphorus (P) due to phosphate ions are deposited with calcium ions (Bano and Fatima, 2009). Some elements, such as Na^+, Cl^-, and B^+, have specific toxic effects to plants. Excessive accumulation of Na^+ in cell walls can quickly lead to osmotic pressure and cell death (Munns, 2002). Soil organic amendments can improve soil physical and chemical properties and microbial activity in saline-sodic *(Luo et al., 2018; Zhen et al*., 2014)

Application of agro-industrial organic wastes as soil amendments such as the Brewers' spent grain, 85 % of the total by-products generated and compost with bacteria inoculation to reclamation saline soil. Organic additives have been used from the residues of the beryl industry, these wastes are inexpensive, and their accumulation causes severe damage to the environment (Hafez *et al*., 2019; Mussatto, 2014; Nassar *et al*., 2014). Spent grain has a negative value for environmental quality due to its accumulation as industrial waste. It has been recently applied as an environment-friendly organic waste to the soil, as well as composting of agricultural biomass, to improve physical and chemical properties of calcareous and sandy soils (Hafez *et al*., 2019; Rashad *et al*., 2016). Spent grain is acidic, rich in macro and micronutrients, amino acids, and vitamins as well as cellulose, hemicelluloses, lipid, protein, arabinoxylan, ash, and lignin (Mussatto and Roberto, 2006). Therefore, it is a good candidate for recycling in agriculture. The environmental systems using a low level of external input have become more important all over the world in the last few years from natural resources, and reduction of environmental systems degradation (Mader, 2002)

In this context, organic wastes incubation with bacteria encourages the use of agricultural practices and alternative technologies, according to economic situation, social and environmental conditions of the region the system is in particular because it refers to fertilization, which is usually

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done by application of organic matter with *Azospirillum* bacteria such as spent grain, compost, plant residues, or manure, a lot of which are produced within the same farm (Hafez *et al*., 2019).

On the other hand, application of eco-friendly organic wastes and bacteria to the saline-sodic soil not only generates a better food situation, but moreover, positively affects other properties such as soil particle assembly, water holding capacity and increase biological activity (Hafez *et al*., 2019; Hayat *et al*., 2010; Scherer *et al*., 2011), contribute to generating high production, even with low application or none of the fertilizer. In the aspect incubation system for organic wastes with micro-organisms are found in soil (Zhuang *et al*., 2007) incubated decreased soil salinity and increased the content of nitrogen, phosphorus, and potassium (Rashad *et al*., 2016; Rojas-Tapias *et al*., 2012). The application of biofertilizers, plant growth promoters and organic amendments enhancing soil fertility, microbial activities and salt-affected. In addition, plant growth-promoting bacteria (PGPB) have been recently applied as a biofertilizer in soil to increase nitrogen fixation and phosphate availability and reduce soil salinity (Hafez *et al*., 2019; Rojas-Tapias *et al*., 2012; Zhen *et al*., 2014;Shrivastava and Kumar, 2015; Zarea *et al*., 2012).

One of the PGPB is the *Azospirillum brasilense*, this is free-living bacteria capable of affecting the growth and seed yield of numerous plant species and many agronomic and ecological significance. Plants treated with PGPB producing bacteria display increased resistance to salinity stress due to improved soil structure (Karagöz *et al*., 2012; Zhuang *et al*., 2007). PGPB can also bind to cations including Na^+ thus making it unavailable to plants under saline conditions. (Zhao *et al*., 2018). Egypt, is located in the arid region therefore, the study of salinity in such dry land area has become of great interest. We hypothesized that the applied spent grain with *Azospirillum* inoculation, for tow period 60 and 150 days of incubation, would reclaimed saline-sodic soil while increasing soil fertility and reduce the ESP. The objectives of this work were to (i) the effect of eco-friendly organic wastes doses with *Azospirillum* inoculation on some chemical and biological properties of saline-sodic soil in the North-Western part of Egypt on reduce soil salinity, (ii) increase the content of beneficial microbes and activity of enzymes, and (iii) increase soil fertility in regards to the SOC improve the availability of macro and micro-nutrients in saline-sodic soil after 60 and 150 days of incubation.

2-MATERIALS AND METHODS

2.1. Site Information

The study sites are located at the farm of El-Bangar village 28, El-Hamam City, Matrouh Governorate, North-Western part of Egypt (30°45'44.47"N 29°25'26.54"E). The soil samples are saline-sodic typically mixed with rocks. Due to its inadequate soil, water regime, salinity in arid regions can occur when the water table is between two and three meters from the surface of the soil. The use and potential effect of soil enhancer materials are important and current raise. The study site

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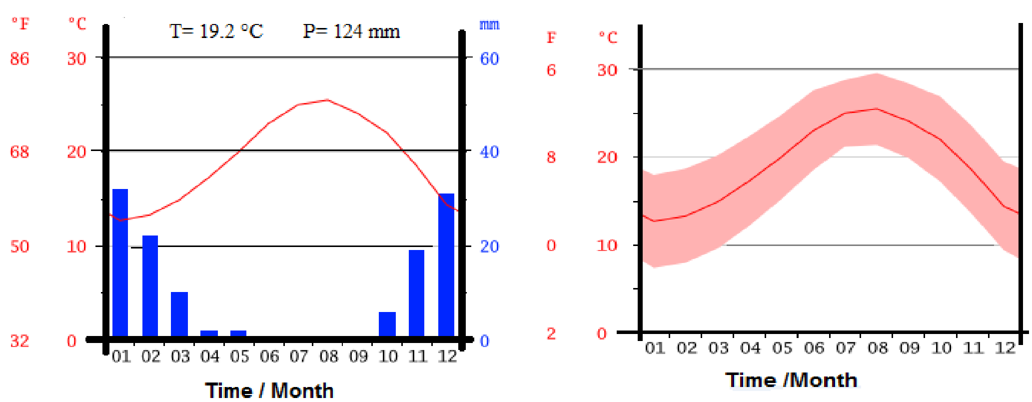
has a desert climate arid, hot summer and semi-cool and semi-wet winters. The climate is classified as BWh by the Köppen-Geiger system (Climate-Data.org, 2019). The average air temperature in El-Hamam is 19.2 °C. In a year, Annual precipitation is 124 mm. The temperatures are higher on average in August, at around 27.5 °C. In January, is the coldest month the average temperature is 12.7 °C. It is the lowest average temperature of the whole year (Fig 1). The soil samples were granules with diameters less than 2- mm, and used after the sieving process. The samples of the airdried soil were used for the physical and chemical parameters of the saline-sodic soil are shown in (Table 1).

(Table.1) Initial physical and chemical parameters of saline-sodic soil used for the study

Soil parameter	**Saline-sodic soil**
pH (1:2.5 w: w)	8.84 ± 0.05
EC_e (dS m^{-1})	5.43 ± 0.10
Total N (gkg^{-1})	0.02 ± 0.001
Available P ($mgkg^{-1}$)	1.20 ± 0.07
Available K^+ ($mgkg^{-1}$)	78.1 ±0.44
Total $CaCO_3$ (%)	18.6 ± 1.35
CEC ($Cmol^+$ kg^{-1})	7.56 ± 0.24
Organic Matter (g kg^{-1})	1.78 ± 0.21
Soil Organic Carbon (g kg^{-1})	1.03 ± 0.01
Total DOC (%)	0.012 ± 0.003
ESP (%)	53.1 ± 1.93
C/N Ratio	49.14 ± 2.1
Sand (%)	31.2 ± 0.11
Silt (%)	23.5 ± 0.21
Clay (%)	45.3 ± 0.18
Texture	Clay Loam
Micronutrients DTPA Extractible ($mgkg^{-1}$)	
Fe^{2+}	0.08 ± 0.01
Zn^{2+}	Nd^*
Mn^{2+}	0.11 ± 0.020
Cu^{2+}	0.003 ± 0.01
B^+	Nd^*
Cl^-	192 ± 0.166

Data correspond to means of four replicates ± standard deviation. Nd^*= not detected



(Fig. 1). Annual precipitation and temperature study area of soil (Climate-Data.org, 2019).
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2.2. Soil Treatments and Azospirillum preparation

Three types of treatments (*Azospirillum*, compost and spent grain) were used. The *Azospirillum* bacteria, it was from Faculty of Agriculture, Ain Shams, Cairo Governorate, Egypt. The compost consisted of plant and animal wastes from the national factory for the production of compost, Alexandria, Governorate, Egypt. The spent grain, a by-product from beer industry, was obtained from Al Ahram Beverages Company, Abu Hammad, Al Sharkia Governorate, Egypt (Schema, 1). The characteristics of the treatments were determined according to the standard procedures shown in (Table 2). *Azospirillum brasilense* (Sp245) was cultured; growth and inoculation were performed as described by (Pii *et al*., 2015b). Briefly, the *Azospirillum* bacterium was grown for 4 days in LB medium (10g L^{-1} triptone, 5g L^{-1} NaCl, 10g L^{-1} yeast extract) with continuous shaking at 30°C. Bacteria were then centrifuged for 15 min at 4500 x g and washed four times with sterile saline-sodic solution (NaCl 0.85% w/v) after that the *Azospirillum* bacteria became ready for inoculation with the soil.

2.3. Experimental design and treatments management

The experiment was carried out during the summer of 2017 laboratory of the Department of Land and Water Technologies, City of Scientific Research and Technological Applications (SRTA-City), and analyses were done between the (SRTA-City) and laboratory of the Department of Soil Science and Soil Ecology, Saint Petersburg State University (SPBSU). The incubation experiment was nine treatments included two levels of spent grain, were referred to 12.8 gkg^{-1} soil (S1) and 25.5 gkg^{-1} soil (S2); two levels of compost, were referred to 23.5 gkg^{-1} soil (C1), and 47 gkg^{-1} (C2); one level of *Azospirillum brasilense* inoculation (A1) was 5 % by weight of the soil; a mix of both sources spent grain and compost (C1S1); a mix of both sources with *Azospirillum* (A1C1) and (A1S1); all treatments were used and compared to the control without amendments. All treatments were incorporated with bottle 400g of saline-sodic soil. To let added treatments to affect soil properties, all bottle treatments were incubated under laboratory conditions, without plants, for five months at 28° C. The bottles were distributed in a randomized complete block design with four replications of each treatment. All bottles were watered with tab deionized-water once to field capacity of the soil. The most relevant chemical and biological parameters were analyzed every month for five months.

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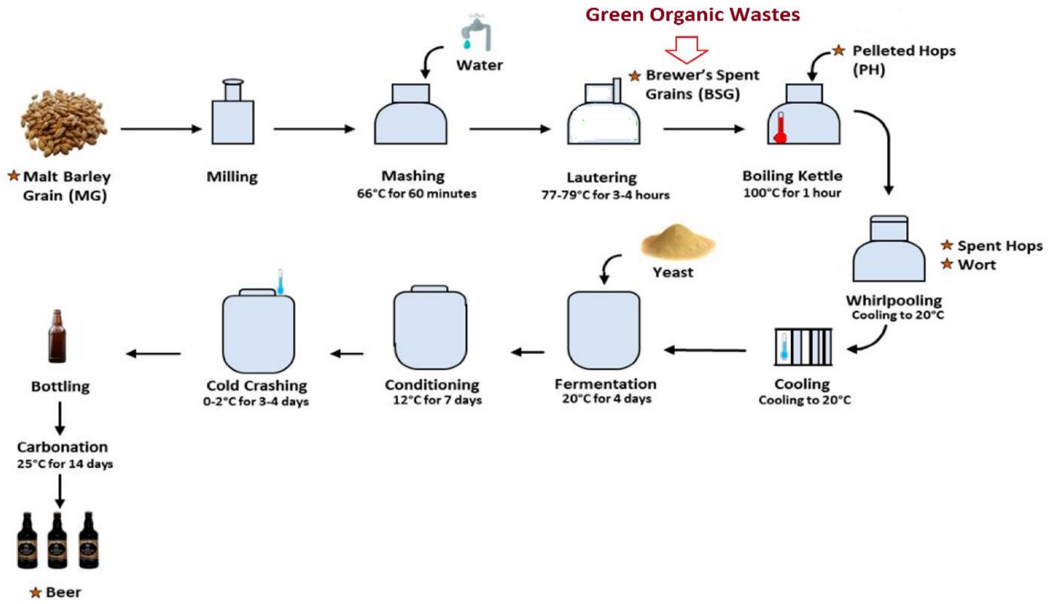

(Schema 1). Schematic beer brewing process and product eco-friendly organic wastes "spent grain" description of extraction method by (Lordan *et al*., 2019).

(Table, 2). Organic wastes characteristics (oven-dry weight basis).

Soil parameter	Compost	Spent grain
pH (1:5 w:w)	7.20 ± 0.01	4.16 ± 0.03
EC (dS/m, 1:5 w:w)	5.81 ± 0.21	1.45 ± 0.21
Organic Matter (g kg^{-1})	332 ±1.23	750 ± 0.57
Total N (%)	2.10 ± 0.32	3.12 ± 0.68
Total P (%)	1.03 ± 0.52	1.86 ± 0.54
Total K (%)	0.57 ± 0.01	1.74 ± 0.63
C: N ratio	9.16 ± 0.35	13.9 ± 0.12
Organic carbon (%)	19.25 ± 0.12	43.5 ± 0.94
Fe^{2+} (mgkg^{-1})	960 ± 2.97	1130 ± 3.87
Zn^{2+} (mgkg^{-1})	220 ± 5.34	368 ± 2.34
Mn^{2+} (mgkg^{-1})	100 ± 2.14	210 ± 1.98
Cu^{+} (mgkg^{-1})	61 ± 1.21	98 ± 1.54

Data correspond to means of four replicates ± standard deviation

3.4. Sampling

3.4.1. Soil physicochemical analyses

The total thirty-six samples referred to nine treatments × four replications, soil samples it was taken from each bottle after the removal of visible roots and fresh litter material, the composite samples were sieved (2 mm) and then stored at room temperature for less than three months until chemical analyses were performed. Samples were air-dried and ready for measured. The soil pH was measured potentiometrically in a soil : water suspension 1:2.5 w/v (Page, *et al*., 1982). The electrical

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conductivity (EC_e) was measured in saturated paste extracts using an EC meter (Corwin and Yemoto, 2017). The total nitrogen (N), available phosphorus (P), and available potassium (K) were measured using (Anderson, *et al*., 1982). Micronutrients (Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and B^{+}) were extracted by DTPA solution as explained by (Soltanpour and Schwab, 1977) then measured with inductively coupled plasma (ICP) atomic emission spectroscopy. Exchangeable sodium percentage (ESP) of soil was determined using the relationship ESP = (exchangeable sodium (Ex Na^{+}) / cation exchange capacity (CEC))*100. Extractable sodium was extracted with 1 M ammonium acetate solution (Normandin *et al*., 1998). Soil CEC was estimated following the Bower saturation method as outlined by (Richards, 1954).

3.4.2 Biological analyses

Dehydrogenase activity was determined by triphenyltetrazolium chloride (TTC) as described by (Casida *et al*., 1964). Soil urease (S-UE) was determined using methods previously published by (Kandeler and Gerber, 1988). Soil organic carbon (SOC) concentration was determined by oxidization with $K_2Cr_2O_7$ (Ouyang *et al*., 2013).

Statistical analysis

Statistical analysis was carried out using of the SPSS v.16 software (Visauta, 2007). Data were submitted to a normality test before the analysis of variance. When statistical significance was found ($P \leq 0.05$), a comparison of the means was carried out by using the Tukey test. Furthermore, a Pearson correlation analysis was carried out to observe the degree of association between some of the studied variables.

RESULTS AND DISCUSSION

Several factors could control eco-friendly organic wastes decomposition. In the present study, organic types, decomposition condition, and soil properties are the dominant factors that control the decomposition processes to reclamation saline-sodic soil. The concentrations of nutrients in the incubated soils were determined as an indication for soil fertility. The chemical and biological properties are discussed and presented separately. Chemical properties, including soil acidity (pH), electrical conductivity in saturated paste extract (EC_e), exchangeable sodium percent (ESP), cation exchange capacity (CEC), exchangeable sodium (Ex-Na^{+}), soil organic carbon (SOC), humic substance (HS), total acidity (TA), available phosphorus (P), available potassium (K^{+}), total nitrogen

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(N), micro-nutrients (Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and B^{+}), dehydrogenase and urease enzymes in saline-sodic soil after 60 and, 150 days of incubation.

3.1. Chemical properties changed as a soil reclamation indicator after the incubation period

The soil chemical properties including, pH, ESP, EX-Na^{+}, EC_e and CEC after 60 and 150 days of incubation, were significantly changed with increasing eco-friendly organic wastes (Table 3). Soil soluble and exchangeable salts content statistically significantly decreased with increasing eco-friendly organic wastes and *Azospirillum* application ($P<0.05$).

Table (3): Soil salinity indicator in saline-sodic soil after 60 and 150 days of incubation

Treatments	Incubation Period	pH	EC_e	Ex-Na^{+}	CEC	ESP
	days		dsm^{-1}	$meq100g^{-1}$	$Cmol+kg^{-1}$	%
Initial	**Zero time**	**8.84±0.05**	**5.43±0.10**	**4.00±0.18**	**7.56±0.24**	**53.12±1.93**
Control	60	8.76±0.05	5.42±0.10	3.39±0.21	7.93±0.24	42.78±1.93
	150	**8.94±0.08**	**5.48±0.02**	**3.83±0.28**	**8.26±0.64**	**46.49±4.16**
S1	60	8.34±0.12	4.11±0.07	3.24±0.29	14.23±0.49	22.76±1.46
	150	**8.03±0.06**	**3.66±0.23**	**2.90±0.26**	**15.03±0.25**	**19.28±1.57**
S2	60	8.10±0.11	3.10±0.12	3.06±0.11	16.20±0.83	18.91±1.73
	150	**7.83±0.10**	**3.00±0.17**	**2.43±0.06**	**17.80±0.2**	**13.66±0.2**
C1	60	8.51±0.04	5.17±0.13	3.96±0.058	12.33±0.31	30.67±0.33
	150	**8.60±0.06**	**5.42±0.10**	**4.46±0.15**	**14.43±0.51**	**30.99±2.01**
C2	60	8.47±0.04	5.41±0.21	4.57±0.44	15.67±0.40	29.34±2.09
	150	**8.58±0.05**	**5.70±0.53**	**4.63±0.56**	**16.03±0.25**	**28.86±3.14**
C1S1	60	8.17±0.36	4.65±0.32	5.50±0.20	14.90±0.20	26.90±0.35
	150	**8.06±0.43**	**4.67±0.02**	**3.50±0.26**	**15.93±0.11**	**21.97±1.76**
A1	60	7.98±0.03	3.84±0.18	2.19±0.30	8.83±0.24	24.89±4.14
	150	**7.86±0.13**	**3.54±0.35**	**1.75±0.18**	**9.26±0.92**	**19.13±3.72**
A1S1	60	7.93±0.06	4.51±0.08	3.06±0.11	15.83±0.29	19.36±0.63
	150	**7.89±0.05**	**4.03±0.07**	**2.07±0.11**	**16.12±0.33**	**12.85±0.49**
A1C1	60	8.24±0.18	4.674±0.03	4.00±0.15	14.25±0.35	28.07±0.83
	150	**8.23±0.01**	**4.485±0.03**	**3.54±0.36**	**14.30±0.42**	**24.72±1.83**
$LSD_{0.05}$	60	0.275	0.892	2.051	0.827	13.72
	150	**0.278**	**0.413**	**0.475**	**0.801**	**4.180**

Data correspond to means of four replicates ± standard deviation. $LSD_{0.05}$= least significant difference (LSD) test at ($p \leq 0.05$).

3.1.1. The soil pH changed after the incubation period

The influences of the eco-friendly organic wastes incubation with *Azospirillum* inoculation on the decrease soil pH compared to initial condition and control treatment followed the order: S2 (spent grain 25.5 gkg^{-1}) > A1 (5% of soil weight) > A1S1 (Mix A1 and S1) > S1 (spent grain 12.8 gkg^{-1}) > C1S1 (Mix C1 and S1) > C2 (47 gkg^{-1}) > C1 (Compost 23.5 gkg^{-1}). Shown (Fig 2) soil pH after 60 days of incubation was higher in the soil initial condition compared to the spent grain (S2) and *Azospirillum* (A1), treatments were 8.10, 7.93 and 7.98, for the treatments S2, A1S1, and A1 respectively, ($P<0.05$). While the corresponding values after 150 days of incubation were 7.83, 7.86 and 7.89, for the S2, A1 and A1S1 respectively, ($P<0.05$). The inherent soil pH values after 150 days

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were between 7.8 and 7.89 are ideal for P-availability, while pH values below 5.5 and between 7.5 and 8.5 limits P-availability to plants due to fixation by aluminum, iron, or calcium (Rashad *et al*., 2016). The S2 treatment affected positively the soil pH greater than compost treatments and control. Accordingly, this reduction in the pH values might be due to increase of the groups of carboxyl and phenol as a result of decomposing of eco-friendly organic wastes and the reduction of bicarbonate in the soil. (Hafez *et al*., 2019) reported that a decrease of pH might be due to the addition of spent grain only or with *Azospirillum* inoculation in calcareous and sandy soils reduces soil pH sharply. The difference in the pH values between the S2 and the A1S1 was a highly significant camper to initial and control. The remediation of saline-sodic soils by organic matter sources has long been known to facilitate the reclamation of saline-sodic soils (Sastre-Conde *et al*., 2015).

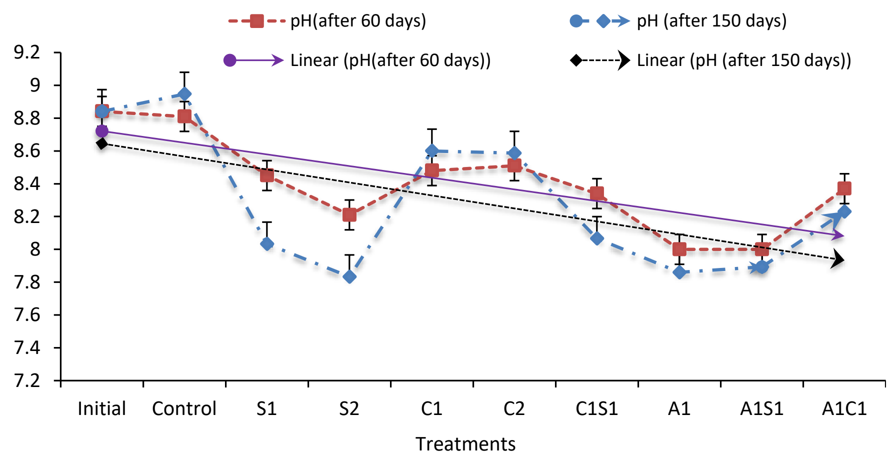

Figure (2): Effect of *Azospirillum* and eco-friendly organic wastes on pH in saline-sodic soil after 60 and 150 days of incubation. Data points and error bars represent means and standard errors (n = 4).

3.1.2. Soil ESP indicator

The soil ESP was decreased with the spent grain and *Azospirillum* increasing (P<0.05). The soil ESP was statistically significantly higher in the inoculation *Azospirillum* with spent grain in saline-sodic soil than that in the compost and control treatments (P<0.05). In the present study, the middle and high salinities exhibited greater salt content and ESP, and less Table (3) and Figure (3). The ESP after 60 days of incubation in the saline-sodic soil was 53.10, 19.35, 20.91 and 23.64 % for the initial condition, A1S1, S2, and S1, respectively. The corresponding percentages for the 150 days was similar to the trend of pH were 53.10, 12.85, 13.66 and 19.28 % for the initial condition, A1S1, S2, and S1, respectively. The addition of spent grain with *Azospirillium* (A1S1) to the saline-sodic soil contributed a statistically significantly lower amount of salt to the soil. The spent grain decreased the amount of exchangeable Na^+ and salinity than the compost and initial condition, this reducing for

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ESP due to mixing *Azospirillium* with spent grain enhancement of the soil activity of carbon degrading extracellular enzymes and microbial decomposition rates. There was a significantly positive correlation between soil salt content and eco-friendly organic wastes amendments. Soil microbes are essential in soil carbon mineralization and accumulation, which determines soil fertility.

The *Azospirillium* increased microbial biomass carbon which decreased the exchangeable Na^+ in soil solution, this result was positively correlated with organic wastes incubation in saline-sodic soil. The addition of organic matter played the very important role for decreased soil salinity and enhancement the soil microbial biomass, this result agreement with (Zhang *et al*., 2015, 2019; Zhen *et al*., 2014) demonstrated that the low soil microbial carbon was attributed to the low soil organic carbon that resulted from little carbon input into the soils for the scarcity of vegetation under high salt condition. (Mahmoodabadi *et al*., 2013; Sastre-Conde *et al*., 2015) attributed the decrease of soil EC, SAR, and ESP by organic wastes and chemical amendments under saline-sodic soil. (Meena *et al*., 2019) further demonstrated that lower organic matter in saline soil is more likely to be due to the decreased organic sources than the increased decomposition rate.

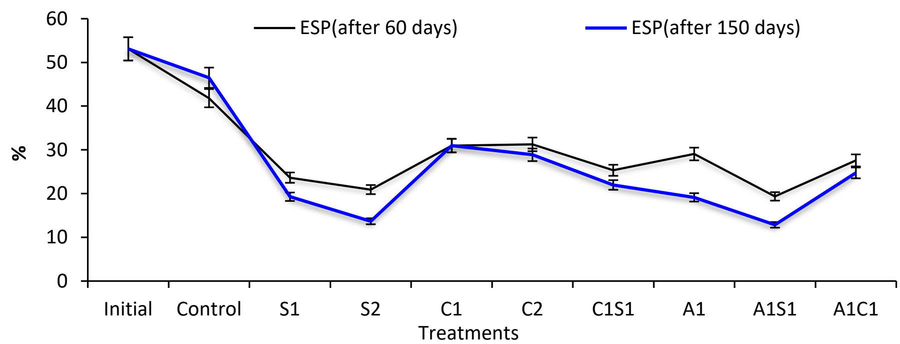

Figure (3): Effect of *Azospirillum* and eco-friendly organic wastes on ESP in saline-sodic soil after 60 and 150 days of incubation. Data points and error bars represent means and standard errors (n = 4).

3.1.3. Soil exchangeable Na^+ and electrical conductivity

The soil Ex-Na^+ was statistically significantly higher in the inoculation *Azospirillum* with spent grain in saline-sodic soil than that in the other treatments (P<0.05). In the present study, the saline-sodic soil before amendments incubation was contained high content of the Ex-Na^+ and ESP, and less organic matter compared to the after amendments incubation (Table 3). The effect of spent grain levels and *Azospirillum* treatments on Ex-Na^+ concentration were consistently higher (P< 0.05) than compost levels, control and the initial (Table 3). The general influences of the *Azospirillum* inoculation with eco-friendly organic wastes incubation on the decrease in the soil Ex-Na^+ concentration compared to initial condition and control treatment, followed the order: A1 >A1S1 >S2 >S1 >C1S1 >control >initial >C2 >C1. Shown (Table 3), the soil Ex-Na^+ for compost treatments after 60 days of incubation were 5.0, 3.80 and 4.0 meq100g^{-1}, for the C2, C1 and A1C1 respectively, ($P<0.05$). While the corresponding values after 150 days of incubation was higher in the compost only and compost with *Azospirillium* treatments compared to the initial condition and control, were 4.6, 4.4 and 3.54 meq100g^{-1} for the treatments C2, C1 and A1C1 respectively. In the control and initial Ex-Na^+ ranged between 3.4 and 4 meq100g^{-1} after 150 days of incubation, while in A1, after 150 days Ex-Na^+ was 1.75 meq100g^{-1}. This confirms that soil salinization was effectively maintained through the control treatment irrigation with saline water, low rainfall since Ex-Na^+ decreased in A1 from 4 in initial to 1.75 meq100g^{-1} for spent grain and *Azospirillium* treatments. The relative reduction in Ex-Na^+ concentration after 150 days corresponded to 56.25 and 48.25% for A1 and A1S1 respectively, compared to the initial condition. Obviously, as the application levels of compost increased, the EC_e and Ex-Na^+ concentrations increased (Table 3). There were significant differences among the treatments in the Ex-Na^+ concentration.

On the other hand, the higher concentrations of EC_e and Ex-Na^+ can lead to deterioration of soil physical properties. The compost treatments was negative a contributed statistically significantly higher amount of EC_e and Ex-Na^+ to the soil compared the control. I think this result due to the compost treatments is contained very high salts content before adding to soil, resulting in increased Na^+ concentration in the compost residues. Therefore, the application of pig compost could increase soil salinity in areas of low precipitation and drainage where the salts are not leached through the soil profile or on borderline saline soils. Therefore, the characterization of organic wastes is mandatory before adding to soil.

On the other hand the *Azospirillum* with spent grain treatments decreased amount of EC_e and Ex-Na^+ than the compost and initial condition, this reducing for the Ex-Na^+ due to spent grain with *Azospirillum* enhancement of the soil microbial activity of carbon-degrading extracellular enzymes, increased humic substance, chelating the sodium in soil to sodium humate form and enhancement of CEC under A1S1 and A1 treatments in saline-sodic soil incubated. Similar results were reported by (Mahmoodabadi *et al*., 2013; Rojas-Tapias *et al*., 2012; Shrivastava and Kumar, 2015; Zhang *et al*., 2015) who reported that the applied organic wastes and PGPB, depending on their chemical composition might have a positive impact on soil properties, especially Na^+ and EC. (Ashraf et al., 2004), who found that inoculation with exopolysaccharide- producing bacteria could restrict Na^+ influx into roots. Further, (Zhang *et al*., 2008) reported that inoculation with *Bacillus* bacteria could mediate the level of salt tolerance in soil.

3.1.4. Cation Exchangeable Sodium Percentage (CEC)

A progressive increase in CEC was observed in all treatments due to the accumulation of Ca^{2+} and Mg^{2+} release with the eco-friendly organic wastes amendments. In particular, CEC

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increased significantly during the spent grain and *Azospirillum* treatments (P < 0.05), when it reached 15.5 and 15.3 Cmol+kg^{-1} in A1S1 and S2 (Table 3) after 60 days of incubation. The A1S1treatment increased CEC and decreased Ex-Na^{+} led to a significant reduction (P < 0.05) for the ESP indicator (Table 3). On the other hand, the corresponding percent for the 150 days was similar to the trend of SOC were 7.50, 17.80, 16.12 and 16.01 Cmol+kg^{-1} for the initial condition, S2, A1S1, and C2, respectively. As a result, in soil, no significant differences were observed between *Azospirillum*, eco-friendly organic wastes, and the control. The effect of *Azospirillum* inoculation with spent grain on the CEC was confirmed when comparing to CEC of the different treatments of compost, (Fig 4). CEC increased by 137.3, 115 and 113.7% in S21, A1S, and C2 respectively, after 150 days of soil incubation. Generally, the reported concentrations in organically treated soil with application rate of *Azospirillum*, spent grain and compost are safe and sufficient for macronutrient consumption. This is results in agreement with other researchers (Shrivastava and Kumar, 2015; Trivedi *et al*., 2017).

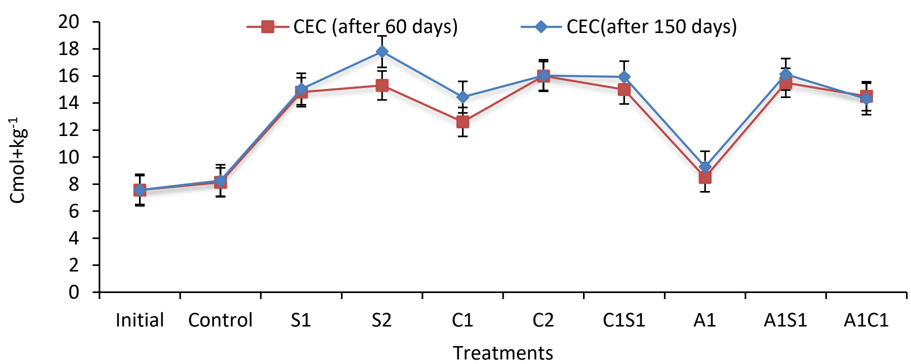

Figure (4): Effect of *Azospirillum* and eco-friendly organic wastes on CEC in saline-sodic soil after 60 and 150 days of incubation. Data points and error bars represent means and standard errors (n = 4).

3.2. Soil biological parameters after incubation period

3.2.1. Dehydrogenase and urease activities

The soil biological parameters dehydrogenase and urease activities showed in Figure (5) and figure (6) revealed that there were significant differences in dehydrogenase and urease activities in saline-sodic soil between all treatments, and also significant differences were recorded when using different levels of eco-friendly organic wastes and *Azospirillum* concentrations followed the order A1S1≥ S2 ≥ A1> C1S1 > C2 > A1C1 ≥ S1 > C1 > control > initial. Also, the differences in dehydrogenase and urease activities were highly significant (P<0.05) when combined with different levels of spent grain with *Azospirillum* or only spent grain, compared with different levels of compost and control. This is results in agreement with other researchers (Liang *et al*., 2014; Meena *et al*.,

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2019), in soils treated with farmyard manure; dehydrogenase activity is increasing. The high organic matter content enhanced the production of microbial activity as a result of soil organic carbon during the mineralization process of organic matter. Generally, the mineralized organic carbon increased as the application rate of spent grain with *Azospirillum* increased. (Liang *et al*., 2014) reported that application of organic amendments increased microbial activity and urease activities in all particle-size fractions as compared to those in control**.** Our results are in the same direction with the findings of (Rojas-Tapias *et al*., 2012; Scherer *et al*., 2011; Shi *et al*., 2019) which proposed that the treatments with high organic amendments might exhibit the greatest microbial activity and dehydrogenase activity. The soil enzyme activities play a very important role in nutrients recycling, making them accessible to plants and microorganisms (Ai *et al*., 2015).

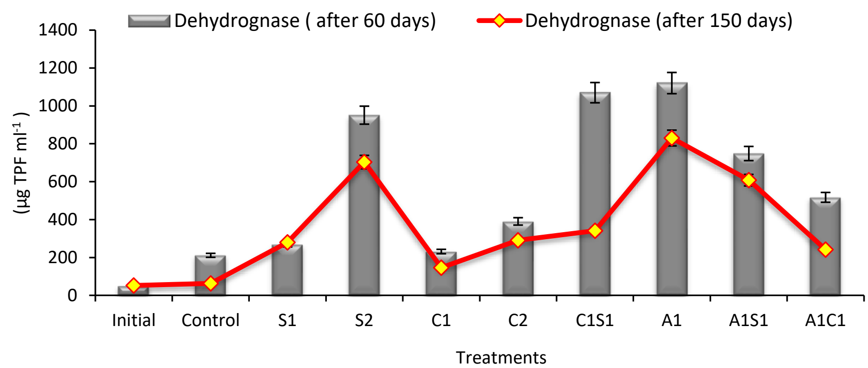

Figure (5): Effect of *Azospirillum* and eco-friendly organic wastes on Dehydrogenase enzyme (µg TPF ml^{-1}) in saline-sodic soil after 60 and 150 days of incubation. Data points and error bars represent means and standard errors (n = 4).

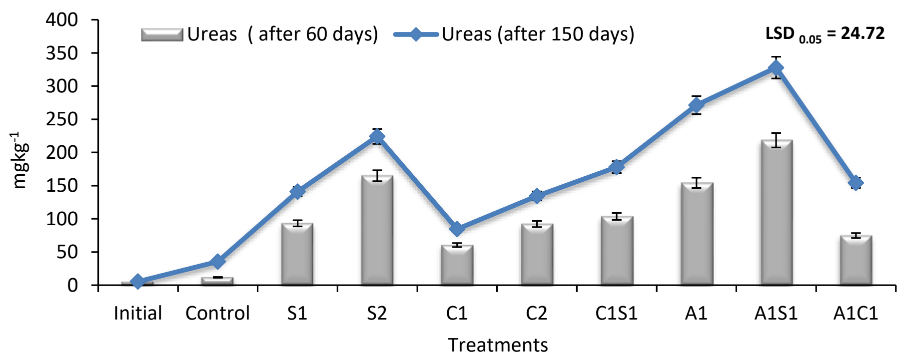

Figure (6): Effect of *Azospirillum* and eco-friendly organic wastes on Urease enzyme in saline-sodic soil after 60 and 150 days of incubation. Data points and error bars represent means and standard errors (n = 4).

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3.2.2. Soil organic carbon

The soil organic carbon (SOC) values are shown in Table (4) and Figure (7). The SOC percentages after 60 days of incubation were 0.1, 0.081, 0.64, 1.16, 0.55, 1.0, 0.98, 0.056, 0.58 and 0.51 for initial condition, control, S1, S2, C1, C2, C1S1, A1, A1S1 and A1C1, respectively. There were significant differences among the treatments. The trend of SOC after 60 days was similar to the trend of SOC after 150 days of incubation, where the SOC percentages were 0.1, 0.0712, 0.54, 1.05, 0.47, 0.88, 0.83, 0.26, 0.50 and 0.39 for initial condition, control, S1, S2, C1, C2, C1S1, A1, A1S1 and A1C1, respectively. The spent grain (S2) possessed the highest values of SOC while the A1 and control showed the lowest value. The spent grain amendments increased the carbon contents in saline-sodic soil up to 12 gkg^{-1}. The use of organic wastes with *Azospirillum* increased saline-sodic soil organic carbon contents. On the other hand, the *Azospirillum* bacteria increased the SOC after 150 days greeter than after 60 days, this result *Azospirillum* increased of decomposition rate was highly significant after 150 days compared with 60 days of incubation. The large increase of SOC and TN amounts with organic amendments application might be attributed to the increased particulate organic matter derived from manure since the organic material added to the soil was initially located in the coarser fraction (Liang *et al*., 2014; Zhang *et al*., 2015). The high organic matter content enhanced the production of soil organic carbon as a result of micro-organisms activity during the mineralization process of organic matter. Generally, the mineralized organic carbon increased as the application rate of spent grain and compost increased**.** The presented results of SOC are in agreement with the results presented by (Luo *et al*., 2018; Nadeem *et al*., 2014; Nassar *et al*., 2014; Scherer *et al*., 2011; Zhang *et al*., 2015)

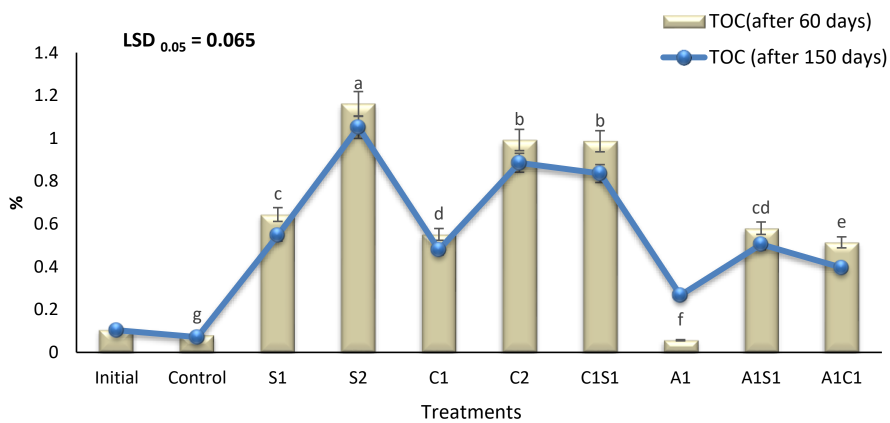

Figure (7): Effect of *Azospirillum* and eco-friendly organic wastes on SOC in saline-sodic soil after 60 and 150 days of incubation. Data correspond to means of four replicates ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) among treatments.

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Table (4): Soil N, P, K and SOC in saline-sodic soil after 60 and 150 days of incubation

Treatments	Incubation Period	Total N	Ava P	Ava K	SOC
	Day	%	mgkg^{-1}		%
Initial	**Zero time**	**0.0021**	**1.2**	**78.0**	**0.1**
Control	60	0.0030	1.95	86.31	0.096
	150	**0.0047**	**2.06**	**92.66**	**0.071**
S1	60	0.0280	3.73	158.47	0.597
	150	**0.0950**	**5.72**	**179.33**	**0.547**
S2	60	0.0633	6.81	268.06	1.175
	150	**0.1482**	**14.28**	**284.33**	**1.052**
C1	60	0.0160	2.66	121.71	0.576
	150	**0.0840**	**3.72**	**145.20**	**0.471**
C2	60	0.0360	5.45	206.24	0.968
	150	**0.1090**	**8.45**	**237.66**	**0.885**
C1S1	60	0.0567	7.22	200.00	1.169
	150	**0.1250**	**10.00**	**237.66**	**0.835**
A1	60	0.0460	8.94	88.366	0.055
	150	**0.1437**	**13.13**	**117.66**	**0.267**
A1S1	60	0.0583	8.81	166.14	0.576
	150	**0.1717**	**9.63**	**195.00**	**0.504**
A1C1	60	0.0324	5.22	139.20	0.501
	150	**0.099**	**7.15**	**180.50**	**0.396**
$LSD_{0.05}$	60	0.005	0.452	11.0	0.208
	150	0.025	0.761	17.74	0.065

3.3. Soil fertility indicator after incubation periods

3.3.1. N, P, and K^+ concentration in saline-sodic soil

The effects of different amendments on soil N are shown in Table (4). *Azospirillum* inoculation not only alleviated the inhibitory effect of salinity on soluble and total macro-nutrients but also induced a marked and progressive increase in N, P and K concentrations. Figure (8) show total N concentrations. Total nitrogen is the sum of nitrate (NO_3^-), nitrite (NO_2), organic nitrogen and ammonia (all expressed as N). The total N percentages for the saline-sodic soil after 60 days of incubation were 0.0021, 0.003, 0.028, 0.063, 0.016, 0.036, 0.056, 0.046, 0.058 and 0.032% for the initial condition, control, S1, S2, C1, C2, S1C1, A1, A1S1 and A1C1 treatments, respectively. The corresponding percentages for the saline-sodic soil after 150 days of incubation were 0.0021, 0.0047, 0.095, 0.148, 0.084, 0.109, 0.125, 0.143, 0.171 and 0.099%. The spent grain with *Azospirillum* inoculation was a good supplier for the total nitrogen at both incubation periods in comparison to the initial total N.

The compost treatment supplied a lower total N in comparison to the *Azospirillum* and spent grain. This is due to the high initial nitrogen percentage in spent grain and enhancements decomposition rate and mineralization process by *Azospirillum* bacteria. The total N was greater after 150 days of incubation than after 60 days of incubation in saline-sodic soil. Is in accordance with the report of (Liang *et al*., 2014; Sastre-Conde *et al*., 2015; Yu *et al*., 2019; Zhang *et al*., 2015), that a majority part of organic fertilizer is released to the soil N, P and K^+ after application of agricultural waste compost and bio-fertilizer in saline-sodic soils. Accordingly, the spent grain with *Azospirillum* used in the present study can supply a growing plant with plentiful of nitrogen after 60 and 150 days of incubation and this period was enough for decomposition organic matter and mineralization of nutrients. Soil microbial communities regulate principal ecosystem processes such as soil nutrient cycling and organic matter formation and decomposition (Sant'Anna *et al*., 2018; Trivedi *et al*., 2017).

The soil available P and K^+ values are shown in Table (4) and Fig (9) were significantly increased by eco-friendly organic wastes with *Azospirillum* treatments compared to the initial condition. The trend of available P and K after 60 days was similar to the trend of available P and K after 150 days of incubation, the available P and K, concentrations followed the order S2 > A1 > A1S1 > C1S1 > C2> S1 > C1 >A1C1 > control > initial respectively. The spent grain (S2) possessed the highest values of P and K while the A1C1 and control showed the lowest value. The available P and K were significantly ($P < 0.05$) increased by spent grain (S2) treatment compared to control treatment after 150 days 12 and 3.64 folds increase in P and K respectively. The spent grain amendments increased the available P and K contents in saline-sodic soil up to 12 $mgkg^{-1}$ in S2 and A1 respectively.

The spent grain application rates with *Azospirillum* were superior compared to the same rate of compost for soil P and K. The high rate of spent grain (S2) significantly produced larger increases in soil P than in the other organic wastes amendment, while A1 was statistically similar to S2 and both significantly increased soil P compared to other organic wastes amendments shown Figure (9). Generally soil N, P and K increased as the application rate of *Azospirillum* or spent grain increased, and all of the organic treatments were effective at adding N, P and K to saline-sodic soil. Furthermore, spent grain and *Azospirillum* should be applied before planting to give sufficient time for natural oxidation of organic wastes materials, which in turn enhances the soil available nutrients. Similar to the results of the present study, incubation of some organic sources resulted high total N. Our results also concur with those of (Barra *et al*., 2019; Ouyang *et al*., 2013; Trivedi *et al*., 2017) who found that the application of composted poultry manure, organic fertilizer, and bio-fertilizers inoculation enhances the benefit of P–fertilization led to the increase of available P in saline-sodic soils. This increase in available P is due to the reduction in pH as a result of organic matter decomposition. Furthermore, the application of bio-fertilizer or FYM significantly increased total N, available P and K for saline soils. (Liang *et al*., 2014; Yu *et al*., 2019).

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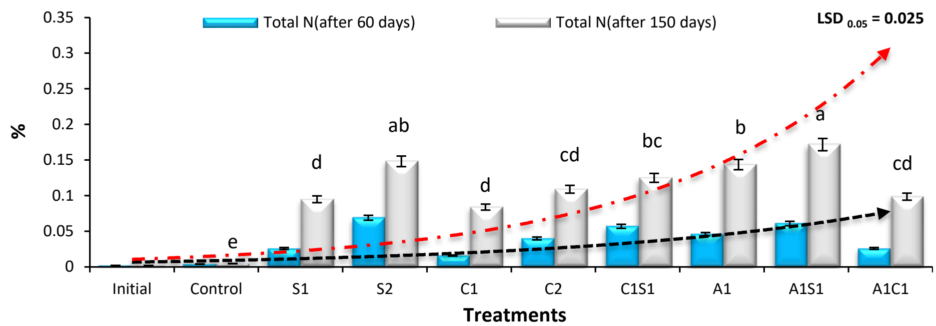

Figure (8): Effect of *Azospirillum* and eco-friendly organic wastes on total (N) in saline-sodic soil after 60 and 150 days of incubation. Data correspond to means of four replicates ± standard error. Different letters indicate statistically significant differences ($p \leq 0.05$) among treatments.

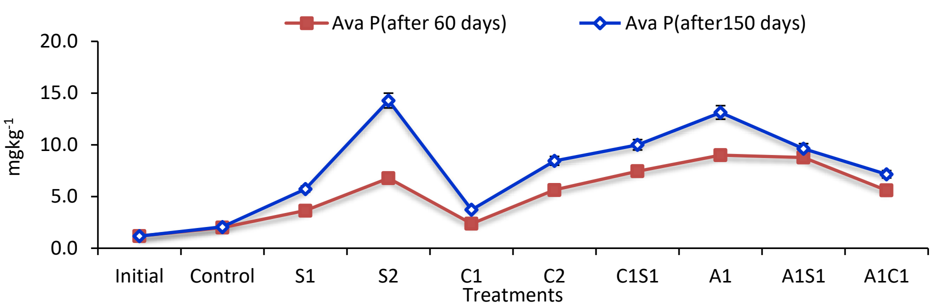

Figure (9): Effect of *Azospirillum* and eco-friendly organic wastes on available (P) in saline-sodic soil after 60 and 150 days of incubation. Data points and error bars represent means and standard errors (n = 4)

Table (5): Soil micronutrients in saline-sodic soil after 60 and 150 days of incubation

Treatments	Incubation Period	Fe^{2+}	Zn^{2+}	Mn^{2+}	Cu^{2+}	B^{+}
	Day	mgkg^{-1}				
Initial	**Zero time**	**0.08±0.01**	**Nd***	**0.11±0.02**	**0.003±0.01**	**Nd***
Control	60	0.74±0.01	0.332±0.06	0.619±0.07	0.093±0.01	0.058±0.01
	150	**1.27±0.08**	**0.477±0.04**	**1.089±0.10**	**0.264±0.02**	**0.081±0.009**
S1	60	3.33±0.19	0.925±0.03	2.049±0.09	0.742±0.08	0.445±0.021
	150	**4.27±0.32**	**1.335±0.13**	**2.625±0.05**	**1.020±0.13**	**0.768±0.151**
S2	60	8.01±0.41	2.633±0.10	5.989±0.66	1.344±0.11	0.716±0.012
	150	**9.15±0.44**	**3.273±0.13**	**7.461±0.72**	**1.776±0.02**	**1.110±0.18**
C1	60	2.62±0.05	0.753±0.03	1.657±0.13	0.403±0.02	0.378±0.04
	150	**3.12±0.06**	**1.059±0.14**	**2.334±0.22**	**0.501±0.08**	**0.510±0.02**
C2	60	4.40±0.14	1.439±0.11	3.522±0.05	0.851±0.02	0.574±0.02
	150	**5.16±0.023**	**2.178±0.07**	**4.719±0.19**	**1.131±0.05**	**0.858±0.05**
C1S1	60	5.96±0.55	1.473±0.07	4.602±1.64	0.868±0.30	0.591±0.18
	150	**6.64±0.65**	**2.010±0.18**	**6.159±0.27**	**1.329±0.10**	**1.143±0.14**
A1	60	4.03±0.03	1.113±0.13	2.595±0.07	0.711±0.11	0.350±0.02
	150	**4.68±0.21**	**1.488±0.12**	**3.183±0.18**	**0.993±0.16**	**0.501±0.07**
A1S1	60	12.58±0.40	2.904±0.04	5.956±0.11	1.531±0.17	0.916±0.04
	150	**13.80±0.36**	**4.125±0.39**	**7.600±0.37**	**1.945±0.24**	**1.275±0.36**
A1C1	60	2.38±0.11	0.865±0.09	1.789±0.11	0.467±0.05	0.439±0.05
	150	**3.81±0.28**	**1.308±0.35**	**2.193±0.16**	**0.660±0.04**	**0.534±0.08**
$LSD_{0.05}$	60	0.484	0.140	1.022	0.228	0.114
	150	0.585	0.361	0.541	0.207	0.271

Data correspond to means of four replicates ± standard deviation. $LSD_{0.05}$= least significant difference (LSD) test at ($p \leq 0.05$). Nd*= not detected

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3.3.2. Soil micro-nutrients (Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} and B^{+})

The soil amendments were found significantly impact on micro-nutrients. Table (5) shows that Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and B^{+} concentrations after 60 days of incubation had similar trend Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} and B^{+} concentrations after 150 days of incubation. Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} and B^{+} concentrations followed the order A1S1 > S2 > C1S1 > C2 > A1> S1 >A1C1> C1 > control > initial. The micronutrients, the largest increases were observed with A1S1 treatment while the lowest increases were observed with C1 and control treatment after 60 and 150 days of saline-sodic soil incubated. Available micronutrients were significantly ($P < 0.05$) increased by *Azospirillum* with spent grain (A1S1) treatment compared to control treatment 18.9, 12.42, 12.66, 20.86 and 21.90 folds increase in Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and B^{+}, respectively. The *Azospirillum* and spent grain application rates (A1S1 and S2) were superior compared to the same rate of compost for Fe^{2+}, Zn^{2+}, and Mn^{2+} (Fig 10). The superiority of the *Azospirillum* with spent grain was due to its effect on lowering soil pH as well as solubility and chelation effect of organic wastes in saline-sodic soil (Pii *et al*., 2015a). Accordingly, the application of A1S1 to enrich soil with micronutrients is highly recommended in saline-sodic soils. The mineralization of eco-friendly organic wastes compounds leads to decrease pH in saline-sodic soil, which increases micronutrient availability in the soil. The presented results are in agreement with the results presented by (Rashad *et al*., 2016; Wang and Cai, 2006; Zhang *et al*., 2019) who reported that the available micro-nutrients are increased by using agro-industrial organic wastes and microbial community.

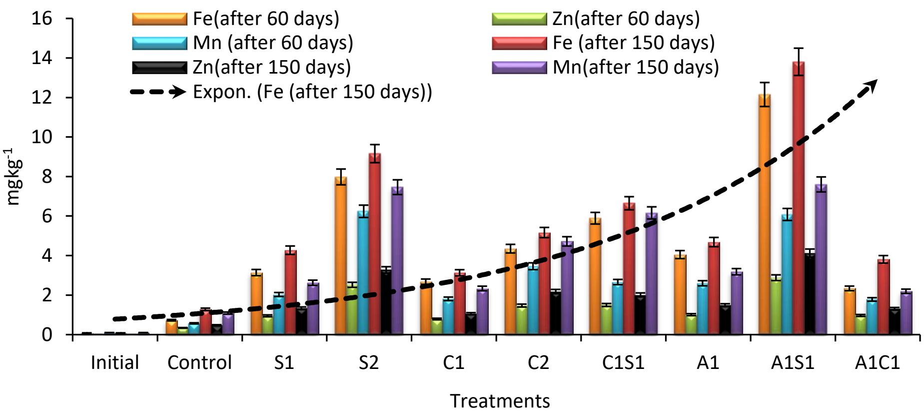

Figure (10): Effect of *Azospirillum* and eco-friendly organic wastes on micro-nutrients (Fe^{2+}, Zn^{2+}, and Mn^{2+}) in saline-sodic soil after 60 and 150 days of incubation. Data points and error bars represent means and standard errors (n = 4).

CONCLUSION

The results of the present study confirm our hypotheses that (i) Application of spent grain doses with *Azospirillum* inoculation reduced the ESP, increased the beneficial microbes and the activity of enzymes from decomposition of the eco-friendly organic wastes and (ii) Soil fertility increased by improvement of macro and micro-nutrients in saline-sodic soil after 60 and 150 days of incubation. The results also showed that the spent grain and *Azospirillum* treatments enhanced the production of soil organic carbon, the activity of enzymes, soil macro and micro-nutrients which decreased soil salinity in saline-sodic soil as a result of micro-organisms activity during the mineralization process of spent grain with *Azospirillum* treatments.

In the saline-sodic soil, the concentration of monovalent total N, available P, and available K^{+} also Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+}, and B^{+} were affected significantly by the application of *Azospirillum* with spent grain under controlled laboratory conditions. In the final application of *Azospirillum* in saline-sodic soil has a negligible effect on the final SOC content but it has a strong effect on soil dehydrogenase, urease, pH, EC, ESP, and Ex-Na^{+}. At the end of experiments, monovalent cations concentrations, as well as soil EC and ESP, were smaller after 150 days than 60 days of incubation. Finally, the soil salinity indicator decreased significantly, even for eco-friendly organic wastes with *Azospirillum*, after 150 days of incubation. Results suggested that the applied spent grain with *Azospirillum*, depending on their biological and chemical properties might have a positive effect on soil properties, especially in the absence of compost.

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