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A NOVEL ENVIRONMENTAL ADDITIVES TO DECREASE NITRATE LEVEL IN AGRICULTURE WASTEWATER AND ENHANCEMENT NUTRIENT STATUS UNDER GREENHOUSE PLANT GROWTH IN CALCAREOUS SOIL

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Abstract

Protecting groundwater from nitrogen contamination such as soluble NO_3-N is an important public health concern and a major national environmental issue in Egypt. The objective of this study was to determine and monitor the influences of bio-spent grain and mineral fertilizers on levels of nitrate leaching and other forms of nitrogen contamination in agricultural wastewater for controlling nitrogen pollution and protecting groundwater safety. Spent grain organic wastes has no value and is available at no cost all year from the beer industry. Eight treatments were established, including spent grain (S2, 20 g kg^{-1} soil); compost (M2, 20 g kg^{-1} soil); mix compost with spent grain (M1S1); *Azospirillium* sp., inoculation (A1); inoculation A1 with S2, (A1S2); inoculation A1 with M2, (A1M2); nitrogen fertilizer (NF), and control (CK, no fertilizer). All treatments were mixed with 30 kg soil pots under greenhouse conditions, *Zea mayz L.* seeds were planted in the soil pots. The most relevant nitrogen leaching forms were collected and analysed every month for four months. The soil drainage was collected under the pots by closed system. The bio-spent grain application effectively increased soil organic matter content, plant nutrients, germination rate, and reduced agricultural wastewater pollution. The Ca^{2+}, K^+ and Na^+ cations in the leaching were not stable, but were present at high levels. Total N, NO_3^-, NH_4^+ and Cl^- were significantly lower in S2, A1, and A1S2 treatments than in NF, M2 and control treatments. The agricultural wastewater in most menial fertilizer's treatments belonged to class III and IV in the Egyptian water standard, which defines water that is unsuitable for human consumption. Our health risk assessments showed that NO_3^--N posed the greatest carcinogenic risk. Therefore, using A1S2 as bio-organic fertilizer reduces the risks of soluble NO_3^--N in agricultural wastewater.

Keywords: Bio-spent grain, agriculture wastewater, Nitrate leaching, carcinogenic risk, greenhouse

Introduction

Agricultural wastewater is the major water supply for drinking and for the domestic, industrial, and agricultural sectors in the North-Alexandria region of Egypt. One serious problem that affects the quality of the region's groundwater is leaching of nutrients from the soil, which is especially evident in areas dominated by agriculture. Nitrogen percolates easily into the groundwater through the soil along with intensive chemical fertilizer practiced. As a result, the mineral fertilizers rapidly dissolve and lose after irrigation (Anim-Gyampo, Anornu, Appiah-Adjei, & Agodzo, 2019) The application of large amounts of nitrogen fertilizers in arid regions of intensive agriculture contributes to excessive nitrogen accumulation in soils and excessive leaching into groundwater bodies. Extensive irrigation and use of nitrogen (N) fertilizers together result in low N-use efficiency and high N loss. Several studies have also reported an increasing incidence of nitrogen pollution and dramatic increases in the nitrate-nitrogen (NO_3-N) concentrations in the groundwater of regions where intensive chemical fertilizer practiced (Anim-Gyampo *et al.*, 2019).

Leaching of nitrate to groundwater water is a major cause of human disease, misery, and death. According to the World Health Organization (WHO), as many as 4 million children die every year as a result of diarrhea caused by nitrate levels infection which contributes to contamination of groundwater. Agricultural pollution is both a direct and indirect cause of human health impacts. The WHO reports that nitrogen levels in groundwater have grown in many parts of the world as a result of the "intensification of farming practice" (WHO, 2008). This phenomenon is well known in parts of Europe.

Nitrate levels have grown in some countries to the point where more than 10% of the population is exposed to nitrate levels in drinking water that are above the 10 mgl^{-1} guidelines. Although WHO finds significant links between nitrate and nitrite and human cancers, the drinking water guideline is established to prevent methaemoglobinaemia which depletes oxygen levels in the blood (WHO, 2008). In addition, increasing rates of stomach cancer caused by increasing nitrate intake have been reported (Vinod, Chandramouli, & Koch, 2015). Therefore, the nitrogen pollution of groundwater to be a problem in developing countries. United Nations' predictions of global population increase to the year 2025 require an expansion of food production of about 40-45% (Anim-Gyampo *et al.*, 2019). Irrigation agriculture of calcareous soil, which currently comprises 17% of all agricultural land yet produces 36% of the world's food, will be an essential component of any strategy to increase the global food supply. Currently 75% of irrigated land is located in developing countries; by the year 2013, it is estimated that 90% will be in developing countries (FAO, 2015).

Therefore, organic farms use recycled sources for 80 percent or more of the nitrogen used to grow food, meaning less reactive nitrogen is released into the atmosphere, groundwater. (Teutscherova *et al.*, 2018) found that organic farming helps prevent nitrogen pollution by recycling or reusing three times more reactive nitrogen than conventional farming. Galloway studies the positive and negative effects of reactive nitrogen on the atmosphere, land-based ecosystems, and freshwater and oceanic eco systems (Lu, Kang, Gao, Chen, & Zhou, 2018). Agriculture is a contributor of nitrate to natural waters and there is concern about the excess nitrogen burden loadings from agriculture on natural waters. Agricultural practices that reduce nitrate leaching from arable land are needed. It is postulated by certain groups that organic farming practices reduce nitrate leaching among other environmental benefits. The application of organic wastes to the soil has the possibility to reduce carbon emission and total N release (Hafez, Popov, & Rashad, 2019) with the high content stable organic carbon in the organic wastes to change the soil properties. Recent studies have organic fertilizers as a possible technique to increase nutrient bioavailability and decrease NO_3-N leaching (Teutscherova *et al.*, 2018) as well as decrease soil CH_4 and N_2O emissions

Organic wastes amendment positively affect the chemical and physical properties of the soil on pH led to direct absorption of NH_4^+ –N and NO_3^-–N, great cation exchange capacity (CEC), improved water holding capacity (WHC) of the soil reducing the volume of leachate (Teutscherova *et al.*, 2018;Zheng *et al.*, 2013). Many early studies stated that effective soil management practices reduce nitrogen contamination especially NH_4^+ –N and NO_3^-–N and reduce pollution of agriculture wastewater (Foufou *et al.*, 2017; Perego *et al.*, 2012) reported a positive effect of bio and organic fertilizers on reducing nitrate levels in wastewater, however, there was an offset by the decreased in N transformation in soil.

Nitrogen losses from organic wastes applications are mostly driven by volatilization or leaching in surface waters and groundwater, with only a small amount utilized by the crop or immobilized by the added organic matter in calcareous soils. Animal organic wastes and compost provide physical, chemical and biological benefits but those can contribute to NO_3-N leaching in groundwater. The effects of their application on nitrate leaching have been evaluated by several researchers. The results of the many studies founds in literature are discording. Other studies report a higher potential risk of NO_3-N leaching for animal compost and organic wastes applications in soils with high soil organic matter (SOM) (Perego *et al.*, 2012). Numerous studies have reported that the yield of a non-legume crop following alfalfa stand increased NO_3-N concentration in agriculture wastewater.

Recent research has focused on a single effect of spent grain and *Azospirillum* on nitrate levels in agriculture wastewater and N leaching in calcareous soils. However, few studies on the combined effects of *Azospirillum* with organic and inorganic amendments under the greenhouse were found (Hafez *et al.*, 2019; Mussatto & Roberto, 2006). It is interesting to conduct research to improve nutrients availability and to reduce NO_3-N leaching by bio-spent grain using the application of spent grain with *Azospirillum* in soil under maize cultivation. The combined application bio-spent grain with chemical fertilizers is considered to be necessary to promote quality productivity and decrease environmental risk by NO_3-N in groundwater.

The hypothesis theory of research was use of bio-spent grain can decrease levels of nitrate and other forms of nitrogen, and based on this decrease, to assess the health risk for agriculture wastewater, thereby providing a scientific basis for controlling nitrogen pollution and protecting groundwater safety. The objectives of this paper are: (1) to compile, summarize and critically analyze information about NO_3-N and other forms of nitrogen leaching from experiment that were managed according to bio-spent grain application; (2) to compare NO_3-N leaching from bio-spent grain application with that from conventional systems by chemical fertilizers. Finally, Leachable amounts of NO_3^--N in soils from two types of organic wastes were compared.

Material and Methods

Study site and Soil Characterization

The field experiment started in June 2017 and is located at the City of Scientific Research and Technological Applications (30° 53' 33.17'' N, 29° 22' 46.43'' E) in Alexandrea, Egypt. The nitrate leaching measurement was from Journey to September 2017 in this study. The site is arid region climate from 2010 to 2018 the mean annual temperature was 18.5 °C, ranging from 27 °C to 35 °C, and the mean annual precipitation was 125.1 mm (Climate-Data.org, 2019). The soil in the study has a sand clay loam texture and can be classified as calcareous cinnamon soil (FAO, 2015 classification).The main soil properties (0- 20 cm depth) are as follows: pH of 8.34 (soil-to-water ratio, 1:2.5); electrical conductivity, 1.74 dSm^{-1}; organic matter content 9.8 g kg^{-1}; total N, 0.3 g kg^{-1}; NO_3–N, 24.5 mg kg^{-1}, NH_4–N, 1.20 mg kg^{-1}; Olsen-P, 4.20 mg kg^{-1}; available K^+, 320.2 mg kg^{-1}; Soil organic carbon, 5.68 gkg^{-1}

Soil Treatments Description and experimental design

Three types of treatments were used. The first one *Azospirillum* bacteria, it was from Faculty of Agriculture, Ain Shams, Cairo Governorate, Egypt. The second was compost consisted of plant and animal wastes from the national factory for the production of compost, Alexandria, Governorate, Egypt. The last one was spent grain, a by-product from beer industry, was obtained from Al Ahram Beverages Company, Abu Hammad, Al Sharkia Governorate, Egypt. The main characteristics of the organic wastes were determined according to the standard procedures shown in (Table 2). Eight treatments were established, including spent grain (S2, 20 g kg^{-1} soil); compost (M2, 20 g kg^{-1} soil); mix compost with spent grain (M1S1); *Azospirillium* sp, inoculation (A1); inoculation A1 with S2, (A1S2); inoculation A1 with M2, (A1M2); nitrogen fertilizer (NF), and control (CK, no fertilizer). All treatments were mixed with 30 kg soil pots under greenhouse conditions for four months, *Zea maize* L. seeds were planted in the soil pots. The most relevant nitrogen leaching forms were collected and analysed every month for four months. The soil drainage was collected under the pots by closed system.

The experiment was conducted in a greenhouse during the (*Zea maize L.*) growing season. Eight treatments with three replicates were carried out, namely S2, M2, A1, A1S2, A1M2, S2M2, NF and Control. Then the experimental area consisted of 24 pots, each post 30 kg soil, and these 24 pots were arranged as split plots in a randomized complete block with a 30 cm isolation strip in order to avoid interference. The control treatment was without fertilization. Maize crop has a high water demand under arid condition. The irrigation period starts in June and ends in early Sept. The number of irrigation events depends on the irrigation method, soil type and the cropping system. The mean water amount which applied per pots cropping season from (June to Sept) was 250 mm from June to Jul, and 500 mm until end experiment.

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

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

***Azospirillum* preparation :** *Azospirillum brasilense* (Sp245) was cultured; growth and inoculation were performed as described by (Pii *et al.*, 2015). 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 solution (NaCl 0.85% w/v) after that the *Azospirillum* bacteria became ready for inoculation with the soil.

Leachate sampling and analysis

The nitrogen loss through water leaching was collected at one-week intervals started at a week after transplanting until two weeks before harvesting. The samples were collected at the leaching pipe of the drainage system then store at 4 °C before analysis and measured using a 50 mL graduated cylinder. The concentration of NO_3^- –N and NH_4^+ –N in the leachate samples were analyzed using a Multi-parameter photometer with COD (HI83399) instrument. NO_3^- –N concentration was analyzed using the cadmium reduction method. NH_4^+ –N concentration was analyze using Nessler Method. The intensity of the color is determined by a compatible photometer and the concentration and based upon the meter will be presented in mg L^{-1} (ppm) of NO_3^- –N and NH_4^+ –N in leachate. The total NO_3^- –N and NH_4^+ –N leaching loss was calculated by multiplying the N concentration to the leachate volume.

Nitrate leaching calculation : In order to calculate the actual nitrate loss through soil pots, we considered the nitrogen concentration of the soil solution at the bottom depth of the explored soil pots and the water draining through it. Nitrate leaching was estimated using the trapezoidal rule proposed by Lord and Shepherd (1993), which assumes that nitrogen concentrations in the extracted soil water solution represented mean flux concentrations. The total nitrogen leached over each sampling interval, in $mgkg^{-1}$, was calculated as: N leached = 0.5(C1 + C2) v / 100 where C1 and C2 are successive pairs of sampling occasions (mg NO_3–N L^{-1}), and v is the drainage volume between sampling occasions (mm).

Analytical methods for soil, spent grain and compost

The total twenty-four samples referred to eight treatments × three 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 Particles-size distribution was determined by the hydrometer as described by Page *et al.*, 1982. Basic characteristics, such as soil pH was measured potentiometrically in a soil : water suspension 1:2.5 w/v Page *et al.*, 1982. The electrical conductivity (ECe) was measured in saturated paste extracts using an EC meter, (Corwin & Yemoto, 2017). The soluble ions were measured in water leaching. The soluble calcium, and chloride were measured by titration method Page, *et al.*, 1982. Soluble sodium and potassium were determined by flame photometer according to Sparks (1987). The total nitrogen (N), was determined by Microkjeldehl method, available phosphorus (P) was extracted with 0.5 N $NaHCO_3$, and available potassium (K) was extracted by 1 N ammonium acetate solution and measured by the flame photometer, the N, P, and K were measured as explained by (Anderson *et al.*, 1982). Total dissolved organic carbon (TDOC) was determined using the TOC analyzer (multi-N/C UV3100, Analytikjena product, Germany) at 1100 °C. Soil total organic carbon (TOC) concentration was determined by oxidization with $K_2Cr_2O_7$.

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

Table 2 (a, b, c, and d) and Figure (1) shows effect of bio-spent grain on concentrations of elements in water leaching from the soil with cropping seasons in the greenhouse . The nutrients included nitrate (NO_3^-), ammonium (NH_4^+) chloride (Cl^-), sodium (Na^+), calcium (Ca^{+2}) and potassium (K^+) these nutrients concentrations were discussed collectively in the following section. Groundwater quality and health risk assessment we selected NO_3-N, and NH_4-N as the assessment index for groundwater nitrogen pollution.

Nitrate leaching losses.

Figure (1) show the trends of nitrate leaching from June to Sept 2017. Nitrate leaching losses in each treatment decreased as the spent grain increased. Table (2) and Figure (1) shows the concentrations of the NO_3^- leaching from June to Sept in the calcareous soil followed the order NF>M2>CK>A1M1>M1S1>S2>A1S1>A1. Additionally, A1 lost more than the control and NF treatments, and S2 lost more than M1S1 did. Nitrate leaching losses in the corn experiment (June, and Jul, 2017) were greater than the losses in the (Aug and Sept, 2017). The trends for each of the four months in the NH_4^+ were very similar withe trend NH_3^-. These results indicated that spent grain and *Azospirillum* application was beneficial for reducing nitrate leaching.

Table 2 : Effects of different treatments on (EC, pH, NO_3^-, NH_4^+, Cl^-, Na^+, Ca^{2+}, and K^+) in water leaching from the soil with cropping seasons in the greenhouse

Treatments	EC	pH	NO_3^-	NH_4^+	Cl^-	Na^+	Ca^{2+}	K^+
	(a) (mg L^{-1}) June 2017							
CK	0.40	8.49	30.52	1.12	47.26	9.48	34.03	12.09
NF	0.71	7.84	61.69	13.00	51.74	8.48	35.43	20.54
S2	0.74	7.41	24.55	0.89	16.12	5.58	19.97	8.32
M2	1.25	7.87	47.79	9.20	62.95	17.04	52.60	38.06
M1S1	0.91	7.86	28.19	3.20	43.83	11.44	35.77	31.90
A1	0.73	7.49	15.40	2.38	32.80	9.96	34.84	17.37
A1S1	0.71	7.94	21.77	2.52	56.21	9.47	30.47	17.97
A1M1	0.85	7.86	29.26	5.04	48.04	13.56	43.79	23.95
$LSD_{0.05}$								
	(b) (mg L^{-1}) July 2017							
CK	0.33	8.12	25.33	0.48	39.22	7.87	28.24	10.01
NF	0.59	7.89	51.21	8.90	42.94	7.04	29.40	17.02
S2	0.61	7.60	20.37	0.38	13.37	4.63	16.58	6.90
M2	1.04	7.94	39.66	5.60	52.25	14.14	43.66	31.58
M1S1	0.76	7.91	23.39	1.37	36.38	9.49	29.69	26.48
A1	0.61	7.50	12.78	1.02	27.23	8.27	28.91	14.28
A1S1	0.59	8.10	18.07	1.08	46.65	7.86	25.28	14.94
A1M1	0.70	8.18	24.29	2.17	39.87	11.25	36.34	19.87
$LSD_{0.05}$								
	(c) (mg L^{-1}) Aug 2017							
CK	0.26	7.87	13.93	0.00	21.57	4.33	15.53	5.51
NF	0.45	7.65	28.16	6.30	23.62	3.87	16.17	9.36
S2	0.47	7.37	11.21	0.00	7.35	2.55	9.12	3.80
M2	0.80	7.71	21.81	3.20	28.74	7.78	24.01	17.37
M1S1	0.58	7.68	12.87	0.00	20.01	5.22	16.33	14.56
A1	0.47	7.28	7.03	0.00	14.97	4.55	15.90	7.86
A1S1	0.46	7.86	9.94	0.00	25.66	4.32	13.91	8.22
A1M1	0.54	7.94	13.36	0.00	21.93	6.19	19.99	10.93
$LSD_{0.05}$								
	(d) (mg L^{-1}) Sep 2017							
CK	0.14	7.79	7.66	0.00	11.86	2.38	8.54	3.03
NF	0.25	7.57	15.49	4.23	12.99	2.13	8.89	5.15
S2	0.26	7.30	6.16	0.00	4.04	1.40	5.01	2.09
M2	0.44	7.63	12.00	2.56	15.81	4.28	13.21	9.55
M1S1	0.32	7.60	7.08	0.00	11.00	2.87	8.98	8.01
A1	0.26	7.20	3.87	0.00	8.24	2.50	8.75	4.32
A1S1	0.25	7.78	5.47	0.00	14.11	2.38	7.65	4.52
A1M1	0.30	7.86	7.35	0.00	12.06	3.40	10.99	6.01
$LSD_{0.05}$								

(a): June, 2017; (b): Jul, 2017; (c): Aug, 2017; (d): Sept, 2017. CK: control; NF: 100% chemical fertilizer; S2: 20 g of spent grain; M2: 20 g of compost; M1S1: mix between M2 and S2; A1: *Azospirillim* sp inoculation; A1S2: *Azospirillim* sp + S2; A1M1: *Azospirillim* sp+M1. The standard deviation (n = 3) was analyzed using a one-way ANOVA. Means followed by the same letter are not significantly different (P<0.05).

Table 2, (a, b, c and d) shows the NO_3^- and NH_4^+ leaching losses. S2 and A1 applications reduced nitrate and ammonium leaching losses in the soil layers. The treatment NF and the control significantly differed (P < 0.05), the exception that no obvious differences between control and S2 in (June, and Jul, 2017) (P > 0.05) were observed. The nitrate leaching losses in June 2017 ranged from 61.69 to 24.55 mgL^{-1} in the NF and S2 respectively, and in Jul to Sept 2017 leaching losses from 51.21–20.37 mgL^{-1} in the NF and S2 respectively. No significant differences were observed between the compost control and treatments. Below the NO_3^- and NH_4^+concentration, no significant differences (P > 0.05) were observed among A1M1, M2, and the CK. The results for the 4 months show that S2 and A1 applications could reduce soil nitrate leaching losses in the soil layer, the losses in both S2 (11.21 mg L^{-1}) and A1 (7.03 mg L^{-1}) significantly differed from those in the NF (28.16 mg L^{-1}) (P < 0.05), and soil NH_4^+ leaching losses decreased 13 mg L^{-1} and 0.89 mg L^{-1} % in NF and S2, respectively The presented results of NH_4^+ and NO_3^- are in agreement to the results presented by (Teutscherova *et al.*, 2018; Anim-Gyampo *et al.*, 2019).

Soil nitrate and Ammonium concentrations

The organic additives nitrate concentration is the key factor influencing nitrate and ammonium leaching losses. Figure (1) shows the course of changes in the soil nitrate concentration in the soil layers at different time points. In the corn pots, the annual mean of the nitrate and ammonium concentrations were clearly higher (P < 0.05) in June and Jul 2017 than in other months (Fig. 1). In the most critical period of leaching was during June because this start time for amendments of organic wastes to soil, and the nitrate concentration was much higher during this time than during the other three months of measurements (P < 0.05). Not surprisingly, the periods of start for organic additive decomposition were also periods of high nitrate leaching. The nitrate concentration in the S2 and A1 treatments was lower than that in the CK and NF treatments in the corn pots compared with that in the M2 treatment, the nitrate concentration in the S2 and A1 treatments both significantly differed (P < 0.05) in Aug, Sept; no significant differences were observed between all treatments in Aug or Sept in NH_4^+ leaching. However, significant differences were observed between the CK and S2 treatments at all-time points (P > 0.05). The results show that spent grain and *Azospirillum* applications were beneficial for reducing the soil nitrate and ammonium concentrations during the early stages of corn growth. However, in the middle and at the end of the corn growing stage, the soil ammonium leaching did not increase despite the soil nitrogen concentration increasing slightly. Although the bio-spent grain application constituted an additional source of nitrogen, the nitrate leaching losses did not increase. The presented results of NH_4^+ and NO_3^- are in agreement to the results presented by (Perego *et al.*, 2012; Teutscherova *et al.*, 2018; Vinod *et al.*, 2015)

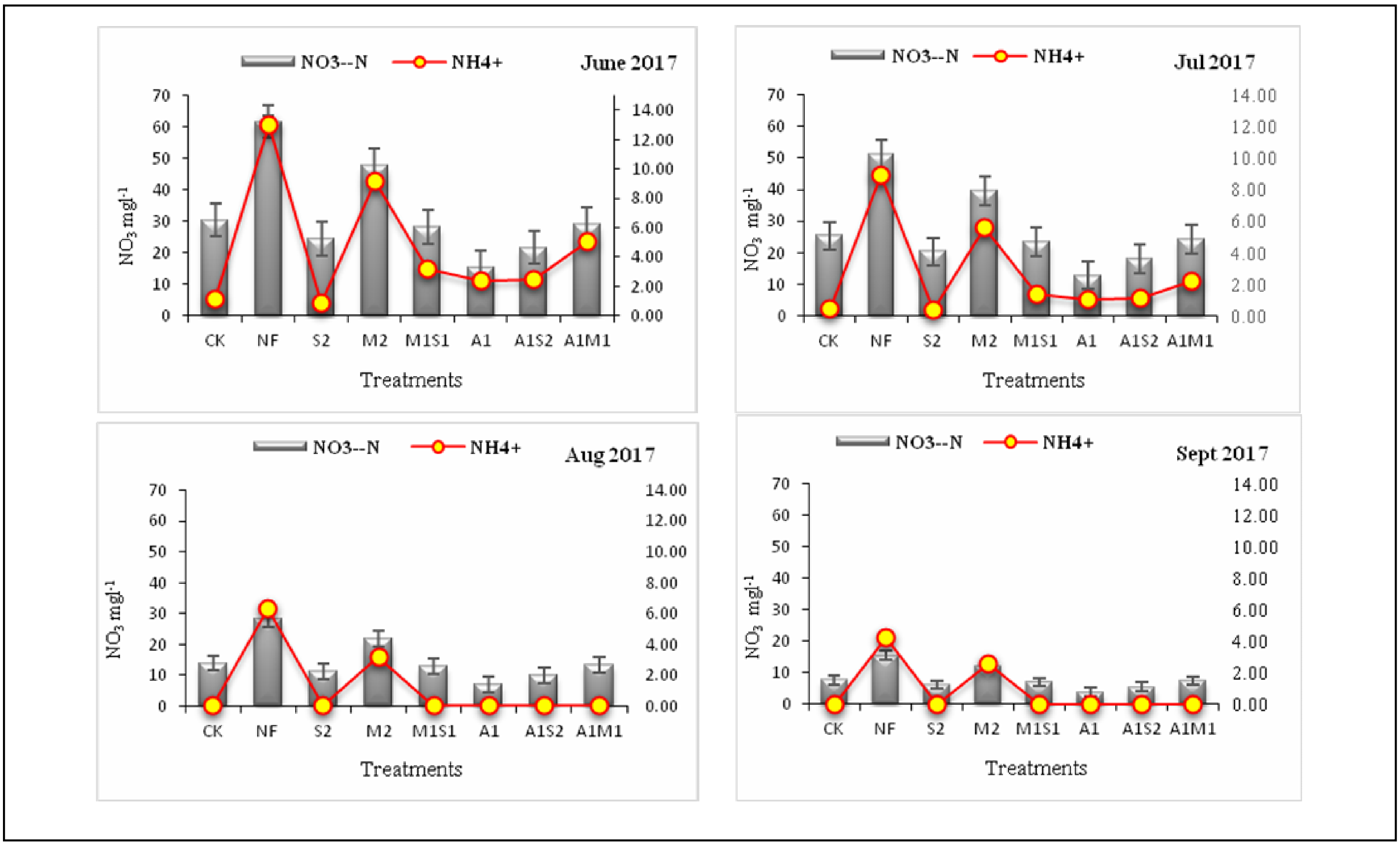

Fig. 1 : Effects of different treatments on Nitrate and ammonium leaching losses from soil with cropping seasons in the greenhouse. CK: control; NF: 100% chemical fertilizer; S2: 20 g of spent grain; M2: 20 g of compost; M1S1: mix between M2 and S2; A1: *Azospirillim* inoculation; A1S2: A1+S2; A1M1: A1+M1. The standard deviation (n = 3) was analyzed using a one-way ANOVA.

The chloride concentrations

The chloride concentrations in the leaching losses are for four months shown Table 2 (a, b, c, and d). The concentrations were 47.26, 51.74, 16.12, 62.95, 32.80, 43.83, 48.04 and 56.21 mg L^{-1} for the CK, NF, S2, M2, A1, M1S1, A1M1, and A1S1, respectively. Figure (2) shows the concentrations of the chloride leaching from June to Sept in the calcareous soil followed the order A1S1>NF>M2> A1M1>CK>M1S1>A1>S2. According to the reported chloride concentrations, the spent grain with *Azospirillum* application rates were lower Cl^- concentration compared to the compost application rates. There were significant differences among the means of the studied treatments. The spent grain and *Azospirillum* did not increase the chloride concentration relative to the other treatments. So, the spent grain and *Azospirillum* could not lead to a salinity hazard and water toxicity by chloride. The compost organic source (M2) possessed high chloride concentration (62.95 mgL^{-1}) in the water draining soil compared to the control condition (47.26 mgL^{-1}) or the NF treatment (51.74 mgL^{-1}). The compost source increased the chloride concentration in the calcareous soil significantly compared to spent grain and *Azospirillum*. The differences in the concentrations of Cl^- were significant among the treatments used. The superiority of the spent grain was due to initially high organic matter, low pH, and the high water holding capacity. The high concentration of chloride provided by the compost raises the risk of salinity hazard for soils. So, the compost applications used in the present study is not favorable in soil fertilization or reclamation compared to the spent grain and *Azospirillum*. Increased concentration of chloride ions in any organic source increases osmotic pressure and decreases water potential, making it harder for plants to take up water. Additionally, high concentrations of chloride could cause toxicity to some plants (Hu & Schmidhalter, 2005). When using an organic source it is important to know the effect of source quality on salt content of the soil solution. All common chlorides are soluble and contribute to the total salt content of soils. However, the chloride is an essential element for plant nutrition but it is needed in a small quantity.

The sodium concentration

The Na^+ concentrations in the water draining shows Table (2) and Figure (2). The concentrations of the Na^+ leaching from June to Sept in the calcareous soil to groundwater followed the order A1S1 > NF > M2 > A1M1 > CK > M1S1 > A1 > S2. According to the reported Na^+ concentrations, the spent grain with *Azospirillum* application rates were lower Na^+ concentration compared to the compost application rates. The trend of Na^+ was similar to the trend of Cl^- content presented earlier. The organic treated soil with a mixture of M2 and NF possessed the highest sodium and chloride concentrations among the treatments. Obviously, as the application level of compost increased, the sodium chloride concentrations increased. There were significant differences among the treatments in the Na^+ and Cl^- concentration. Although the bio-spent grain application constituted an additional source of salts, the Na^+ and Cl^- leaching losses did not increase (Hafez *et al.*, 2019). High sodium chloride can lead to deterioration of soil physical properties and increase water salinity and toxicity. Soluble sodium and chloride can adversely affect soil structure by making the soil very susceptible to crusting, impeding water infiltration and hindering root growth (Hu & Schmidhalter, 2005; Shi, Liu, & Zhang, 2019).

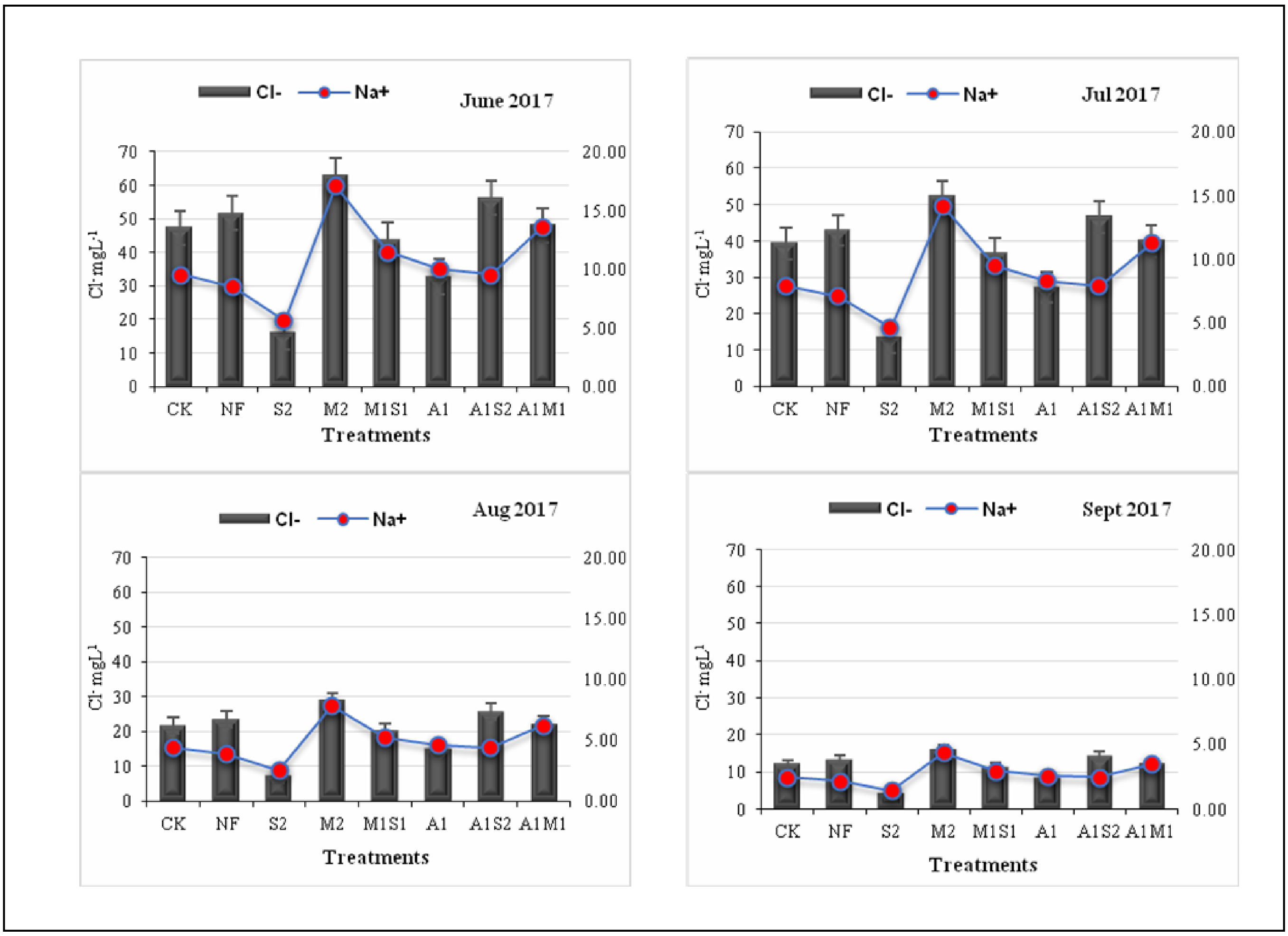

Fig. 2 : Effects of different treatments on Cl^- and Na^+ leaching losses from soil with cropping seasons in the greenhouse. CK: control; NF: 100% chemical fertilizer; S2: 20 g of spent grain; M2: 20 g of compost; M1S1: mix between M2 and S2; A1: *Azospirillim* sp inoculation; A1S2: A1+S2; A1M1: A1+M1. The standard deviation (n = 3) was analyzed using a one-way ANOVA.

The Calcium and Potassium concentrations

Figure (3) and Table 2(a, b, c and d) show the calcium (Ca^{2+}) and Potassium (K^+) concentrations in water draining. The concentrations of the Ca^{2+} leaching from June to Sept 2017 in the calcareous soil. The concentration was lower in the bio-spent grain treatments than in the compost and other treatments. The Ca^{2+} leaching concentrations in water draining were followed the order M2 > M1S1 > A1M1 > A1 > NF > CK > A1S1 > S2, respectively. The additive spent grain affected the concentration positively. The S2 treatment supplied the lowest calcium concentration while the M2 supplied the highest in soil. Ca^{2+} efficiency due to higher soil Ca^{2+} is rare, but may occur on alkaline and calcareous soils. Calcium is generally not deficient in soils when soil pH is 7.5 or above. So, the soil used in the present study have sufficient of calcium needed by a growing plant. On the other hand the concentration pf K+ was similar to the trend of Ca^{+2} content presented earlier. The organic treated soil with a mixture of M2, A1MA and NF possessed the highest K^+ concentration among the treatments. Obviously, as the application level of compost increased, the K^+ concentrations increased in the water draining. The results show that spent grain and *Azospirillum* applications were beneficial for reducing the soil calcium and potassium concentrations during the early stages of corn growth in June to Sept (Mussatto & Roberto, 2006). The application of spent grain with *Azospirillum* was significantly increased soil fertility and plant growth this results agreement with (Hafez *et al.*, 2019; Rashad *el al.*, 2016).

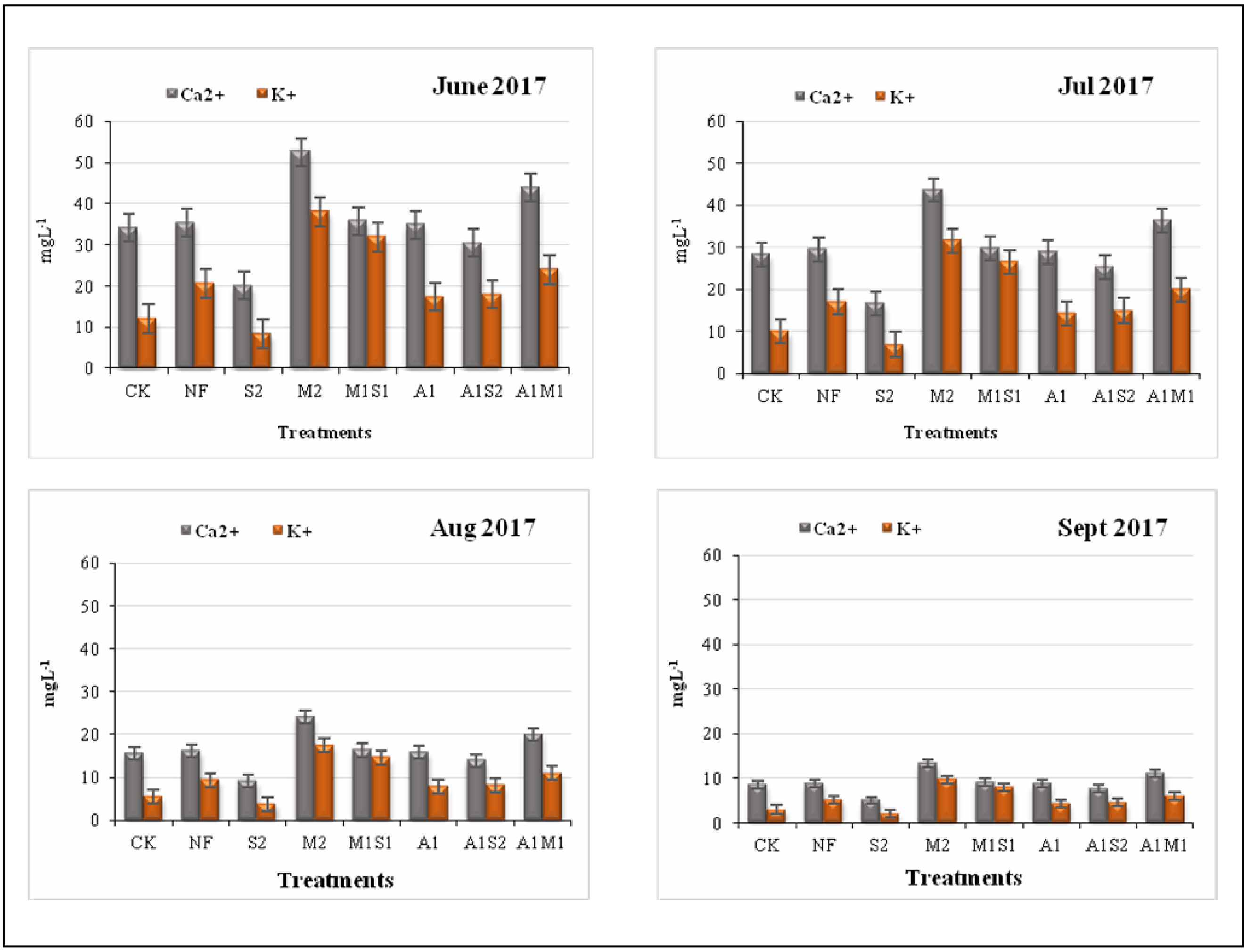

Fig. 3 : Effects of different treatments on Calcium and Potassium leaching losses from soil with cropping seasons in the greenhouse. CK: control; NF: 100% chemical fertilizer; S2: 20 g of spent grain; M2: 20 g of compost; M1S1: mix between M2 and S2; A1: *Azospirillim* inoculation; A1S2: *Azospirillim* + S2; A1M1: *Azospirillim* sp+M1. The standard deviation (n = 3) was analyzed using a one-way ANOVA.

Conclusions

Bio-spent grain fertilizers significantly decreased NO_3^--N and NH_4^+ concentrations in agriculture wastewater compared with compost from June to Sept 2017, but mineral fertilizers and compost applications may cause serious environmental risk. The highest nitrogen use efficiency with the least nitrate losses in soil was also found in S2 and A1 treatments. Moreover, under this bio-organic fertilization way, nitrate concentration in soil leachate was outside of danger of damaging the environment. Thus, spent grain with *Azospirillum* ware suggested to be the optimal fertilizer with the best yield, quality and the least environmental risk under the "*Zea Maiz. L*" organic system.

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