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Combined land application of treated wastewater and biosolids enhances crop production and soil fertility

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ABSTRACT

Land application of treated wastewater (TWW) and biosolids is considered a cost-effective reuse method and valuable source of organic matter and nutrients. However, it may pose an adverse impact on the environment and human health. The main objective of this study is to determine the ability of land application of TWW and biosolids to enhance crop production and soil fertility. The study compared the effect of biosolids, commercial fertilizer, and manure on forage crop production (*Zea mays*). The soil and applied materials were analyzed before the experiment for major characteristics. Application rates were determined and calculated based on the crop nitrogen requirement, which is approximately 135 kg ha^{-1}. Applied materials were incorporated uniformly in the top soil. Drip irrigation system was installed and *Z. mays* were sowed, and the plants were irrigated weekly with TWW to reach field capacity water content. At harvest, the crop yield and yield components were determined. Soil and plant samples were taken at the end of the experiment. Soil samples were analyzed for physical, chemical, and microbiological parameters. Plant samples were analyzed for essential nutrients, heavy metals, and biological analysis. The results have shown that, compared with the control, the crop yield increased similarly with the application of biosolids, commercial fertilizer, or manure. The yield increase was attributed to the increase in both leaves number and plant height that were affected similarly as the yield did. Plant macronutrient and micronutrient concentrations were not significantly affected by the treatments. However, the plant uptake of macronutrients and micronutrients similarly increased with biosolids, commercial fertilizer, and manure. On the other hand, Cd and Pb levels were below the detection limits. The results of soil analysis indicated that soil pH, EC, and SAR were not significantly affected by the treatments. However, CEC and OM significantly increased in the top soil with the application of biosolids, commercial fertilizer, or manure. DTPA-extractable micronutrients were not affected by the treatments. Soil and plant microbiological analysis indicated that *Salmonella* spp., IPN, and total fecal coliform count were not affected by the treatments and all samples were tested negative for enteric viruses. It can be concluded from this study that combined land application of TWW and biosolids improves crop production and enhances soil fertility level without significant impact on the environment and human health.

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1. Introduction

Reusing of treated sewage sludge (biosolids) and treated wastewater (TWW) is an important environmental issue in many countries as being a potential source for contamination of the ecosystem [1,2]. Wastewater treatment plants (WWTP) in Jordan are of the secondary type, utilizing activated sludge treatment processes, and thus generate large quantities of biosolids. The estimated annual amount of biosolids production in Jordan is about 1,682,640 m^3/y (liquid sludge after thickening). Most of this amount is generated from Samra WWTP, the largest in the country (Ministry of Water and Irrigation, MWI, Jordan, 2009) [3]. Moreover, large amounts of biosolids are accumulating inside the premises of Samra (WWTP) since plant started operating (1985).

Disposal of generated biosolids at dumping sites and landfills is the major currently practiced method in Jordan. However, this could be accepted only as a temporary solution. High and real potentials for health hazards and soil and water contamination are associated with such inappropriate disposal [1,4]. This may cause serious unfavorable consequences due to leachate and biogas miss-management. On the other hand, proper use of biosolids can alleviate pollution problems and health hazards. A sustainable and acceptable option for the long-term management of biosolids must be environmentally friendly, economically viable, and socially acceptable [5]. There is general consensus among sanitary engineering professionals that municipal wastewater and biosolids is not a "waste," but a potential source of valuable resources [6]. [7] Biosolids must be regarded as a recyclable resource that can be put to beneficial uses. Although there are different biosolids beneficial use options, application to agricultural land is of primary importance and has been widely practiced in different countries for many years [8–11]. Biosolids [1] and TWW [4,12] proved to be a valuable source for plant nutrients and organic matter needed for maintaining fertility and productivity of arid soils. On the other hand, these sources contain pathogens and some undesirable substances and therefore, their reuse may create environmental problems if not properly treated and managed [13–15]. Best management practices of biosolids (BMPs) are essential for maximizing their beneficial effects and minimizing their adverse effects [16]. BMPs include agronomic loading rates, slope limitations, soil pH limitations, buffer zones, public access restrictions, grazing deferments, soil conservation practices, restrictions for saturated and frozen soils, protection of endangered species, and other site restrictions [17].

The application of sewage sludge (50 t ha−1) had highly significant ($p < 0.01$) positive effects on cation-exchange capacity and organic matter, total nitrogen, phosphorus, iron, and manganese diethylene triamine-penta-acetic acid extractable in soil and nitrogen, phosphorus, iron, and manganese in plant. However, these benefits were limited by the presence of some potentially toxic trace metals in biosolids [18].

The Jordanian regulations for biosolids reuse and disposal No. 1145/2006 [19] classify biosolids according to some microbiological, chemical, and physical aspects into three types: Type I is used as a fertilizer; Type II is used as soil amendment; and Type III is to be disposed of in sanitary landfills. Currently, there are no advanced treatment techniques of sludge to produce Type I biosolids that have been certified as acceptable in Jordan. Few studies have been conducted to evaluate the reuse of biosolids and/or TWW in agriculture. However, the effect of combined application of both biosolids and TWW on the soil–plant system has not been investigated. The goal of this study is to determine if the use of biosolids as fertilizer for forage crop production is an environmentally sustainable management method. The specific objective of the project is to determine the effect of combined land application of biosolids (anaerobically digested) and TWW on plant growth, soil chemical and microbiological parameters of the soil, and consequently on the soil fertility and productivity.

2. Materials and methods

The field pilot experiment was conducted during 2012 (between April and July) at the Zarqa region near the Samra WWTP (40 km northeast of Amman). The average temperature ranges from 7.7 ˚C in January to 26.1 ˚C in August with an average annual rainfall of 147.2 mm/y (1968–2003). Two shallow cultivations were carried out using chisel plow (duck foot model). The experiments have been established in randomized complete block design with four replications. Experimental unit (plot) of 4 m × 5 m was established. The following treatments were investigated: T-1: biosolids (air dried anaerobically digested biosolids obtained from Samra Waste Water Treatment Plant) at a rate of 21 wet tons per dun; T-2: commercial fertilizer (di-ammonium phosphate DAP and urea) at the recommended fertilizer rate of 135 kg N/dun; T-3: manure (obtained from local animal farms) at a rate of 22 wet tons per dun; and T-4: control (nothing was added). All treatments were incorporated uniformly with soil to a depth of 0–15 cm on 1 May 2012. An irrigation system was installed. *Zea mays* was sowed manually at a rate of 100 seeds m-2 (10 rows, 40 cm row spacing) on 22 June 2012. Plants were irrigated once per week. Weeds were controlled manually throughout the growing period.

2.1. Sampling and analysis

2.1.1. Soil analysis

Composite soil samples were collected prior to treatments application at two different depths, 0–15 and 15–30 cm. Samples were air dried and grounded to pass 2-mm sieve. Soil samples were then analyzed for pH and electrical conductivity (EC) in the saturation paste [20]; for total Kjeldahl nitrogen [21]; for phosphorus by extraction with 0.5 M $NaHCO_3$ [22]; for $CaCO_3$ by acid neutralization method [23]; for exchangeable cations by extraction with 1 M NH_4OAc [24]; for cation-exchange capacity (CEC) by the method described by Polemio and Rhoades [25]; and for soil texture by hydrometer method [26]. Soil organic matter was measured by rapid oxidation [27], and Fe, Zn, Cu, Mn, Cd, and Pb by extractions with Diethylene triamine pentaacetic acid (DTPA) [28]. In addition to some biological analysis (*Salmonella* spp., total fecal coliform count (TFCC), and intestinal pathogenic nematodes eggs), results are shown in Table 3. Chemical and microbial analyses were carried out using Methods of Soil Analysis, Parts 2, 3, and 4—SSSA Book Series No 5. After harvesting, composite soil samples at two different depths (0–15 and 15–30 cm) were collected from each plot and analyzed for the same mentioned parameters.

2.1.2. Biosolids and manure analysis

Biosolids used were collected from the accumulated biosolids generated from Samra Waste Water Treatment Plant (2 years old). The biosolids were grounded and applied according to the treatments at the calculated agronomic rates. As for manure used, it was obtained from local animal farms from Al-Dulial area and applied at the calculated rates.

Biosolids and manure composite samples were analyzed for total solid, pH, EC, organic matter, mineral nitrogen (NH_4), total Nitrogen, total Kjeldahl nitrogen, total phosphorus, total potassium, micronutrients, and trace metals (Ca, Na, Mg, Fe, Zn, Mn, Cu, Cd, and Pb), in addition to some biological analyses (*Salmonella* spp., TFCC, and intestinal pathogenic nematodes eggs). Chemical analysis was carried out using Standard Methods for the Examination of Water and Wastewater, online. Microbial analyses were carried out using EPA methods number 1682/2006, 1682/2006, and 625R92/013. These analyses were conducted prior to application.

2.1.3. Crop measurements

At maturity, plants were manually harvested. Plants from each plot were weighed and the fresh yield was calculated. Twenty plants from each plot were sampled randomly to determine the numbers of cones and leaves, stem diameter, and plant height. About 1.5 kg plant materials were taken from each plot to determine dry weight/yield.

2.1.4. Plant analysis

Representative samples of the harvested plant parts were collected, dried, and ground. A representative sample was taken from the ground plant material for analysis. Plants were analyzed for total nitrogen, total phosphorus, total potassium, and trace metals (Zn, Cu, Mg, Fe, Cd, and Pb), in addition to some biological analyses (*Salmonella* spp., TFCC, and intestinal pathogenic nematodes eggs). Plant samples were analyzed for total N using a modified micro-kjeldahl digestion procedure [21] and for total P after dry ashing with the ascorbic acid-molybdate blue method [29]. Total K was also determined in the dry ashed samples digestion using flame emission photometry. Micronutrients and heavy metals in the dry ashed samples were determined using the atomic absorption spectrophotometer. As for the microbial analysis, it was carried out using in-house based on compendium of methods and for the microbiological examination of food (AHA) 1984. Analysis of variance (ANOVA) was used to determine the effect of each treatment. When the *F* ratio was significant, means were tested using Fisher's Least Significance Test (0.05 probability level). Statistical analyses were performed with Systat statistical program [30].

Soil, plant, biosolids, and manure samples were checked against the presence of enteric viruses (six types of viruses: adenovirus, human rotavirus, human hepatitis a, human adenovirus, human enterovirus 71, human coxsackievirus, and human poliovirus). Detection of viruses in plants was carried out using reverse transcription-polymerase chain reaction (RT-PCR) following the protocols of Bianchi et al. [31]. Viruses detection in soil, biosolids, and manure was carried out using ASTM Method D-4994-89, ASTM Method D 4994-89, "Standard Practice for Recovery of Viruses From Wastewater Sludge", Annual Book of ASTM Standards: Section 11, Water and Environmental Technology, 1992.

2.1.5. Irrigation water

The effluent of Samra WWTP is the source of irrigation. It has been characterized against the Jordanian standard No. 893/2006 "Water-reclaimed domestic wastewater" for agricultural purposes (Table 1). Prior to the sowing phase, a sample was taken from the irrigation water and analyzed to determine its N content to help determine the correct application rates for the different treatments. Moreover, during the course of the growing season (irrigation period April–September 2012), six samples of the irrigation water were collected and analyzed. Results show that water used for irrigation complies with the JS No. 893/2006 for irrigating forage crops, industrial crops, and forest.

2.1.6. Statistical analyses

Data were subjected to ANOVA using the General Linear Model procedure of MSTATC Program (Michigan State University). To determine the main effect of each factor, the LSD 0.05 (least significant difference at probability 0.05) was used to separate treatments mean.

3. Results and discussion

3.1. Preliminary soil analysis

Soil's physical, chemical, and microbial properties were determined at two depths: 0–15 and 15–30 cm. The soil has a loam texture with average CEC of 19.3 and 17.2 Cmol/kg at the two depths, respectively; pH 1:2 was moderately alkaline (7.6 and 8.1 SU) at the two depths, respectively; EC (1:2) value was 415 and 456 μS/cm at the two depths, respectively; total N was 0.14 and 0.08% at the two depths, respectively; mineral nitrogen NO_3-N was below detection limit and NH_4-N was 6.9 and 6.7 mg/kg at the two depths, respectively; and exchangeable-K was 727 and 695 mg/kg at the two depths, respectively.

Exchangeable-Na was 341 and 335 mg/kg at the two depths, respectively; level of OM was medium 3.8 and 1.6% at the two depths, respectively; and DTPA extractable of Fe, Zn, Mn, Cu, Cd, and Pb at the two depths were 0.54 and 0.51 mg/kg, 7.4 and 5.3 mg/kg, 1.4 and 1.3 mg/kg, 0.80 and 0.73 mg/kg, 0.024 and 0.012 mg/kg, and 0.34 and 0.28 mg/kg, respectively.

Table 1
Characterization of water used for irrigation (Effluent of Samra WWTP)

Parameter (mg/l)*	Effluent of Samra WWTP**	JS No. (2006/893) Forage, industrial crops and forest
pH (SU)	7.2	6–9
Temp(℃)	30	…
Total dissolved solids TSD	1,108	1,500
Total suspended solids TSS	11	300
Biological oxygen demand BOD_5	3	300
Chemical oxygen demand COD	51	500
Total Kelda nitrogen TKN	4.5	…
NO_3-N	13.4	15.8
NO_2-N	0.032	…
TN	18	100
PO_4	7.2	30
Fats oil and grease FOG	<8	8
Methylene blue active substances MBAS	0.15	100
Phenol	<0.002	<0.002
Total fecal coliform count (MPN)	8.2E + 02	…
Intestinal pathogenic nematodes eggs IPN (Egg/ 5L)	Not seen	<1

Note: MPN: Most probable number.
*Units are in (mg/l) unless otherwise mentioned.
**Average during irrigation period (April–September 2012).

Soil microbiological analysis showed that *Salmonella*, TFCC, IPN, and Enteric viruses were not detected for both soil depths; except for *salmonella* at 15–30 cm, soil depth was detected at a very low level (Table 2). So, in general, the soil is characterized by being calcareous and alkaline, non-saline with relatively high CEC. In addition, the soil is low in N and micronutrients, and relatively high in P and K.

3.2. Biosolids and manure analysis

Table 3 shows biosolids and manure characteristics. The Jordanian regulation for biosolids use and disposal No. 1145/2006 classifies biosolids according to some microbiological, chemical, and physical aspects into three types: Type I that can be used as a fertilizer; Type II that can be used as soil amendment; and Type III, which is to be disposed of in sanitary landfills. By comparing the analyses results for the biosolids sample with requirements of the JS No. 1145/2006, it can be concluded that the trace metals' concentrations and microbiological results meet Type I biosolids criteria. Biosolids, manure, and commercial fertilizer rates were based on the agronomic nitrogen requirement of corn (*Z. mays*), which is approximately 135 kg dun-1. It is noted that if the target yield is between 5.0 and 7.0 tons per acre for corn (*Z. mays*), the application rate of biosolids should be 21 wet tons per dun. As for manure, it should be 22 wet tons per dun for the same target yield. In general, the proper rate is one that maximizes yield while ensuring protection of the environment [32]. However, application rate of biosolids has commonly been based on its nitrogen content, nitrogen requirement of the crop, and its cadmium content [33].

3.3. Plant analysis

3.3.1. Crop measurements

Table 4 shows the effect of different treatments on different crop measurements. The following parameters were determined: fresh yield, dry yield, No. of cones, No. of leaves, stem diameter, and plant height.

Table 2
Preliminary analysis of the soil

Parameter	Result 0–15	 15–30
Soil texture	Loam	Loam
pH (1:2)	7.6	8.1
EC (1:2), μS/cm	415	456
CEC, CMOL/kg DW	19.3	17.2
OM, %	3.8	1.6
TN, %	0.14	0.08
TKN, %	0.15	0.08
NH_4-N, %	6.9	6.7
NO_3-N, mg/kg DW	<0.226	<0.226
Available P (PO_4), mg/kg DW	219	250
Soluble Na, mg/kg DW	189	93
Soluble Mg, mg/kg DW	39	7.2
Soluble Ca, mg/kg DW	72	15
SAR, mg/kg DW	4.4	4.9
Exchangeable-K, mg/kg DW	727	695
Exchangeable-Na, mg/kg DW	341	335
DTPA-extractable Fe, mg/kg DW	0.54	0.51
DTPA-extractable Zn, mg/kg DW	7.4	5.3
DTPA-extractable Mn, mg/kg DW	1.4	1.3
DTPA-extractable Cu, mg/kg DW	0.80	0.73
DTPA-extractable Cd, mg/kg DW	0.024	0.012
DTPA-extractable Pb, mg/kg DW	0.34	0.28
Salmonella spp., MPN/4 g DW	<0.14	0.33
TFCC, MPN/g DW	<0.036	<0.036
IPN (Intestinal pathogenic nematodes) egg/4 g DW	Not seen	Not seen
Enteric viruses, PFU/ 4 g DW	<1	<1

Note: MPN: Most probable number; PFU: Plaque-forming unit; DW: Dry Weight.

Table 3
Biosolids and manure characteristics

Parameter	Biosolids	Manure
Moisture content, %	9.2	23.9
Volatilization loss (TVs), %	38.5	43.4
pH (1:2)	6.84	9.37
EC (1:2), μS/cm	12,090	25,630
Organic carbon (OM), %	39	41
N, %	2.7	1.6
TKN, %	2.7	1.7
P, %	1.6	0.9
K, %	0.12	2.1
NH_4-N, mg/kg DW	10.0	18.6
Ca, %	13.8	6.9
Na, %	0.2	0.6
Mg, %	0.92	1.25
Chloride, %	0.5	1.5
Fe, %	1.0	0.9
Mn, mg/kg DW	193	327
Cd, mg/kg DW	2.6	2.1
Cu, mg/kg DW	171	34
Pb, mg/kg DW	70	7.5
Zn, %	0.16	0.02
Salmonella spp., MPN/4 g dry weight	<0.16	<0.013
TFCC, MPN/g dry weight	0.05	<0.03
IPN (Intestinal pathogenic nematodes) egg/4 g DW	Not seen	Not seen
Enteric viruses, PFU/4 g DW	<1	<1

Table 4 shows that the fresh and dry yield/plot was affected by the addition of different treatments during the first season. Minimum fresh and dry yield was obtained at control treatment (71.4, 19.1 kg/plot, respectively), while the maximum was obtained at manure treatment (91.8, 26.9 kg/plot, respectively). The ANOVA shows significant increase in fresh and dry yield at manure and biosolids treatments over the control. However, they are insignificantly different from each other or from fertilizer treatment.

Number of cones was slightly affected by the addition of different treatments during the first season (Table 4). The crop was harvested before cone formation, which resulted in the minimal number of cones. The minimum number of cones was obtained at control treatment (0.75), while the maximum was obtained at fertilizer treatment (1.05). The ANOVA shows insignificant differences between different treatments. Number of leaves increased significantly by the addition of biosolids treatment over the control. However, there were no significant differences between biosolids treatment and both manure and fertilizer treatments. Corn (*Z. mays*) stem diameter was not affected by the addition of different treatments during the first season. The ANOVA shows insignificant differences between different treatments. Plant height increased significantly at biosolids and manure treatments over the control. There were no significant

Table 4
The effect of different treatments on different crop measurements

Treatment	Fresh yield kg/plot	Dry yield kg/plot	No. of cones	No. of leaves	Diameter (cm)	Plant height (cm)
Control	71.4 b	19.1 b	0.75 a	14.15 b	9.6 a	216 b
Biosolids	90.0 a	26.4 a	0.77 a	15.52 a	9.9 a	243 a
Manure	91.8 a	26.9 a	1.02 a	14.52 ab	9.9 a	246 a
Fertilizer	84.8 ab	23.8 ab	1.05 a	14.52 ab	9.7 a	238 ab
LSD 0.05	15.07	4.889	0.4772	1.035	1.318	23.08

Note: a, b, and ab show the significance between different treatments.

differences between biosolids, manure, and fertilizer treatments. Minimum plant height was obtained at control treatment (216 cm), while the maximum was obtained at manure treatment (246 cm). It can be inferred from the yield results that the application of biosolids was as effective as the manure and fertilizer application in increasing the plant growth and crop production. Other researchers have reported positive effects of biosolids and TWW on crop production [1,4,34]. The positive effects of biosolids and TWW land application on plant growth are attributed to the their nutrient contents as well as to their favorable effect on soil chemical and physical properties of the soil [13,14,35,36].

3.3.2. Plant chemical analysis

Effects of different treatments on macronutrient concentration: Table 5 shows the effect of different treatments on macronutrients concentration in corn (*Z. mays*). Nitrogen concentration was slightly affected with different treatments application. There were significant increases in N concentrations at fertilizer treatment over the control; the minimum concentration was obtained at manure application (1.34%), while the maximum was obtained at fertilizer application (1.69%); however, there were no significant differences between biosolids treatment and both manure and fertilizer treatments. Phosphorous concentration significantly increased at manure treatment over the control. The minimum concentration was obtained at control and fertilizer treatments, while the maximum was obtained at manure treatment. There were no significant differences between other treatment (biosolids, fertilizer, and control).

Potassium concentration significantly decreased at biosolids treatment over the other treatments. The minimum concentration was obtained at biosolids treatment (1.29%), while the maximum was obtained at fertilizer treatment (1.39%). There were no significant differences between manure, fertilizer, and control treatments. Magnesium concentration was not affected by different treatments application. There were no significant differences between biosolids, manure, fertilizer, and control. Effects of different treatments on micronutrient concentration: Table 6 shows the effect of different treatments on micronutrients concentration in corn (*Z. mays*). There were no significant differences in zinc, iron, and copper concentrations between different treatments; all treatments were not significantly different over the control. As for cadmium and lead concentration, analysis results were below the detection limit.

Table 5
The effect of different treatment on macronutrients concentration in corn (*Z. mays*)

Treatment	N%	P%	K%	Mg%
Control	1.39 b	0.25 b	1.38 a	0.285 a
Biosolids	1.48 ab	0.26 b	1.29 b	0.293 a
Manure	1.34 b	0.28 a	1.37 a	0.291 a
Fertilizer	1.69 a	0.25 b	1.39 a	0.289 a
LSD 0.05	0.23	0.15	0.38	0.0628

Note: a, b, and ab show the significance between different treatments.

3.3.3. Plant microbiological analysis

Table 7 shows the microbiological analysis of corn (*Z. mays*) at harvesting stage. The results indicate that *Salmonella* and IPN were not detected and levels of TFCC were only slightly above detection limits and the values were not affected by different treatments. All samples were tested negative for rotaviruses, eneteroviruses (human enterovirus, human poliovirus, human echovirus, and coxsackievirus), and hepatitis virus A. Additionally, all samples were tested negative for adenoviruses using PCR. Accordingly, corn samples were free of the tested viruses based on PCR assays.

3.4. Soil analysis

3.4.1. Soil chemical analysis

Soil samples were chemically analyzed for soil texture, pH, EC, CEC, SAR, organic matter, mineral nitrogen (NH_4 and NO_3), available phosphorus (PO_4-P), soluble Na, soluble Ca, soluble Mg, exchangeable K, exchangeable Na, DTPA extractable micronutrients, and trace metals (Fe, Zn, Mn, Cu, Cd, and Pb). The effects of different treatments on soil properties are presented in Tables 8–10.

Soil pH 1:2 values show no significant differences between different treatments at two soil depths, 0–15 and 15–30 cm. The pH value ranged between 8.1 for control and 8.3 for biosolids at 0–15 cm soil depth. Also, it ranged between 8.1 for fertilizer and 8.3 for control at a 15–30 cm depth. EC values (EC 1:2) show no significant differences between different treatments at two soil depths, 0–15 and 15–30 cm. EC values were ranged from 593 μS/cm (fertilizer treatment) to 616 μS/cm (biosolids treatment) at 0–15 cm soil depth. As for the 15–30 cm soil depth, it ranged between 502 μS/cm (fertilizer treatment) and 531 μS/cm (manure treatment). CEC values show significant increase at biosolids, manure, and fertilizer treatments over the control at 0–15 cm soil depth. However, there were no significant differences between different treatments at

Table 6
The effect of different treatment on micronutrients concentration in corn (*Z. mays*)

Treatment	Zn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
Control	62 a	437 a	8.4 a	<0.25	<4.5
Biosolids	61 a	319 a	7.0 a	<0.25	<4.5
Manure	55 a	311 a	7.9 a	<0.25	<4.5
Fertilizer	56 a	341 a	7.7 a	<0.25	<4.5
LSD 0.05	6.7	142	2.8	..	..

Note: a, b, and ab show the significance between different treatments.

Table 7
The effect of different treatment on microbiological analysis of corn (*Z. mays*) at harvesting stage

Treatment	TFCC (MPN/g)	*Salmonella* spp. (present/absent/25g)	IPN (Egg/150g)	Viruses (PFU/g)
Control	67	Absent	Not seen	<1
Biosolids	28	Absent	Not seen	<1
Manure	1	Absent	Not seen	<1
Fertilizer	4	Absent	Not seen	<1

Table 8
The effect of different treatment on soil chemical properties

Treatment	pH (1:2) SU		EC (1:2) μS/cm		CEC Cmol/kg		OM%		SAR	
Soil depth cm	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30
Control	8.1 a	8.3 a	603 a	528 a	20 b	19 b	2.1 b	2.8 a	4.9 a	5.8 a
Biosolids	8.3 a	8.2 a	616 a	522 a	22 a	21 ab	3.6 a	3.4 a	4.5 a	5.3 a
Manure	8.2 a	8.2 a	611 a	531 a	23 a	23 a	2.7 ab	3.0 a	6.1 a	5.3 a
Fertilizer	8.1 a	8.1 a	593 a	502 a	23 a	22 ab	2.8 ab	2.7 a	5.9 a	5.2 a
LSD 0.05	0.9327	0.2529	112.2	60.15	1.813	3.232	1.382	1.33	2.317	1.166

Note: a, b, and ab show the significance between different treatments.

15–30 cm soil depth except for manure treatment as it increased significantly over the control. CEC values ranged from 20 to 23 Cmol/kg at 0–15 cm soil depth and from 19 to 23 Cmol/kg at 15–30 cm soil depth. Organic matter concentrations (OM) show that there is a slight increase within all treatments over the control. However, it increased significantly in the biosolids treatment at 0–15 cm soil depth. Moreover, there were no significant differences between other treatments over the control. As for 15–30 cm depth, there were no significant differences for all treatments. OM value was medium and ranged from 2.1% (control treatment) to 3.6% (biosolids treatment) at 0–15 cm soil depth, and from 2.7% (manure treatment) to 3.4% (biosolids treatment) at 15–30 cm soil depth.

Sodium absorption ratio (SAR) values show no significant differences between different treatments at two soil depths, 0–15 and 15–30 cm. The SAR value ranged between 4.5 for biosolids and 6.1 for manure at 0–15 cm soil depth. Also, it ranged between 5.2 for fertilizer and 5.8 for control at 15–30 cm depth. Plant available nitrogen (nitrate NO_3-N and ammonium NH_4-N) and nitrate concentration at two soil depths were relatively low. At soil depth 0–15 cm, no significant differences were observed between different treatments. At soil depth 15–30 cm, nitrate concentration slightly increased with biosolids and manure treatments over the control; however, the increase was not statistically significant. On the other hand, nitrate concentration was significantly increased at biosolids and manure treatments over the fertilizer treatments. Ammonium (NH_4-N) concentrations were ranged from 8.3 to 6.7 mg/kg at 0–15 cm soil depth and from 7.6 to 6.8 mg/kg at 15–30 cm soil depth, which is considered typical. At soil depth 0–15 cm, NH_4-N significantly increased in biosolids treatment over all other treatments. At soil depth 15–30 cm ,there was no significant difference between different treatments (Table 8).

Plant available phosphorus (PO_4-P) results show no significant differences between different treatments at two soil depths, 0–15 and 15–30 cm. PO_4-P values were high and ranged from 365 mg/kg (biosolids treatment) to 306 mg/kg (fertilizer treatment) at 0–15 cm soil depth. As for the 15–30 cm soil depth, it ranged between 220 mg/kg (biosolids treatment) and 156 mg/kg (control treatment).

Soluble sodium values (soluble-Na) show no significant differences between different treatments at two soil depths, 0–15 and 15–30 cm. Soluble-Na values ranged from 133 to 110 mg/kg at 0–15 cm soil depth and from 103 to 110 mg/kg at 15–30 cm soil depth. Soluble calcium values (soluble-Ca) increased significantly in biosolids treatment over manure treatment at two soil depths, 0–15 and 15–30 cm. At soil depth 0–15 cm, there was no significant increase between biosolids, manure, and fertilizer treatments over the control treatment. At 15–30 cm, soil depth soluble-Ca concentration increased significantly in biosolids and manure treatment over the control treatment.

Soluble magnesium values (soluble-Mg), at soil depth 0–15 cm, significantly increase in biosolids treatment over all other treatments (control, manure, and fertilizer). On the other hand, there were no significant differences between different treatments at 15–30 cm soil depth. Exchangeable potassium (exchangeable-K) results show no significant differences between different treatments at 0–15 cm soil depth. At 15–30 cm soil depth, exchangeable-K concentration increased significantly in manure treatments over the control and fertilizer treatments, and there was no significant increase over the biosolids treatment. Exchangeable sodium (exchangeable-Na) results show no significant differences between different treatments at soil depths 0–15 and 15–30 cm. Exchangeable-Na values ranged from 266 mg/kg (fertilizer treatment) to 327 mg/kg (manure treatment) at 0–15 cm soil depth. As for the 15–30 cm soil depth, it ranged between 281 mg/kg (biosolids treatment) and 314 mg/kg (control treatment). Micronutrients concentration in soil results show that Fe, Zn, Mn, Cd, and Pb were not significantly affected by different treatments application at two soil depths. Cu concentration was not significantly affected at 0–15 cm soil depth. On the other hand, at 15–30 cm soil depth, it increased significantly in manure treatment over other treatments (Table 9).

Mohammad and Mazahreh [2] reported that secondary treated WW improved soil fertility parameters such as soil organic matter and plant nutrients contents. On the other hand, they reported an adverse impact on the soil salinity parameters. Therefore, they recommend to adopt an appropriate irrigation management of and to use an efficiently TWW to reduce salt content. In addition, proper irrigation management and periodic monitoring of soil quality parameters are required to minimize adverse effect on the soil. In addition, Mohammad and Athamneh [1] and Rusan and Athamneh [2] have reported positive impact of sewage sludge land application of some soil fertility parameters such as soil, organic matter, and plant nutrients, but they, at the same time, reported potential negative impact on heavy metal accumulation in the soil. Khai et al. [16] reported that the use of biosolids as a fertilizer significantly increased total organic carbon and total nitrogen in the soil. Mahdy et al. [37] reported that the effective co-application ratio of biosolids to water treatment residuals (WTRs), for increasing corn yield and minimizing the potential for bioavailable P in runoff, was approximately 1:1 at the application rate of 3% biosolids, and 4% WTRs in the alkaline soils.

3.4.2. Soil microbial analysis

Table 11 shows the microbiological analysis of soil at tow depth 0–15 and 15–30 cm. The results indicate that *Salmonella* and IPN were not detected, and levels of TFCC were only slightly above detection limits and the values were not affected by different treatments. All soil samples were tested negative for rotaviruses, enteroviruses (human enterovirus, human poliovirus, human echovirus, and coxsackievirus), and hepatitis virus. Accordingly, soil samples were free of the tested viruses based on ASTM Method D-4994-89, ASTM Method D 4994-89, "Standard Practice for Recovery of Viruses From Wastewater Sludge", Annual Book of ASTM Standards: Section 11 - Water and Environmental Technology, 1992.

Zerzghi et al. [38] evaluated the influence of 20 annual land applications of Class B biosolids (US EPA [Rule 503]) on the soil microbial community. Data showed that land application of Class B biosolids had no significant long-term effect on indigenous soil microbial numbers including bacteria, actinomycetes, and fungi compared with un-amended control plots. Importantly, no bacterial or viral pathogens were detected in soil samples collected from biosolids-amended plots.

Table 9
The effect of different treatments on soil chemical properties*

Treatment	NO_3-N		NH_4-N		PO_4-P		Soluble Na		Soluble Ca		Soluble Mg		Exchangeable K		Exchangeable Na	
Soil depth cm	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30
Control	2.0 a	3.1 ab	6.8 b	6.8 a	316 a	156 a	110 a	110 a	24 ab	15 c	9.0 b	7.4 a	562 a	517 b	310 a	314 a
Biosolids	5.0 a	5.4 a	8.3 a	6.9 a	356 a	210 a	119 a	108 a	32 a	17 a	13.2 a	8.4 a	577 a	543 ab	296 a	281 a
Manure	5.1 a	5.9 a	6.9 b	7.6 a	312 a	218 a	125 a	103 a	18 b	16 b	8.9 b	7.3 a	618 a	579 a	327 a	305 a
Fertilizer	2.0 a	2.1 b	6.7 b	6.8 a	306 a	190 a	133 a	103 a	21 ab	15 c	9.9 b	8.5 a	555 a	508 b	266 a	292 a
LSD 0.05	3.135	2.393	1.452	0.8629	49.91	81.83	50.25	20.22	10.35	0.9231	3.203	1.507	83.37	54.99	63.93	54.42

Note: a, b, and ab show the significance between different treatments.
*Units are in (mg/kg dry weight) unless otherwise mentioned.

Table 10
The effect of different treatments on DTPA extractable micronutrients and trace metals*

Treatment	Fe		Zn		Mn		Cu		Cd		Pb	
Soil depth cm	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30
Control	6.2 a	8.4 a	9.7 a	12.5 a	67 a	91 ab	1.06 a	1.24 a	0.032 a	0.037 a	0.66 a	0.86 a
Biosolids	6.7 a	9.5 a	10.3 a	13.2 a	70 a	95 a	1.12 a	1.26 a	0.035 a	0.039 a	0.68 a	0.93 a
Manure	6.0 a	7.9 a	9.2 a	12.6 a	64 a	86 b	0.97 a	1.06 b	0.030 a	0.035 a	0.72 a	0.93 a
Fertilizer	7.0 a	8.6 a	9.9 a	12.0 a	70 a	92 ab	1.03 a	1.19 ab	0.029 a	0.035 a	0.67 a	0.87 a
LSD 0.05	1.457	1.947	1.808	1.492	9.431	8.775	0.1959	0.1518	0.016	0.016	0.0112	0.1893

Note: a, b, and ab show the significance between different treatments.
*Units are in (mg/kg dry weight) unless otherwise mentioned.

Table 11
The effect of different treatment on microbiological analysis of soil

Treatment	TFCC (MPN/g)		*Salmonella* spp. (MPN/4g DW)		IPN (Egg/4g DW)		Viruses (PFU/4g DW)	
Soil depth cm	0–15	15–30	0–15	15–30	0–15	15–30	0–15	15–30
Control	0.09	0.09	Absent	Absent	Not	Not	<1	<1
Biosolids	0.05	0.21	Absent	Absent	Not	Not	<1	<1
Manure	0.02	0.11	Absent	Absent	Not	Not	<1	<1
Fertilizer	0.01	0.04	Absent	Absent	Not	Not	<1	<1

4. Conclusion

The research demonstrates the usage of biosolids as a fertilizer and gives results similar to that of using commercial fertilizer or manure with no adverse impact on the environment. Therefore, land application of biosolids enhances crop production and reduces the use of chemical fertilizer and cost of production. Moreover, such practice would demonstrate a sustainable management option and avoid the risk of unsafe and uncontrolled methods of biosolids disposal.

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