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ORIGINAL RESEARCH

Application of sewage sludge for cereal production in a Mediterranean environment (Lebanon)

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Abstract

Purpose Management of sewage sludge generated from wastewater treatment plants is a big challenge for its potential reusage in agriculture. Most of the Lebanese local sludge is discarded in the water or in landfills (most wastewater plants are partially functional). The objective of this research was to assess the effect of the application of different sewage sludge rates on the wheat production as an alternative of chemical fertilizer.

Method Field trials were conducted, for one-year study, in IAAT village in the Bekaa valley-Lebanon. The considered treatments of 4, 8 and 16 kg.m^{-2} rates were compared to a control treatment. Physicochemical and microbiological analysis were performed on sludge and soil samples (pre cultivation and post-harvest). The harvested wheat was also analyzed for several parameters as mineral content.

Results Results presented significant differences between control and treatments. pH values decreased with biosolids additions; organic matter rose in the amended soils, macronutrients levels increased. Heavy metals outcome increased significantly after amendment, microbiological analysis didn't show any contamination by *Salmonella*, *E.Coli*, *Staphylococcus Aureus* and Helminth eggs. As for wheat plants' evaluation, fiber and protein contents presented an increase similar to nitrogen and phosphorus.

Conclusion These results are a key component that identifies the role of biosolids as pH regulator and soil conditioner which improves the physicochemical properties of soil without any risk of microbiological contamination. These results are promising and they encourage the use of biosolids as agriculture amendment.

Keywords Soil conditioner, Sewage sludge, Macronutrients, Micronutrients, Grain yields

Introduction

Biosolids are an inevitable byproduct of urban wastewater treatment plants. Municipalities and other operators search for best disposal and usage for biosolids. Like animal manure, biosolids occupy a large part of the nature cycle (Jacobs and McCreary 2001). One of the most appropriate management techniques of biosolids are agricultural usage (Bittencourt et al. 2014). Biosolids have been described as crop yield enhancer (Eid et al. 2020) that supply valuable nutrients to plants. On average, organic content in biosolids can reach 80%, and significant amounts of macronutrients such as nitrogen, phosphorus and potassium can be supplied. Indeed, a common environmental profit is the recovering of phosphorus in the food chain which provides to the preservation of mineral phosphorus reserves, consequently lowers cadmium inputs present in phosphate rocks (Ahmed et al. 2010). Besides being rich in organic matter, nitrogen and especially phosphorus (Harrison et al. 2003), its advantages reside in improving soil properties which will provide an advantage for sustained crop production. Lu et al. (2012) in their review on land application of biosolids reported that soil properties (porosity, moisture, electrical conductivity, texture…) are improved when land is amended with biosolids. In addition, they have mentioned that biosolids can be used as a substitute to chemical fertilizers. Some more benefits of biosolids usage in agriculture is the sequestration of a portion of the recycled carbon into the soil which will reduce greenhouse emissions

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and thus climate change. In addition, the replacement of chemical fertilizer by biosolids is claimed to reduce nitrous oxide emissions associated with fertilizers (Suanon et al. 2016). Nontheless, the concern of heavy metals uptake by plants and its cycling between the system biosolids-soil-plant is a real health threat to be monitored without forgetting the negative impact of existing pathogens on soil and environment (Du et al. 2012). In Brazil, the Resolution of the National Environment Council (Conama 375/06) and in the State of Paraná the Resolution of the State Environment Cabinet (Sema 021/09) established strategies for agricultural use of biosolids, which resulted in economic and agricultural benefits (Bittencourt et al. 2014). As for the United States of America, protective regulations and continuous monitoring of biosolids applications on land is required (Lu et al. 2012). In Saudi Arabia, the barley growth was boosted when they used the sewage sludge as soil amendment (Eid et al. 2020). However, in Lebanon, although a proposition for Lebanese guidelines on sludge reuse in agriculture is being established (FAO 2010), there was no studies on the effects of applying biosolids on lands and on plants' nutrient status (Romanos et al. 2019). In that context, the aim of this study was to conduct a field trial for the assessment of the use of sewage sludge as an alternative to the use of mineral fertilizers in cereal production in the Bekaa valley of Lebanon. The impact of using such biosolids on physicochemical and biological properties of the agricultural soil will be also considered. In addition, *Triticum aestrum* Var Tal Aamara" was chosen to evaluate the effectiveness of the application of biosolids on the nutrient profile of the plant.

Material and methods

Experimental site and climate

The field experiment was carried out during the growing season, 2019, in the village of IAAT located in the Bekaa Valley, Lebanon (34.048410N lat., 36.143973E long.). The experiment was conducted in a field near the Wastewater Treatment Plant (WWTP) that serves the village. The WWTP is treating municipal wastewater according to a secondary treatment through activated sludge process, followed by disinfection by chlorination. The sludge was treated conventionally by drying it under solar rays (UNDP 2013). The soil of the study area is sandy clay loam containing 50.62% sand, 32.44% clay and 16.94% silt (Romanos et al. 2019). The climate is typically Mediterranean, characterized by a cold winter and a hot-dry season from April to October. The main weather parameters were obtained from a standard agro-meteorological station located in the region. The weather regime, in terms of minimum and maximum temperatures (Tmin and Tmax), rainfall (R), and reference evapotranspiration (ETo) during the growing season are given in Fig 1. In general, the average maximum air temperature during the growing cycle in 2019 was 22.36 °C while the average minimum temperature was 5.41 °C. The total precipitation was 529 mm.

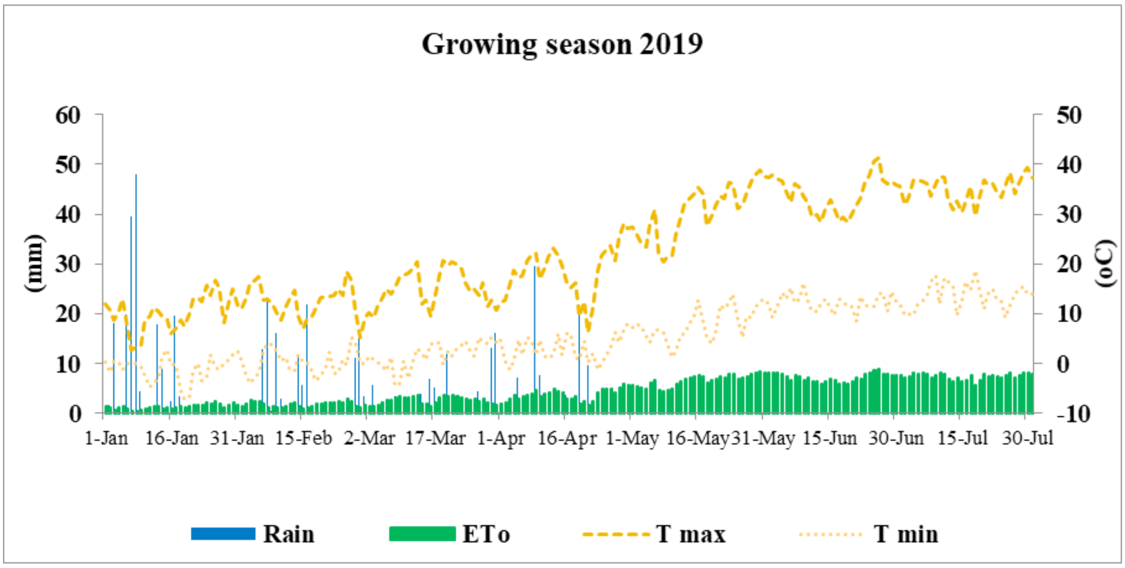

Fig. 1 Rainfall (mm), maximum and minimum temperatures (°C) and reference evapotranspiration (mm) for the growing season 2019

Treatments and agronomic management

Durum wheat (*Triticum durum* L.) was sown in rows 15 cm apart. The seeding rate was 250 kg ha^{-1}, according to the standard practices in the Bekaa valley. The response of the crop was assessed under different sewage sludge (SS) rates. Sewage sludge was collected from the local WWTP of IAAT. Sludge doses of 4, 8 and 16 $kg.m^{-2}$ were used, reflecting the maximum allowable doses of sewage sludge according to the Lebanese guidelines for sludge reuse in agriculture (FAO 2010). In total, the experiment consisted of four treatments with four replicates per treatment. These treatments were: C- control soil without sewage sludge but a rate of 10 $g.m^{-2}$ urea amendment was added; SS_4- sewage sludge added to the soil at a rate equivalent to: 4 $kg.m^2$; SS_8- sewage sludge added to the soil at a rate equivalent to: 8 $kg.m^{-2}$; SS_{16}- sewage sludge added to the soil at a rate equivalent to: 16 $kg.m^{-2}$. Treatments were arranged in a randomized complete block design. Each plot replicate was 2 x 2m. Sowing time occurred on January 1st, 2019 while harvest was on July 30th. The sewage sludge was added in the soil before sowing of seeds. The appropriate dose of sewage sludge in each treatment was incorporated into the topsoil up to 10-15 cm depth (Fig 2).

Fig. 2 Sludge incorporation as soil amendments

Soil and sewage sludge analysis

Before sowing, the soil was analyzed before the application of sewage sludge. In addition, an analysis of the sewage sludge was also conducted. At the end of the season and after harvesting the crop, soil samples were collected from the different treatments for analysis. Soil and SS samples were collected, air dried, and sieved.

Physico-chemical analysis

Soil sampling was done according to the ICARDA (International Center for Agricultural Research in the Dry Areas) manual (Estefan et al. 2013). From every site, the collection of three subsamples was performed. With the use of an auger the samples were taking, from each 4 m^2 plot, at a depth of 20 cm. After sampling, soils were air dried in labelled trays. After drying, samples were crushed and sieved with the use of a special soil grinder (ELE international Fb523-100-01) and then distributed in special labelled containers.

The first physico -chemical analysis was soil texture determination as per Bouyoucos Hydrometer Method, soil pH and electrical conductivity (EC). In summary, 50 g of dried, fine-textured soil were weighed into a baffled stirring cup. The sample is treated with 40 mL sodium hexametaphosphate (50 $g.L^{-1}$) and 5mL hydrogen peroxide 35%. The mixture is stirred for 15 minutes until soil aggregates are broken down. Then the mixture is transferred to a settling cylinder and filled to the lower mark with room temperature distilled water while the hydrometer is in the liquid. Then, after shaking the suspension vigorously in a back-and-forth manner, the hydrometer (R1 and R2) and temperature (T1 and T2) readings are recorded, and the mixture is settled down for 2 hours before another reading again. Corrections are made for the density and temperature of the dispersing solution (ISO 2006).

The calculations are deducted from the equations:

$$\%Clay + Silt = \frac{R1 + [(T1 - 20)x0.36]}{weight}x100$$
$$\%Clay = \frac{R2 + [(T2 - 20)x0.36]}{weight}x100$$
$$\%Silt = (\%Clay + Silt) - \%Clay$$
$$\%Sand = 100 - \%(Clay + Silt)$$

The texture is determined from the USDA textural triangle (Estefan et al. 2013).

As for pH and EC determination, the analysis method was following the British Standard Institute (Das et al. 2013); 20 g of soil were suspended in 100 mL of distilled water and shacked vigorously using a special shaker (Orbit Shaker, Labline Junior 3521). The value is recorded with a pH meter and Electrical conductivity (Orion Star pH meter from Thermo Scientific) after buffer calibration.

Organic matter and total limestone were determined as per ISO methods. Organic matter was determined according to the Walkley-Black method (ISO 14235 1998). 10 mL of potassium dichromate (1 N) and 20 mL of concentrated sulfuric acid were added to approximately 0.5 g of sieved and grinded soil. After 30 minutes, total organic carbon was determined by titration with ferrous ammonium sulphate (0.5 N) in the presence of sodium fluoride and the indicator diphenylamine hydrochloride. Colour will turn from dark blue to green.

Total limestone or carbonate is determined by the method of Bernard Calcimeter (ISO 2010). Briefly, Hydrochloric acid (6 N) is added to 0.5 g of soil to decompose the carbonate present in the soil. The volume of carbon dioxide is measured using the apparatus. The percentage of carbonate is determined by the equation:

$$\%CaCO_3 = \frac{(V_1 - V_0) \times 0.4}{weight}$$

Where V_0: volume of carbon dioxide before reaction with soil carbonate

V_1: volume of carbon dioxide when total reaction with carbonate is finished

For the active limestone, Drouineau method is used to determine active Das et al. (2013). 250 mL of ammonium oxalate (0.2 N) are added to 2.5 g of soil. The solution is shacked for 2 hours and then filtrated. 10 mL of the filtrate is heated up to 70 °C with 5 mL of concentrated sulfuric acid. The titration is realized with potassium permanganate (0.2 N) until a persistent pink colour is obtained.

Table 1 summarizes some physico-chemical properties of the experimental soil and the sewage sludge used for this study

Then macronutrients were analysed: total nitrogen, available phosphorus, potassium, sodium, calcium and magnesium.

Total nitrogen was digested, then measured by Kjeldahl method. Briefly, 0.5 g of soil is digested with aqua regia solution in a microwave digester (Ethos Easy – Milestone). 50 mL of the digested solution is distilled with sodium hydroxide and recovered in an acid boric solution that is back titrated with the use of hydrochloric acid (ISO 1995).

Available phosphorus is determined according to the “Olsen method”. 2.5 g of soil is extracted in 50 mL of 0.5 N sodium hydrogen carbonate, then filtrated and 10 mL were added to a mixture of 8 mL single reagent containing ammonium molybdate, ascorbic acid and a small amount of antimony. The intensity of the blue colour is spectrophotometrically measured at a wavelength of 882 nm (ISO 1994).

Extractable potassium, calcium and magnesium are determined according to Estefan et al. 2013. In brief, 5 g of soil are extracted in 50 mL of 1 N ammonium acetate solution. The concentration of potassium is determined by flame photometer. As for calcium and magnesium, their levels are calculated with the use of atomic absorption technique (iCE 3000 series AA spectrometers -Thermo Fisher Scientific brand).

Heavy metal analysis

Thirteen heavy metals were determined: mercury, lead, chromium, nickel, zinc, copper, cadmium, aluminum, iron, boron, molybdenum, cobalt and manganese. Heavy metals’ content was determined according to the international organization for standardization (ISO 2012). Briefly, 0.5 g of soil sample is weighted and digested in aqua regia solution. Digested soil is then analyzed for heavy metals determination with the use of Atomic Absorption technique iCE 3000 series AA spectrometers (Thermo Fisher Scientific).

Pathogens analysis

The detection of *Salmonella* in soil samples was conducted following ISO 6579 method : an incubation period at 37 °C for 18± 2h in a non - selective growth in peptone water was followed by adding 0.1 mL of the obtained suspension that were transferred to 10 mL of selective medium with acidic sodium selenite , and 1 mL of suspension was transferred to 10 mL of MKTTn selective medium. The selective multiplication was performed at 37 °C for 24± 2h. Afterward, the solid growth media XLD agar was inoculated with the selective colonies of *Salmonella* at 37 °C, for 24±2h. When characteristic colonies were found, polyvalent serological latex tests were conducted to confirm the presence of *Salmonella* (ISO 2002).

The quantification of *E. coli* was elaborated according to ISO 16649-2 method (ISO 2001). Duplicate plates of 15 mL tryptone-bile-glucuronic medium (TBX) are inoculated with 1 mL of the initial suspension. Under the same conditions, using decimal dilutions of the initial suspension, two plates per dilution are inoculated. The dishes are incubated for 18 h to 24 h at 44 °C ± 1 °C then examined to detect the presence of colonies which, from their characteristics, are considered to be β-glucuronidase-positive *Escherichia coli*.

As for the detection of *Staphylococcus Aureus*, the enumeration of *staphylococci* involves the inoculation of a 10^{-1} dilution (or other appropriate dilutions) of the sample on a selective agar medium and other appropriate decimal dilutions of the test sample followed by incubation at 37°C for 48 h. Calculation of the number of *staphylococci* (CFU per g or mL) is made from the number of typical and/or atypical colonies obtained on the selective medium and subsequently confirmed by coagulase and DNase testing (Fnes 2016).

In addition, helminth eggs were detected by the method of Amoah et al. 2018: The main four steps were by order: desorption, flotation, extraction and incubation with sieving samples at 100 µm and then N sulfuric acid for 28 days after detected the eggs through a microscope.

Plant analysis dry biomass and yield determination

At physiological maturity, aboveground biomass, and the grain yield (grains per m^2) were measured by harvesting a sample area of 1 m^2 at the center of each plot. The dry weight was determined by oven drying samples at 70°C until constant weight was reached. The dried plant parts were grounded and kept for further estimates (Du et al. 2012).

Plant mineral and metal analysis

Similar to the metals analyzed in soil samples, the same macronutrients, micronutrients and heavy metals were determined in plants: total nitrogen, available phosphorus, potassium, sodium, calcium, magnesium, mercury, lead, chromium, nickel, zinc, copper, cadmium, aluminum, iron, boron, molybdenum, cobalt and manganese. According to the ICARDA manual of: Methods of Soil, Plant, and Water Analysis (Estefan et al. 2013), approximately 0.5g of dried plant material was weighed and transferred into appropriate tube for microwave assisted digestion. 9mL of concentrated sulfuric acid and 3mL of hydrogen peroxide were added to the sample and digested with Milestone Ethos Easy microwave digestion system. After digestion heavy metals and micronutrients were determined by Atomic Absorption using iCE 3000 series AA spectrometers (Thermo Fisher Scientific). Nitrogen was determined by Kjeldahl method. 50mL of the digested sample is brought to boiling with 100 mL 5 N NaOH. The collected distillation with boric acid and colored indicator is back titrated with 0.01N hydrochloric acid. Phosphorus is measured according to Olsen method (ISO 1994). Spectrometically color development of 1 mL of digested sample with 10 mL 0.5 N sodium bicarbonate and 8mL of reagent B is read at 882 nm wavelength. Potassium is directly determined by the use of flame photometer (Estefan et al. 2013).

Grain fiber and protein content

The plant protein was determined as described in the method of Lowry et al. (Mæhre et al. 2018). Briefly, 500 mg of grain powder was extracted with 10 mL phosphate buffer (pH 6.6), then centrifuged for 30 minutes at 3000g. 1 mL of the supernatant was transferred to a test tube followed by 5 mL alkaline copper sulphate solution (a mixture of: 48 mL reagent A: 2% sodium carbonate in 0.1 N sodium hydroxide and 1 mL reagent B: 0.5% copper sulphate in water and 1 mL reagent C: 0.5% sodium potassium tartrate in water), mixed and then allowed to stand for 15 minutes. Afterward, 0.5 mL of Folin Ciocalteau reagent (dilution 1:1) was added, mixed rapidly, stand for 30 minutes till a blue color was developed and then the absorbance was read at 700 nm. A standard stock solution of 100 ppm of Bovine Serum Albumin (BSA) was used for plotting a standard curve of respective concentrations: 0, 20, 40, 60, 80, 100 ppm. For the fiber determination, approximately 1g of grain samples were weighed and enzymatically digested in three phases :100 µL α-amylase, incubated at 100 °C, afterward 100 µL protease then 200 µL amyloglucosidase and incubated at 60 °C. After digestion, the total fiber content was precipitated with 3 sample volumes ethanol 95%. The solution was then filtered and fiber was collected, dried and weighed (Ozoliņa et al. 2009).

Statistical analysis

Significant differences between the measured parameters at different biosolids amendment doses were determined using a one-way ANOVA. Tukey's significant difference (HSD) test at $p<0.05$ was used to indicate the significant differences between the means of the applied biosolids amendment doses. The statistical package SPSS 23 was used to process all the statistical analyses, at $p< 0.05$.

Results and discussion

Composition of sewage sludge and pre-cultivation soil

The composition of the used sewage sludge and the pre-cultivation soil in terms of physico-chemical parameters, micronutrients and metal content, and pathogens presence is provided in Table 1.

Concerning the sewage sludge, the results of the physico-chemical analysis showed that the pH is slightly acid with a mean value of 6.68±0.02. In addition, the sewage sludge has a high content of minerals and organic matter (52.32±0.47%) which means that it is good for agricultural use as alternative to mineral fertilizers. For pathogen analysis, the results revealed that the sludge was free from pathogenic bacteria and helminth eggs. Considering the metal content of sludge, the following metal elements were studied: Pb, Cr, Ni, Zn, Al, Fe, Cu, Cd, B, Mo, Co, Mn and Hg. According to their found concentrations that presented values within the admissible limits, the sludge was classified as Class A, referring to the Lebanese guidelines for sludge reuse in agriculture (FAO 2010). That means that there are no restrictions on use, and the sludge can be applied for agricultural use except for the production of vegetables that are eaten raw. Consequently, the physicochemical and metal characteristics of the analyzed sludge confirm that it has a potential to be considered as a significant fertilizer replacement (Dai et al. 2006). The findings agree with the study of Romanos et al. (2019) that assessed the sewage sludge from the same wastewater treatment plant in year 2018.

Concerning the pre cultivation soil, it is noted that the control site was conventionally cultivated using typical practices of monoammonium phosphate fertilizer. The results show that the analyzed soil contains1.78±0.14% organic matter. It also has a sufficient content of phosphorus (66.38±16.15 ppm) and potassium (567.52±37.66 ppm). However, it is deficient in some micronutrients, such as Ni and Co. All analyzed metals were below the maximum admissible levels based on the normal limits found in agricultural soils according to Lebanese guidelines for sludge reuse in agriculture (FAO 2010).

Physico-chemical properties of the amended soils after harvest

After collecting the postharvest soil from the different treatments, the physicochemical properties of collected samples were analyzed. The results are presented in Table 2.

Concerning the pH of the soil in the different treatments, the results showed that the pH value decreased with the increase of the amount of applied sewage sludge to the soil. The pH value of the postharvest soil decreased from 7.65 (control soil) to 7.50 ± 0.01 in T_8 treatment. For the EC, it recorded a value of 0.13 $dS.m^{-1}$, which is normal for such soil types. Usually, the pH of soil influences the uptake of nutrients and heavy metals by the plants (Kabata-Pendias and Pendias 2001). It should be highlighted that the pH values are not within the acidity range, therefore, the bioavailability of metals for plant uptake, which is usually enhanced at lower pH, is not a restrictive factor as described in the study of Mtshali et al. 2014. Biosolids have slightly lowered soil pH which can control metal absorption – desorption on surfaces, along with the steady state of electrical conductivity (Wiechmann et al. 1997). However, the effect of sludge application on soil properties must be observed at long-term (more than one season) in order to understand such process.

Concerning the organic matter, the results indicate that the OM in the different treatments increased significantly and linearly with the increase of the sewage sludge rate. The OM increased from 1.11 ± 0.14%

Table 1 Properties of pre cultivation soil and sewage sludge

		Agricultural soil		Sewage sludge	
Parameter	**Unit**	**Value±SD**	**Average limit[a]**	**Value±SD**	**Allowable limits[b]**
Physico-chemical					
pH	_	7.71±0.06	_	6.68±0.02	_
EC	dS/m	0.23±0.03	_	1.46±0.02	_
OM	%	1.78±0.14	_	52.32±0.47	_
N	%	0.11±0.01	_	3.24±0.10	_
P_2O_5	ppm	66.38±16.15	_	383.60±17.50	_
K_2O	ppm	567.52±37.66	_	2595.64±192.50	_
CaO	ppm	5558.06±877.67	_	60983.04±1646.95	_
MgO	ppm	1049.64±45.58	_	15678.12±961.55	_
Na	ppm	26.88±11.29	_	557.37±33.91	_
Metals and micronutrients					
Hg	ppm	0.03±0.01	1	0.58±0.06	4
Pb	ppm	3.96±0.53	100	38.74±6.48	150
Cr	ppm	31.24±8.76	100	0±0	250
Ni	ppm	43.28±2.81	60	33.64±2.54	125
Zn	ppm	78.65±7.71	200	597.67±79.26	700
Cu	ppm	23.72±4.26	100	148.50±13.59	375
Cd	ppm	0.15±0.19	1	0±0	5
Al	ppm	5701.76±609.61		14357.24±6706.27	
Fe	ppm	1769.48±362.80		5601.40±2249.61	
B	ppm	174.74±9.84		1340.20±548.18	
Mo	ppm	85.78±9.47		895.77±491.38	
Co	ppm	18.50±0.64		26.41±12.06	
Mn	ppm	382.05±24.37		349.70±22.86	
Pathogens					
Salomnella	CFU/g	0±0	_	0±0	3//4g
Helminth Eggs	CFU/g	0±0	_	0±0	1//5g
E. Coli CFU/g	CFU/g	0±0	_	0±0	<1000
Staphylococcus aureus	CFU/g	0±0	_	0±0	_

[a] (Moffat 2006)

[b] Allowable limits according to the Lebanese guidelines for sludge reuse in agriculture (FAO 2010)

(Treatment C) to 1.93 ± 0.06% at the sludge rate of 16 kg/m^2. In general, Mediterranean agricultural soils are known to be poor in organic matter. It is consequently adequate to use bio solids as soil amendments in addition for being a suitable option for the management of the sludge (Roig et al. 2012). The organic matter can also control the bioavailability of heavy metals; it is an important medium for metals' adsorption, so, a high content of organic matter will decrease the mobility of metals in soil (Hamdi et al. 2019). Most studies have described and upsurge in organic matter content after SS application to agricultural soils (Ahmed et al. 2010; Lu et al. 2012; Nkoa 2014). This statement indicates the improvement in biological and physicochemical features of agricultural soils (Alvarenga et al. 2016; Hamdi et al. 2019). Organic matter application has the possibilities of improving water holding capacity and moisture of soils, recirculation of air in the land, thus soil porosity and its structure, in addition to the expansion of soil humus, and reduction of bulk density and exposure to erosion (Suanon et al. 2016; Mohajerani et al. 2019). The essential trait of organic material structure is its ability to make complexes with metals, hence reducing their availability (Walter et al. 2006). But still, the most advantageous attribution to organic matter addition to soils is the enhancement of soil biotic diversity, and nutrients' storages and accessibility (Petersen et al. 2003).

The major nutrients (nitrogen, phosphorus, and potassium) were not significantly different in respect to the control treatment, for which urea fertilizers have been amended, indicating that the sewage sludge can be potentially a good alternative replacement to the application of mineral fertilizers for cereal production. There is not any fluctuation in limestone and active limestone levels after addition of the amendments in all treatments.

Micronutrients and metals in the amended soils after harvest

Micronutrients that are essential for plant growth and human nutrition (Cu, Zn, Fe, Mn, Mo, Co, B, etc.) and metals that can pose serious problems for the environ-

Table 2 Physico-chemical properties of postharvest soils at different sewage sludge amendment rates (means ±standard error)

			Sludge treatment rate			
Variables	**Unit**	**Significance**	**C**	**SS_4**	**SS_8**	**SS_{16}**
pH		****	7.65 ± 0.00 a	7.56 ± 0.02 ab	7.50 ± 0.01 b	7.54 ± 0.01 ab
EC		**	0.13 ± 0.01 a	0.12 ± 0.01 b	0.14 ± 0.01 a	0.14 ± 0.01 a
M.O.	(%)	****	1.11 ± 0.14 c	1.57 ± 0.15 b	1.81 ± 0.13 a	1.93 ± 0.06 a
N	(%)	ns	0.31 ± 0.05	0.31 ± 0.02	0.29 ± 0.04	0.26 ± 0.01
P_2O_5	(ppm)	ns	388.22 ± 146.62	431.84 ± 102.54	486.57 ± 37.3	502.67 ± 65.86
K_2O	(ppm)	ns	205.17 ± 16.06	188.92 ± 18.04	193.54 ± 14.54	178.27 ± 6.88
Cal.T	(%)	ns	26.11 ± 3.92	25.21 ± 2.14	26.88 ± 3.2	25.52 ± 2.7
Cal Act.	(%)	ns	7.34 ± 0.95	6.92 ± 0.54	6.82 ± 0.56	6.56 ± 0.27
CaO	(ppm)	***	5053.73 ± 82.93 ab	4798.95 ± 97.4 c	4961.7 ± 60.9 b	5170.71 ± 22.02 a
MgO	(ppm)	**	422.2 ± 60.65 b	470.2 ± 22.31 b	468.03 ± 23.4 b	552.39 ± 26.45 a
Na	(ppm)	****	49.27 ± 1.21 d	55.64 ± 1.08 c	58.19 ± 0.27 b	60.52 ± 0.78 a

Means in the same row followed by different letters are significantly different at $p < 0.05$

Table 3 Micronutrients and metals in the amended soils after harvest

		Sludge treatment rate			
Variables (ppm)	**Significance**	**C**	**SS_4**	**SS_8**	**SS_{16}**
Cr	ns	5.41 ± 0.83	5.56 ± 1.19	6.68 ± 0.96	6.18 ± 0.44
Al	**	5201.71 ± 326.39 b	4799.3 ± 749.88 b	6482.23 ± 765 a	6785.75 ± 704.29 a
B	****	102.74 ± 6.12 c	110.11 ± 7.42 c	148.14 ± 4.59 b	165.74 ± 11.22 a
Cd	****	0.14 ± 0.02 c	0.32 ± 0.15 b	0.38 ± 0.03 b	0.66 ± 0.04 a
Co	ns	16.15 ± 2.04	18.92 ± 0.96	19.73 ± 2.4	20.12 ± 5.06
Cu	*	17.01 ± 4.28 a	17.25 ± 2.68 a	13.18 ± 3.32 ab	10.00 ± 1.22 b
Fe	***	1487.02 ± 63.87 c	1598.62 ± 77.95 c	2017.38 ± 119.62 a	1827.88 ± 163.18 b
Hg	****	0.034 ± 0.00 b	0.032 ± 0.00 ab	0.044 ± 0.00 ab	0.046 ± 0.00 a
Mn	****	184.51 ± 2.20 a	133.14 ± 0.62 b	151.62 ± 0.99 ab	180.95 ± 1.98 ab
Mo	****	86.06 ± 3.34 b	97.76 ± 3.76 ab	118.7 ± 2.67 ab	267.66 ± 31.94 a
Ni	****	22.25 ± 1.42 a	12.26 ± 0.28 ab	8.07 ± 0.42 b	9.97 ± 1.35 ab
Pb	****	3.24 ± 0.08 b	5.75 ± 0.40 ab	6.95 ± 0.21 ab	7.91 ± 0.32 a
Zn	****	28.16 ± 1.15 a	13.3 ± 3.82 c	20.85 ± 1.64 b	20.17 ± 2.17 b

Means in the same row followed by different letters are significantly different at $p < 0.05$

ment were monitored in the postharvest soils collected from the different treatments. Their concentrations are provided in Table 3.

The results indicate that most of the concentrations of analyzed micronutrients and metals increased in the postharvest soils with increasing the sewage sludge rate. However, all tested elements were below or within the allowable limits for agricultural soils (Suchkova 2014). The decrease in heavy metals' concentrations after harvesting is essentially attributed to two major reasons: heavy metals leaching (Conde-Suárez et al. 2004) and their uptake by plant (Eid et al. 2020). The overuse of fertilizers are adding more heavy metals to the soil than the uses of biosolids as soil amendments (Atafar et al. 2010). In addition crop rotation and planting crops for soil phytoremediation could be a promising solution for heavy metals accumulation problems (Wyrwicka and Urbaniak 2018).

Wheat biomass, yield, fiber, and protein content

The above ground biomass, the yield as well as the fiber and protein content of the grains are presented in Table 4, as obtained for the different sludge treatments.

Table 4 Wheat biomass, yield, fiber and protein content in the different treatments

Variables		Significance	Sludge treatment rate			
			C	SS_4	SS_8	SS_{16}
Wheat total biomass	(t/ha)	*	11.39±1.51 a	9.26±0.86 b	11.58±0.53 a	12.72±1.76 a
Grain yield	(t/ha)	ns	4.35±0.44	4.97±0.70	4.88±0.08	5.02±0.61
Fiber	(%)	****	2.28±0.00 ab	2.27±0.00 b	2.37±0.00 a	2.33±0.00 ab
Proteins	(%)	****	17.92±0.00 ab	17.44±0.00 b	18.57±0.00 a	17.69±0.00 ab

Means in the same row followed by different letters are significantly different at $p < 0.05$

In general, there was a significant difference among the treatments in terms of biomass production, with the highest mean values obtained in the treatment SS_{16} (12.72±1.76 t. ha^{-1}). In fact, this treatment showed a biomass that was 8.97, 27.20, and 10.46%, respectively higher than that obtained in SS_8, SS_4, and C. Such result highlights that the sludge dose of SS_8 and SS_{16} could constitute a suitable practice to be recommended as an alternative for the use of chemical fertilizers. Moreover, the results showed that there was no significant difference among the treatments in terms of grain yield which further confirm that the application of sewage sludge did not negatively compromise wheat production in that area. It should be mentioned that the obtained yields are in the range of values reported in the work of Abi Saab et al. 2019 in the Bekaa valley.

Finally, concerning the grain quality, the highest fiber and protein content were obtained in SS_8 and SS_{16}, and were significantly very close from the treatment C. As reported by Samara et al. (2017), application of SS on soil leads to a positive effect on wheat growth and production.

Mineral and metal content in wheat

The macronutrients, as well as the micronutrients and the metals, and their respective concentrations in wheat grain yield, are presented in Table 5.

According to the obtained results, the essential macronutrients nitrogen and phosphorus have increased after addition of SS. It is well known that nitrogen is very exposed to leaching and denitrification, thus its availability is related to its amendment into the soil (Thanh Binh 2017). The addition of SS increased nitrogen in plant by approximately its double level, which can be related to the increasing effect of its availability (Mohamed et al. 2019). Phosphorus concentrations increased because SS is known as a potential source of phosphorus (Song and Lee 2010). Potassium decreased gradually from 1243 to 883 ppm after addition of different rates of SS. According to Sullivan et al. 2015, SS doesn't contain a high amount of potassium comparing to other macronutrients and the contribution of SS is insignificant when it comes to providing potassium. On the other hand, and as expected, sodium and calcium have increased due to their originally high amount in SS (50% sodium increase and 14% calcium).

As for the heavy metals, most of them have intensified after the addition of SS, which is very normal due to

Table 5 Mineral and metal content in wheat as obtained in the different treatments

		Sludge treatment rate			
Variables	**Significance**	**C**	**SS_4**	**SS_8**	**SS_{16}**
N(%)	****	0.38±0.01 b	0.63±0.02 ab	0.69±0.01 a	0.67±0.03 ab
P_2O_5(ppm)	****	15598.42±177.90 ab	13931.02±325.69 b	16968.42±500.15 a	15618.73±480.10 ab
K_2O (ppm)	***	1243.93±59.85 a	1078.23±25.67 ab	1044.99±37.84 ab	883.35±79.09 b
MgO (ppm)	****	2898.04±27.57 a	2700.49±93.64 b	2480.88±67.49 c	2403.31±84.73 c
CaO(ppm)	****	6052.23±156.07 ab	5318.47±211.69 ab	2170.04±75.63 b	7560.65±358.53 a
Na (ppm)	*	18.63±0.65 b	31.96±5.93 a	32.60±9.60 a	26.35±0.60 ab
		Metals and micronutrients			
Pb (ppm)	***	9.39±0.63 b	11.12±0.98 b	13.01±1.16 ab	15.26±1.22 a
Ni (ppm)	****	5.20±0.22 d	7.33±0.27 b	5.99±0.25 c	8.44±0.60 a
B (ppm)	ns	7.19±0.84	8.28±0.42	7.74±0.42	7.90±0.30
Mo (ppm)	ns	212.16 ±6.77	202.92 ±17.06	226.23 ±9.95	210.96 ±8.09
Co (ppm)	ns	51.83 ±1.57	50.55±3.25	54.70±4.26	48.85±2.26
Al(ppm)	****	814.77±18.95 ab	695.39±3.19 b	756.44±7.87 ab	3199.38±13.83 a
Fe (ppm)	****	85.83±1.35 ab	61.29±1.09 b	77.65±0.74 ab	205.18±3.30 a
Cu (ppm)	***	3.44±0.04 a	2.79±0.42 ab	0.73±0.14 b	3.49±0.51 a
Cd (ppm)	***	0.528±0.02 b	0.794±0.02 ab	0.837±0.00 a	0.858±0.04 a
Hg (ppm)	****	0.009±0.00 ab	0.018±0.00 ab	0.006±0.00 b	0.026±0.00 a
Cr (ppm)	**	0.00±0.00 b	0.00±0.00 b	0.00±0.00 b	0.864±0.05 a
Zn (ppm)	****	17.20±0.26 ab	8.93±0.29 b	14.84±0.11 ab	18.14±0.16 a
Mn (ppm)	****	22.16 ±0.17 b	12.47 ±0.95 ab	17.72 ±0.13 ab	39.46 ±0.75 a

Means in the same row followed by different letters are significantly different at p <0.05

the increase of heavy metals in the soil after SS amendment.

Conclusion

Sewage Sludge application as soil amendments is becoming a concern since its global expansion as a green solution for sewage sludge disposal. However, this study in the Bekaa region of Lebanon, and that focused on three amendment levels, showed a slight increase in the electrical conductivity of biosolids' amended soils, but that remained in the safe limit for crop cultivation and production. pH decreases also marginally and gradually within the biosolids dose applied. The global texture did not change, but the content in Clay, Silt and Sand varied to some extent. All these variations did not affect the soil properties negatively but positively in an agricultural point of view especially when the soil had an increment in macronutrients (N, P, K), micronutrients (Ca, Mg) and organic matter. The risk of contamination of heavy metals has been determined to be low with a safe margin for the use of these biosolids as agricultural soil amendments. Additionally, another trial on additional weight of biosolids per square meter may be considered for further investigation since the 16Kg amendment is still safe and beneficial on both soil and plants.

Also, this study showed the importance to add organic matter to soil, the soil becomes richer in macro and micronutrient after six months of adding the sludge without passing the allowed threshold of the presence of heavy metals in the soil. Also, the effect of adding biosolids each year will be taken in consideration in a future study.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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