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Changes in Soil Fertility and Plant Uptake of Nutrients and Heavy Metals in Response to Sewage Sludge Application to Calcareous Soils

Munir Jamil Mohammad and Bayan M. Athamneh
Department of Natural Resources and the Environment, Faculty of Agriculture,
Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan

Abstract: Pot experiment was conducted to evaluate the response of lettuce to application of Sewage Sludge (SS) to calcareous soils. The following treatments were investigated zero SS application (control), 20, 40, 80 and 160 t SS ha^{-1}. Additional treatment (80 kg P_2O_5 ha^{-1}) as diammonium phosphate (DAP) was included to represent the recommendation rate. Lettuce was grown for 8 weeks then the plant dry and fresh weights were recorded. Grounded plant tissue and soil were analyzed for chemical and physical parameters. Results showed that application of SS increased the fresh and dry weights over fertilizer and control treatments. High rates (80 and 160 t ha^{-1}) of SS gave similarly the highest plant dry and fresh weights while the DAP fertilizer treatment and the two lowest SS rates (20 and 40 ha^{-1}) resulted in similar plant growth. Macronutrients, microelements and heavy metals were increased with SS application over the control and the DAP fertilizer treatments. SS addition decreased soil pH and increased electrical conductivity, organic matter and soil P. Addition of SS increased DTPA-extractable micronutrients and heavy metals over the DAP fertilizer and the control treatments. Addition of 40 SS t ha^{-1} was equivalent to the recommended fertilizer rate in increasing lettuce growth. Higher rates of SS resulted in farther increase in lettuce growth but resulted in higher plant heavy metal content. Therefore, addition of 40 t ha^{-1} to calcareous soil is recommended to achieve acceptable level of plant growth with minimal adverse effect on plant and soil qualities.

Key words: Sewage sludge, soil fertility, lettuce, heavy metals, calcareous soil

INTRODUCTION

The use of SS as fertilizer becomes increasingly attractive due to its high fertilizer value and its low costs[1]. SS can be a source of plant essential nutrients such as N, P, K, Mg, Zn, Co, Mn, Fe and B but may also contain heavy metals that are non-essential and even toxic to plants such as Cd and Pb[2]. Therefore, improper application of SS may result in excessive accumulation of heavy metals and resultant phytotoxicity[3]. Accumulation of heavy metals in edible parts of the plants is a pathway into the food chain of man[4]. It has been reported that SS application significantly increased soil organic matter[5], water holding capacity and soil aggregation[6], soil macronutrients[1]. On the other hand, Samaras and Kallianou[7] found that SS application resulted in significant increases of Zn, Cu, Cd and Ni extracted by DTPA.

Proper management of SS application is essential for maximizing its beneficial effects and minimizing its adverse effects[3]. Such management should consider several aspects such as content of heavy metals and other contaminants, the crop type and its nutrient requirement, the amount, form of nutrients contained in the SS and soil chemical and physical properties. All these will help to define the appropriate rate, time and method of application[2]. In general, the proper rate is one that maximizes yield while ensuring protection of the environment[8]. However, application rate of SS has commonly been based on its N content, N required of the crop and its Cd content[9]. The objective of this study was to determine the influence of application of different rates of SS on calcareous soil on lettuce growth and nutrient and heavy metal uptake.

MATERIALS AND METHODS

Two greenhouse pot experiments were conducted to evaluate the effect of application of different rates of SS to highly calcareous soils on lettuce growth and nutrient uptake and on quality parameters of lettuce and soil. The following treatments were investigated in a Randomized

Corresponding Author: Munir Jamil Mohammad, Department of Natural Resources and the Environment, Faculty of Agriculture, Jordan University of Science and Technology, P.O. Box 3030, Irbid, Jordan
Tel: 962 2 7201000 Ext: 22258 Fax: 962 709569 E-mail: mrusan@just.edu.jo

Complete Block Design with four replications: zero SS application (control), 20, 40, 80 and 160 t SS ha^{-1}. In addition, one fertilizer treatment with an application of 80 kg diammonium phosphate (DAP) ha^{-1} was included which represents the recommended fertilizer rate. The soil used in this experiment is classified as fine-loamy, mixed, thermic, calcic paleargid[10]. Seven liter-pots were used as the experimental units. Each pot was filled with 7 kg air dry and sieved through a 5 mm screen surface soil (top 20 cm). In each pot the amount of SS according to the treatments was mixed with soil. Lettuce (*Lactuca sativa* L.) seedlings were planted at rate of one plant per pot. Lettuce plants were grown for 8 weeks. Plants were watered alternate day to maintain water content at approximate field capacity.

Before conducting the experiment a soil subsample was sieved through 2 mm screen and was analyzed for general characteristics; texture by hydrometer method[11]; soil pH was measured on 1:1 soil: water suspension[12]; soluble salts were determined by measuring the electrical conductivity of 1:1 soil: water extracts[13]. Organic matter content was determined by the Walkley-Black method[14]; available pp by extraction with sodium bicarbonate[15] and heavy metals (Cu, Cd, Fe, Zn, Pb, Mn) by DTPA extraction[16]. Preliminary analyses of the soil characteristics are presented in Table 1. At the end of each experiment, the soil was also analyzed for the same parameters.

The SS used was collected from the drying peds, from Irbid Mechanical Treatment Plant, Irbid, Jordan. The SS was air dried and grounded to about 5 mm granules and applied as such to each soil according to the treatments. The dried SS (H_2O 11.1%) was analyzed for chemical properties and ion contents. The pH of the SS was determined in suspension (the suspension was prepared by weighing 3.2 g of well-mixed sample of dry solid SS in to a 150 mL beaker, then add 96.8 mL of deionized water, shake the sample for 30 min at 150 oscillations per minute) as suggested by Doty *et al.*[17]. Total N was determined by Kjeldahl digestion of the sample[18]. Total P, Na, K, Zn, Cu, Pb, Cd, Fe, Mn, Mg and Mn were determined in the dry ashed digestion. Total P was determined using Vanadate-Molybdate-Yellow method, K and Na by flame photometer and Zn, Cu, Pb, Cd, Fe, Mn, Mg and Mn by atomic absorption spectroscopy[17]. Lettuce was harvested at the end of growing period. Fresh weight was recorded. Plants were oven dried at 70°C for 48 h and the dry weight was recorded. Oven dries plants were ground to a fine powder using a laboratory mill with 0.5 mm sieve. The ground plant samples were analyzed for the total N using a modified micro-Kjeldahl digestion procedure[18]. Total P, Na, K, Zn, Cu, Pb, Cd, Fe, Mn, Mg and Mn were determined in the dry ash digestion. Total P was determined using Vanadate-Molybdate-Yellow method, K and Na by flame photometry and Zn, Cu, Pb, Cd, Fe, Mn, Mg and Mn by atomic absorption spectroscopy. All data were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of SAS (Statistical Analysis System. 1999-2000, SAS Institute, Cary, N. C.) to determine the main effect of each factor and the interaction effect among factors. The $LSD_{0.05}$ was used to separate treatments mean.

RESULTS AND DISCUSSION

The soil used is a silty clay loam with slightly basic and alkaline reaction (Table 1). The electrical conductivity value, organic matter and available P are relatively low. The soil is highly calcareous with values of DTPA-extractable micronutrients and heavy metals (Zn, Cu, Pb, Cd, Fe and Mn) lower than the normal concentration ranges[19]. The SS used has a basic pH with slight salinity level (Table 1). The SS has a relatively higher CEC than the soil while the OM% of SS is much higher than that of the soil. The SS is quite rich in N, P and K. DTPA-extractable micronutrients and heavy metals in the SS are within the acceptable levels for application to agricultural soils.

Lettuce yield: The addition of high rates (80 and 160 t ha^{-1}) of SS gave similarly the highest plant dry and fresh weights while the control treatment (no SS application) on the other hand resulted in the lowest plant

Table 1: Selected properties of the soil and Sewage Sludge (SS) used in the experiment

Soil		SS	
pH	8.18	pH	7.13
EC (dS m^{-1})	0.61	EC (dS m^{-1})	1.09
CEC (cmol kg^{-1})	34.32	CEC (cmol kg^{-1})	51.37
O.M (%)	0.72	O.M (%)	62.30
N (%)	0.01	N (%)	4.50
P (mg kg^{-1})	7.10	P (%)	2.40
K (mg kg^{-1})	452.00	K (%)	0.27
$CaCO_3$ (%)	13.38	Ca (%)	5.64
-	-	Mg (%)	0.51
-	-	Na (%)	0.12
Fe (mg kg^{-1})	3.56	Fe (mg kg^{-1})	731.30
Mn (mg kg^{-1})	5.58	Mn (mg kg^{-1})	685.00
Zn (mg kg^{-1})	1.88	Zn (mg kg^{-1})	621.00
Cu (mg kg^{-1})	1.22	Cu (mg kg^{-1})	132.40
Pb (mg kg^{-1})	0.68	Pb (mg kg^{-1})	62.50
Cd (mg kg^{-1})	0.06	Cd (mg kg^{-1})	1.88
Texture	Silt clay loam	Moisture content (%)	11.12

Table 2: Effect of Sewage Sludge (SS) application rates on lettuce yield

SS rate (t ha^{-1})	Dry weight (g/plant)	Fresh weight (g/plant)
0	18.00d*	214.5e
20	29.50c	440.0d
40	41.75b	596.0cb
80	52.25a	636.3ab
160	51.75a	721.3a
DAP**	36.25bc	527.0cd

* Means with different letter(s) within each column are significantly different at 0.05 probability levels. ** DAP = diammonium phosphate

Table 3: Effect of Sewage Sludge (SS) application rates on macronutrients uptake by lettuce

SS rate (t ha^{-1})	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	N (mg/plant)	P (mg/plant)	K (mg/plant)
0	1.93c*	0.357e	3.10bc	359.11e*	79.21d	556.9d
20	3.30b	0.510c	4.60a	972.96c	176.94c	1343.3b
40	3.66a	0.729b	3.40b	1520.85b	303.76b	1422.0b
80	3.67a	1.106a	3.30b	1912.69a	577.82a	1702.1a
160	3.78a	1.101a	2.53c	1953.38a	570.10a	1321.7b
DAP**	1.99c	0.443d	2.68c	703.50d	130.11cd	978.3c
LSD 0.05	0.19	0.069	0.59	199.13	67.83	267.0

* Means with different letter(s) are significantly different at 0.05 probability levels (by columns). ** DAP = diammonium phosphate

dry and fresh weights (Table 2). The application of the recommended chemical fertilizer as diammonium phosphate (DAP) resulted in plant growth equivalent to that obtained by the application of the two lowest SS rates (20 and 40 ha^{-1}). Improved growth and yield of vegetable crops with SS application has been reported by other researchers[20] and attributed the yield increase to the N and P provided by the added SS. Linden *et al.*[21] reported that the yields on the SS amended soil have been better than on the fertilized control areas. Moreover, the three years of consecutive applications of SS on calcareous and basic soil showed that large amount of N and P supplied by SS did not adversely affect crop yields by creating imbalances of nutrients within the plants. Samaras and Kallianou[7] found that SS application in the first year of applications significantly increased yield at rate of 77 t ha^{-1} and stated that due to residual effect the yield in the second year was increased by lower rate (42 t ha^{-1}) of SS application.

Concentration and uptake of nitrogen, phosphorus and potassium

Nitrogen: Addition of SS increased N concentration in lettuce shoot over the control and the DAP fertilizer treatments (Table 3). N concentration increased from 1.93% in the control treatment to 3.7% in the 160 t ha^{-1} SS. There was no significant difference between the DAP fertilizer and the control treatments. Moreover, there was no significant difference between 40, 80 and 160 t ha^{-1} treatments. N uptake increased with SS application where the highest value was obtained similarly with the application of 80 and 160 t ha^{-1}. The DAP fertilizer treatment increased N uptake higher than the control but lower than any of the SS rates. These results may be attributed to the increase in soil N as the application rate of SS increased. A similar result was reported by Buchanand and Gliessnan[22].

Phosphorus: P concentration in lettuce shoot increased with increasing SS application rate. It increased from 0.357% at the control treatment to 1.101% at 160 t ha^{-1} SS. There was no significant difference in P concentration between the two highest rates of SS application (80 and 160 t ha^{-1}). P concentration in the control and the DAP fertilizer treatments were lower than those obtained by any rate of the SS treatments. Morel *et al.*[23] reported similar results and predicated that the efficacy of P uptake by plants was controlled predominantly by the concentration of HPO_4^{-2} and H_2PO_4 in the soil solution, which was in turn affected by the addition of SS to soil. Sommers[24] stated that SS contains appreciable P concentrations to meet fertilizer P recommendations, therefore, applying SS at a rate to provide the entire N needed by a crop could add P more than crop requirement for optimum yields. P uptake increased from 79.21 mg $plant^{-1}$ in the control treatment to 577.82 mg $plant^{-1}$ in the 80 t ha^{-1} SS rate. There were no significant differences between the DAP fertilizer treatment and both the control and the lowest rate of SS application 20 t ha^{-1}. A similar results were obtained by Candelaria *et al.*[2] who reported that N provided by the applied SS had positive synergistic effect on the P uptake. Such synergistic effect of N on P was reported by others and was attributed to the root proliferation in the root zone of N placement which will assist the P uptake[25] and to the acidification of the rhizosphere and nitrification that facilitate P uptake[26].

Potassium: The highest K concentration (4.60%) was observed at 20 t ha^{-1}. There were no significant differences among other treatments. It is not clear why K concentration increased only by the lowest SS rate but generally there was no clear effect for SS application on K concentration. This can be attributed to the fact that SS is low in K since K is water-soluble and mostly was removed through treatment process[27]. The addition of SS increased K uptake by lettuce shoot over the control. K uptake increased from 556.9 mg at the control treatment to 1702.1 mg at the 80 t ha^{-1} SS rate. There were no significant differences among other rates of SS. Moreover, addition of SS increased K uptake over the DAP fertilizer treatment. Such pattern in K uptake which was quite different from that of K concentration can be attributed to the pattern of dry weight increase with increasing SS rates[28,29].

Concentration and uptake of iron, manganese, zinc and copper: The lowest Fe concentration was obtained by the control treatment which was not significantly lower than

Table 4: Effect of Sewage Sludge (SS) application rates on microelements concentration in lettuce

SS rate (t ha^{-1})	Zn (μg g^{-1})	Cu (μg g^{-1})	Pb (μg g^{-1})	Cd (μg g^{-1})	Fe (μg g^{-1})	Mn (μg g^{-1})
0	38.68d*	4.95c	9.50cb	0.425b	106.38c	10.00c
20	75.35c	8.55b	8.50cd	0.500b	141.35bc	47.75c
40	76.03c	8.28b	10.00ab	0.525b	150.95b	58.50c
80	113.85b	8.18b	11.00a	0.525b	326.63a	158.25b
160	126.98a	10.68a	10.25ab	0.673a	343.10a	241.00a
DAP**	35.60d	4.08c	8.00d	0.450b	180.40b	58.75c
$LSD_{0.05}$	12.06	0.92	1.43	0.110	41.17	25.01

* Means with different letter(s) are significantly different at 0.05 probability levels (by columns). ** DAP = diammonium phosphate

that obtained by the lowest SS rate (20 t ha^{-1}). The highest Fe concentration was obtained by addition of the highest two SS rates (80 and 160 t ha^{-1}) as shown in Table 4. Other treatments resulted in no significant differences in Fe concentration. Addition of DAP fertilizer gave Fe concentration higher than the control treatment.

Addition of DAP fertilizer and all rates of SS except the lowest rate increased Fe uptake compared to the control treatment. Fe uptake increased from 1.91 mg in the control treatment to 17.74 mg in the 160 t ha^{-1}. The addition of the highest SS rates resulted similarly in the highest Fe uptake values (Table 5).

Mn concentration in lettuce shoot increased by addition of the highest two SS rates (80 and 160 kg ha^{-1}) where the highest value of Mn concentration was obtained by the highest SS rate (Table 4). All other treatments resulted in no significant differences in Mn concentration. Mn uptake by lettuce increased by addition of 40, 80 and 160 t SS ha^{-1}, where the higher the SS rate the higher the Mn uptake was (Table 5). There were no significant differences among all other treatments. This agrees with results reported by Jarausch-Wehreheim *et al.*[1].

Addition of SS increased Zn concentration from 38.68 mg kg^{-1} in the control treatment to 126.98 mg kg^{-1} in the 160 t ha^{-1} SS with no significant differences between the 20 and 40 t ha^{-1} SS rates (Table 4). Addition of DAP fertilizer resulted in no significant increase in Zn concentration compared to other treatments. Hanlon[30] reported significant increases in Zn concentration in the edible portion of most of growing crops: wheat, potato, lettuce, cabbage and rye grass due SS application. Addition of DAP fertilizer and the control treatment resulted in the lowest values of Mn uptake. Increasing the rate of SS application increased Zn uptake where the highest two SS rates similarly gave the highest value of Zn uptake. Similar result has been reported by Hanlon[30].

Addition of SS increased Cu concentration over the control and DAP treatments. The highest Cu concentration was obtained with application of 160 t ha^{-1} while no significant differences were obtained among the 20, 40 and 80 t ha^{-1} SS rates (Table 4). Chu and Wong[31] stated that concentration of Cu in the leaves showed slight increase with the amount of SS applied. Cu uptake by lettuce shoot increased significantly with increasing SS rate. There was significant increase in Cu uptake between the control and the DAP fertilizer treatments (Table 5).

Concentration and uptake of lead and cadmium: Compared to the control and lowest SS rate (20 kg SS ha^{-1}) treatments, Pb concentration increased similarly by addition of the highest three SS rates (40, 80 and 160 kg ha^{-1}) and by addition of DAP fertilizers (Table 4). The highest values of Pb uptake were obtained similarly when 80 and 160 t ha^{-1} SS rates were added. There was no significant difference in Pb uptake between the control and the 20 t ha^{-1} rate, which gave the lowest Pb uptake values and between the 20 and the DAP fertilizer treatments (Table 5). The increase in Pb uptake followed the increase in plant dry weight with increasing the application rates of SS[20,28]. Ross[19] mentioned that normal range of Pb in plant material is 0.1 to 10 μg g^{-1} fresh weight, whereas concentration in contaminated plants is 30 to 300 μg g^{-1}.

The concentration of Cd in lettuce increased only by addition of the highest SS rate (160 t ha^{-1}). No significant differences were obtained among all other treatments (Table 4). However, the concentration of Cd for all treatments remained within the normal range (0.2 to 0.8 μg g^{-1}) reported by Ross[19] who also reported that the normal range of Cd in contaminated plants is 5 to 30 μg g^{-1}. The highest value of Cd uptake were obtained when 80 and 160 t ha^{-1} of SS were added and the lowest for the control and the lowest SS rate treatments (Table 5). This agrees with results reported by others[30,32]. Hanlon[30] reported that significant increases in Cd, Ni, Cu and Zn contents in the edible portion of most of growing crops: wheat, potato, lettuce, red beet, cabbage and rye grass due SS application. Frost and Ketchum[33] found higher Cd in leaves than in the fruit with SS application while Oudeh *et al.*[6] found that Zn and Cu but not Cd, contents were accumulated more in the roots than in shoots. Jing and Long[34] mentioned that the most important factors in determining plant uptake of Cd (in addition to Cd application rate, soil pH and plant species) is, in part, SS Cd chemistry, specifically SS Cd content and in part, to differences in SS-Cd bioavailability.

Table 5: Effect of Sewage Sludge (SS) application rates on microelements uptake by lettuce

SS rate (t ha^{-1})	Zn (μg/plant)	Cu (μg/plant)	Pb (μg/plant)	Cd (μg/plant)	Fe (μg/plant)	Mn (μg/plant)
0	699.6d *	88.58e	171.75d	7.80e	1.91d	0.72d
20	2222.1c	252.33d	251.25cd	14.68ed	4.18dc	1.41cd
40	3177.9b	347.50c	415.00b	22.08cb	6.24bc	2.43c
80	5932.7a	424.08b	572.50a	27.85ab	17.02a	8.30b
160	6575.2a	552.75a	531.25a	34.85a	17.74a	12.49a
DAP**	1298.3d	147.23e	289.25c	16.38cd	6.54b	2.13cd
LSD 0.05	827.9	64.09	83.90	7.21	2.33	1.62

Table 6: Effect of Sewage Sludge (SS) application rates on soil planted with lettuce

SS rate (t ha^{-1})	pH	Electrical conductivity (dS m^{-1})	Organic matter (%)	P (mg kg^{-1})
0	8.15a*	0.51e	0.72e	7.66e
20	7.94b	1.57d	1.00d	12.44de
40	7.65c	1.75c	1.48c	25.75c
80	7.46d	2.80b	1.76b	45.60b
160	7.22e	4.96a	1.89a	77.13a
DAP**	8.22a	0.50e	0.70e	19.00d
$LSD_{0.05}$	0.088	0.14	0.057	6.60

* Means with different letter(s) are significantly different at 0.05 probability levels (by columns). ** DAP = diammonium phosphate

Soil pH, soil salinity, soil organic matter and soil phosphorus: Addition of SS decreased soil pH but increased soil salinity, as measured by Electrical Conductivity (EC) of the soil solution, soil organic matter and soil phosphorus content (Table 6). Soil pH reduced with addition of SS. The soil pH decreased from 8.15 in the control treatment to 7.22 in the 160 t ha^{-1} SS treatments. These may be attributed to the acidity generated from the mineralization of the added SS[35]. Decomposition of organic matter would increase CO_2 levels which may be also responsible for the decrease in pH of the SS-treated soil[36]. The DAP fertilizer treatment did not affect the soil pH. On the other hand, Mulchi *et al.*[37] reported that the soil pH values were increased, decreased or remained largely unchanged following the application of SS depending on technology used to produce the SS material.

The decrease in EC with SS addition ranged from 0.508 dS m^{-1} for the control treatment to 4.955 dS m^{-1} in the highest SS treatment rate 160 t ha^{-1}. Jamjoum[38] reported that at zero SS treatment the EC was 0.82 dS m^{-1}, while by addition of 60 t ha^{-1} SS the EC had increased to 2.28 dS m^{-1}. Who also reported that addition of 60 t ha^{-1} decreased soil pH value from 7.93 to 7.64 and increased soil EC from 0.82 to 2.28 dS m^{-1}.

Addition of SS at all rates increased soil organic matter over the DAP fertilizer and control treatments. Organic matter increased from 0.72% in the control treatment to 1.89% in the 160 t ha^{-1} SS treatment (Table 6). This may be attributed to increase in organic carbon as SS rate was increased[39]. Sommers[24] and Hernandez *et al.*[40] stated that decomposition of SS organic matter in soils releases N in plant-available forms for several years after SS application. He *et al.*[41] evaluating the nutrient release from SS decomposition, found that N mineralization rate was rapid. Barbarick and Lppolioto[9] reported that 50% of SS N mineralized within three weeks after application. On the other hand, Fine and Mingelgrin[42] emphasized that excessive amount of released N and P by high rates of SS application can be detrimental to crop production and the environment

The extractable P increased from 7.66 mg kg^{-1} in the control treatment to 77.13 mg kg^{-1} in the 160 t ha^{-1} SS. There was no significant increase in P concentration between the control 7.66 mg kg^{-1} and the lowest rate of SS application 20 t ha^{-1} 12.44 mg kg^{-1}. Soil P level increased by the DAP fertilizer treatment as high as that obtained with an application of 20 t SS ha^{-1}. Similar result was obtained by Jamjoum[38] who reported that the soil extractable P in the 0-20 cm soil depth had increased by 78, 110 and 92% over the control treatment by applying SS at rates of 20, 40 and 60 t ha^{-1}, respectively. Rubin *et al.*[43] applied SS at a rate consistent with plant's N requirement and found the amount of P was excessive while the K was insufficient. Moreover, he reported that balancing the amount of N applied in the SS with the amount needed for plant growth given specific soils and crops is an important management factor. Frossard *et al.*[44] reported that many sources of SS can supply all the P requirements for plants. Debosz *et al.*[45] stated that SS amendment had increased the amount of available P by a factor of 1.6.

DTPA-extractable iron, manganese, zinc and copper: Addition of SS increased soil Fe by all SS rates over the DAP fertilizer and the control treatment. Fe increased from 3.82 mg kg^{-1} at the control treatment to 13.83 mg kg^{-1} in the 160 t ha^{-1} SS treatment (Table 7). Application of 60 t ha^{-1} of SS to a clay loam soil had increased extractable Fe by 14% over the control[38]. McCaslin *et al.*[46] reported that in a three year experiment comparing two rates 33.6 and 67.2 t ha^{-1} of SS applied to calcareous soil found that SS had improved soil Fe levels.

Addition of SS increased soil Mn by all SS rates. Mn increased from 7.19 mg kg^{-1} in the control treatment to 31.63 mg kg^{-1} in the highest SS rate 160 t ha^{-1}, which still below the normal range (Table 7). Mn concentration showed no significant differences between the MAP

Table 7: Effect of Sewage Sludge (SS) application rates on DTPA-extractable micronutrients and heavy metals in the soil

SS rate (t ha^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Cd (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})
0	1.68e*	1.330d	0.710d	0.048e	3.82e	7.19e
20	5.45d	1.900c	1.070d	0.068d	6.27d	12.95d
40	9.80c	2.480b	1.540c	0.088c	8.02c	18.05c
80	15.32b	3.780a	2.220b	0.118b	11.18b	27.03b
160	24.08a	3.870a	2.870a	0.180a	13.83a	31.63a
DAP**	0.99e	1.510d	0.810d	0.055de	3.68e	16.91c
$LSD_{0.05}$	1.64	0.224	0.434	0.018	0.66	1.60

* Means with different letter(s) are significantly different at 0.05 probability levels (by columns). ** DAP = diammonium phosphate

fertilizer and the 40 t ha^{-1} SS application. Hue *et al.*[47] stated that DTPA-extractable heavy metal concentrations (Mn and Zn) had increased with SS application in three soils studied.

Addition of SS at all rates increased soil Zn compared to DAP fertilizer and the control treatments. Soil Zn increased from 1.68 mg kg^{-1} in the control treatment to 24.08 mg kg^{-1} in 160 t ha^{-1} (Table 7). Ross[19] mentioned that normal range of Zn in soil is 10-300 µg g^{-1} dry weight. Increased the level of soil Zn with SS application has been reported by other researchers[7,30]. Jamjoum[38] stated that application of 60 t ha^{-1} of SS to a clay loam soil had increased soil extractable Zn by 27% and extractable Fe by 14%.

Cu concentration in soil increased significantly up to the 80 t ha^{-1} SS application rate. There was no significant increase between high rates 80 and 160 t ha^{-1}. Cu increased from 1.33 mg kg^{-1} in the control treatment to 3.87 mg kg^{-1} in the 160 t ha^{-1} SS (Table 7). All SS treatment rates increased significantly over the commercial fertilizer and the control treatment. Similar result was obtained by others[7,39]. Ross [19] mentioned that the normal range of Cu in soil is 2 to 100 µg g^{-1} dry weight, whereas concentration in soil considered toxic is 60-125 µg g^{-1} dry weight.

DTPA-extractable cadmium and lead: Addition of SS at all rates increased soil Cd over the control treatment. Cd content increased from 0.048 mg kg^{-1} at the control treatment to 0.180 mg kg^{-1} at the 160 t ha^{-1} SS treatment; while there was no significant differences between the DAP fertilizer treatment and the 20 t ha^{-1} SS application rate (Table 7). Similar results were reported by Samaras and Kallianou[7]. DTPA-extractable Pb in the SS-treated soil increased significantly with increasing SS application rates. It ranged in the soil from 0.71 mg kg^{-1} in the control treatment to 2.87 mg kg^{-1} in the 160 t ha^{-1} SS application rate. While there was no significant effect between the control treatment 0.71 mg kg^{-1} and the 20 t ha^{-1} treatment 1.07 mg kg^{-1}. Ross[19] mentioned that normal range of Pb in soil is 2-200 µg g^{-1} dry weight, whereas concentration in soil considered toxic is 100 to 400 µg g^{-1} dry weight.

El-Demerdashe *et al.*[48] stated that long-term application of SS can gradually accumulate heavy metals in the soil to levels that may be toxic to some plant species. Samaras and Kallianou[7] found that SS application resulted in significant increases in DTPA-extractable Zn, Cu, Cd and Ni and were strongly positively correlated with organic matter and were negatively correlated with pH. Moreover they mentioned that total Cd was gradually increased with the increase of SS application. However, Chaudri *et al.*[49] reported that soluble Cd gave a more linear relationship with grain Cd than total soil Cd.

Welch and Lund[35] reported that soils pH is the main factor affecting heavy metal solubility because it controls reactions of soil properties with heavy metals and precipitation and dissolution of minerals in soil. McBride[50] mentioned that the strongest evidence for a protective effect of SS in limiting Cd uptake by leafy crops (lettuce and corn leaves) was for low Cd loading and non acid soil conditions. Bevacqua and Mellano[28] reported that heavy metals increased in soil as a function of SS application, but below levels that would cause plant toxicity. Ross[19] mentioned that normal range of Cd in soil is 0.01-7 µg g^{-1} dry weight whereas concentration in soil considered toxic is 3 to 8 µg g^{-1} dry weight.

Based on the results of this study it can be concluded that addition of SS to calcareous soil at a rate of 40 t ha^{-1} is equivalent to the addition of the recommended DAP fertilizer rate in increasing lettuce growth. Higher rates of SS resulted in farther increase in lettuce growth but resulted in higher heavy metal contents in lettuce and soil. Therefore, addition of 40 t ha^{-1} to calcareous soil is recommended to achieve acceptable level of plant growth while minimizing the adverse effect on plant and soil qualities parameters.

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