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EFFECT OF SEWAGE SLUDGE AS AMENDMENT ON SOIL MICROBIAL POPULATIONS AND THEIR ACTIVITIES IN TWO DIFFERENT SOILS

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ABSTRACT: Sewage sludge may improve soil fertility but there is concern about effects of sludge metals on soil microorganisms and their activities. Laboratory studies were conducted to determine the effect of two levels of sewage sludge (2% and 4% w/w) on the microbial populations and their activities in two different soils (sandy clay loam and sandy soil) during 42 days periods. The application of sewage sludge with the rate of 2% increased the total counts of bacteria, fungi, actinomycetes, nitrifiers, aerobic and anaerobic nitrogen fixers significantly in both kinds of soil than the unamended and the amended soil with the rate of 4% sewage sludge. The treatment with the high rate of sewage sludge (4%) caused a reduction in the total counts of the tested microorganisms and the activities of some soil enzymes (dehydrogenase, phosphatase, and urease) than the treatment with 2% sewage sludge. However the values obtained with 4% sewage sludge was still higher than those found in the unamended soil (control). Sewage sludge with the rate of 4% caused an increase in total nitrogen reaching 634 and 378 mg kg^{-1} dry soil after 7 days of incubation in sandy clay loam and sandy soils, respectively. Generally, the addition of sewage sludge to both kinds of soils with the rate of 2% increased the values of ammonia and nitrate nitrogen than the unamended and amended soil with the rate of 4% sewage sludge. No harmful effect to microbial population and their activities was observed when sandy clay loam and sandy soils were amended with sewage sludge with the rate of 2% consequently this rate can be used safely in agricultural soils.

Key words: Sewage sludge, microbial population, dehydrogenase, phosphatase, urease, ammonia, nitrate, total nitrogen

INTRODUCTION

Many problems are associated with environmental pollution especially those concerning with heavy metals. Although metals such as Cu, Ni, and Zn are essential nutrients for living organisms (Alloway, 1995) they may be toxic at high concentration. Heavy metals are being added to the environment from a variety of sources including municipal, industrial, and agricultural wastes as well as dredge spoils (Huisingh, 1974, Austin, *et al.,* 1977 and Timoney *et al.,* 1978)

The addition of sewage sludge to agricultural soil improves physical characteristics and acts as a valuable source of nutrients for growing crops (Smith, 1996). Also the addition of sewage sludge to soil, especially soils of low organic matter, significantly increased the population and the activity of soil microorganisms (Pichtel and Hayes, 1990, Barakah *et al.,* 1995 and Banerjee *et al.*, 1997) and also increased soil enzymes-activities especially dehydrogenase activity (Crecchio *et al.,* 2004) and promoted higher values of phosphatase and urease activity (Qiling *et al.,* 2002, Awad and Fawzy, 2004 and Crecchio *et al.*, 2004).

However, there is concern over the use of sludge that has been contaminated with potentially toxic elements (PTEs) from both industrial and domestic sources. The concentration of PTEs in sludge is usually considered the main determinant in restricting its application to agricultural soils. Application of sewage sludge to agricultural land can lead to an accumulation of metals in the surface layers with estimated residence times of the metal contaminants in the order of thousands of years (Mc Grath, 1987). Moreover contamination with heavy metals arises also where sewage sludge containing pesticides or fertilizers are used extensively as amendments since it can affect the activities of the beneficial soil microorganisms, and consequently affect the cycling of plant nutrients in the soil (Giller *et al.,* 1998). Heavy metal contamination can also inhibit soil functions, but it is often difficult to determine the degree of pollution. Enzyme assays offer potential indicators of biological functioning of soil (Belen, *et al.,* 2004).

Several studies have shown that metals influence microorganisms by adversely affecting their growth, morphology, and biochemical activity, resulting in decreased biomass, dehydrogenase and urease activities by increasing rates of sewage sludge addition (Reddy *et al.*, 1987, Fliessbach and Reber, 1990, Chander and Brookes, 1991, Fliessbach *et al.,* 1994, Kandeler *et al.,* 1996, Roane and Kellogg, 1996, Baath *et al.,* 1998, Rodriguez *et al.,* 2004 and Belyaeva, *et al.,* 2005).

The present work aimed to study the effect of amending two kinds of soils (sandy clay loam and sandy) with two levels of sewage sludge (2% and 4% w/w) on the microbial populations and the soil-enzyme activities such as (dehydrogenase, phosphatase and urease activities) as well as total nitrogen, ammonia, and nitrate nitrogen under controlled conditions in the laboratory.

MATERIALS AND METHODS

In this experiment, the effect of the treatment of the soil with different rates of sewage sludge on the enumeration and activities of different microbial groups was studied in two types of soil which have never received sewage sludge before. These types were sandy clay loam and sandy soils (sandy clay loam soil has been collected from El- Hosseinia district, Sharkia governorate, and sandy soil has been collected from Fakous district, Sharkia governorate).

The two soils were air dried, pulverized to pass through a 4- mm stainless steel sieve. The soils were analyzed for their physical and chemical properties (Table 1). Plastic pots 15cm in diameter were filled with 1.5 kg of sandy clay loam soil or sandy soil. The two soils were first treated by mixing thoroughly with sludge before putting them in the pots, as follows.

1- Soil without sludge.
2- Soil treated with sewage sludge (2% w/w).
3- Soil treated with sewage sludge (4% w/w).

The chemical properties and the heavy metals content of the sludge used in this experiment are shown in (Table 2) by plasma optical emission mass spectrometer (POEMS 3)

Three replicates for each treatment were used. The experiment was designed and the pots were placed in the incubator under controlled conditions (in the dark at 28± 2°C. WHC 60%). The microbiological and chemical measurements have been carried out at intervals zero, 7, 14, 28, 42 days of incubation.

Microbiological Analysis

Determination of the total counts and some specific groups of microorganisms in soils during the time course of this study were carried out as follows:

An amount of 10 g of soil sample was accurately weighed

Table 1. Physical and chemical properties of the soils under investigation.

Type of soil analysis	Properties	Soil	
		Hossenia	Fakous
Physical analysis	pH	7.6	8.7
	EC dsm^{-1}	2.53	3.17
Mechanical analysis	Sand %	55.89	93.16
	Silt %	16.80	5.20
	Clay %	27.31	1.64
	Type of soil	Sandy clay loam	Sandy
Chemical analysis (meq / 100 g soil)	Cation		
	Ca^{++}	2.5	1.8
	Mg^{++}	1.5	0.9
	Na^{+}	0.8	2.85
	K^{+}	0.06	0.12
	Anion		
	HCO_3^{-}	0.63	2.8
	Cl^{-}	0.46	1.2
	SO_4^{--}	1.32	0.81
	CO_3^{--}	Traces	Traces

Table 2. Heavy metals content of sewage sludge and the soils under investigation (mg/L)

parameter	Sewage sludge		Sandy clay soil		Sandy soil	
	Total	Available	Total	Available	Total	Available
Aluminum	2801	0.559	5013	1.960	1883	0.820
Boron	84.83	1.510	214.2	0.765	26.54	0.082
Calcium	14070	165.2	17410	113.7	2012	305.0
Cadmium	0.435	0.033	0.260	<0.001	0.333	<0.001
Cobalt	26.92	0.559	2.418	0.261	1.745	0.035
Chromium	49.46	0.292	19.80	0.166	9.011	<0.007
Copper	52.99	26.28	38.88	6.629	15.38	0.330
Iron	38010	345.0	16910	84.67	2254	14.57
Magnesium	878.9	80.97	13580	916.0	569.3	55.39
Manganese	133.8	37.25	739.0	82.14	42.37	5.511
Molybdenum	139.8	0.700	9.798	0.391	5.975	0.064
Nickel	55.27	5.929	25.50	0.888	7.661	0.057
Lead	26.46	12.99	<0.01	<0.01	2.303	0.255
Strontium	171.5	3.193	71.41	2.011	18.93	2.648
Vanadium	143.1	7.305	12.40	0.375	7.546	0.258
Zinc	189.1	92.27	98.16	1.076	30.85	1.033

and transferred to a bottle containing 90 ml of sterile phosphate saline solution (pH 7.3) according to Page (1982). The bottles were vigorously shaken for 10 minutes on a mechanical shaker. From each bottle, serial dilutions were made, and one ml from each desired dilution was used according to plate count technique and the dilution frequency method.

Plate count technique:

It was used to count bacteria, fungi and actinomycetes in soil samples using the following media:

1-Soil extract agar medium for counting the bacteria (Baruah and Barthakur, 1997).

2- Martin's rose bengal medium for counting the fungi (Baruah and Barthakur, 1997).

3-Dextrose- nitrate agar medium for counting the actinomycetes (Black, 1965).

The media were sterilized at 121°C for 20 minutes. In all cases. triplicate plates were prepared and inoculated with 1 ml of each desired soil solution under aseptic condition, and plates were incubated at 30 °C. The counting was carried out after 5, 7, and 14 days of incubation for plates of bacteria, fungi, and actinomycetes, respectively. The counts were then calculated to obtain a number of organisms per gram dry soil.

Most probable number (MPN) methods:

This technique was used according to Cochron Tables, (Alexander, 1982).to determine the densities of nitrifiers (*Nitrosomonas* and *Nitrobacter*), aerobic N_2- fixing *Azotobacter* and spore formers clostridia. In this connection, the following media were used:

1-Ammonium- calcium carbonate medium (Black, 1965).

2-Nitrite- calcium carbonate medium (Black, 1965).

3-Modified Ashby's medium for *Azotobacter* (Abdel- Malek & Ishac, 1968).

4-Modified Winogradsky's medium for anaerobic spore former nitrogen fixer *Clostridium* (Allen, 1959).

Ammonium oxidizers:

Ammonium oxidizers counting tubes were incubated at 30 °C for 30 days. After incubation period, spot test for nitrite was carried out using Griess- Ilosvay reagent according to Black (1965).

Nitrite oxidizer:

Nitrite oxidizer counting tubes were incubated at 30 °C for 30 days. After this time, nitrate spot test was carried out using Griess-Ilosvay reagent according to Black (1965).

Azotobacter:

After 3 weeks of incubation at 30 °C, cultures were considered positive when turbidity and presence of characteristic pellicle were observed, and detection of the organism in the stained preparations.

Spore formers clostridia:

After subjecting the soil solution to pasteurization to get rid of the vegetative cells, the anaerobic spore former nitrogen fixer *Clostridium*, using the above mentioned selective liquid media, was determined. After inoculation, the tubes were covered with a sterile layer of vaspar and incubated at 30 °C for 3 weeks. The presence of clostridia was detected by the accumulation of gases and by observation of non mistakable plectridial sporangia microscopically.

Chemical Analysis of the Soil

Chemical analysis including ammonium, nitrate, total nitrogen, and the activities of some soil important enzymes, such as dehydrogenase, phosphatase and urease, were also determined periodically in the soil according to Page (1982).

RESULTS AND DISCUSSION

In this study two kinds of soil textures were used for studying the effect of amending the soil with sludge as a source of heavy metals on the microbial densities, soil enzyme activities and the changes in the chemical properties of the soil represented as total N, ammonia and nitrate nitrogen. Sewage sludge was used as an organic amendment (2% and 4%) besides an unamended soil as a control.

Effect of Application of Different Rates of Sewage Sludge to the Soil on the Microbial Counts.

Total bacterial counts:

Data in Table 3 show the effect of adding two different rates of sewage sludge to sandy clay loam and sandy soils on the total bacterial counts during 42 days of incubation. In both soils the treatment with 2% of sewage

Table 3.Total counts of bacteria, fungi, and actinomycetes in sandy clay loam and sandy soils as affected by applications of two rates of sewage sludge (2% and 4%)

	Sandy clay loam soil					Sandy soil				
Days	0	7	14	28	42	0	7	14	28	42
Treat.	**Bacteria (x 10^5 CFU g^{-1} dry soil)**									
Control	82.02a	122.94b	110.67c	96.62b	75.27c	39.97b	51.95b	57.80a	43.90b	31.46b
2%	82.59a	140.27a	176.35a	128.37a	116.62a	43.29a	56.24a	53.90b	51.46a	51.29a
4%	83.78a	98.37c	153.51b	125.67a	85.81b	40.97b	49.39c	51.46c	49.63a	49.63a
	LSD 3.280					**LSD 1.928**				
	Fungi (x 10^2 fungal propagule g^{-1} dry soil)									
Control	119.31b	205.13c	208.64c	200.16b	198.41b	52.43b	71.09b	57.56b	49.75c	46.21c
2%	120.67b	239.45b	244.59a	245.13a	229.72a	53.04b	82.07a	72.43a	89.63a	62.56a
4%	123.64a	270.27a	215.13b	178.24c	167.97c	57.19a	60.24c	56.46b	72.19b	55.48b
	LSD 1.998					**LSD 1.621**				
	Actinomycetes (x 10^4 CFU g^{-1} dry soil)									
Control	83.41a	92.43c	95.43c	132.43c	128.10b	53.04a	53.29b	59.39c	62.80b	60.60c
2%	84.45a	111.62a	128.78a	145.13a	147.16a	50.60b	52.19b	73.78a	77.32a	80.24a
4%	81.48b	98.24b	115.81b	141.21b	120.81c	49.14b	58.90a	69.39b	77.07a	75.97b
	LSD 1.107					**LSD 1.564**				

sludge caused a significant increase in the total counts of bacteria comparing with either of the unamended soil or treatment received 4% sewage sludge. These results were in agreement with those obtained by Mikanova *et al.*, (2001) and Speir *et al.*, (2004) who found that significantly higher numbers of total countable bacteria were found at the least-polluted sites. Numbers decreased with proximately to the pollution site (with increasing concentration of pollutants). Data also show that the highest bacterial count was obtained after 14 days of incubation in sandy clay loam soil (1.7×10^7 CFU g^{-1} dry soil). On the other hand, sandy soil gave the highest bacterial counts (5.6×10^6 CFU g^{-1} dry soil) after incubation time of 7 days, when treated with 2% sewage sludge. Generally, the total counts of bacteria in sandy clay loam soil increased faster after 7 days of incubation reaching about 150%, 171% and 120% over the total counts of bacteria obtained at zero time as influenced by the application of 0, 2 and 4% sewage sludge, respectively. The same trend was obtained in sandy soil after 7 days of incubation time reaching 130%, 141% and 124% over the control when 0, 2 and 4% of sewage sludge were used, respectively. Regardless the incubation time, the data in Table 3 show also that the amended sandy clay loam and sandy soils with 2% of sewage sludge enhanced the rate of the bacterial counts. These results were in a harmony with those obtained by Barakah *et al.*, (1995) and Stoven *et al.*, (2005) who mentioned that amendment of loamy soil with sludge at the common rate (2%) enhanced the microbial flora with no harmful effects to plant growth or soil fertility and can be used safely in agricultural land. However, Kelly *et al.*, (2003); Smejkalova *et al.*, (2003) and Rajapaksha *et al.*, (2004) found that heavy metals caused a decrease in CFU in the case of oligotrophic bacteria.

Our results show also that the application rate of 4% sewage sludge to the soil caused higher counts of bacteria on both kinds of soils than the unamended soils (control), since the higher total counts obtained were 1.5×10^7 CFU g^{-1} dry soil and 5.1×10^6 CFU g^{-1} dry soil in the 14^{th} day of incubation time in the sandy clay loam and sandy soils, respectively. This indicates that there was no harmful effect on the total bacterial counts in both soils, under the use of sewage sludge (4%) to the soils.

Total fungal counts

Data in Table 3 show the density of the total fungal counts in the sandy clay loam and sandy soils as affected by sewage sludge application. From this Table we can realize that sandy clay loam soil which was amended with 4% sewage sludge gave the highest value of fungal counts reaching 2.7×10^4 fungal propagule g^{-1} dry soil after 7 days of incubation time. Although the highest value of the total counts of fungi was observed after 7 days of incubation when the soil was amended with 4% sewage sludge, the counts were significantly decreased gradually from the second sampling time of incubation until the end of the experiment reaching 1.7×10^4 fungal propagule g^{-1} dry soil. On the other hand, the amended sandy clay loam soil with 2% sewage sludge gave a significant higher values of fungal counts through the 42nd day of incubation comparing with the unamended sandy clay loam soil.

Concerning the sandy soil, the data in Table 3 show that the fungal counts were significantly higher in the soil amended with 2% sewage sludge than either the unamended or the amended with 4% sewage sludge.

In conclusion, the application of sewage sludge with the rate of 2% to sandy clay loam and sandy soils was useful concerning the density of fungi. These results were in harmony with those obtained by Kacprzak and Stanczyk (2003), Awad and Fawzy (2004), Pisarek and Moliszewska, (2004) and Rajapaksha *et al.*, (2004) who found that fungi activity initially increased with the level of metal contamination, being up to 3 and 7 times higher than that in the control samples during the first week at the highest level of Zn and Cu addition, respectively. The positive effect of metal addition on fungal activity was observed then decreased, but fungal activity was still higher in contaminated soil than in control soil after 35 days. Also Banerjee *et al.*, (1997) found that single and repetitive applications of different amount of sludge significantly increased the amount of soil microbial biomass but reduce the functional diversity of microbial community at the highest rate of application. On the other hand, these results were in disagreement with those obtained by Ropek and Para (2002), Kelly *et al.*, (2003), Gharieb *et al.*, (2004) and Stoven *et al.*, (2005) who found that heavy metals ions inhibited fungal growth.

Total actinomycetal counts

Data in Table 3 show the effect of the addition of different rates of sewage sludge to sandy clay loam and sandy soils on the actinomycetal counts.

Generally, the amended soil with 2% sewage sludge increased the counts of actinomycetes significantly than both of unamended soil (control) or the amended soil with 4% sewage sludge in sandy clay loam as well as in sandy soil. Data also show that the highest numbers of actinomycetes were obtained after the 42^{nd} day of incubation time in both sandy clay loam and sandy soils treated with 2% sewage sludge reaching 1.47×10^6 and 8.02×10^5 CFU g^{-1} dry soil, respectively. These results were in harmony with those obtained by Awad and Fawzy (2004) and Stoven *et al.,* (2005) who found that the addition of sewage sludge with low heavy metal content as well as the addition of heavy metal polluted sludge showed increase counts of actinomycetes compared to the control. On the other hand, these results were disagreement with those obtained by Kelly *et al.,* (2003) who stated that soils with higher levels of metals contamination showed decrease in actinomycetes.

Aerobic nitrogen fixers (*Azotobacter* spp)

Treatment of sandy clay loam and sandy soils with either 2% or 4% sewage sludge amendments increased the populations of aerobic nitrogen fixers (*Azotobacter* spp.) significantly as compared with the control (unamended soil) through the experimental periods (Table 4). Data also show that in sandy clay loam soil, the highest counts of aerobic nitrogen fixer were obtained in the 14^{th} day of incubation period with the two levels used from the sewage sludge (2% and 4%) reaching 4.72×10^3 cells g^{-1} dry soil. Comparing the counts of *Azotobacter* spp in sandy clay loam and sandy soils, it was clear that these counts were lower in the latter soil.

Concerning the sandy soil, the highest counts of *Azotobacter* spp was found at the 7^{th} day of incubation period when 4% sewage sludge was applied reaching 1.45×10^3 cells g^{-1} dry soil. This count, decreased sharply in the 14^{th} day since the counts reached approximately half of the value obtained at the 7^{th} day.

The data show also that there is a some stimulation in *Azotobacer* spp counts after 14 days of

Table 4. Counts of aerobic and anaerobic N_2 fixers (*Azotobacter* spp and *Clostridium* spp) in sandy clay loam and sandy soils as affected by applications of two rates of sewage sludge (2% and 4%)

	Sandy clay loam soil					Sandy soil				
Days	0	7	14	28	42	0	7	14	28	42
Treat.	*Azotobacter* spp ($x10^3$ cells g^{-1} dry soil)									
Control	2.78a	3.09b	3.09b	2.32c	1.45c	0.51a	0.56c	0.46c	0.46c	0.36b
2%	2.32b	4.24a	4.72a	3.64a	2.47a	0.52a	0.71b	1.13a	1.08a	0.93a
4%	2.63a	4.18a	4.72a	2.91b	1.96b	0.56a	1.45a	0.71b	0.50b	0.35b
	LSD 0.214					**LSD 0.072**				
	Clostridium spp ($x10^3$ cells g^{-1} dry soil)									
Control	3.72a	4.18a	3.64b	3.25b	2.91b	0.78a	0.91c	0.89b	0.59b	0.58a
2%	3.78a	4.24a	6.16a	3.64a	3.31a	0.80a	1.96a	2.08a	1.03a	0.52a
4%	3.25b	2.91b	2.31c	2.16c	1.89c	0.63b	1.40b	0.71c	0.52b	0.46a
	LSD 0.125					**LSD 0.137**				

incubation period even though in control treatment. This increase in *Azotobacter* spp counts decreased sharply after 28 days of incubation time till the end of the experiment reaching 1.45 x 10^3 cells g^{-1} dry soil in sandy clay loam soil when no sludge was added. On the other hand, the counts of *Azotobacter* spp reaching 2.47 x 10^3 and 1.96 x 10^3 at the end of the experimental periods when the sludge was added with the levels of 2% and 4%, respectively.

Although the counts of *Azotobacter* spp were lower in sandy soil, the application of sewage sludge with the level of 2% gave a significantly higher values comparing with an unamended one. These results were in agreement with those obtained by Badran, (1983) who reported that the sludge may also enhance growth of aerobic N2 fixers. And these results also were in agreement with those obtained by Barakah *et al.*, (1995) who found that the amended of the soil with sewage sludge (2%) significantly enhanced N_2 fixers.

By contrast the higher level of sewage sludge (4%) gave lower counts of *Azotobacter* spp reached about half of the counts obtained when the low level (2%) was used. These results were in harmony with those obtained by Balzer and Ahrens (1991), Athar and Masood (2002) and Vasundhara *et al.*, (2002) who reported that numbers of *Azotobacter* in soils of treatments receiving 2.5 t ha^{-1} of sewage sludge regularly at Giessen were tenfold greater than in soil receiving NPK; by contrast, they were only one-tenth of the numbers in the NPK control in a treatment receiving sludge at 5 t ha^{-1}.

Anaerobic nitrogen fixers *(Clostridium* spp)

Data in Table 4 show that the highest number of anaerobic spore formers bacteria was obtained at the 14^{th} day of incubation period reaching 6.16 x 10^3 cells g^{-1} dry soil when sandy clay loam soil was treated with 2% of sewage sludge. Concerning the sandy soil, the highest number of anaerobic spore formers bacteria was obtained at the 14^{th} day of incubation period reaching 2.08 x 10^3 cells g^{-1} dry soil. Data also show that at the end of the experiment, the counts of anaerobic spore formers bacteria in the treatment of 4% of sewage sludge were lower than those obtained with the control in both sandy clay loam and sandy soils, this decrease was significant in sandy clay loam and was non significant in sandy soil.

Generally, data show that in sandy clay loam soil the counts of anaerobic spore formers bacteria with the treatment of 2% sewage sludge were increased significantly in the 14th day and then decreased until the end of the experiment. These results were in agreement with those obtained by Barakah *et al.*, (1995) who found that the amended soil with sewage sludge (2%) significantly enhanced N_2 fixers. On the other hand, application of sewage sludge (4%) in sandy clay loam soil caused a reduction in the counts of the anaerobic spore formers bacteria reaching about 50% of the counts obtained in unamended one. This reduction was observed in the 7th day and continued until the end of the experiment. Our results were in agreement with those obtained by Smejkalova *et al.*, (2003), Xu *et al.* (2003) and Heras *et al.,* (2005) who found that supplementing of the Cu (II)-exchanged montmorillonite in the diet of broilers decreased the numbers of *Clostridium*. By contrast in sandy soil, the treatments with unamended as well as the addition of 4% sewage sludge caused an increase in the counts of anaerobic spore formers bacteria in the 7th day of incubation period and then decreased gradually until the end of the experimental periods.

Nitrifiers bacteria

Data in Table 5 show the effect of application of sewage sludge on *Nitrosomonas* spp counts in sandy clay loam and sandy soils. The data show that the highest counts of *Nitrosomonas* spp was observed in the 14th day in the amended sandy clay loam soil with 2% sewage sludge reaching 2.35×10^3 cells g^{-1} dry soil. Data also show that the counts at the rate of 2% sewage sludge were higher than those unamended or 4% sewage sludge. Also, in sandy soil higher counts of *Nitrosomonas* spp was found with the treatment of 2% sewage sludge compared with unamended or 4% sewage sludge.

Data also show that the highest number of *Nitrosomonas* spp in sandy soil was obtained at the 7th day of incubation period reaching 1.40×10^3 cells g^{-1} dry soil when the soil was amended with 2% sewage sludge. Generally in sandy soil, the counts of *Nitrosomonas* spp increased significantly in the 7th day comparing with the control and then decreased until the end of the experimental periods.

However, a great increase was observed in sandy soil at the 7th day of incubation time when it was treated with 2% sewage sludge

Table 5. Counts of nitrifying bacteria (*Nitrosomonas* spp and *Nitrobacter* spp) in sandy clay loam and sandy soils as affected by applications of two rates of sewage sludge (2% and 4%)

	Sandy clay loam soil					Sandy soil				
Days	0	7	14	28	42	0	7	14	28	42
Treat.	*Nitrosomonas* spp ($x10^3$ cells g^{-1} dry soil)									
Control	0.89a	1.21b	1.32b	0.94c	0.72b	0.40a	0.52c	0.48b	0.42b	0.35b
2%	0.94a	1.41a	2.35a	1.85a	1.08a	0.42a	1.40a	0.94a	0.60a	0.50a
4%	0.94a	1.32ab	1.17c	1.08b	1.06a	0.39a	0.59b	0.42c	0.39c	0.31b
	LSD 0.136					**LSD 0.065**				
	Nitrobacter spp ($x10^3$ cells g^{-1} dry soil)									
Control	0.54ab	0.84b	0.93c	0.66c	0.66c	0.17a	0.31b	0.37b	0.29a	0.26a
2%	0.64a	1.63a	1.85a	1.41a	1.13a	0.20a	0.87a	0.60a	0.35a	0.31a
4%	0.52b	1.56b	1.32b	0.93b	0.84b	0.23a	0.35b	0.32b	0.32a	0.24a
	LSD 0.109					**LSD 0.076**				

reaching 3.5 folds of the counts obtained at the beginning of the experiment.

Also data in Table 5 show the influence of two levels of sewage sludge applied to sandy clay loam and sandy soils on *Nitrobacter* spp counts. Data show that the highest value of *Nitrobacter* spp counts was found at the 14th day in sandy clay loam soil which was amended with 2% of sewage sludge reaching 1.85 x 10^3 cells g^{-1} dry soil. On the other hand, in sandy soil, the highest number was found at the 7th day with the application of 2% sewage sludge reaching 8.7 x 10^2 cells g^{-1} dry soil. Data also show that, the amended soil with 2% caused a significant increase in the number of *Nitrobacter* spp comparing with either the control or the application of 4% of sewage sludge in both sandy clay loam and sandy soils. These results and the results obtained with the *Nitrosomonas* spp were in agreement with those obtained by Yang *et al.*, (2005) who found that the addition of cadmium at low levels stimulated the nitrifying activity in soil, while inhibitory influence were shown at high levels. Nitrifying bacteria was proved to be the most sensitive one.

In conclusion, the application of sewage sludge with the rate of 2% caused a significant increase in *Nitrobacter* spp counts in sandy clay loam soil as well as in sandy soil reaching 1.85 x 10^3 and 8.7 x 10^2 cells g^{-1} dry soil, respectively. Our results concerning nitrifiers bacteria were in agreement with those obtained by Barakah *et al.*, (1995), Furczak and Joniec (2002) and Jezierska and Frac (2005) who found that the amended soil with sewage sludge (2%) significantly enhanced nitrifying bacteria. On the other hand, the measurement of the potential ammonia oxidation indicated that ammonia oxidizing bacteria were completely inhibited in the contaminated soil with heavy metals.

Assay of the Soil-Enzyme Activities

Dehydrogenase activity:

Data in Table 6 show the effect of addition of sewage sludge to the soil on the dehydrogenase activity in sandy clay loam and sandy soils. The data show that dehydrogenase activity was higher in the soil amended with 2% of sewage sludge than those of the unamended and amended with 4%

Table 6. Dehydrogenase and urease activities in sandy clay loam and sandy soils as affected by applications of two rates of sewage sludge (2% and 4%)

	Sandy clay loam soil					Sandy soil				
Days	0	7	14	28	42	0	7	14	28	42
Treat.	**Dehydrogenase activity (micro mole H_2 / g dry soil / day)**									
Control	3.83	4.29	5.63	4.46	3.70	1.39	1.63	1.86	0.90	0.77
2%	4.07	5.57	5.86	5.06	4.72	1.41	1.99	2.60	1.91	1.62
4%	3.83	4.46	5.35	4.59	3.76	1.40	1.94	1.97	1.09	0.94
	Urease activities (ppm utilized urea / g dry soil / day)									
Control	60.05	62.32	57.76	47.94	43.66	14.47	18.69	20.10	20.91	19.63
2%	61.11	65.51	66.51	57.32	47.47	16.02	32.84	26.98	24.91	23.72
4%	59.06	65.40	61.98	51.36	43.78	16.80	27.40	22.15	17.83	15.62

of sewage sludge. These results might be due to the lower concentrations of heavy metals which could be reflected on the total counts of bacteria as well as dehydrogenase activity. In addition

the data show that the highest values of dehydrogenase activity were observed in the 14th day in amendment with 2% of sewage sludge in both kind of soils reaching 5.86 and 2.60 micro mole H_2 g^{-1} dry soil/day in sandy clay loam and sandy soils, respectively. It was obvious that dehydrogenase activity increased sharply in the 14th day then decreased gradually. These phenomena appeared in both sandy clay loam and sandy soils in all the treatments used. These results were in harmony with those obtained by Furczak and Joniec (2002),Crecchio *et al.*, (2004) and Stoven *et al.*, (2005) who found that the addition of municipal solid waste to cropped plots increased dehydrogenase activity by 9.6%.

Concerning the application of sewage sludge with the level of 2%, it was obvious that this treatment increased the dehydrogenase activity in both kinds of soils. On the other hand, the application of sewage sludge with 4% to sandy clay loam and sandy soils caused a reduction in the dehydrogenase activity comparing with the unamended soil (with no sludge). This reduction in dehydrogenase activity with the application of 4% sewage sludge might be due to the higher concentrations of heavy metals (Table 2). Similar results were obtained by Chander and Brookes, (1991), Mikanova *et al.,* (2001), Lee *et al.*, (2002) and Ying *et al.,* (2002) who mentioned that the lowest activity of dehydrogenase was found in contaminated soil with heavy metals. Also Reddy *et al.,* (1987) and Fliessbach and Reber, (1990) found that dehydrogenase was more sensitive to sewage sludge.

As a conclusion, dehydrogen-ase activity at 42nd day of incubation time was still higher when the soil was amended with 2% sewage sludge than either of the treatment with 4% sewage sludge or unamended one.

Urease activity:

Data in Table 6 show the effect of addition of sewage sludge to the soil on the urease activity in sandy clay loam and sandy soils. Data also show that the highest value of urease activity was observed in sandy clay loam soil at the 14th day in the treated soil with 2% of sewage sludge reaching 66.51 ppm utilized urea g^{-1} dry soil/day. On the other hand, in sandy soil, the highest value of urease activity was observed at the 7th day in amendment with 2% of sewage sludge reaching 32.84 ppm utilized urea g^{-1} dry soil/day. These results were in harmony with those obtained by Qiling *et al.*, (2002) who found that the activity of urease in soil increased with increasing rates of sewage sludge. At the same time, Stuczynski *et al.* (2003) demonstrated that Cd has stimulatory effect on urease activity in soil.

The data in Table 6 show also that the treatment of sandy soil with 4% sewage sludge stimulates the activity of urease at the beginning of the experiment at the 7th day then caused an inhibition effect reaching 15.6 ppm utilized urea g^{-1} dry soil / day at the end of the experimental period. This value was lower than those obtained with the unamended or the amended soil with 2% sewage sludge which reaching 19.63 and 23.72 ppm utilized urea g^{-1} dry soil/day , respectively. From this data we can conclude that higher percentage of sewage sludge application caused an inhibitory effect than the lower percentage of sewage sludge. The latter caused a stimulatory effect. These results were in agreement with those obtained by Giridhara and Siddaramappa, (2002),Wen *et al.*, (2002), Liu-Chung *et al.*, (2002), Liu-Xia, *et al.*, (2003) and Belyaeva, *et al.*, (2005) who found that urease activity in the soil treated with heavy metals was decreased compared with the control. On the other hand, Renella *et al.*, (2005) found that urease activity was not affected by sludge amendment.

Phosphatase activity:

Data in Table 7 show the effect of the addition of different concentrations of sewage sludge to sandy clay loam and sandy soils on the phosphatase activity. Generally, it could be observed that alkaline phosphatase gave lower values than those of acidic phosphatase activity in sandy clay loam soil. By contrast, no clear different values were obtained between the two kinds of phosphatase activity in sandy soil. On the other hand, alkaline phosphatase measurements gave the higher values in sandy soil comparing with those obtained in sandy clay loam soil. Data also show that, in sandy clay loam soil the highest values of acidic phosphatase were observed at the 14th day in either of unamended or an amended soil with 4% of

Table 7. Phosphatase activity (µg P / g dry soil / day) in sandy clay loam and sandy soils as affected by applications of two rates of sewage sludge (2% and 4%)

Days	0		7		14		28		42	
Treatment	**Acidic**	**alkaline**	**Acidic**	**alkaline**	**Acidic**	**alkaline**	**Acidic**	**alkaline**	**Acidic**	**alkaline**
					Sandy clay loam soil					
Control	40.21	2.39	41.94	3.36	43.28	3.51	40.31	3.11	33.36	2.13
2%	40.03	2.68	41.34	3.21	42.25	3.26	40.09	3.27	34.95	2.58
4%	39.32	2.31	42.24	3.52	43.26	3.91	41.21	3.68	33.67	2.35
					sandy soil					
Control	6.66	8.36	7.03	10.20	6.83	10.33	6.72	10.36	5.93	9.63
2%	7.41	8.21	6.49	10.51	6.95	10.50	6.34	10.41	6.41	10.11
4%	6.35	8.96	5.44	11.31	8.08	10.42	7.03	10.32	6.52	10.23

sewage sludge reaching 43.28 and 43.26 μg P g^{-1} dry soil/day, respectively. Also, in sandy soil the highest value of the acidic phosphatase was obtained at 14th day when 4% of sewage sludge

was applied reaching 8.08 μg P g^{-1} dry soil/day. From these results it could be concluded that in both soils no harmful effect was observed when sewage sludge was applied even though at the high level of application.

Data also show that the highest value of alkaline phosphatase was observed at the 7th day in an amended soil with 4% of sewage sludge in sandy soil reaching 11.31 μg P g^{-1} dry soil /day. On the other hand, in sandy clay loam soil the highest value was found in the 14th day in an amended soil with 4% of sewage sludge reaching 3.91 μg P g^{-1} dry soil /day. These results were in agreement with those obtained by Awad and Fawzy, (2004), Crecchio *et al.*, (2004) and Speir *et al.*,(2004) who found that the increasing rates of sewage sludge promoted higher values of phosphatase activity. By contrast, Lee *et al.*, (2002); Wang *et al.*, (2002) and Stuczynski *et al.*, (2003) found that the pollution with heavy metals (Cu and Zn) caused an inhibitory influence on phosphatase activity. However Brookes *et al.*, (1984), Reddy *et al.*, (1987) and Gzik *et al.*, (2003) stated that phosphatase activity was not affected with increasing metals.

Effect of Different Concentrations of Sewage Sludge on Total Nitrogen, Ammonia and Nitrate of the Soil.

Total nitrogen

Data in Table 8 show the influence of the addition of two rates of sewage sludge to sandy clay loam and sandy soils on total soil nitrogen content. Total nitrogen in two kinds of soils increased with the addition of sewage sludge to both soils.

Concerning the application of 2% sewage sludge, it was clear that this treatment caused an increase in the total nitrogen comparing with the control treatment. These values reaching 131, 136, 137, and 135% over the values obtained with the control after 7, 14, 28, and 42 days of incubation time. Data also show that in sandy clay loam soil, the highest value of total nitrogen was obtained after 7 days of incubation time in the soil which was treated with 4% sewage sludge reaching 634 mg kg^{-1} dry soil. On the other hand, the values of the total

Table 8 . Total nitrogen (mg kg^{-1}) in sandy clay loam and sandy soils as affected by applications of two rates of sewage sludge (2% and 4%)

Treatments	Days				
	0	7	14	28	42
			Sandy clay loam soil		
Control	435	438	448	420	407
2%	510	572	609	577	550
4%	547	634	630	612	580
			Sandy soil		
Control	286	249	261	236	199
2%	320	332	340	323	273
4%	361	378	368	336	311

nitrogen decreased gradually with increasing the incubation time, which reaching 580 mg kg^{-1} dry soil at the end of the experimental period In sandy soil, total nitrogen was increased with the addition of 2% as well as 4% sewage sludge. Although the highest value was obtained when sewage sludge was used at the rate of 4% reaching 378 mg kg^{-1} dry soil after 7 days only, but when 2% sewage sludge was added, the highest value was obtained after 14 days reaching 340 mg kg^{-1} dry soil. These values with the two rates were decreased gradually until the end of the experimental time reaching 273 and 311 mg kg^{-1} dry soil when 2% and 4% sewage sludge were used, respectively.

The increase in total nitrogen as influence of application of 2% and 4% sewage sludge might be due to the increasing numbers of aerobic and anaerobic nitrogen fixers (*Azotobacter* spp and *Clostridium* spp) (Table 4). Our results were in agreement with those obtained by Balzer and Ahrens (1991) who found that nitrogen increased with increasing application of sewage sludge. Also Barakah *et al.,* (1995) found that total nitrogen in rhizosphere and nonrhizosphere of alfalfa and wheat significantly increased with application of organic amendment. Also they added that the amendment of the soil with sewage sludge (2%) significantly enhanced nitrogen fixers.

Our results also were in harmony with those obtained by Brofas *et al.,* (2000) who found that the addition of sewage sludge at seven rates (0, 10, 20, 40, 60, 80, and 120 tonnes ha^{-1}) in a field experiment increased total nitrogen. Also, they found that the total nitrogen concentration decreased with the time after sewage sludge application. Generally, our results show that the total counts of bacteria, fungi and actinomycetes in sandy clay loam soil (Table 3) were higher when sewage sludge was applied comparing with the control. This increase might be due to the higher production in sugar and organic acid which were necessary for growing *Azotobacter* spp and hence stimulate the nitrogen fixation in the soil (Table 8). These results were in agreement with those obtained by Barakah (1995); Crecchio *et al.,*(2004), Speir *et al.,* (2004), Stoven *et al.,* (2005) and Heras *et al.,* (2005) who mentioned that the amendment of the soil with sewage sludge 2% significantly enhanced nitrogen fixers.

Ammonia and nitrate nitrogen

Data in Table 9 show the effect of the addition of two levels

Table 9. Ammonia and nitrate nitrogen (mg kg^{-1}) in sandy clay loam and sandy soils as affected by applications of two rates of sewage sludge (2% and 4%)

Days	0		7		14		28		42	
Treat.	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
					Sandy clay loam soil					
Control	18.7	30.7	18.8	32.7	17.0	30.1	14.9	25.6	12.1	20.0
2%	18.6	30.5	19.2	42.0	21.6	44.3	16.8	40.3	15.3	36.8
4%	19.1	30.5	20.5	39.2	16.8	36.4	15.1	35.6	14.2	34.2
					sandy soil					
Control	10.0	15.3	10.6	13.2	11.2	11.2	10.2	9.6	8.4	8.6
2%	10.2	16.6	14.9	20.0	14.2	28.0	13.1	22.3	10.2	18.6
4%	10.3	16.9	14.0	26.1	13.1	22.6	11.2	20.1	9.3	15.2

of sewage sludge (2% and 4%) on ammonia and nitrate nitrogen in sandy clay loam and sandy soils. The data show also that in sandy clay loam soil the highest values of ammonia nitrogen were found at the 14th day of incubation periods when the rate of sewage sludge application was 2% reaching 21.6 mg kg^{-1}. This value decreased gradually until the end of the experiment reaching 15.3 mg kg^{-1}. Concerning sandy soil, the peak of ammonia was obtained during 7-14 days being 14.9 and 14.2 mg kg^{-1}, respectively when 2% sewage sludge was applied. Generally the addition of sewage sludge to the two soils with the rate of 2% increased the values of ammonia nitrogen than the unamended and the amended soil with the rate of 4% sewage sludge. Although, the application with 4% sewage sludge increased the values of ammonia nitrogen than the control, but these values were lower than those obtained with the application of 2% sewage sludge in both kinds of soils. The highest values of ammonia nitrogen were observed at the 7th day in sandy clay loam and sandy soils when the soils were amended with 4% sewage sludge reaching 20.5 and 14 mg kg^{-1}, respectively. These values decreased gradually until the end of the experimental periods reaching 14.2 and 9.3 mg kg^{-1} in sandy clay loam and sandy soils, respectively. These results were in agreement with those obtained by Furczak and Joniec (2002) and Yang *et al.,* (2005) who found that the addition of cadmium at low levels stimulated the ammonifying activity in soil, while inhibitory influence were shown at high levels. Also Jezierska and Frac (2005) found that fertilizer application to the soil with diary sewage sludge resulted in the stimulation of the number of ammonifying bacteria and ammonium nitrogen concentration.

The data also show that the highest value of nitrate nitrogen was found at the 14th day of incubation period with the rate of 2% sewage sludge in sandy clay loam soil reaching 44.3 mg kg^{-1}. The same trend was obtained in sandy soil which gave the highest value reaching 28 mg kg^{-1}. It's obvious that the incubation more than 14 days caused almost plateau line until the end of the experiment in both kinds of soils. The data also show that the addition of sewage sludge to the sandy clay loam and sandy soils with the rate of 2% increased the values of nitrate nitrogen. These results were true in both kinds of soil. These results were in agreement with those obtained by Furczak and Joniec (2002) who stated that strongly crumbled sludge stimulated most biochemical activities of the soil (ammonification and nitrification). By contrast, these results were in disagreement with those obtained by Kelly *et al.*, (2004), Gremion *et al.*, (2004) and Wilke *et al.*, (2005) who found that ammonia oxidizing bacteria were completely inhibited in the contaminated soil with heavy metals.

The results of this investigation clearly showed that, no harmful effect to microbial population and their activities was observed when sandy clay loam and sandy soils were amended with sewage sludge with the rate of 2% consequently this rate can be used safely in agricultural soils.

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تأثير حمأة المجارى على المجاميع الميكروبية وأنشطتها فى نوعين مختلفين من التربة

سمير حماد سالم – تحية عبدالعزيز خاطر – ناهد أمين الوفائى

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استخدمت من قبل حمأة المجارى كبديل للسماد العضوى لتحسين خصوبة التربة ولكن هناك تخوف من تأثير المعادن الموجودة فى الحمأة على ميكروبات التربة وكذلك على نشاط هذه الميكروبات. وفى هذا البحث تم استخدام مستويين من حمأة المجارى مقدرة وزن / وزن بنسبة ٢% و ٤% من خلال تجربة معملية لدراسة تأثير هذه الحمأة على كل من أعداد ونشاط الميكروبات وذلك من خلال استخدام نوعين من التربة إحداهما كانت رملية طميية طينية والأخرى كانت تربة رملية حيث أن كلاهما لم يتعرضا لأى إضافة من حمأة المجارى . تم أخذ عينات من هذه التجربة على فترات بعد التحضين على درجة حرارة ٢٨ ± ٢°م واستمر ذلك لمدة ٤٢ يوم . واستخلص من هذه التجربة ما يلى : وجود زيادة فى الأعداد الكلية لكل من البكتريا والفطر والاكتينوميسيتات وكذلك أعداد كل من بكتريا التأزت والبكتريا المثبتة للنيتروجين اللاتكافلية سواء كانت هوائية أو لا هوائية عند استخدام معدل إضافة بمقدار ٢% من الحمأة فى كلا النوعين من التربة المستخدمة مقارنة بالتربة غير المعاملة بالحمأة أو تلك التى عوملت بمعدل ٤% . كما لوحظ أن المعاملة بالمعدل الأعلى (٤% من الحمأة) تسبب فى انخفاض أعداد الميكروبات التى تم تقديرها فى هذه التجربة وكذلك انخفضت الأنشطة الخاصة ببعض إنزيمات التربة مثل (الديهيدروجينيز ، الفوسفاتيز واليورييز) عن تلك التى عوملت بمعدل ٢% من الحمأة وعلى الرغم من انخفاض أعداد وأنشطة الميكروبات فى التربة المعاملة بالتركيز الأعلى إلا أن تلك الأعداد لهذه المجاميع الميكروبية وكذلك أنشطتها كانت فى مستوى أعلى مما وجد فى التربة غير المعاملة.

وقد أدى استخدام الحمأة بالمعدل الأعلى الى زيادة فى النيتروجين الكلى وصلت الى ٦٣٤ و ٣٧٨ مللجم / كجم تربة جافة بعد ٧ أيام فقط من التحضين فى كل من التربة الرملية الطميية الطينية والرملية على التوالى.

وبصفة عامة فإن إضافة حمأة المجارى الى كلا النوعين من التربة المستخدمة فى هذه التجربة بمعدل ٢% حمأة قد أدت الى زيادة القيم المتحصل عليها من الامونيا والنترات عن التربة الاخرى غير المعاملة أو المعاملة بالمعدل الأعلى ولم يلاحظ من هذه التجربة أى تأثير ضار على أعداد الميكروبات أو نشاطها عند استخدام المعدل المتوسط من الحمأة . ونخلص من هذه التجربة أن هذا المعدل (٢%) من الحمأة يمكن استخدامه بأمان فى التربة الزراعية .