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Soil Enzymes Activities as Bio-indicators for Soil Contamination by Heavy Metals from Sewage Sludge Application.

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ABSTRACT

Incubation and field experiments studies were carried out to estimate urease and arginine ammonification activities in soil treated with sewage sludge as bioindicators. The amounts of DTPA - extractable Cd, Cr, Cu, Pb, Ni, and Zn in the original soil were 0.03, 0.64, 5.3, 2.3, 0.35 and 1.3 mg/Kg, respectively. The incubation study was carried out in the laboratory at controlled conditions (23±3 C° and 50 % water holding capacity) at different periods. The obtained results showed that urease and arginine ammonification activities increased with increasing sludge in soil relative to the control soil. In experimental field study, soil samples (0-15 cm) were collected from fields cultivated with winter season as wheat and faba bean and summer season crops as corn and soybean. The results obtained showed that urease and arginine ammonification activities were higher in sewage sludge-treated soil than in those of the control. Urease and arginine ammonification activities were almost lower in soil samples collected from fields cultivated with winter crops than those of summer crops. These data showed that the amounts of DTPA – extractable Cd, Cr, Cu, Pb, Ni, and Zn from the sludge treated soil of the four fields did inhibit both urease activity and arginine ammonification. These findings suggest that recycling of sewage sludge, and applied at a rate of 200 ton /hectare for three successive growth seasons did not lead to accumulation of heavy metals in soil to the levels which could hinder soil biochemical activity.

Keywords: Sewage sludge, heavy metals, urease activity, arginine ammonification.

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INTRODUCTION

The activities of soil enzymes can be sensitive tools and indicators of both natural and anthropogenic contaminations of the soil. Heavy metals contamination results in significant adverse effects on soil biochemical activities and in turn soil enzymes [1-7]. Sewage sludge application to agricultural land provides not only essential plant nutrients but also heavy metals which are potentially toxic to the biochemical processes in the soil ecosystem. The presence of heavy metals in high concentration levels in sewage sludge limits its reuse as an organic fertilizer.

Alexandria general organization for sanitary drainage is currently producing half million ton dried sewage sludge annually. Part of this quantity is subjected to an aerobic composting and is used as an organic fertilizer by farmers. Current interest in assessing the quality of soil is increasing in order to protect the agricultural ecosystem from degradation. Soil biology is a significant component of soil quality and microbiological activity. It is a vital factor of soil fertility [1,4, 8, 9]. The presence of heavy metals in soil treated with sewage sludge possibly influences the biochemical activity in soil. The objective of this study was to evaluate urease and arginine ammonification activities as bioindicators for sewage-sludge treated soil by using incubation and field experiment studies.

MATERIALS AND METHODS

The soil was used in this study is lacustrine, collected from Abis Village (Latitude 31.21337 N and Longitude 29.98203 E), Alexandria, Egypt. and the sewage sludge compost was obtained from site 9N, Alexandria General Organization for Sanitary Drainage. The soil and sewage sludge were analyzed for the determination of EC (1:1 soil-water extract), and pH in 1:2.5 soil-water suspension [10], organic carbon [11], dissolved organic carbon [12], total nitrogen [11] and ammonium nitrogen [13], nitrite-nitrogen and nitrate-nitrogen [14], available P and heavy metals (ammonium bicarbonate-DTPA reagent) [15] and the particle size distribution: sand, silt and clay, bulk density and the water hold capacity [9]. The results obtained are shown in Table1.

Table 1: The main characteristics and elements content of the used soil and sewage sludge compost.

Parameter		Soil	Sewage sludge		Parameter		Soil	Sewage sludge
pH		8.1	7.9		Soil texture		clay loam	–
EC	dS/m	1.7	5.4		Bulk density	g/cm^3	1.49	0.75
SAR		1.3	–		WHC	%	66.2	76.8
Organic matter	%	1.4	35.3		Total P	Mg/Kg	370	1790
Total nitrogen	%	0.125	1.76		″ K	″ ″	200	1750
C/N ratio		5.3	11.6		″ Cd	″ ″	1.7	7.4
Total carbonate	%	13.6	18.0		″ Cr	″ ″	19.0	37.2
Particle size distribution:					″ Cu	″ ″	15.0	133.8
Sand	%	36	–		″ Pb	″ ″	20.0	144.0
Silt	%	22	–		″ Ni	″ ″	21.8	26.0
Clay	%	42	–		″ Zn	″ ″	56.3	1760

Incubation study:

Sewage sludge treatments were 0, 80 and 160 g sludge per Kg soil. Four Kg of the sludge-treated soil were placed in PVC pot watered with distilled water and soil moisture was maintained at 60% WHC throughout the incubation period. Each treatment was repeated in three replicates. The soil pots were incubated at 23 ± 2°C for 0, 10, 20, 40 and 80 days and soil samples were taken at each incubation period for determination of AB-DTPA extractable heavy metals [15], Urease activity [16], Arginine ammonification [17], inorganic N [14], organic carbon [11], and dissolved organic carbon [12].

Field experiments: were carried out at Agriculture Researsh Station, Abis, Faculty of Agri., Alexandria University, in two growing seasons. Both faba been (*Vicia faba L*. variety Renablanka) and wheat (*Triticum aestivum L,* variety Gemiza 7) were cultivated in winter season while soybean (*Glycine max L* variety crow ford) and corn (*Zea maize L*, variety 10) were cultivated in summer. These plant crops were grown in sewage sludge-treated and untreated soils in a factorial experiment with two factors in randomize complete block design. Three sewage sludge rates (0, 80 and 160 ton/ Fed) were used. Sample of soils were collected after 65 days from plant sowing and after crop harvest. The soil samples were prepared for analysis as required by Van Reeuwijk [10]. The soils were also analyzed for the determination of urease activity and arginine ammonification. The data obtained were statistically analyzed according to Snedecor and Cochran [18].

RESULTS AND DISCUSSION

Incubation Study:

During incubation, there were significant decreases in soil pH especially at the highest sewage sludge rate with each incubation period. Generally, the soil pHs were almost higher than 7.0 and lower than 8.08 which indicates the higher buffer capacity of the soil. It was mainly related to high carbonate content. On the other hand, levels of OC increased significantly with increasing sewage sludge in soil from 0.98 % (control soil) to 1.54 % (160g sludge-mixed soil) during incubation period. Also, the levels of DOC in soil were significantly higher in soil mixed with the highest sludge rate than that of the control soil.

The amounts of NO_3^--N were significantly higher in soil-sludge mixture especially with sludge rate of 80 g Kg^{-1} soil than the control soil (Table 2). while, the concentrations of NO_2^--N were significantly lower in soil-sludge mixture in all sludge rate, than those of the control soil (Table 2). Another study showed that the levels of NO_3^--N were decreased in soil sludge mixed soil during the incubation period of 12 weeks [19]. Table 2 showed that the levels of NH_4^+-N in soil-sludge mixture were higher than that of the control. However, there were significant sharp decreases in the levels of NH_4^+-N in soil-sludge mixture during incubation periods. Another study reported neither NH_3 volatilization nor NH_4^+ accumulation were detected throughout the incubation period of soil-sludge mixture [19].

Table 2: Means value of NO_3^--N, NO_2^--N and NH_4^+-N during incubation periods of sludge-treated soil.

Sludge g /Kg soil	Incubation period (day)					$LSD_{0.05}$
	0	10	20	40	80	
			NO_3^- – N (µg/g soil)			
0	6.61	49.45	66.95	56.43	49.91	
80	22.15	94.10	132.75	76.95	86.85	15.25
160	26.64	31.02	37.90	76.10	117.65	
$LSD_{0.05}$			14.26			
			NO_2^- – N (µg/g soil)			
0	0.32	0.25	0.21	0.14	0.33	
80	0.18	0.12	0.28	0.26	0.37	NS
160	0.21	0.16	0.23	0.44	0.60	
$LSD_{0.05}$			NS			
			NH_4^+ – N (µg/g soil)			
0	8.8	2.1	2.1	1.3	2.0	
80	166.7	5.3	3.7	3.9	1.9	2.5
160	262.7	14.0	7.3	5.5	3.2	
$LSD_{0.05}$			5.7			

Figure 1 illustrated that with increasing sewage sludge rates in soil-sludge mixture Urease activity was significantly decreased of the un-incubated soil. This may be due to either (i) the effect of heavy metals in the sludge, (ii) decreased amount of free urease in soil-sludge due to binding of urease inside the sludge floc which reduced urease activity, (iii) high soil pH, or all of which would create unfavorable condition for microbial growth and N cycles [20]. However, during incubation, urease activity increased significantly with the three sludge rates which suggested the presence of high substrate concentration in the soil-sludge mixture under favorable wet conditions. Wetting the dry soil-sludge mixture (before incubation) caused lysis of microbial cells which released urease in soil [20, 21]. During incubation, urease activity significantly increased which could be a result of the supply of nutrients from the sludge. Comparing urease activity in the dry soil-sludge mixture (Zero incubation) with that of during incubation is shows that, wet soil had high urease activity than dry soil.

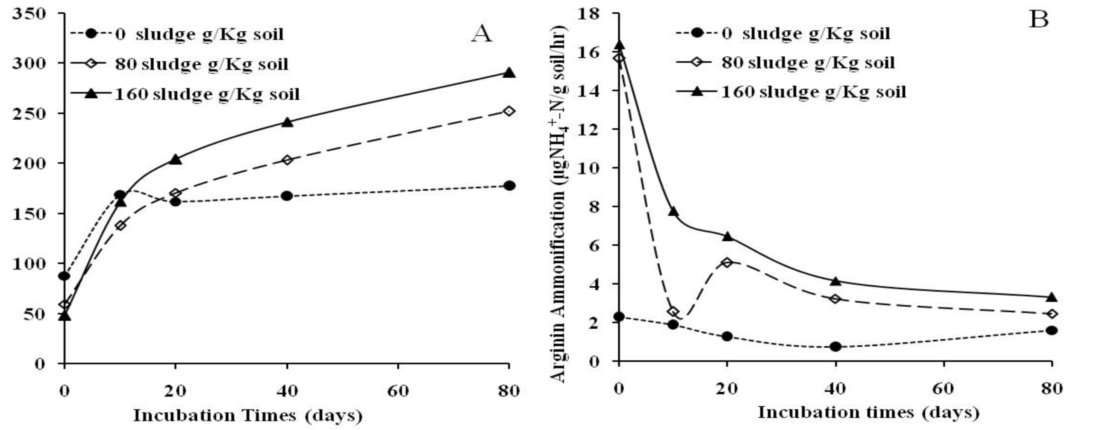


Figure 1: Effect of incubation periods of sludge-treated soil on Urease (A) and arginine (B) ammonification activity with $LSD_{0.05}$ 12.2 and 0.65 respects

Table 3: Amounts of AB-DTPA extractable P and heavy metals (mg/Kg soil) from sewage sludge-treated soil before incubation (zero time).

Sewage sludge (g/Kg soil)	P	Cd	Cr	Cu	Ni	Pb	Zn
0	5.96	0.03	0.64	2.3	0.35	2.3	1.3
80	14.81	0.05	0.64	3.2	0.48	4.0	5.6
160	22.48	0.06	0.69	4.2	0.64	5.4	10.2

Arginine ammonification also was significantly higher in soil-sludge mixture than the control soil (Figure 1). During incubation, significant increase in arginine ammonification was associated with sludge increase in the soil-sludge mixture. It has been showed that arginine ammonification assay provides a good index of gross N mineralization rate by microorganisms in soil [7]. They also reported that arginine ammonification rate activity correlated strongly with heterotrophic microbial activity. It is reported that sewage sludge-soils have lower mineralization rates as a result of the effect of heavy metal content in the sludge-treated soil. Chemicals pollutants entering soil destabilize ammonification, which has microbiological and chemical consequences for soil environment [22]. It may due to heavy metal contamination of soil [23]. Significant inhibition of arginine ammonification activity was also found in sludge-treated soil containing high concentration of Cd [24]. However, in the present study, incorporation of sewage sludge stimulated both urease and arginine ammonification activities. This indicates that heavy metals of the soil-sludge mixture system had no adverse effects on microbiological activities in this system. However, the level of metals in soil-sludge mixture was low (Table 3) that did not cause inhibition of soil microorganisms.

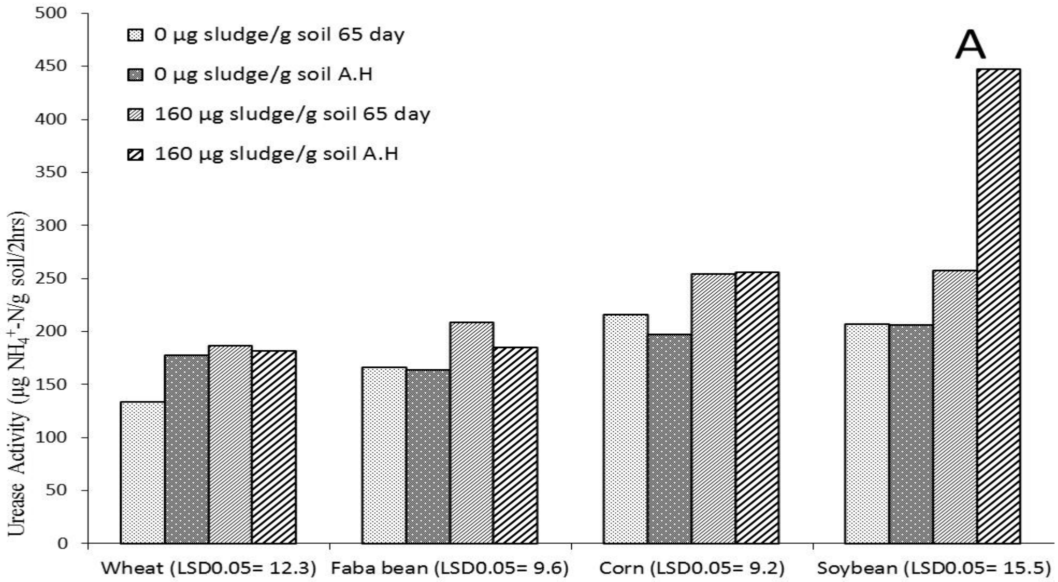
Field Study:

Table 4: Means value of NO_3^--N, NO_2^--N, and NH_4^+-N and OC in soil sewage sludge-treated (0-15 cm) that collected from fields cultivated with wheat, faba bean, corn, and soybean after 65 days from sowing.

Plant crop	Sludge (ton/Fed)			$LSD_{0.05}$		Sludge (ton/Fed)			$LSD_{0.05}$
	0	80	160			0	80	160	
	NO_3^- – N (µg/g soil)					NH_4^+-N (µg/g soil)			
Wheat	1.7	49.5	47.6	7.1		11.46	8.20	11.13	3.39
Faba bean	7.7	9.1	40.4	13.9		11.36	8.79	12.46	0.50
Corn	36.3	97.0	128.8	26.7		3.55	2.25	2.86	N.S.
Soybean	47.2	70.7	96.6	14.5		4.88	6.39	5.82	1.88
	NO_2^- – N (µg/g soil)					OC (%)			
Wheat	0.18	0.62	0.21	0.14		1.04	1.45	1.91	0.23
Faba bean	2.34	0.80	2.31	0.30		1.21	1.89	2.20	0.34
Corn	0.27	0.28	0.41	0.11		0.74	1.37	1.42	0.27
Soybean	0.12	0.21	0.15	0.09		0.93	1.66	1.68	0.26

Sewage sludge application increased significantly the levels of NO_3^--N in soil especially in soil cultivated with wheat and faba bean (winter crops) as compared with those of corn and soybean (summer crops). However, there were no specific trend with respect to levels of NO_2^--N and $NH4^+$-N. On the other hand, the amounts of OC were higher in sludge-treated soil and were very close in soils cultivated with the four crops (Table 4).

Urease activity was significantly higher in sludge-treated soil than in the control soil whether in soil samples collected after 65 days from sowing or after plant harvest (Figure 2A). Also, urease activity was almost higher in corn and soybean soils (summer season) than those of wheat and faba bean (winter season). Also, arginine ammonification was significantly higher in sewage sludge-treated soil than the untreated soil (Figure 2 B) and, its activity was higher in soils cultivated with corn and soybean (summer season) than in those cultivated with wheat and faba bean (winter season). These results indicated that the concentration levels of heavy metals in sludge treated soils (Table 5) are too low to cause adverse effects on soil biochemical activities under the used sludge rates.



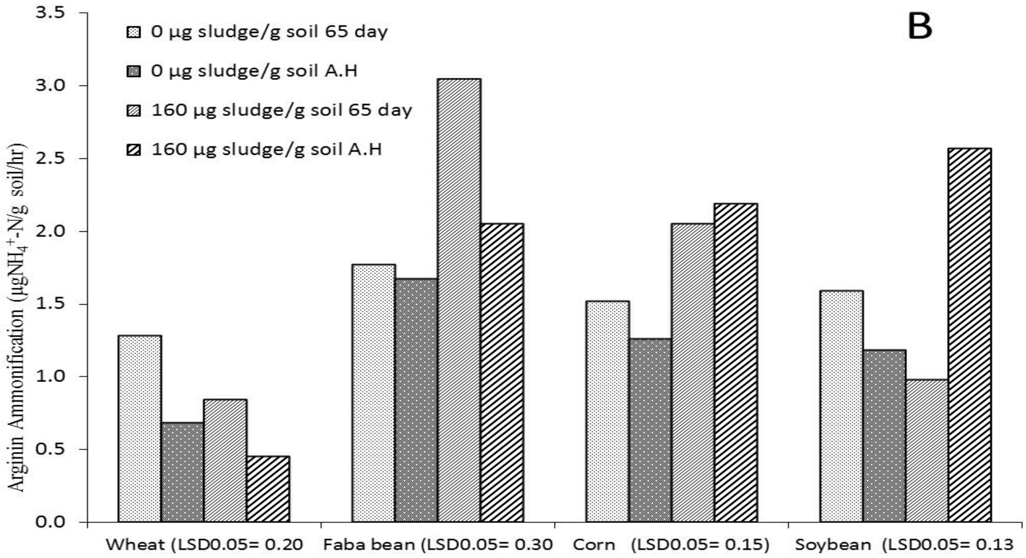


Figure 2: Mean value of urease (A) and arginine ammonification (B) activity of sewage sludge-treated soil (0-15 cm) collected from fields cultivated with wheat, faba bean corn and soybean, after 65 days from sowing and after plant harvest (A.H).

Table 5: Amounts of OC (%), inorganic-N (N_i) and AB-DTPA extractable P and heavy metals (mg/Kg soil) from sewage sludge-treated soil and cultivated with wheat, faba bean, corn and soybean in soil collected after 65 days from sowing (0-15 cm upper soil layer).

Plant crop	Sludge (ton/Fed)	O.C	N_i	P	Cd	Cr	Cu	Pb	Ni	Zn
Wheat	0	1.04	14	10.0	0.04	0.25	4.47	3.43	0.66	4.11
Faba bean	0	1.21	12	11.2	0.06	0.25	2.47	3.92	0.70	6.98
Corn	0	074	42	4.1	0.21	0.35	6.27	2.53	0.26	2.08
Soybean	0	093	53	3.9	0.07	0.45	3.99	1.34	0.28	2.83
Wheat	80	1.45	58	9.8	0.05	0.20	7.58	4.30	0.97	9.15
Faba bean	80	1.89	18	13.7	0.05	0.25	7.95	5.53	0.52	16.03
Corn	80	1.37	101	23.6	0.13	0.45	9.31	4.80	0.46	17.18
Soybean	80	1.66	77	10.5	0.07	0.42	5.03	3.40	0.27	6.30
Wheat	160	1.91	59	7.4	0.07	0.21	5.80	5.80	1.15	14.41
Faba bean	160	2.20	55	18.2	0.08	0.35	9.86	8.55	0.69	37.48
Corn	160	1.42	132	22.8	0.17	0.48	11.43	5.57	0.49	16.64
Soybean	160	1.68	104	10.1	0.08	0.25	5.65	351	0.30	12.33

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