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INFLUENCE OF SEWAGE SLUDGE APPLICATION ON SOME SOIL BIOLOGICAL CHARACTERS

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ABSTRACT

Soil samples were treated with different rates of sewage sludge (10, 20, 40, 80 and 120 tons/fed). The total bacterial count and the count of microorganisms adapted to different rates of sewage sludge were recorded after 1, 2, 4, 8, 12 and 14 weeks. Also, tolerance of some bacterial isolates to five heavy metals detected in sewage sludge was studied. In addition, urease, phosphatase activities and CO_2 evolution were assayed during the incubation period. Results showed that when soil was treated with sewage sludge, the total count was increased from 3.6×10^6 to 3.12×10^7 CFU g^{-1} dry soil throughout the 14 days. Compared to the control treatment, sewage sludge at the rate of 10 tons/fed proved to be toxic to the soil microbial population. Moreover, addition of high rates of sewage (20, 40 80 and 120 tons/fed), the counts were less and ranged between 5.0×10^2 and 3.0×10^4 CFU g^{-1} dry soil. As a result of adding different rates of sewage sludge, twenty seven bacterial isolates were picked from the agar plates as resistant bacteria. These isolates were purified and classified into four groups (G^+ cocci, G^+ spore former, G^+ short rods and G^- short rods). The data recorded about the enzymes activities (urease and phosphatase) detected that urease and phosphatase activities were increased throughout the 14 days followed by a decrease in its activities until the end of the incubation period. The same observation was recorded with CO_2 evolution, where its value was decreased with the incubation period increased.

Keywords: Sewage sludge, Bacterial, Cocci, Spore former, Short rods, Urease, Phosphatase, CO_2 evolution

INTRODUCTION

Current interest in assessing the quality of soil resources due to the increasing awareness of soil as a component of the earth. This component plays a role not only in the production of human and animal foods, but also in the maintenance of environmental quality. Thus, it is critical to define and evil of soil recourses. Conceptually, soil quality is defined as the capacity of soil to function within ecosystem boundaries to sustain biological production of the environment and to promote plant and animal health (Doran and Parkin, 1994).

Also, Karlen *et al.* (1997) stated that, soil biology is a significant component of soil quality and an important agent responsible for many of the transformations occurring in soil, most notably the reduction involved biological aspects of soil quality within the context of overall system function.

There is limited information available on how sewage sludge application can influence soil microbial quality. The effects of heavy metal on the soil microbial community, with emphasis on specific microbial activities, have been reported by Reber (1992).

Knight *et al.* (1977) and Fliebbach *et al.* (1994) detected that, the application of low metal sludge's had beneficial effects on microbial biomass, organic C and on the soil microbial activity, where metal contamination of soil resulted in considerable decrease in biomass carbon.

In addition, Kandeler *et al.* (1996) found that microbial biomass and enzyme activities decreased with increasing heavy metal pollution using salts of heavy metals but the amount of decrease was depending on the enzymes. They also observed that, heavy metal pollution severally decreased the functional diversity of the soil microbial community. However, Sastre *et al.* (1996) showed that sewage sludge applications at recommended rates increased microbial activity in soil. It appears from various studies that sewage sludge tie up the heavy metals making them unavailable to plants and soil. Thus metals contained in sludge have less effect than equivalent salts. The availability of metals in sludge depends upon the concentration of heavy metal present in the sewage sludge and the nature of the metals.

Although the effects of trace elements on bio-chemicals transformation have been studied extensively, a very little information is available on the influence of sewage sludge's on some transformations occurred in soil. For example, the rate and amount of evolution of carbon dioxide (CO_2) from soils has been used as an indicator of the effects of heavy metals and other pollutants on the overall heterotrophic activity of microorganisms (Babick and Stotzky, 1985).

The present work was carried out to study: 1) the effects of sewage sludge application which containing high levels of heavy metals on count as well as adaptation of microorganisms to heavy metals. 2) isolation, purification and classification of some tolerance bacterial isolates to various concentrations of sewage sludge heavy metals. 3) determination the toxic effect of heavy metal content in sewage sludge on soil enzymes (urease and phosphatase) as well as CO_2 evolution.

MATERIALS AND METHODS

Soil sample

The soil samples used in this study was clay silty. The soil sample was sieved (mesh size, < 2 mm) and sorted to remove plant debris and any soil animals and then allowed to stabilize for 7 days at 25 °C before analysis. The chemical and physical properties of the soil used were determined by method of Page (1982), Table (1).

Table (1): Physical and chemical properties of the soil sample used

Physical properties		Chemical properties	
Sand (%)	10.00		
Silt (%)	28.00		
Clay (%)	53.00		
pH	7.80	Available P (mg kg^{-1})	18.20
EC (dSm^{-1})	0.56	Total N (%)	0.32
Organic matter (%)	1.44	Fe (mg kg^{-1})	3.87
C (%)	0.56	Cu (mg kg^{-1})	1.64
Cation exchange capacity (c mol kg^{-1})	24.00	Zn (mg kg^{-1})	0.44
		Mn (mg kg^{-1})	6.01
		Ni (mg kg^{-1})	0.52
		Cd (mg kg^{-1})	0.07
		Cr (mg kg^{-1})	0.05

Sewage sludge sample

The sewage sludge sample was collected from the Abo-Rawash wastewater treatment plant, Giza governorate. The treatments applied in this plant were recorded in Table (2 a and b), such as treatment process, sewage flow and biological oxygen demand (BOD).

Table (2 a): The major characters of the sewage sludge used in this investigation

Physical properties		Chemical properties	
pH	7.15	Available P (mg kg^{-1})	534.10
EC (dSm^{-1})	1.82	Total N (%)	1.72
Organic matter (%)	22.15	Fe (mg kg^{-1})	24.62
C (%)	12.31	Cu (mg kg^{-1})	11.80
Cation exchange capacity (c mol kg^{-1})	61.00	Zn (mg kg^{-1})	89.40
		Mn (mg kg^{-1})	75.10
		Ni (mg kg^{-1})	1.53
		Cd (mg kg^{-1})	0.39
		Cr (mg kg^{-1})	0.41

Table (2 b): Description of sewage sludge treatment plant

Governorate	Type of sludge	Average daily sewage flow (m^3/day)	Sludge cake (tons /day)	BOD (tons/day)
Giza	Anaerobic digested	220136	286.9	74.5

Treatment used

The soil sample was air dried, ground, sieved at < 2 mm sieve and put in polyethylene bags at the rate of 3.0 kg soil bag^{-1}. The soil used was amended with the sewage sludge at the rate of 10, 20, 40, 80 and 120 ton fed^{-1} and thoroughly mixed very well. The previous treatments were replicated five times for each treatment which gave a total of 200 bags. The untreated soil and treated soils were incubated at 25 °C and the water holding capacity was maintained at 60% throughout the investigation period (0, 1, 2, 4, 8, 12 and 14 weeks).

Microbiological determination

Adaptation of soil microorganisms to heavy metals

The effect of adding sewage sludge (0, 10, 20, 40, 80 and 120 ton/fed) on the soil microbial population and possible development of metal-tolerance microorganisms were carried out. Where, a three replicates of each treatment was made and a one week intervals was used for microbiological determination to calculate the total count and metal—tolerant microorganisms count.

Tolerance of some bacterial isolates to various concentrations of heavy metals

Total isolates from the previous experiments were purified by the streak-plate method. The pure isolates were arranged into groups according to the morphological characteristics and Gram-reaction.

The spot inoculation technique was applied where a loopful of 24-hours soil bacterial culture was inoculated on the surface of agar plates supplemented with different concentrations of the heavy metals (50, 75, 100, 150, 200 and 250 ppm). The plates were incubated at 30 °C and daily examined for the presence or absence of microbial growth.

Chemical analysis

Assay of urease and phosphatase activity was determined by use the method of Hofmann and Hoffman (1966). Also, the soil respiration (mg CO_2 100 g^{-1} soil day^{-1}) was determined by titration of the NaOH by HCl in an excess of $BaCl_2$. During the incubation periods from 0 to 14 weeks, all tests were carried out as a triplicate assay.

RESULTS AND DISCUSSION

The general characteristics of the sewage sludge used in this study were found in Table (2). Where, the sludge used was anaerobically digested and contained 175 mg NO_3^{-2} kg^{-1} soil. Also, the sewage samples were slowly to moderately dry and the NH_4 contents losses during air drying.

Adaptation of soil microorganisms to sewage sludge application

The harmful effect of heavy metal pollution on soil microorganisms have been studied by many investigators (Brookes, 1995 and Diaz-Ravina and Baath, 1996). Most investigations were focused on the effect of heavy metals on microbial numbers, biomass and biochemical activities of soil microorganisms. So that, the presence and development of microbes resistant to such toxic levels of heavy metals is a very important point to study. For that, Nordgren *et al.* (1988) and Baath (1989) found that, soil pollution with heavy metals can encourage the growth and development of some microorganisms able to tolerate the toxic compounds of heavy metals.

To detect the effect of clay soil amended with different rates of sewage sludge on the microbial population in soil, this experiment was carried out and extended to 14 weeks. Periodical changes in total population and counts of heavy metal resistant bacteria are presented in Table (3). The data recorded showed that, the total microbial counts increased from 3.6 X 10^6 to 3.12 X 10^7 CFU g^{-1} dry soil throughout the first two weeks. While, at the beginning of the third week, the microbial population decreased during the incubation period to reach their minimal values (3.2 X 10^5 CFU g^{-1} dry soil) at the end of 14 weeks incubation period.

Differences between treatments resulting from the effect of the rate of sewage sludge applied mostly clear during the incubation period (14 weeks). Compared to the control treatment, sewage sludge applied in soil at the rate of 10 ton fed^{-1} proved to be a toxic to the soil microbial population. Where, the counts reached to 1.0 X 10^5 CFU g^{-1} dry soil (68.70% decreases) at the end of experiment period. Moreover, adding of 20, 40, 80 and 120 tons fed^{-1} sewage sludge was comparatively more effect on soil populations giving a low numbers where, the counts ranged from 3.0 X 10^4 to 1.0 X 10^3 to CFU g^{-1} dry soil at the 14 weeks incubation period respectively.

Table (3): Periodical changes in total population (CFU X 10^5 g^{-1} soil) and percent of soil microbial tolerant counts to different rates of sewage sludge

Amount of sewage sludge amended to the Soil	Incubation period (weeks)						
	0	1	2	4	8	12	14
0.0 (control)	36.0	215.0	312.0	240.0	132.0	17.0	3.2
10.0 (ton/fed)							
Total Count	30.0	198.0	232.0	174 0	84.0	8.5	1.0
Tolerant bacteria (%)	83.3	92.1	74.6	72.5	63.6	50.0	31.3
20.0 (ton/fed)							
Total Count	30.0	170.0	210.0	140.0	30.0	3.0	0.3
Tolerant bacteria (%)	83.3	79.1	67.3	58.3	22.7	17.6	9.4
40.0 (ton/fed)							
Total Count	29 0	106.049.3	142.0	73.0	14.0	2.0	0.1
Tolerant bacteria (%)	80.6		45.5	30.4	10.6	11.8	3.1
80.0 (ton/fed)							
Total Count	29.0	82.0	109.0	51.0	6.0	0.2	0.04
Tolerant bacteria (%)	80.6	38.1	34.9	21.3	4.5	1.2	1.3
120.0 (ton/fed)							
Total Count	26.0	67.0	79.0	23.0	1.0	0.01	0.01
Tolerant bacteria (%)	72.2	31.2	25.3	9.6	0.8	0.10	0.2

With respect to the development of tolerant bacteria as a result of toxicity of heavy metals found in sewage sludge, it found that the trend of tolerant bacteria was similar to the trend detected with total viable counts. Also, the counts of tolerating bacteria to high rate of sewage sludge were significantly lower than that recorded at low rate of sludge application. Moreover, it could be stated that a rapid development of tolerant bacteria took place within the first and second weeks.

It is clear from data obtained that the heavy metal resistant microorganisms increased after one week of incubation period and then remained either constant or fluctuated up to the 2^{nd} - 4^{th} week periods. From the fifth week, the counts decreased throughout the incubation period. This result is in agreement with Yamamoto *et al.* (1985) who stated that selection of some heavy metals tolerance among microorganisms may proceed rapidly.

Tolerance of some bacterial isolates to sewage sludge heavy metals application

This experiment was carried out to assess the adaptation of some soil bacterial strains to heavy metals detected in sewage sludge. Colonies growing on agar medium were isolated as resistance ones. The data in Table (4) show a number of isolates picked from agar plates (twenty seven isolates). The isolates were purified and classified according to the microscopic examination as well as Gram stain reaction to four groups. These groups are G^+ cocci form (3 isolates, 11.1%), G^+ spore forming bacteria (7 isolates, 25.9%), G^+ short rods (5 isolates, 18.5%) and G^- short rods (12 isolates, 44.5%) respectively.

Table (4): Different groups of heavy metal tolerant bacteria

Tolerant to sewage sludge heavy metal (ton/fed)	G^- short rods	G^+ short rods	G^+ spore former	G^+ cocci	Total isolates
10.0	4	1	2	2	9
20.0	4	1	2	1	8
40.0	2	2	1	-	5
80.0	1	-	1	-	2
120.0	1	1	1	-	3
Total	12	5	7	3	27

In addition, more advanced study was carried out on the twenty seven isolates. Due to the cultural and morphological characteristics, fourteen bacterial isolates were selected. Each of the selected isolates was tested for its ability to tolerate different concentration of five heavy metals (ppm) detected in sewage sludge tested (Cd, Cu, Ni, Pb and Zn).

The results presented in Table (5) detected that only a one isolate of G^+-cocci, and G^+-short rods as well as three isolates of G^--short rods and two isolates of G^+-spore former were able to tolerate the five tested metal. While, a bacterial isolates tolerating four metals were three groups belonging to G^+ cocci (1 isolate), G^+-short rods (1 isolate) and G^+-spore former (1 isolate). In addition, the isolates tolerating to 3 heavy metals were three isolates (two for G^+-spore former and one for G^--short rods) and only one isolate was tolerate to 2 heavy metal (G^--short rods), respectively.

Table (5): Resistance of different bacterial groups to different concentrations of heavy metals (ppm)

Isolates	Maximum tolerable concentrations					Number of
characters	Cd	Cu	Ni	Pb	Zn	tolerated metals
G^+ cocci						
Ni-C1	250	150	100	-	100	4
Pb-C2	150	75	50	200	150	5
G^+ spore former						
Cd-S1	100	75	75	50	150	5
Cu-S2	-	150	100	100	-	3
Ni-S3	250	150	100	250	75	5
Pb-S4	-	75	100	150	-	3
Zn-S5	-	200	100	150	100	4
G^- short rods						
Cd-SH1	100	100	100	200	100	5
Cu-SH2	-	100	100	-	75	3
Ni-SH3	-	100	100	-	-	2
Pb-SH4	150	150	75	150	100	5
Zn-SH5	200	100	100	100	150	5
G^+ short rods						
Cu-Sh1	-	200	100	100	200	4
Ni-Sh2	100	150	100	100	50	5

Assays of potential enzyme activity are important in estimating the effects of heavy metal pollution on the soil environment. It is difficult; however, the low enzymatic activity is because of metal inhibition or forms a low concentration of the enzyme, resulting from impeded microbial growth (Tyler, 1974).

Several measurements of enzyme activities have been used in relation to heavy metal pollution in soil. The activity of phosphatase used as an indicator of pollution, but urease activity appears in many cases to be equally or even more sensitive to heavy metal pollution as phosphatase (Tyler, 1981).

Nevertheless, soil enzymatic activity is believed to be a sensitive indicator for the effect of environmental factors on microbial population. Thus because of their role in nutrient cycling, enzyme like arylsulphatase, acid phosphatase and alkaline phosphatase are suggested of potentially beneficial or harmful effects on the ecosystem (Dick, 1994).

Urease activity

Data recorded about the urease activities in sewage sludge-amended soil was illustrated in Figure (1). The results showed that the enzyme activity was increased with incubation period increased until the second week. After that, the urease activity was decreased until the end of the incubation period. Also, the inhibition of enzyme was observed when the soils were amended with a low and medium rate of sewage (0, 10 and 20 ton fed^{-1}). But the enhancement of urease activity was occurred when the rate of sewage sludge increased (40 and 80 ton fed^{-1}).

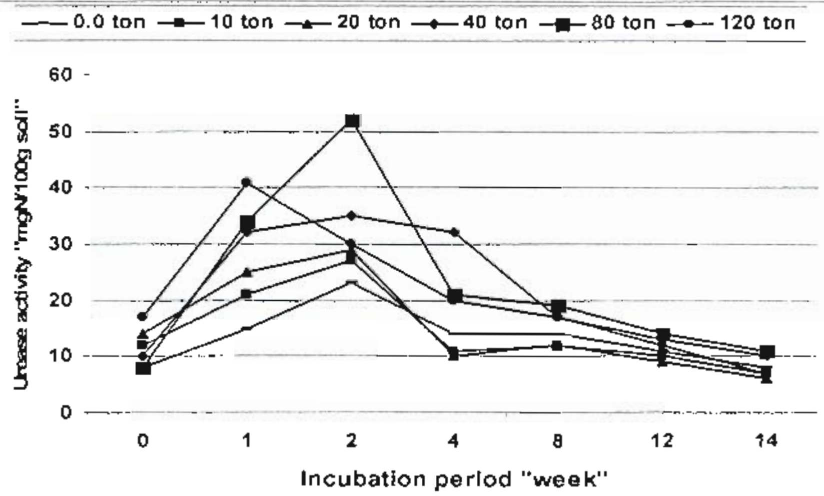

Fig. (1): Effect of application of different sewage sludge application rates on soil urease activity

The reduction detected in urease activity due to application of sewage sludge in soil was studied by Shaw and Raval (1961). They reported that metal ion inhibition was non-competitive and postulated that the inhibition involved the reaction of the sulphydryl groups in the catalytic site of urease. Also, Ataman and Arcak (2000) detected that, inhibition of urease activity was probably due to several metals and not to single component. Also they detected that the increased activity of enzyme in the sludge amended soil at the highest rate may be due to the additional sources of organic matter and nutrients supplied by the sludge which stimulated microbial urease synthesis.

Phosphatase activity

The results of phosphatase activity observed in Figure (2) revealed that the enzyme activity behave the same trend which was observed with urease activity. Where, the activity of phosphatase increased with the rate of sludge applied increased. So that, the activity of enzyme increased until the second week of incubation period. On the other hand, the incubation period increased, the activity of phosphatase activity was flucuated until the end of experiment (14 weeks).

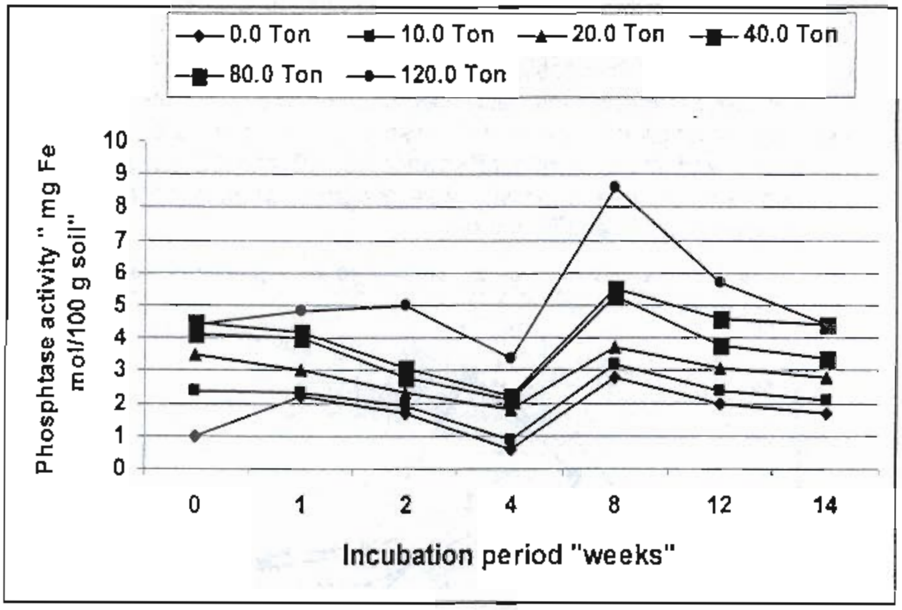

Fig. (2): Effect of different rates of sewage sludge amended in soil on phosphatase activity

So, Tyler (1981) detected that the low enzymatic activity in soil can be due to low concentrations of the enzyme or metal inhibition of the enzyme masking of active groups, denaturation by the other effects on enzyme configuration or by a competition with activating metal ions.

While, Ataman and Arcak (2000) detected that, sludge application had relatively little effect on activity of enzyme studied, and potential soil enzyme activities were generally increased or not affected by the sludge application.

Also, Reddy *et al.* (1987) found that dehydrogenase and phosphatase enzymes activities were inhibited in all their experimental sludge soils. Increasing rates of sludge application reduced urease activity in some soils, but increased it in others. The decline in enzyme activity in several investigations is probably mainly an effect of a decreased enzyme synthesis associated with inhibition microbial growth than to do by the metals.

CO_2 evolution

Soil respiration rate is easy to measure and appears to be a sensitive measurement with to detect heavy metal pollution, especially conditions (Baath, 1989). CO_2 evolution in the sewage sludge amended soil increased with increasing incubation period. While, a decreased in CO_2 evolution in the soil amended with high rate application was observed. Also, the results in Figure (3) detected that the CO_2 evolution increased until the second week of incubation period tested when the soil amended with 20 ton fed^{-1} sewage sludge. Also, the amount of CO_2 evolution was decreased with the application rate increased.

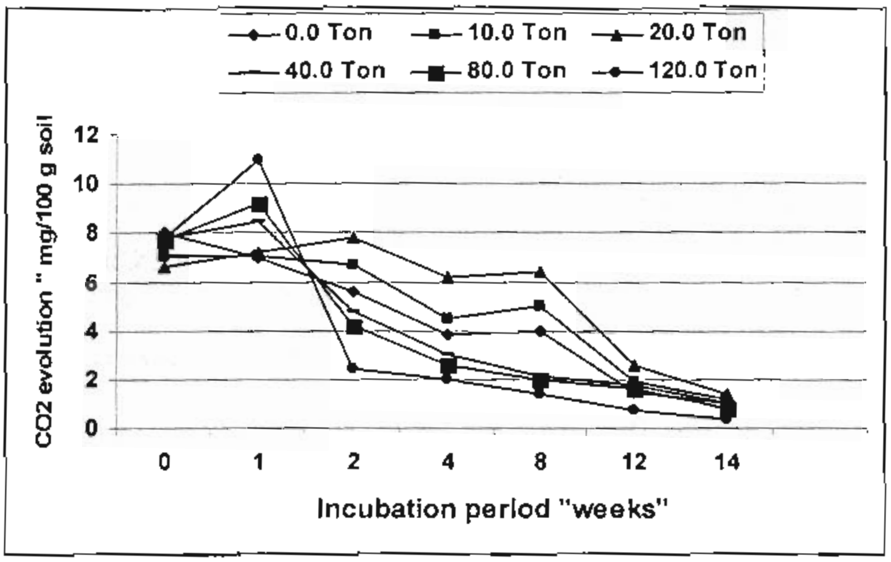

Fig. (3): Effect of sewage sludge application on CO_2 evolution in soil

More or less similar results have been reported by some investigators who tested for the effect of various rates of sewage sludge amended to soil on microbial respiration as an index for microbial activity. Where, Ghorbani *et al.* (2002) showed that, a clear suppressive relationship between the presence of heavy metals and soil respiration. Also, Hattori (1992) stated that, the impact of heavy metals in relation to CO_2 evolution varied among the metals and between soils tested. He added that, the effect of Cd^{2+}, Cu^{2+} and Ni^{2+} was highly significant while, that of Pb^{2+} was the least significant effect. Moreover, Tyler (1974) reported that, decreasing the CO_2 evolution is because of the inhibition of the active microorganisms

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تأثير إضافة كميات مختلفة من الحمأة النشطة على بعض الصفات الحيوية للتربة

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تم فى هذا البحث دراسة تأثير معاملة عينات تربة بمعدلات مختلفة من مخلفات الحمأة النشطة (١٠، ٢٠، ٤٠، ٨٠، ١٢٠ طن/فدان). حيث تم تقدير الأعداد الكلية وكذلك أعداد الميكروبات المحتملة لمختلف تركيزات الحمأة النشطة خلال فترات زمنية مختلفة (١، ٢، ٤، ٨، ١٢، ١٤ أسبوع) •وكذلك مدى مقاومة بعض العزلات البكترية لخمسة من المعادن الثقيلة التى ثبت وجودها في عينة الحمأة النشطة المختبرة• إضافة إلى ذلك تم تقدير كل من نشاط انزيمى اليورياز، الفوسفاتيز وأيضا كمية ثانى أكسيد الكربون المنتجة خلال فترة التحضين•

ولقد أظهرت النتائج المتحصل عليها بأن إضافة الحمأة النشطة إلى التربة يؤدى إلى زيادة الأعداد الكلية للميكروبات من ٣,٦ X ١٠^{٤} إلى ٣,١٢ X ١٠^{٧} مجموعة بكترية / جم تربة جافة خلال الأسبوعين الأوليين•

وبمقارنة التربة المعاملة الحمأة النشطة بالتربة الغير معاملة وجد أن إضافة ١٠ طن من الحمأة النشطة/ للفدان كان لها تأثير ساما للمجتمع الميكروبى فى التربة بل أكثر من ذلك زيادة معدلات الإضافة من الحمأة النشطة إلى ٢٠، ٤٠، ٨٠، ١٢٠ طن/فدان أدى إلى زيادة تناقص الأعداد الكلية حيث تراوحت الأعداد مابين ٥,٠ X ١٠^{٢} إلى ٣,٠ X ١٠^{٤} مجموعة بكترية / جم تربة جافة •

ونتيجة لإضافة معدلات مختلفة من الحمأة النشطة تم عزل سبعة وعشرون عزلة بكترية (كبكتريا مقاومة) مثل هذه العزلات ثم تنقيتها وتقسيمها إلى أربعة مجاميع رئيسية (كرويات موجبة لجرام – عصويات متجرثمة موجبة لجرام – عصويات قصيرة موجة لجرام – عصويات قصيرة سالبة جرام). ولقد أظهرت النتائج المتعلقة بالنشاط الأنزيمى أن معدل نشاط كلا الأنزيمين (يورياز– فوسفاتيز) يزددان خلال الأربعة عشرة يوما الأولى تلاها انخفاض معدل النشاط حتى نهاية فترة التحضين •ونفس الاتجاه ثم ملاحظته فى تقدير ثانى أكسيد الكربون حيث تناقصت قيمتها خلال فترة التحضين•