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MICROBIAL ACTIVITIES IN CHEMICALLY POLLUTED SOIL AMENDED WITH ORGANIC MATERIALS AND CULTIVATED WITH LEGUME AND CEREAL CROPS

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ABSTRACT: *A pot experiment was carried out in order to evaluate the efficiency of organic amendments, namely humic acid, biogas manure and compost, on certain microbial activities in an alluvial clay soil polluted with a mixture of some heavy metals, i.e. Zn+ Pb+ Cu, and cultivated with faba bean "Vicia faba" (legume) and maize "Zea mays" (cereal). Two rates of each of the organic materials (0.5 & 1.0 % C) and pollutants mixture (the permissible safe limit of each element & duplicate of those limits). Number of bacterial root nodules and nitrogen content in the shoots of faba bean plants (as measures of symbiotic diazotrophy), as well as dehydrogenase activity (as a general microbial measure) in the soil under each of faba bean and maize crops, had been determined at two growth periods after planting, i.e. 45 & 90 days for faba bean and 30 & 60 days for maize.*

The results obtained revealed that the heavy metals mixture added, in absence of organic supplements, inhibited the measured microbial activities. On the other hand, application of organic materials, to the un-polluted soil, generally enhanced the microbial activities assayed. Elevating the dose of either heavy metals mixture or organic additives exaggerated the influence of each, in an opposite trend between both. Combination among the applied treatments depended on the positive role of organic amendments in limiting the hazardous action of chemical pollution in soil. Humic acid, at the first growth period, and compost at the second , were superior in their beneficial effect, whereas biogas manure was intermediate in all cases. Microbial activities in soil under faba bean plants excelled those under maize ones regarding their susceptibility to pollution harm.

***Key words**: Heavy metals, humic acid, biogas manure, compost, diazotrophy, dehydrogenase, faba bean, maize.*

INTRODUCTION

Pollution is the most serious of all environmental problems. Heavy metals are prime soil pollutants, especially wherever present above the highest permissible safe limit of each of them. However, such limit if applicable to growing plants, might be injurious to micro inhabitants in soil including the beneficial agents. Heavy metals reach the agricultural soil mainly through industrial drainage and sewage. Biological control of heavy metal pollution in soil, using natural organic materials, have been recommended as a safe practice to overcome this environmental problem. Formation of organo-mineral complexes is thus behind reducing the availability of those metals in soil to the favour of growing plants (Chaney, 1994,; Soccol *et al.*, 2003 and Walker *et al.*, 2003).

Soil microorganisms are major component of the life cycle in nature. They mainly contribute, directly and indirectly, to plant growth and crop production (Paul and Clark.1996). Heavy metals in soil could be deleterious to the beneficial microbial community, as well as to the growing plants. Extent of such inhibition depends on type of the metal, its concentration and other factors involving soil conditions.

The present work was therefore designed in order to clear up the impact of some organic amendments, namely humic acid, biogas manure and compost, on microbial activities in soil polluted with a mixture of certain heavy metals, i.e. Zn+ Pb+ Cu, and cultivated with faba bean (legume) and maize (cereal) plants.

MATERIALS AND METHODS

1. Materials

1.1. Soil

Samples of an alluvial soil were collected from the surface layer (0-30 cm) of the Experimental Farm of the Faculty of Agriculture, Minufiya University (Shibin El-Kom, Egypt). Data of initial features of such soil are presented in Table (1).

1.2. Organic Sources

Compost, biogas manure and humic acid represented the organic amendments applied to the soil under consideration . Data of chemical analyses of those materials are shown in Table (2). Such materials and their analytical data were provided by the Dept. of Agric. Microbiol., Agric. Res. Center (Giza, Egypt).

1.3. Plants

Two different major crops were used as test plants, namely faba bean "*Vicia faba*", as a winter legume (deep tap-rooted crop) and maize"*Zea mays*", as a summer cereal (shallow fibrous rooted crop).

1.4. Chemical Pollutants

Acetate salts of each of zinc " Zn", lead "Pb" and copper "Cu" were added together in a mixture, to the examined soil. Such heavy metals are among the most chemical agents polluting soils in Egypt (Abou-El Naga *et al.*,1999).

2. METHODS

2.1. Treatments

- One soil, three organic materials, two crops and one chemically polluting mixture consisted of three heavy metals.
- Controls (without organic materials " O_0 " and without heavy metal pollutants "P_0", as well as the dual controls e.g. without both organic materials and pollutants " O_0P_0 ").

Table (1). Initial analytical data of the studied soil.

a. Physical Properties

Moisture Content, at the field capacity (%)	Fraction (%)			Textural Class
	Sand	Silt	Clay	
34.4	11.4	33.7	54.9	Clayey

b. Chemical Properties

Organic Matter	$CaCO_3$	ESP	pH (1:2.5, soil: water Susp.)	EC (dSm^{-1})	Soluble Ions (meq/100g soil)							
					Cations				Anions			
					Ca^{2+}	Mg^{2+}	Na^+	K^+	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}
(%)				(1:5, soil : water extract)								
1.8	2.6	4.6	7.6	0.4	0.9	0.4	2.4	0.5	–	0.8	2.6	0.8

c. Nutrient and Heavy Metal Contents

Major Nutrients						Heavy Metals (mg/kg soil)					
Total (%)			Available (mg/kg)			Total			DTPA-Extractable		
N	P	K	N	P	K	Zn	Pb	Cu	Zn	Pb	Cu
0.15	0.10	0.60	58.1	9.2	270	42	10	28	7.2	4.4	6.4

Table 2

- **Sub-treatments (actual experimental variables).**

.. Organic materials: Three (compost, biogas manure and humic acid), each at two rates (0.5 and 1.0 % organic carbon basis).

.. Plants : Two (faba bean and maize).

.. Chemical pollutants: Three heavy metals, namely Zn, Pb & Cu, at two levels of each element, were added as a mixture. According to Alloway (1995); Blaylock and Huang (2000) and Adriano (2001), the permissible safe levels of the heavy metals, particularly Zn, Pb and Cu, are 120, 70 and 50 mg kg^{-1} soil, respectively. Consequently, the levels assigned for such sub-treatment, in the present study, were a collection of the allowed limits of such elements, and another containing a duplicate level of each element in the mixture, i.e.:

P1= 120 Zn, 70 Pb and 50 Cu mg kg^{-1} soil.
P2= 240 Zn, 140 Pb and 100 Cu mg kg^{-1} soil.

2.2. Experimental Technique

A greenhouse experiment had been conducted, using plastic pots (25×30cm). Seven kg of the soil were packed in each pot after being thoroughly mixed with the calculated amount of each organic material, i.e. rates of 0.5 & 1.0 %C. All pots were then moistened to 60% of the water-holding capacity of the soil for one week before planting.

The experimental sub-treatments were arranged in a complete randomized block design with 6 replications of each. Seeds of faba bean were inoculated, before sowing, with the specific diazotrophs, namely *Rhizobium Leguminosarum*. Eight seeds of faba bean or maize, were planted in each pot, according to the cultivation season (on 15^{th} October and 15^{th} April, respectively). After complete germination, seedlings were thinned to 4 plants /pot, and Mixtures of pollutant treatments (P_1& P_2) were then added with irrigation water. The experimental sub-treatments were fertilized with superphosphate for each crop (at a rate of 200 kg/ fed.) before planting, and both ammonium nitrate (at 50 and 200 kg/ fed., for faba bean and maize, respectively) and potassium sulphate (at a rate of 100 kg/ fed. for each crop) after thinning, according to the recommendations of the Egyptian Ministry of Agriculture. Soil moisture content had been maintained, at the field capacity in all pots during the experimental period, via regular compensation every three days. Faba bean plants of three pots of each sub-treatment were carefully uprooted at each of two growth periods after planting. For faba bean, the first sampling took place after 45 days, through full tillering, and the second followed by 45 more days . For maize, the first sampling occurred after 30 days and the later was performed on the 60^{th} day. Nodules of the faba bean fresh roots were counted directly after taking the intact plants carefully off the pots.

2.3. Laboratory Determinations

2.3.1. Initial soil analysis:

❖ **Physical properties..(Klute, 1986):**

- Mechanical analysis was determined by the universal pipette method, using sodium oxalate as a dispersing agent.

❖ **Chemical properties.. (Page *et al.*,1982):**

- Organic matter content was assessed by means of Walkly and Black method.
- Soil pH value was measured, in the 1:2.5 soil-water suspension, using standard glass electrodes (pH meter).
- Calcium carbonate content was determined volumetrically, using a calcimeter.
- Total salinity (EC) was determined in the 1:5 soil/water extract, using an electrical conductivity meter (salt bridge).
- Soluble ions, i.e. the cations Ca^{2+}, Mg^{2+}, K^+ & Na^+ and the anions CO_3^{2-}, HCO^{3-}, Cl^- & SO_4^{2-} were determined in the 1:5 soil-water extract, following the traditional standard methods.
- Total and bioavailable amounts of each of Zn, Pb & Cu were determined by atomic absorption spectrophotometer.

2.3.2. Biochemical Assay..(Casida *et al.*, 1964):

- Dehydrogenase activity "DHA" in soil, was determined colourimetrically, for the 2,3,5-triphenyl formazan (TPF) produced from the reduction of 2,3, triphenyl tetra zolium chloride (TTC), using acetone for extraction. DHA had been assayed in the soil under the growing plants. at two periods after planting, i.e. 45 & 90 days for faba bean and 30 & 60 days for maize.

2.3.3. Plant analysis:

- A portion of faba bean shoots was oven dried at 105 C^o to estimate the dry weight, whereas another portion was dried at 70 C^o, then digested using a mixture of 1:3 concentrated perchloric acid : sulphoric acid.
- Nitrogen content in the acid digest of plant shoots, was determined, following the conventional method by means of a semi-micro-steam Kjeldahl distillation apparatus **(Cottenie *et al.*, 1982)**.

2.3.4. Calculations:

Raw results (analytical data of the replicates means of the various sub-treatments) were further calculated on the dry weight basis of the plants and/or soil, as convenient.

Rates of the relative changes of the final results (as percent) "RC%" were calculated for the result tabulated for a particular sub-treatment, referring to the result of the specific dual control (without both organic amendments and pollutants "O_0P_0").

$$\mathbf{RC\%} = \frac{\text{Result of a particular sub treatment} - \text{Result of the dual control "}O_0P_0\text{"}}{\text{Result of the dual control "}O_0P_0\text{".}} \times 100$$

RESULTS AND DISCUSSION

1.Bacterial Nodulation on Faba Bean Plant Roots

The effect of organic amendments (compost, biogas manure or humic acid) on number of bacterial nodules formed on the roots of faba bean plants grown on the chemically polluted soil with heavy metals mixture (Zn+ Pb+ Cu), at the first growth period (45 days) and the second (90 days), appeared in Table (3). Data show that, application of the organic materials to the un-polluted soil, increased the number of bacterial root nodules with increasing the applied rate up to 1.0% O.C.. Humic acid revealed a superiority, in such connection, above the other additives at the first growth period. However, at the later one, action of humic acid was replaced with compost. Biogas manure had occupied the second rank in all cases. Related results were obtained by Abdel-Wahab *et al.* (2009). In this regard, Saad (2013) reported that, application of organic matter to soils generally increased the microbial activity, and subsequently a greater number of bacterial root nodules in legume plants were detected.

Concerning the influence of pollutants mixture (Zn+ Pb+ Cu), added to the soil at two levels (P_1 & P_2), on number of bacterial nodules formed on the roots of faba bean plants, data of Table (3) exhibited that, at the lower level (P_1) nodulation capacity very slightly diminished, whereas elevating the pollution level (P_2) actually inhibited such bacterial process. Organic materials are the sole depot of carbon and energy for the chemoheterotrophic microbial community in soil including the diazotrophy *Rhizobium* (Paul and Clark, 1996). Accordingly, abundant invasion takes place into the faba bean roots. The narrower C/N ratio of humic acid than the other organics applied (Table 2a) facilitated the nutritional supply generally for both soil microorganisms and growing plants at the first time of detection. However, such advantage of humic acid had not stood longer because of its early breakdown. Consequently, compost, with its wider C/N ratio excelled the others later on.

Addition of heavy metal mixtures generally decreased the numbers of faba bean root nodules, at both growth periods, very slightly at the lower level (P_1) (Table 3). Such nodule counts indicated a probable moderate resistance or tolerance of that specific bacterial agent to the heavy metals harm, supported by favourable soil conditions, organic additives and growing plants (Wild and Russell, 1988; Alloway, 1995 and Pool, 2013).

Table (3): Influence of organic amendments on the number of nodules formed on roots of Faba Bean plants grown on the chemically polluted soil at both growth periods.

Organic Amendments and their Application Rate (%OC)		First Growth Period (45 days)				Second Growth Period (90 days)			
		Levels of Pollutants Mixture (Zn+ Pb+ Cu)*							
		P_0	P_1	P_2	Mean	P_0	P_1	P_2	Mean
		Number of Root Nodules (No/ plant)							
Control	O_0	55	50	30	45	68	54	35	52
Compost	0.5	61	59	52	57	90	85	74	83
	1.0	70	70	62	67	94	90	80	88
	Mean	62	60	48	–	84	76	63	–
Biogas manure	0.5	67	67	64	66	84	72	66	74
	1.0	74	73	64	70	88	80	70	79
	Mean	65	63	53	–	80	69	57	–
Humic acid	0.5	76	74	68	73	72	70	55	66
	1.0	82	84	72	79	86	77	60	74
	Mean	71	69	57	–	75	67	50	–

* P_0 = Control (no addition of heavy metals).
P_1 = 120 Zn+ 70 Pb+ 50 Cu mg kg^{-1} soil.
P_2 = 240 Zn+ 140 Pb+ 100 Cu mg kg^{-1} soil.

2. Nitrogen Content in Faba Bean Plant Shoots

Data noted in the preceded Table (4) display that, application of the organic amendments each alone (compost, biogas manure or humic acid) to the clay soil, without heavy metals addition, generally enhanced the faba bean plant growth, and subsequently concentration and uptake of nitrogen besides that biologically fixed, at both detection periods(45 and 90 days), compared to the un-amended soil (control). Direct relation was obtained by elevating the rate of application (1.0% C). Higher contents of nitrogen determined in the shoot tissues, at the later plant sampling, was actually a result of accumulation of the nutrient by advancing the growth period. Humic acid was the most encouraging and followed by biogas manure and then compost. This was the order at the first plant sampling, but as the time elapsed, compost was the uppermost treatment. This was confirmed by the rates of relative changes "RC %". Noteworthy that, the RC values of the organic additions at P_0 decreased at the second sampling due to a lower rate of absorption. Similar results were obtained by El-Shouny (2011) and Sadek and Sallam (2012).

Addition of pollutants mixture (Zn + Pb + Cu) at both levels (P_1 & P_2), generally diminished the content of nitrogen at the first and second growth periods (Table 3). The higher level (P_2) severally decreased such N values. The lower RC values indicated the inhibition power among the various treatments. Related results were obtained by Sheppard (1998) and Chen *et al.* (2007).

Table 4

Application of organic supplements in combination with the heavy metals mixture to the examined soil, reduced the inhibitory effect of such pollutants to the benefit of plant growth. This was besides that organic materials, in general, and legume diazotrophy, in particular, are main supplies of plant nitrogen. Thus, such sources are available in the present study. Opposite relation existed between the influences of both organic substances and pollutants rates, on N contents in the plants. Again, humic acid and compost occupied the most effective situation, respectively, between the first and second growth periods, whilst the biogas manure had remained at the middle. Chelation reaction for heavy metals by organic materials is the bioremediator against the deleterious activities in the minerally polluted soil, had been reported by (Masil and John,2002; Julio *et al.,* 2007; Trigo *et al*., 2009 and Angelova *et al.,* 2010).

3. Dehydrogenase Activity in the Soil Planted with Faba Bean and Maize

Dehydrogenase activity (DHA) is frequently used as a measurement of the overall microbial activity in soil. Results of DHA in the rhizosphere soil of faba bean plants are declared in Tables (5&6). Data revealed that, application of organic amendments, without heavy metal pollution, enhanced the rate of dehydrogenase activity in a direct relation with the rate applied (0.5 to 1.0%C), as compared with the control "O_0". Superiority was observed to be for humic acid, at the first growth period, and compost, at the second, while biogas manure was in between at both periods. The RC values verified such detection. DHA of the soil under faba bean plants appeared to be higher than that under maize plants. The activity values gained at the first soil sampling seemed to be lower than the later one. Early degradation of humic acid, due to its narrower C/N ratio (Table 2) (shorter persistence), resulted in its superiority at the first enzyme assay, and inferiority later on, in an opposite position of compost (longer persistence).

Organic substances are polymers of compounds, consisting of nutritional elements needed for living beings. Reduction reactions are behind the polymerization processes, thus to make use of the tied nutrients, such polymers must be depolymerized via oxidation or dehydrogenation processes. This is achieved by a catalytic reaction drived by the enzyme dehydrogenase produced by soil microorganisms (Poole, 2013). Through the breakdown of organic materials, many metabolites are transitionally formed. Among these intermediary products are poly carboxylic organic acids and poly phenols. Such metabolites are involved in the so-called chelation reactions, by which heavy metals (polyvalent cations) are captured and consequently their bioavailability and pollution action in soil become limited (Wild and Russell, 1988 and Alloway, 1995).

Regarding the effect of the added heavy metals composite (Zn+ Pb+ Cu), in absence of organic additives on DHA in the soil planted with faba bean and maize, the results exhibited that, such enzyme activity slightly decreased at the lower level (P_1), and severely at the higher one (P_2). Application of organic supplements largely improved the passive action of the mineral pollutants on the enzyme activity in soil, with the higher application rate of the organics giving better results. Humic acid, alternatively with compost, between both sampling times, were the fittest, whilst biogas manure had positioned second consistently. Microbial activities in soil under faba bean plants were less susceptible than those under maize ones. Hence, plant kind and its root exudates are involved in such concern (Curl and Truelove, 1986; Wild and Russell, 1988; Tomoyoshi *et al.,*2005 and Wang *et al.*,2007). Values of the relative changes "RC%" clearly support the results gained (El-Howeity *et al*., 2008 and Shalaby *et al.,* 2010).

A number of heavy metals are considered as micronutrients for plants and microorganisms, as they are required at low amounts to play specific roles in various metabolic processes, mainly as co-enzymes. Particularly, zinc contributes to the

Table 5

Table 6

synthesis of the enzymes dehydrogenase, proteinases, peptidases and phosphor hydrolases. Zinc also influences the permeability of membranes and that stabilizes cellular compounds and systems of microorganisms. Copper plays vital functions in N-reduction and diazotrophy and cell wall metabolism. Both zinc and copper are involved in the metabolism of DNA, RNA, protein and carbohydrates in both plants and microorganisms. No evidence has declared necessity of lead to plants or microorganisms, otherwise it could be toxic even at low concentrations (Masil and John, 2002; Zeig and Taiz, 2010 and Poole, 2013).

The ability of organic substances and their metabolites to bind and chelate heavy metal ions in soil may be due to their high content of oxygen- containing functional (reactive) groups within various structures (Table 2), and thus reducing the bioavailability of such hazardous minerals, for the favour of pollution control or "bioremediation".

REFERENCES

Abd El-Wahab, A.F., A.M. Biomy and W.M. El- Farghal (2009). Composting of plant residues and their utility with micronutrients to enhance productivity of faba bean- *Rhizobium* symbiosis under soil conditions. Egypt. J. App. Sci., 24:343-368.

Abou-El Naga, S.A., M.M. El-Shinnawi, M.S. El- Swaaby and M.A. Salem (1999). Chemical pollution of soils, waters and plants at the industrial area of Helwan City in Egypt. Egypt. J. Soil Sci., 39(3): 263-280.

Adriano, D.C. (2001). Trace Elements in Terrestrial Environments. Biogeochemistry, Bioavailability, and Risks of Metals. 2nd ed. Springer-Verlag, New York, USA.

Alloway, B. J. (1995). Heavy Metals in Soils. Blackie Acad. and Profes. 2nd ed. London, U.K.

Angelova, V., R. Ivanova, G. Pevicharova and K. Ivanov (2010). Effect of organic amendments on heavy metals uptake by potato plants. 19th World Congress of Soil Science, Soil Solutions for a Changing World. Brisbane, Australia.

Blaylock, M.J. and J.W. Huang (2000). Phytoextraction of Metals in: Phytoremediation of Toxic Metals, Rosking and Ensley (Eds.). John Wiley and Sons. New York, USA.

Casida, L.E., D.A. Kiein and T. Santovo (1964). Soil dehydrogenase activity. Soil Sci., 8: 371-376.

Chaney, R.L. (1994). Soil bioremediation and heavy metal contamination. J. Environ. Quality, 23:1151-1157.

Chen, C., C.M. Kao, C.F. Chen and C.D. Bong (2007). Distribution and accumulation of heavy metals in the sediments of Kaohsing harbor. Taiwan Chemosphere, 66: 1431-1440.

Cottenie, A., M. Verloo, L. Kiekens, G. Velghe and R. Camerlyck (1982). Analytical Problems and Methods in Chemical Plant and Soil Analysis. Handbook, Ed. A. Cottenie. Gent, Belgium.

Curl, E.A. and B. Truelove (1986). The Rhizosphere. Springer Verlag. Berlin, Germany.

El-Howeity, M.A., M.M. Shinnawi and S.A. Abdel-Gawad (2008). Enzyme activities in composted soil treated with nematicides. Minufiya J. Agric. Res., 33: 211-230.

El-Shouny, M.M. and E.M.K. Behiry (2011). Influence of some soil amendments on some characteristics of calcareous soil and its productivity of faba bean and maize. Minufiya J. Agric, Res., 36 (3):775-793.

Julio, A. L., W. Katarzyna, A. Scott, A. Joseph, G .C. Felix and W. Kazimierz (2007). Effect of some heavy metals and soil humic substances on the phytochelatin production in wild plants from silver mine areas of Guanajuato, Mexico. Chemosphere, 70: 2084–2091.

Klute, A. (1986).Method of Soil Analysis. Part 2: Physical and Mineralogical Properties. Amer. Soc. Agron. Madison, USA.

Masil, K. and S. John (2002). Effects of metal (Cd, Cu, Ni, Pb or Zn) enrichment of sewage-sludge on soil microorganisms and their activities. Appl. Soil Ecol., 20(2): 145 -155.

Page, A.L., R.H. Miller and D.R. Keeney (1982). Methods of Soil Analysis. Part 2, 2nd ed. Amer. Soc. Agron., Inc. Mad., Wisc., USA.

Paul, E. and F. Clark (1996). Soil Microbiology and Biochemistry. Academic Press. New York, USA.

Poole, R.K. (2013). Advances in Microbial Physiology. Elsevier Sci. Pub. Amest., The Netherlands.

Saad, Shimaa M. (2013). Effect of compost, compost tea and N applied on soil fertility, cucumber growth and resistance of some diseases. M.Sc. Thesis, Fac. Agric., Tanta Univ. Egypt.

Sadek, Jacklin G. and Amany M. Sallam (2012). Effect of grains soaking with micronutrients and foliar nutrition with humic acid on the productivity and quality of barley plant under saline soil conditions. Minufiya J. Agric. Res., 37(4-2): 1049-1068.

Shalaby, O.E., M.A.El- Howeity and M.M. El-Shinnawi (2010). Wheat yield and microbial activities in newly reclaimed soil as influenced by foliar application of bio-organic agents. Minu. J. Agric. Res., 35(2): 769-783.

Sheppard, S.C. (1998). Geophagy: Who eats soil and where do possible contaminants go? Environ. Geology, 33: 109-114.

Soccol, C.R., L.P. Vandenberghe, A.L. Woiciechowski, V. Thomaz C.T. Correia and A. Pandey (2003). Bioremediation: an important alternative for soil and industrial wastes clean-up. Indian J. Exp. Biol., 41(9):1030-1045.

Tomoyoshi, M., K.K. Masami and T. Takejiro (2005). Effects of Pb, Cu, Sb, In and Ag contamination on the proliferation of soil bacterial colonies, soil dehydrogenase activity, and phospholipid fatty Acid profiles of soil microbial communities. water, air, and soil pollution , 164(1-4): 103-118.

Trigo, C., R. Celis, M.C. Hermosin and S. Cornejo (2009). Organoclay-based formulations to reduce the environmental impact of the herbicide Diuron in olive groves. Soil Sci. Am. J., 73:1652-1657.

Walker, D.J., R. Clemente, A. Roig and M. Bernal (2003). The effect of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. Environ. Pollution, 22: 303-312.

Wang, Y., J. Shi, H. Wang, Q. Lin, X.Chen and Y. Chen (2007). The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. Ecotoxicol. Environ. Saf., 67(1):75 -81.

Wild, A. and E.W. Russel (1988). Russells Soil Condition and Plant Growth. Harlow Longman. London, UK.

Zeig, E. and L. Taiz (2010). Plant Physiology. Sinauer Associates. CA, USA.

الأنشطة الميكروبية في أرض ملوثة كيميائيا مضافا اليها مواد عضوية و منزرعة بنبات بقولي و أخر حبوب

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الملخص العربي

أجريت تجربة أصص لتقويم تأثير حامض الهيوميك و سماد البيوجاز و الكمبوست علي أنشطة ميكروبية محددة في أرض رسوبية طينية لوثت عمدا بمخلوط من العناصر الثقيلة هي " الزنك +الرصاص+ النحاس)" و منزرعة بكل من الفول البلدي (كمحصول بقولي) و الذرة (كمحصول حبوب). و كانت معدلات كل من المواد العضوية هي "٠.٥، ١.٠% كربون" و مخلوط المعادن الثقيلة هي " الحد الأمن المسموح به من كل عنصر، و ضعف هذا الحد". هذا بجانب معاملتي الكونترول بدون مواد عضوية أو ملوثات كيماوية. و أجريت قياسات التجربة علي فترتي نمو نباتي الفول "٤٥ و ٩٠ يوم" و الذرة" ٣٠ و ٦٠ يوم" من الزراعة، حيث تم تقدير عدد العقد البكتيرية علي جذور الفول و محتوي مجموعه الخضري من النيتروجين "كمقياسين لتثبيت النيتروجين الجوي تكافليا" ، و كذلك نشاط انزيم الديهيدروجينيز في الأرض "كمقياس ميكروبي عام" تحت المحصولين.

و أظهرت النتائج التأثير المثبط لمخلوط العناصر الثقيلة للأنشطة الميكروبية المختبرة في غياب الإضافات العضوية. و من ناحية أخري شجعت المواد العضوية تلك الأنشطة الميكروبية بصفة مطلقة، و ذلك في غياب المعادن الثقيلة المضافة. و كان تأثير معدل إضافة كل من المعاملتين عكسيا مع الملوثات المعدنية و طرديا مع المواد العضوية. هذا و قد أدت الاضافات العضوية إلي الأرض الملوثة كيميائيا إلي الحد من خطورة تلك العناصر الثقيلة. و كان حامض الهيوميك في فترة النمو الأولي، و الكمبوست في الثانية، هما الأكفأ في هذا الشأن، بينما احتفظ سماد البيوجاز بموقع وسطي في جميع الحالات. و أظهرت الأنشطة الميكروبية في أرض الفول قلة حساسيتها لضرر الملوثات عنها في أرض الذرة.

Table (2). Chemical analysis of the tested organic materials.

Organic Fertilizers																				
Organic Fertilizers	pH (1:5,water suspension)	EC (dSm^{-1}) (1:5, water extract)	O.C	O.M	Total N	C/N Ratio	Major Elements (%)				Minor Elements ($mg\ kg^{-1}$)							
			(%)				P	K	Ca	Mg	Fe	Mn	Zn	Mo	Cu	Co	Cd	Pb
Compost	7.8	1.1	31.6	54.3	1.1	28.7	1.2	1.3	0.1	0.7	130	90	80	6	46	6	3	6
Biogas manure	6.6	2.8	29.1	50.0	1.5	19.4	0.8	0.9	-	-	556	264	72	12	34	8	4	4

Humic Acid																			
Element Contents (%)					Minor Elements ($mg\ kg^{-1}$)			Element Ratio			Ash (%)	Total Acidity	Total COOH	Total OH	Phenolic OH	Alcoholic OH	Carbonyl C=O	CEC
C	N	H	O	P	Zn	Pb	Cu	C/N	C/H	C/P		(meq 100 g^{-1})						
46.5	3.1	6.3	43.6	0.5	60	2	24	15.0	7.4	93.0	3.1	890	440	840	450	390	290	692

Table (4): Influence of organic amendments on Nitrogen uptake by Faba Bean plants grown on the chemically polluted soil, at both growth periods.

Organic Amendments and their Application Rate (%OC)		First Growth Period (45 days)										Second Growth Period (90 days)									
		Levels of Pollutants Mixture (Zn+ Pb+ Cu)*																			
		P_0			P1			P2			Mean	P_0			P1			P2			Mean
		Content of Nitrogen in Plant Shoots and its Relative Changes (RC%)**																			
		%	mg/pot***	RC%	%	mg/pot	RC%	%	mg/pot	RC%	mg/pot	%	mg/pot	RC%	%	mg/pot	RC%	%	mg/pot	RC%	mg/pot
Control	O_0	1.90	250.80	0.00	1.80	219.6	-12.44	1.50	139.50	-44.38	203.30	2.60	426.40	0.0	2.10	289.80	-32.04	1.60	166.40	-60.98	294.20
Compost	0.5	1.96	278.32	10.97	1.82	240.24	-4.21	1.60	198.40	-20.89	238.99	3.10	759.50	78.12	2.80	610.40	43.15	2.90	562.60	31.94	644.17
	1.0	1.98	302.94	20.79	1.86	247.38	-1.36	1.62	208.98	-16.67	253.10	3.20	812.80	90.62	3.00	693.00	62.52	2.98	649.64	52.35	718.48
	Mean	–	277.35	–	–	235.74	–	–	182.29	–	–	–	666.23	–	–	531.07	–	–	459.55	–	–
Biogas manure	0.5	2.12	373.12	48.77	2.00	296	18.02	1.80	248.40	-0.96	305.84	2.98	691.36	62.14	2.50	472.50	10.81	2.30	388.70	-8.84	517.52
	1.0	2.18	403.30	60.81	2.20	349.8	39.47	1.82	269.36	7.40	340.82	3.00	714.00	67.45	2.60	530.40	24.39	2.80	501.20	17.54	581.87
	Mean	–	342.41	–	–	288.47	–	–	219.09	–	–	–	610.59	–	–	430.90	–	–	352.10	–	–
Humic acid	0.5	2.22	446.22	77.92	2.20	360.8	43.86	2.00	306.00	22.01	371.01	2.66	563.92	32.25	2.20	378.40	-11.26	1.88	315.84	-25.93	419.39
	1.0	2.28	478.80	90.91	2.22	388.5	54.90	2.20	360.80	43.86	409.37	2.80	627.20	47.09	2.40	451.20	5.82	2.00	352.00	-17.45	476.80
	Mean	–	391.94	–	–	322.97	–	–	268.77	–	–	–	539.17	–	–	373.13	–	–	278.08	–	–

* P_0 = Control (no addition of heavy metals).
P_1 = 120 Zn+ 70 Pb+ 50 Cu mg kg^{-1} soil.
P_2 = 240 Zn+ 140 Pb+ 100 Cu mg kg^{-1} soil.

$$** \; RC\% = \frac{\text{Result of a particular sub treatment – Result of the dual control "}O_0P_0\text{"}}{\text{Result of the dual control "}O_0P_0\text{".}} \times 100$$

*** Each pot contained 4 plants.

Table (5): Influence of organic amendments on activity of dehydrogenase enzyme in the chemically polluted soil cultivated with Faba Bean plants, at both growth periods.

Organic Amendments and their Application Rate (%OC)		First Growth Period (45 days)							Second Growth Period (90 days)						
		Levels of Pollutant Mixture (Zn+ Pb+ Cu)*													
		P_0		P_1		P_2		Mean	P_0		P_1		P_2		Mean
		Dehydrogenase activity (µg TPF/g dry soil) and its relative changes (RC%)**													
		µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}	µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}
Control	O_0	26.0	0.0	24.0	-7.7	18.0	-30.7	22.7	34.0	0.0	28.0	-17.6	12.0	-64.7	24.7
Compost	0.5	45.0	73.1	40.0	53.9	37.0	53.9	40.7	80.0	135.3	72.0	111.8	70.0	105.9	74.0
	1.0	50.0	92.3	46.0	76.9	45.0	73.1	47.0	86.0	152.9	80.0	135.3	75.0	120.6	80.3
	Mean	40.3	—	36.7	—	33.3	—	—	66.7	—	60.0	—	52.3	—	—
Biogas manure	0.5	60.0	130.7	50.0	92.3	45.0	73.1	51.7	70.0	105.9	64.0	88.2	60.0	76.5	64.7
	1.0	66.0	153.9	58.0	123.1	50.0	92.3	58.0	72.0	111.8	70.0	105.9	64.0	88.2	68.7
	Mean	50.7	—	44.0	—	37.7	—	—	58.7	—	54.0	—	45.3	—	—
Humic acid	0.5	70.0	169.2	65.0	150.0	64.0	146.2	66.3	60.0	76.5	55.0	61.8	50.0	47.1	55.0
	1.0	82.0	215.4	70.0	169.2	68.0	169.2	73.3	69.0	102.9	60.0	76.5	52.0	52.9	60.3
	Mean	59.3	—	53.0	—	50.0	—	—	54.3	—	47.7	—	38.0	—	—

* P_0 = Control (no addition of heavy metals).
P_1 = 120 Zn+ 70 Pb+ 50 Cu mg kg^{-1} soil.
P_2 = 240 Zn+ 140 Pb+ 100 Cu mg kg^{-1} soil.

$$** \ RC\% = \frac{\text{Result of a particular sub treatment – Result of the dual control "}O_0P_0\text{"}}{\text{Result of the dual control "}O_0P_0\text{".}} \times 100$$

Table (6): Influence of organic amendments on activity of dehydrogenase enzyme in the chemically polluted soil cultivated with Maize plants, at both growth periods.

Organic Amendments and their Application Rate (%OC)		First Growth Period (30 days)							Second Growth Period (60 days)						
		Levels of Pollutant Mixture (Zn+ Pb+ Cu)*													
		P_0		P_1		P_2		Mean	P_0		P_1		P_2		Mean
		Dehydrogenase activity (µg TPF/g dry soil) and its relative changes (RC%)**													
		µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}	µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}	RC%	µg g^{-1}
Control	O_0	22.0	0.0	20.0	-10.0	14.0	-36.4	187	30.0	0.0	26.0	-13.3	10.0	-66.7	22.0
Compost	0.5	44.0	100.0	38.0	72.7	35.0	72.7	39.0	56.0	86.7	53.0	86.7	45.0	50.0	51.3
	1.0	45.0	104.6	39.0	77.3	37.0	81.8	40.3	62.0	106.7	60.0	100.0	50.0	66.7	57.3
	Mean	37.0	—	32.3	—	28.7	—	—	49.3	—	46.3	—	35.0	—	—
Biogas manure	0.5	48.0	118.2	40.0	81.8	39.0	90.9	42.3	50.0	66.7	48.0	60.0	40.0	33.3	46.0
	1.0	56.0	154.6	45.0	104.6	42.0	118.2	47.7	50.0	66.7	47.0	66.7	45.0	50.0	47.3
	Mean	42.0	—	35.0	—	31.7	—	—	43.3	—	40.3	—	31.7	—	—
Humic acid	0.5	60.0	172.7	50.0	127.3	47.0	127.3	52.3	44.0	46.7	42.0	40.0	30.0	0.0	38.7
	1.0	70.0	218.2	59.0	154.6	56.0	154.5	61.7	48.0	60.0	44.0	46.7	31.0	3.3	41.0
	Mean	50.7	—	43.0	—	39.0	—	—	40.7	—	37.3	—	23.7	—	—

* P_0 = Control (no addition of heavy metals).
P_1 = 120 Zn+ 70 Pb+ 50 Cu mg kg^{-1} soil.
P_2 = 240 Zn+ 140 Pb+ 100 Cu mg kg^{-1} soil.

$$** RC\% = \frac{\text{Result of a particular sub treatment – Result of the dual control "}O_0P_0\text{"}}{\text{Result of the dual control "}O_0P_0\text{".}} \times 100$$