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# IF THE SOIL HEAVY METALS HAD AFFECT ON THE MICROBIAL BIOMASS IN DIFFERENT ECOSYSTEMS

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## ABSTRACT

The microbial population as well as the soil enzymes activities was recorded in four different ecosystems in Egypt with a wide range of heavy metal concentrations. The metal concentrations were ranged between 126.22 to 3555.42 mg $kg^{-1}$ dry soil (As, Cd, Cr, Cu, Ni, Pb and Zn). The total microbial population express as bacterial, yeast and fungal counts, $CO_2$ evaluation, nitrogenase activity, soil enzymes activities were determined.

The results about microbial biomass detected that the counts were lower in moderate and high polluted soils. The high reduction in the enzymes activities were closely paralleled with the increase in the concentrations of heavy metals detected in such soils.

Also, the data obtained reveled that increasing the concentrations of heavy metal in soils has a positive effect on the growth and activity of microorganisms as well as on the roles played on the organic matter decomposition and nutrient cycling in such site.

**Keywords:** Heavy metal, Microorganisms, Enzymes, Pollution, $CO_2$ production and Nitrogenase activity

## INTRODUCTION

The military operations often release toxic chemicals into the environment. The relative effects of heavy-metal-contamination of soils on ecosystem can be determined by calculate the microbial biomass and its activities, degradation of organic matter and nutrient release in the soil.

More advanced researches have been done on microbial biomass or soil enzymes activities especially in acidic soil were done by Nordgren *et al.* (1986) and Baath (1989) or in agricultural soils amended with sewage sludge which containing a heavy metal (Fliessbach *et al.*, 1994 and McGrath *et al.*, 1995).

The physico-chemical characteristics of an ecosystem into which metals are deposited determine the chemical speciation forms as well as hence the bioavailability and toxicity of the metals to the indigenous micro biota. Such a biotic environmental factors include pH, aeration, inorganic anions and cations, hydrous metal oxides, organic matter, temperature and hydrostatic pressure (Babich and Stotzky, 1982 & 1983 and Stotzky and Babich, 1980 & 1984).

Babich and Stotzky (1985) detected that microorganisms, like other components of the biota, were sensitive to heavy metals. They recorded that, there was a two general microbial aspects of the environments contaminated with heavy metals (1): a reduction in the numbers and species diversity of the biota and (2): the development of metal-resistant microbial populations. Also, Tyier (1981) found that soil containing 28.000 ppm Pb, 972 ppm As, 599 ppm Cu and 151 ppm Cd had lower numbers of bacteria including actinomycetes

In addition, a few studies have focused on the influence of pH on heavy metal toxicity to microbial-mediated ecological processes that occur in natural environments. Neither 1000 ppm $Pb^{2+}$ or $Zn^{2+}$ had no effect on nitrification in a naturally acidic soil (pH 6.0), but in pH 7.0, both metal slightly increased nitrification, while in pH 7.7, nitrification was significantly reduced, with $Pb^{2+}$being more toxic than $Zn^{2+}$ (Bhuiya and Cornfield, 1974).
Also, Baath *et al.* (1989) studied the effect of heavy metals containing sewage sludge on the soil microbial community in two agricultural soils, which had been contaminated separately with $Cu^{2+}$ $Zn^{2+}$ and $Ni^{2+}$ at two different levels.They suggested that microbial community tolerance increased in all metal treatments compared to untreated soil.

Bacterial proliferation in soil amended with sewage sludge to which a various metals were added was studied, by Zibiliski and Wagner (1982). They found that, the growth of bacteria in soils containing sludge supplemented with 11 or 78 ppm Cu was stimulated initially (> 5 to 20 days), but, thereafter, decreased. Moreover, sludge containing 556 ppm Cu greatly suppressed bacterial growth.

The rate and amount of evaluation of carbon dioxide ($CO_2$) from soil has been used an indicator about the effects of heavy metals and other pollutants on the overall heterotrophic activity of microorganisms involved in the decomposition of organic matter (Environmental Protection Agency, 1979 a,b and c). $CO_2$ evaluation during 12-weeks incubation was reduced in an acidic sandy soil amended with 1000 ppm of various metals, with the sequence of toxicity being Ni> Pb> Cu> Zn (Bhuija and Cornfield, 1972).

In addition, nitrogen fixation was also inhibited in a soil amended with 5 m M Cd/kg (Lighthart, 1980); Cu (20 ppb) inhibited N-fixation by a spring bloom of the *cyanobacterium* (Wurtsbaugh and Home, 1982). Moreover, Cd (18 µm) caused a 30% decrease in the N-ase activity of soybeans inoculated with *Rhizobium japonicum* (Huang *et al.*, 1974) and Cd (10 Urn) reduced N-fixation, nodule weight and nodule number in bean plants inoculated with *Rhizobium phaseoli* (Vigue *et al.*, 1981).

Extracellular enzymes derived from microbial cells, were presumably responsible for many biochemical process in soils and sediments. Such activity of many of these enzymes is inhibited by metals, with inhibition being the results of the masking of catalically active groups, protein denaturation, effects on enzyme conformation and competition with activating cations involved in the formation of enzyme-substrate complexes (Tyler, 1981). The same author detected that Cd, Pb and Zn at 50 to 500 ppm, inhibited the synthesis of amylase, cellulose and urease. Moreover, the activities of urease and acid phosphatase, but not of B-glucosidase, were depressed in soil contaminated with Cd and Zn (Tyler, 1974 and 1975). Also, phosphatase activity was depressed in soils contaminated with V derived from the combustion of fuel oil (Tyler, 1976).

The purpose of this study was if the military waste disposal operation as well as adding the sewage sludge which containing heavy metals had affect on the microbial population, soil respiration and enzymes activities in four different soil sites.

# MATERIALS AND METHODS

## Soil Collection

Soil samples were collected from four sites, which is covered different sources of contamination. The first sample was collected from North Sinai (as a source of military operation), the second sample was collected from Helwan (as a sources of soil contaminated with factories wastes), the third sample was collected from Abo-Rwash, (as a soil fertilized with a sewage sludge from many years ago) and the fourth sample was collected from farm of Faculty of Agriculture, Cairo University, Giza (as a untreated soils or as a reference site).

Soil from each sites were collected from five randomly selected quadrates (2 x 2) using a 5 cm-dia. soil corer to a depth of 20 cm. Soil samples were kept in a cooler during transportation and stored at 4-8 °C until prepared for analysis.

## Metal determination

Total As, Cd, Cr, Cu, Ni, Pb and Zn were extracted by nitric-per choric acid digestion and quantified by atomic emission spectroscopy. While, potassium, calcium and magnesium were detected by using ammonium acetate extraction method (Bary, 1988). Also phosphorus was detected by means of the Bary method (1988). Organic matter content was determined by loss on ignition. Soil pH was calculated by adding equal volume of water to the soil, lifting to stand for 10 mins and measuring.

## Enzymes assays

Soil samples were stored in a freezer at 25 °C until assayed. Samples were thawed at room temperature for 2 h and then placed in the refrigerator at 6-8 °C during subsequent analysis.

The activities of the extracellular enzymes such as, cellulase, N-acetylglucosaminidase and phosphatase (acidic and alkaline) were quantified for each soil sample. Enzymes determination were made at 25 °C by using 10 grams soil (wet) suspended in 150 ml acetate buffer (50 mM, pH 5). But, in case of alkaline phosphatase, the soils were suspended in 100 mM tris buffer at pH 9.5. Incubation period were 5 hours for acidic phosphatase, 6 hours for alkaline phosphatase and N-acetylglucosaminidase and 9 hours for endocellulase. The enzymes activities were measured by using the spectrophotometric method of Sinsabaugh and Linkins (1990). While, endocellulase assay was a viscometic method (Aimin and Eriksson, 1967).

In addition, the substrates used in these methods were N-acetylglucosaminide and p-phosphate. But for the endocellulase assay carboxymethlcellulose used as a substrate. Every enzyme test were repeated three time and the activity was expressed as umol substrate converted $g^{-1}$ dry wt $h^{-1}$ soil and Endocellulase activity is expressed as viscometric units $g^{-1}$ dry wt $h^{-1}$ soil. The total phosphatase activity was calculated by summing the mean activities for acid phosphatase and alkaline phosphatase for each soil sample.

## Microbial determination

Microbial activities were recorded by determined the counts of bacteria, yeast and fungi as colony count on solid medium. Respiration

($CO_2$ production) was also used as an indicator of microbial biomass and calculated by method of Cheng and Virginia (1993). Acetylene reducing activity (ARA) was also measured.

**Statistical analysis**

Statistical analyses were made among the four soil samples by the least significant differences using MSTAT microcomputer statistical program (Power, 1982).

## RESULTS

**Heavy metal content**

The results obtained showed that the total heavy metal concentration for four sites was 3555.4, 2120.1, 960.3 and 126.22 mg $K^{-1}$ dry soil respectively. The concentrations of heavy metals content in soil samples were 28.2 times greater in sample collected from North Sinai, 19.2 times greater in soil of Helwan and 7.6 graters in soil of Abo-Rwash than found in soil sample collected from Giza as a reference s ite (Table 1). The concentration order of these heavy metal in the two area tested (north Sinai and Helwan) were Zn> Pb> Cu> Cr> Ni> As and Cd. While in Abo-Rawash site, the order of heavy metals concentration was Zn> Pb> Cr> Cu> As> Ni> Cd. But in the reference site the order of heavy metals was Pb > Zn > Cr > Ni > Cu > As and Cd.

**Table 1: Concentrations of heavy metals recorded in soils collected from four sites of Egypt**

| Heavy metal ($mg kg^{-1}$ dry soil) | Giza (reference) | Abo-Rawsh | Helwan | North Sinai |
|---|---|---|---|---|
| As | 4.60 | 6.20 | 6.80 | 12.20 |
| Cd | 0.18 | 1.14 | 2.84 | 4.02 |
| Cr | 20.34 | 64.18 | 102.52 | 162.40 |
| Cu | 10.00 | 10.8 | 328.20 | 534.00 |
| Pb | 22.00 | 450.00 | 913.00 | 1380.00 |
| Ni | 4.40 | 12.80 | 15.70 | 20.20 |
| Zn | 60.70 | 315.20 | 1049.20 | 1442.60 |
| Total Greater % | 126.22 | 960.32<br>7.60 | 2420.06<br>19.20 | 3555.42<br>28.17 |

*** Values are average of five sub sample for each site**

Also, values of the other chemical factors were significantly varied among different sites (Table 2). Hydrogen concentration expressed as pH and were 6.4 for Abo-Rawash area, 6.6 for Helwan area and 7.8 for north Sinai when compared with the reference site (6.1). In addition, the organic matter content was high in reference area (4.95 %) and the percent decreased in other three sites (2.60, 1.80 and 1.30%) respectively. In addition,the calcium level was also highest in Giza area (682 mg $g^{-1}$respectively. In addition, the calcium level was also highest in Giza area (682 mg $g^{-1}$ dry soil) and lowest in north Sinai site (335 mg $g^{-1}$ dry soil). Also, the other factors tested (cation exchange capacity, Mg, P and K) were mush higher in reference site than the other three sites tested and the values were depended upon the site tested and amount of pollution. While, the heavy metal concentrations were also found in a low quantity in Giza area (126.22) when compared with the other three areas tested.

**Table 2: Main chemical characteristics of the four soil samples tested collected from different Egyptian areas**

| Soil characteristics | Giza | Abo-Rawsh | Helwan | North Sinai |
|---|---|---|---|---|
| pH | 6.10 | 6.40 | 6.60 | 7.80 |
| Organic matter % | 4.95 | 2.60 | 1.80 | 1.30 |
| Cation exchange capacity meq 100 $g^{-1}$dry wt soil) | 7.10 | 6.00 | 5.10 | 4.40 |
| Ca (mg $g^{-1}$dry wt soil) | 682.00 | 508.00 | 456.00 | 335.00 |
| Mg (mg $g^{-1}$dry wt soil) | 62.00 | 100.10 | 102.40 | 198.40 |
| P (mg $g^{-1}$drywtsoil) | 5.40 | 8.80 | 7.10 | 35.20 |
| K (mg $g^{-1}$ dry wt soil) | 66.10 | 52.10 | 47.40 | 42.10 |

## Enzymes assay

Comparing the results recorded for the reference site with the other three soil samples, the activities of carbon, hydrogen and phosphorus enzymes were significantly lower in the other three sites tested (Table 3). The differences in total soil enzymes activities may be due to the lower of organic matter content recorded in the areas exposed to moderate or high level of heavy metals.

**Table 3: Soil enzymes activities in four soil samples collected from different sites of Egypt**

| Enzymes | Giza | Abo-Rawsh | Helwan | North Sinai |
|---|---|---|---|---|
| Endocellulasde (viscometic unit $g^{-1}$dry wt $h^{-1}$) | 67.400 | 16.800 | 11.600 | 5.300 |
| Acetylglucosamindase (mmoles substrate converted $g^{-1}$dry wt soil $h^{-1}$) | 0.245 | 0.052 | 0.034 | 0.012 |
| Acid phosphatase (mmoles substrate converted $g^{-1}$dry wt soil $h^{-1}$) | 1.871 | 0.517 | 0.312 | 0.113 |
| Alkaline phosphatase (mmoles substrate converted $g^{-1}$dry wt soil $h^{-1}$) | 0.614 | 0.194 | 0.068 | 0.064 |
| Total phosphatase (mmoles substrate converted $g^{-1}$dry wt soil $h^{-1}$) | 2.485 | 0.711 | 0.380 | 0.177 |

Also, the results obtained showed that, in soil of north Sinai, the endocellulase,alkaline phosphatase, acid phosphatase and acetylglucoseamindase activities were represented 7.86, 7.64, 6.04 and 4.90% of the enzyme activities in Giza area respectively. Moreover, The previous data also detected that increasing heavy metal content were led to, decreasing organic matter content as well as decreasing the pH values which had a positive effect on the degree of enzymes activities in such soil tested.

## Microbial determination

The results recorded (Table 4) proved that, increasing the total heavy metal content in soil led to decreases the microbial populations. Where, the bacterial, yeast and fungal counts recorded in site with a high concentrations of heavy metal were represented only 4.2, 7.3 and 9.3% respectively of the population densities occurred in reference site. While, the microbial count of

Giza soil sample was reached to 4.30 x $10^4$fu $g^{-1}$soil for fungi, 6.31 x $10^6$cfu $g^{-1}$ soil for yeast and 3.18x $10^6$cfu $g^{-1}$ soil for bacterial count.

Moreover, the $CO_2$ production and nitrogenase activity (acetylene reducing activity) as indicators of microbial biomass was also detected. The $CO_2$ and N-ase values were lower in area which have a high contamination of heavy metal and reached to 4.3% and 3.7% of the values assayed in control area.

**Table 4: Microbial population (x$10^4$ cfu $g^{-1}$ soil), COz production and N-ase activity recorded in four different soil samples of Egypt**

| Microbial biomass | Giza | Abo-Rawsh | Helwan | North Sinai |
|---|---|---|---|---|
| Fungi Yeast Bacteria | 4.30<br>63.10<br>318.00 | 8.60<br>24.40<br>70.30 | 10.50<br>22.10<br>43.40 | 0.40<br>4.60<br>13.20 |
| COa<br>N-ase | 2.80<br>8.40 | 0.62<br>3.78 | 0.54<br>2.46 | 0.12<br>0.31 |

## DISCUSSION

The study detected that the changes occurred in soil conditions caused in the soil sites study have a positive effect on soil biomass and enzymes activities. The microbial content decreased as did in the activities of enzymes tested which play an important roles in breakdown of organic matter, nitrogen, carbon and phosphorus. In addition, the $C0_2$ measurement as indepent of microbial biomass had been highly effected that the microbial processes i.e., decomposition of organic matter as well as nutrient cycling have been severely reduced by the military war occurred in this area (north Sinai).

The concentration range of heavy metals estimated in such sites is similar to results detected by Baath, 1989 and Kuperman and Carreiro, 1997. The chemical analysis of such soil which contaminated with high content of heavy metals showed that this soil was low in organic matter and alkaline but the reference site (Giza area) was much higher in organic matter and near to nutrient in ph.

As results of the high concentration of heavy metals, the relative effects of heavy metals on microbial population as well as enzymes activities between different sites tested in this study. In the sites which had a high content of heavy metal, amylase activity was decreased by 25% (Ebergt and Boldewijin, 1977), phosphatase by 30% and urease by 35% (Tyler, 1974). Also, Tyier and Westman (1979) found that at some areas, where total soil heavy metal concentration of only 239 mg $kg^{-1}$, phospgatase and urease activities were reduced by 28%. In addition, phosphatase activity was reduced by 69% in the area containing 2600 mg Cu $kg^{-1}$ and 1900 mg Ni $k^{-1}$ (Freedman and Hutchinson, 1980).

The relative contribution of the organic matter%, pH, $Ca^{2+}$ and heavy metals content on the reduction of soil microbial biomass and enzyme activities is so difficult because the main factors were changed from one site to another. The explanation is the different heavy metal concentrations and the lower of organic matter content of tested soils occurred in the contaminated areas. Low content of organic matter can be reduce the soils microbial densities and of course, immobilize heavy metals (Duxbury, 1985; Tyier *et al.*, 1989 and Gadd, 1993). So, the lower OM content in sites tested may have a reduction in enzyme activity which could caused by increasing in heavy metal content or reducing in plant biomass.

The results done by Ebregt and Boldewijin (1977) showed that Ca was negatively correlated with heavy metals where, Ca replacement by heavy metals in soils. Also, the data made by Kuperman and Carreiro (1997) detected that, the increased of heavy metal content as well as increased in soil pH was responsible for the decrease in microbial biomass and enzyme activity. Either factor could produce a reduction in microbial population may be due to the effect of pH on heavy metals which increase its toxicity (Starky, 1973; Gadd and Griffiths, 1980; Gadd and White, 1985; Collins and Stotzky, 1992 and Gadd, 1993).

Nordgren *et al.* (1983) and Bardgett *et al.* (1994), observed that pH were positive correlated with heavy metals. So they, suggests that the very high concentration in heavy metals occurred in site with alkaline pH may have been responsible for the decrease in the microbial biomass.

Activities of five enzymes tested were detected that severally reduction was occurred as heavy metals content increased. The five enzymes were more sensitive to the soil conditions found in contaminated soil and its activities were depressed by 92 to 95% (Fig. 1). This strongly suppressive effect on enzymes activities were observed by several researchers (Tyler, 1974; Eivazi and Tabatabai, 1990 and Aoyamaetal., 1993).

Alkaline phosphatase activity from' our results showed that the rate of its activity were 78, 63 and 67% lower than acid phosphatase in the low pH sites. The same phenomenon was observed by Haynes and Swift (1988) who said that relative alkaline and acid phosphatase activities were correlate with pH.

Moreover, Chrost (1991) detected that the concentration of magnesium, which can stimulate alkaline phospatase activity and could have been partially responsible for increasing its activity. Also, Dick (1991) and Fliessbach *et al.* (1994) reported that the increased alkaline phosphatase activity at high concentrations of metal could reflect a change in the composition of the microbial community.
Such changes may be as a result from the environmental selection of bacterial strains resistance to the heavy metal toxicity.

In addition, the increase in alkaline phosphatase can be related to the increase in P concentrations. This increase could have resulted from hydrolysis of some chemical agents under alkaline soil conditions (Nemeth, 1989).

The relationship between microbial biomass and enzymes activities indicated the decrease in enzyme activity is caused by direct suppression of microbial growth in the contaminated soils. The same fact was detected by Gadd (1993), who said that

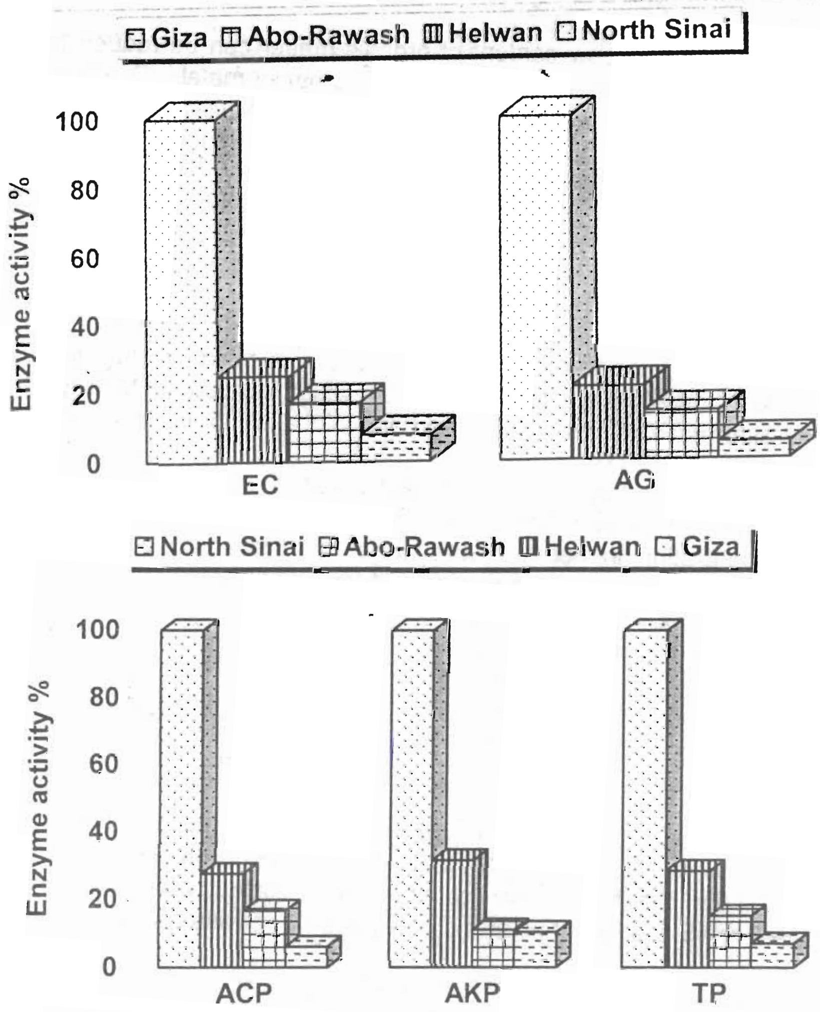


Fig. 1: Relative activities of soil enzymes in soil samples collected from four areas of Egypt
EC = endocellulase, AG = acetylglucosaminidase,
ACP = acid phosphatase, AKP = alkaline phosphatase
and TP = total phosphatase

## CONCLUSION

Our study detected that the contamination of soil with a heavy metals have a ct effect on soil microbial population, microbial types and as a results on enzymes cities. So, determination of microbial count, $C0_2$ production and enzymes cities were very important to give a full picture on structure and function of soil imunities and ecosystem processes found in these areas.

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## هل تواجد المعادن الثقيله يكون له تأثير واضح على الكتله الميكروبيه فى مختلف الاوساط البيئيه ؟

**محمد زكريا صديق**

**قسم الميكروبيولوجيا الزراعيه_كلية الزراعه _جامعة القاهره**

تم تقدير الاعداد الميكروبيه و كذلك معدل نشاط انزيمات التربه فى اربع اوساط بيئيه مختلفه فى مدى احتوائها على المعادن الثقيله .ومن النتائج المتحصل عليها وجد ان تركيز المعادن الثقيله (ممثله فى الاسترنشم ،الكادميوم ،النحاس ،النيكل،الرصاص،الزنك)يتراوح فى الاراضى المختبره ما بين ١٢٦،٢٢ الى ٣٥٥٥،٤٢ ملليجرام / كيلو جرام تربه جافه .

لقد تم تقدير الاعداد الكليه للميكروبات ممثله فى تقدير اعداد البكتريا ،الخمائر ،الفطريات كذلك تقدير كمية ثانى أكسيد الكربون المنتج ،معدل نشاط انزيم النتروجينيز و أيضا نشاط انهزيمات التربه الاخرى .

و لقد اظهرت النتائج المتحصل عليها بأن الاعداد الكليه للميكروبات ممثله فى الكتله الميكروبيه تكون قليله فى التربه ذات معدلات متوسطه او عاليه من التلوث. وأن أعلى معدل تناقص فى نشاط الانزيمات يكون مرتبطه ارتباطا وثيقا بزيادة تركيز المعادن الثقيله فى التربه.

أيضا أوضحت النتائج أن زيادة تركيز المعادن الثقيله يكون لها تأثيرا ايجابيا على نمو ونشاط الكائنات الحيه الدقيقه و بالتالى الدور الذى تلعبه فى تحليل الماده العضويه و بالتالى دورة العناصر فى التربه.