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MICROBIAL ACTIVITIES IN CHEMICALLY POLLUTED SOIL UNDER CEREAL AND LEGUME CROPS

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ABSTRACT: ***The influence of heavy metals, sources of irrigation water and cultivated crops on some microbial processes in soil was studied in a pot experiment. Two soils, i.e. non-polluted and polluted with heavy metals, cultivated with wheat or faba bean plants, and irrigated with different sources of water, namely, fresh Nile water, treated sewage effluent and treated industrial aqueous wastes were under consideration. The microbial processes, particularly, soil respiration (evolution of CO_2), dehydrogenase activity, nitrogenase activity, Azotobacter numbers in wheat rhizosphere, and faba bean root nodulation were measured at different times of plant growth, throughout the experiment.***

The results demonstrated that, the rate of CO_2 evolution was highest from the non-polluted soil irrigated with the Nile water, planted with either wheat or faba bean, and the lowest rate was recorded for the polluted soil irrigated with the industrial waste water under either cultivated plant.

The treatment of sewage effluent significantly increased the dehydrogenase activity in the non-polluted soil planted with either wheat or faba bean, comparatively with the other irrigation sources. Irrigation with the industrial waste water significantly diminished the dehydrogenase activity in the soils tested. In the polluted soil, the Nile water surpassed the sewage effluent, in this regard. The other treatments denoted intermediate varying figures.

Nitrogenase activity in the rhizoplane of wheat cultivated in the non-polluted soil irrigated with the Nile water was significantly greater, followed by the sewage and industrial waters in a decreasing order. The sewage effluent came prior to the Nile and industrial waters in such concern, as to favour the N_2 ase in the faba bean root system in the non-polluted soil, but oppositely between the Nile water and sewage effluent in the polluted soil. Likewise, the non- polluted soil surpassed the polluted one in all circumistances.

Azotobacter numbers, of wheat rhizosphere, in all treatments irrigated with the different water sources, revealed significant augmentations up to the 30^{th} day, of sowing, thereafter began to decline. Treatments of the Nile water and sewage effluent attained the highest numbers of Azotobacter, followed by the industrial liquid wastes, decreasingly, in both soils .

The faba bean root nodules in the non-polluted soil irrigated with the Nile water or sewage effluent were large, pink in colour, few in numbers and largely confined to the upper part of the root system (effective in N_2-fixation).

Whereas, the treatments irrigated with the industrial waste water in the non-polluted soil, as well as, all treatments of the polluted soil, exhibited small, colourless, numerous nodules and evenly spread throughout the entire root system (uneffective nodules).

Key words: ***Waste water, Wheat, Faba bean, Diazotrophy, Dehydrogenase, Nitrogenase, CO_2 evolution, Heavy metals, Soil pollution.***

INTRODUCTION

Heavy metal pollution resulting from human industrial and agricultural activities or sanitary landfills affects the biological systems in general. Several reviews provide an up-to date picture of persistence, turnover and environmental effects of heavy metals release to the aquatic and terrestrial ecosystems. Industrial effluents often contain considerable amounts of potentially toxic metals, such as Fe, Mn, Zn, Ni, Cu, Pb, Co and B. Heavy metals are known to be persistent in the cultivated layers of soil (Collins and Stotzky, 1989; Gupta, 1992 and Harmsen, 1992) and can have ecotoxicological effects on plants and soil microorganisms (Martensson and Witter, 1990; Chander *et al.,* 1995).

There is an increasing evidence of adverse effects of heavy metals on soil microbial activities. Rühling and Tyler (1973) found significant decreases in soil microbial activities, i.e. respiration rate, dehydrogenase activity and decomposition of spruce needle litter in soil polluted with Cu and Zn. Reddy *et al.* (1987) showed, also, that dehydrogenase activity was significantly ($P<0.01$) inhibited in soil contaminated by Cu, Zn, Ni, Cd, Fe and Mn. As well as, Castro *et al.* (1997) reported a decrease in effectiveness and genetic diversity of *Rhizobium leguminosarum* population present in contaminated soils. Soils have long been used as disposal "sinks" for municipal refuse. "Sanitary landfills" are widely employed to dispose a variety of wastes from our cities. Unfortunately, sanitary landfills are sometimes not so sanitary. Leaching and run off from these sites can contaminate both surface water and ground water.

Discharging the aqueous wastes derived from industrial activities into water streams makes such water polluted and progressively becomes unsuitable either for irrigation purposes or as potable for humans and animals (Abdel-Tawab, 1985). On the other hand, disposal of sewage wastes onto land or their application to soil as a source of nutrients and/or irrigation water contributes to arising of numerous environmental hazards resulting from accumulation of detrimental materials, main of which are heavy metals (Hinsely *et al.,* 1979; Williams *et al.,* 1980).

It is known that the biological activity and genetic pools of microorganisms present in soils contaminated by heavy metals can be used as indicators of the deleterious effects of this kind of pollution. In Egypt, there

is a lack of information about the ectoxicological effects of industrial pollutants on soil microorganisms. For this study, soils from an area with known pollution problems where heavy metals and other pollutants have been emitted by industry for nearly 30 years, were chosen. This area is particularly affected by the release of liquid wastes from different factories such as iron & steel, coal, metal smelting, spinning & weaving, cement and others. Many researchers have studied the impact of urbanization and industrialization on pollution of the biosphere, in air, water and soil and the consequences on the various living beings (Badawi, 1993 and Abou El-Naga *et al.,* 1996).

The aim of the present work was to assess the effect of irrigation with waste water on microbial processes, namely, soil respiration, dehydrogenase activity, and dinitrogen fixation in non-polluted and heavy metal polluted soils cultivated with wheat and faba bean crops.

MATERIALS AND METHODS

Soils

Surface soil samples (0-30 cm) were collected from two sites of agricultural areas at the satellite of Helwan city, Cairo Governorate. The first site is an ordinary non-polluted soil, located away from factory complex (Iron & Steel, Coal, Spining & Weaving and others). The second site is a chemically polluted soil within the location of such industrial complex. Soil samples of each site were air-dried, ground to pass through a 2-mm sieve, thoroughly mixed, and subjected to laboratory analyses for their physical and chemical properties (following the methods described by Page *et al.,* 1982); data obtained appear in Table (1, A thru C).

Waters

Three sources of irrigation water were used, namely, ordinary River Nile's fresh water (as a control), treated sewage effluent, and treated industrial liquid wastes (from Iron & Steel factory's outlet). Chemical analysis of the applied waters was carried out according to the methods reported by APHA (1975). Data in Table (2, a & b) revealed that the industrial liquid wastes contained high amounts of soluble cations, anions, phosphorus and heavy metals, whilst the sewage effluent possessed intermediate levels, and the Nile water came least in such concern.

Experiments

Greenhouse experiment was carried out using two agricultural (arable) soils, i.e., ordinary non-polluted and polluted, two kinds of plants, namely wheat (*Triticum aestivum L.*) representing cereals and faba bean (*Vicia faba L.*) representing legumes. Three sources of water were selected for irrigation, i.e. fresh Nile water, sewage effluent and industrial aqueous wastes.

48 earthenware pots (30 cm wide & 40 cm high), each was filled with 4 kg of the soil employed. Each pot received 25 mg P_2O_5 (as superphosphate) kg^{-1} soil and similar amount of each of K_2O (as potassium sulphate) and N (as ammonium sulphate), and all mixed well with the soil crumbs. Each pot was planted with twenty grains of wheat (*Triticum aestivum* L.) or ten seeds of faba bean (*Vicia faba L.*), as convenient. Two weeks after sowing, seedlings were thinned to ten or four healthy wheat or bean plants, in respect, per pot. The pots were watered with the Nile water regularly every two days to maintain 60% of the water-holding capacity (WHC) of the soil, until germination, thenceforth, the various irrigation water sources had been introduced, regularly at intervals of 0, 7, 15, 30, 45, 60, 75 and 90 days. Each treatment was replicated four times.

Determinations

A. For both crops:

1. Soil respiration : was done by measuring the carbon dioxide evolved from the soil during mesophilic incubation at 15-day intervals along 90 days, according to the method of Anderson (1978), using 10 g soil mixed with glucose solution (2.5 mg g^{-1} soil) in a glass container. Moisture content of the soil was kept constant at 60% WHC. The container was placed in a 500 ml conical flask stoppered with a rubber blug having two pores; passing through each a tubing, one serving as an inlet for CO_2 free air and the other serving as an outlet for CO_2 evolved from the soil, which was trapped into a conical flask containing 0.5 N Na OH. The system was incubated at room temperature (22-25 °C) for 8 hours. The sodium hydroxide solution was back titrated with 0.5 N HCl in the presence of Ba Cl_2 (40%) and phenolphthalein indicator. Amount of carbon dioxide was then calculated.

2- Dehydrogenase Activity in soil (DA): was determined at 15 day intervals up to 90 days, by the method of Casida *et al.* (1964) in which TPF (2, 3, 5, triphenylformazan) resulted after 24 h of incubation at 37 °C, from the anaerobic reduction of TTC (2, 3, 5, triphenyltetrazolium chloride), was measured colorimetrically.

3- Nitogenase activity (NA): was determined following the method of Hardy *et al.* (1973), as follows: whole plant samples were used, at intervals of vegetative growth for wheat and only at 60 days for faba bean. The plants from each treatment were carefully uprooted without tearing the root hairs and the plants were separated into shoots and roots by cutting at the cotyledonary nods. Bean roots were carefully washed free of soil with distilled water several times, but the wheat roots were left unwashed. Ten wheat roots and four faba bean roots, of each replicate, were placed in glass jars (300 ml) and tightly covered with rubber suba seal. 10% of the gas phase of the glass jar were replaced by acetylene (C_2H_2) using disposable plastic syrings, then the jars were incubated in darkness at 30 °C for 24 and

2 hours for wheat and bean roots, respectively. Ethylene (C_2H_4) produced was measured by injecting 1.0 or 0.5 ml gas samples (wheat or bean, respectively) into a gas chromatograph (GC) "model HP 6890 equipped with hydrogen flame ionization detector (HFID), poropak N of 100-200 mesh size, and a stainless steel column, 1.5 m long 3 mm in diameter". Finally, to each jar containing sample of wheat root, CO was added to give 0.5% concentration, the jars were incubated again for another 6 h. Thereafter, the headspace air was analyzed using the same chromatography technique to measure the endogenous C_2H_4 formation. Difference in C_2H_4 production between the two treatments was ascribed to a spontaneous C_2H_4 formation (Martensson, 1993).

B. For each particular crop

a. Wheat :

***Azotobacter* numbers in rhizosphere soil : were counted according to Page *et al.* (1982), at 0, 15, 30 and 60 days, using decimal dilutions of the soil sample, each to inoculate five test tubes of a N-free liquid medium with the following composition, Ca Cl_2, 20 mg; Fe_2 SO_4, $7H_2O$, 50mg; K_2HPO_4, 1.0 g; Mg SO_2-$7H_2O$, 0.2 g; Mn Cl_2, 2 mg; Na_2 MoO_4; 1 mg; mannitol, 10 g; redistilled water, 1 L. The most probable number (MPN) was calculated from Cochran's Table (Page *et al.,* 1982).**

b. Faba bean:

Root nodulation : the root samples of faba bean were removed from jars (of NA determination) and the occurrence, number, size, and colour of root nodules were assessed by observation and scoring system.

The obtained experimental data were statistically analysed, according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Results gotten for the influence of various experimental treatments on the assigned microbial processes in soils, are reported and interpretted as follows:

I. Overall Microbial Activities in Soil

A) Soil Respiration (Evolution of CO_2)

Rates of CO_2 evolution from the soils irrigated with the different sources of water and measured at various times are illustrated in Fig. (1). Extent of CO_2 evolution was highest from the non-polluted soil irrigated with the Nile water planted with either wheat or faba bean, and lowest from the polluted soil irrigated with the industrial waste water under either cultivated plant. CO_2 evolution also increased from all treatments up to the 45^{th} day, then began to decline. Irrigation with the Nile water (control) attained the highest levels of CO_2 evolution, followed by sewage and industrial waters, in a decreasing

order. An (O) order kinetics was shown, for both soils and crops, up to 30 and 45 days. Similar results were presented by Jacek and Dirk (2000), Krishan and Joergensen (2001), and Irha *et al.* (2003).

Hattori Hiroyuki (1992), Burkhardt *et al.* (1993) and Abaya *et al.* (2005) also obtained similar results and ascribed the inhibition of CO_2 evolution to the heavy metals, depending mainly on the degree of toxicity of the metal on soil bacterial growth and the amount of water soluble heavy metals in soils. As well as, Germund (1974); Roman and Carreirs (1997) and Cecilia *et al.* (1998) reported that the reduction of CO_2 evolved from polluted soil was due to the effect of Cu + Zn concentration, which lowered the carbon mineralization basal respiration and substrate induced respiration. Ken *et al.* (1998) and Kools *et al.* (2005) concluded that the high concentration of chloride in soil solution increased the solubility of heavy metals and consequently increased their toxicity on soil microorganisms.

B) Dehydrogenase Activity in Soil (DA)

Data of dehydrogenase activity (DA), in the non-polluted soil irrigated with the different sources of water, presented in Fig. (2), illustrated that the treatment of sewage effluent significantly promoted ($P<0.05$) the activity of such enzyme in the soils planted with either wheat or faba bean, comparatively with the other irrigation sources, i.e. the Nile water (as a control) and industrial liquid wastes. This might be attributed to the presence of appreciable amounts of suspended organic materials and nutrients in the sewage effluent that suit the heterotrotrophic microbial population in soils (Chander and Brookes, 1991). Irrigation with the industrial waste liquids significantly decreased ($P<0.01$) the DA. The inhibitory action of such waste water was actually due to the presence of heavy metals (see Table, 2 a & b) injuring the soil microbial inhabitants.

In the polluted soil, results of DA were significantly lower ($P<0.05$) than those of the non-polluted soil (Fig. 2), under both crops wheat and faba bean, but with the Nile water occupying the upper rank in place of the sewage effluent. These findings are in agreement with those reported by Doelman and Haanstra (1979), Jacek (1995), Ken *et al.* (1998) and Jacek and Dirk (2000). Those investigators had referred their results to accumulation of heavy metals, as well as to the poor conditions of such polluted soils. Irha *et al.* (2003) also, concluded that contamination of the soil by heavy metals, especially Pb, which is locally very common in industrialized countries, may decrease the total number of microorganisms, the level of respiration and dehydrogenase activity. This is especially true for soils having a low cation-exchange capacity. As well as, Chander and Brookes (1991) reported that the decreases, in dehydrogenase activity due to some physical or chemical reaction of TPF with soil Cu was occurring, causing a diminished absorbance. In addition, Cu may also cause a small genuine decrease in the biomass in addition to a biological reaction between Cu and TPF. Deng and Tabatabai (1995) noted that heavy metals may inactivate enzyme reactions by

complexing the substrate, by reacting with protein-active groups of enzymes, or by reacting with the enzyme-substrate complex, or indirectly by altering the microbial community which synthesizes enzyme (Kandeler *et al.,* 2001).

Likewise, data of DA in the polluted soil of the present work, planted with wheat were lower than in the same soil planted with faba bean (Fig. 2); this is probably due to the higher microbial proliferation and activity in root zone (rhizosphere), being encouraged with the leguminous plant roots (Oliveira and Pampulha, 2006).

II. Biological Diazotrophy

a) Nitrogenase activity (NA)

Fig. (3 a) illustrated the results of NA in the rhizoplane of wheat planted in the non-polluted soil irrigated with the different sources of water, indicating that the treatment of Nile water was significantly ($P<0.05$) greater comparatively with the other irrigation sources, i.e. sewage and industrial waters, which significantly decreased the NA. This was actually due to the presence of detrimental chemical compounds and/or heavy metals (see Tables, 1 & 2). The inhibitory action of heavy metals on nitrogenase, found by several investigators, is probably referred to a diminished rate of enzyme synthesis being associated with inhibited microbial proliferation rather than to a direct enzyme suppression by the heavy metals (Dick, 1994, Banerjee *et al.,* 1997 and Ataman and Arcak, 2006).

Nitrogenase activity also decreased in all treatments of the polluted soil planted with wheat up to the end of experiments. Irrigation of the polluted soil with the Nile water (control) gave the highest figures of NA, followed exchangeably by sewage and industrial waters in a decreasing order. These results confirm the finding of Sabine *et al.* (1992), that a clear inhibition of heterotrophic acetylene reduction assay was observed in soil having high heavy metal contamination. Martensson and Torstensson (1996), Krishan and Joergensen (2001), Banger (2003), Wilke *et al.* (2005) and Vasquez *et al.* (2006), explained the inhibition of potential N_2-fixing activities, by the increasing concentration of heavy metals in polluted soil, that such elements may inactivate enzyme reactions by complexing the substrate by reacting with protein-active groups of enzymes, or by reacting with the enzyme-substrate complex or indirectly by altering the microbial community which synthesizes enzymes (Kandeler *et al.,* 2000 and Christopher *et al.,* 2005).

Results of Fig. (3 b) also showed the values of nitragenase activity (NA) in the root system of faba bean planted in the non-polluted and polluted soils and irrigated with the different water sourcens at 60 days after sowing. In the non-polluted soil irrigated with the sewage effluent, NA rcorded the greatest rate (29.2 µM C_2H_4) per unit ($g.h^{-1}$) dry weight of roots, followed by the Nile water, whilst the industrial water was least. In the polluted soil, in vitro NA of

faba bean roots exerted rates somewhat lower than those recorded for the non-polluted soil, irrespective of irrigation water source, however, the Nile water herein relatively surpassed the sewage effluent. The extent of difference between NA rates in the non-polluted soil and those of the polluted soil was actually attributed to the high concentrations of heavy metals, particularly Cd and Pb, which were associated with the changes in the ulrastructure of the root nodules, in which the active dinitrogen fixing area was reduced and the N_2-fixing cells in the area were also reduced (Amar *et al.*, 1992, Ken *et al.,* 1998 and Chen *et al.,* 2003). Likewise, Smith and Giller (1992) and Castro *et al.*(1997) reported that the effect of heavy metals from industrial

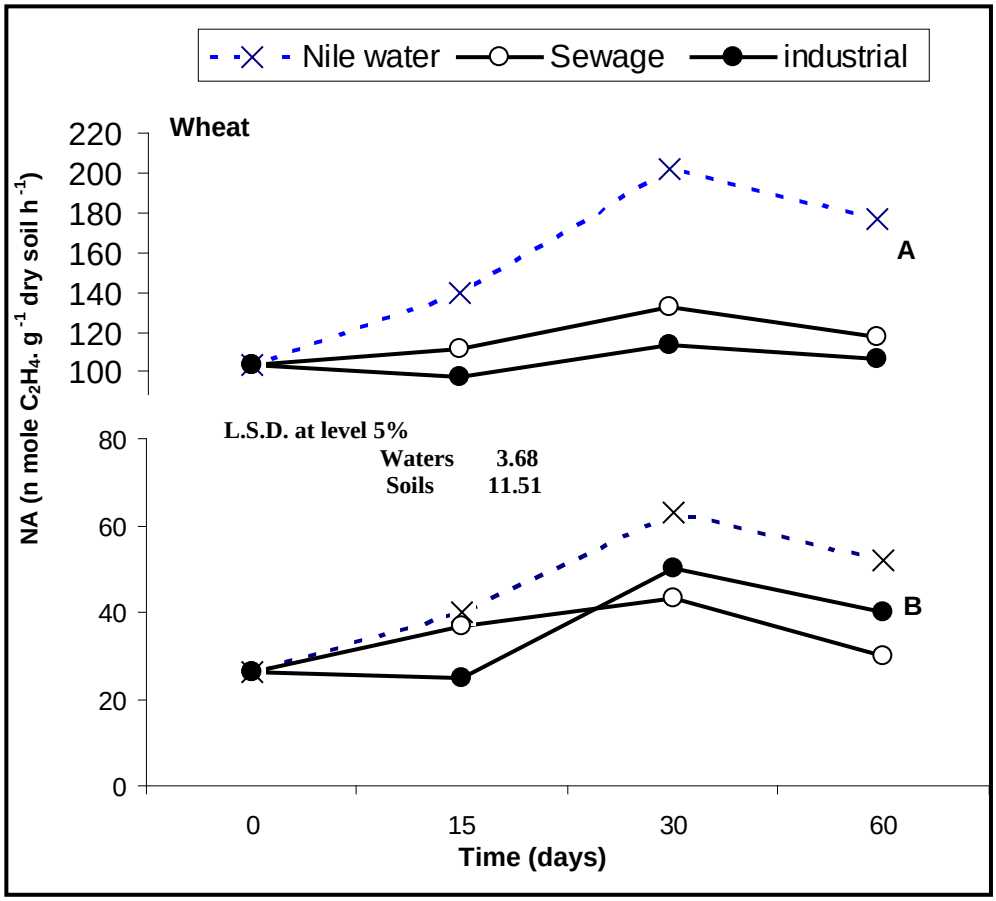

Fig. (3a) Nitrogenase activity (NA) in wheat rhizoplane, as affected by the different irrigation water sources in the non-polluted soil (A) and polluted soil (B), at intervals of vegetative growth.

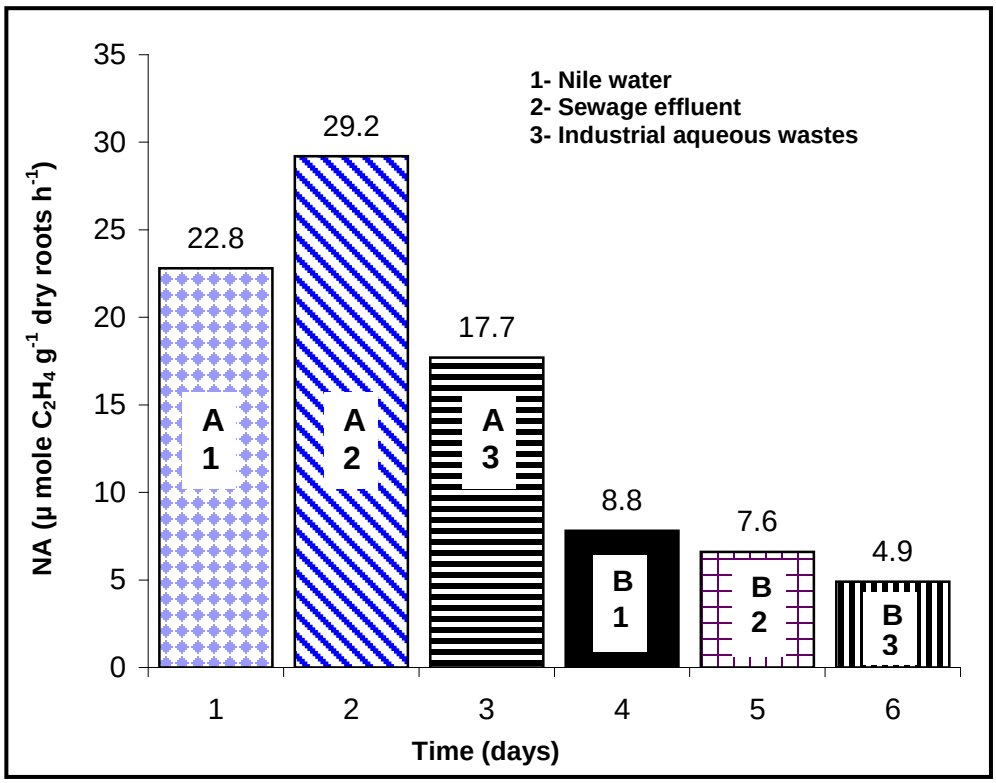

Fig. (3 b) Nitrogenase activity (NA) in faba bean root system, as affected by the different irrigation water sources in the non-polluted (A) and polluted (B) soils, at 60 days after sowing.

effluents decreased in effectiveness and genetic diversity of *Rhizobium leguminosrum*. Also, the deleterious effects of Cd on the nodulation and N_2-fixation and the decreasing toxicity of metal to nodule formation and symbiotic N_2-fixation had been ranked in a decreasing order as Cd> Co> Cu> Zn, respectively.

b) Aerobic Chemoorganotrophic Agnts

b1. Free-living "Associative" Bacteria (with cereals)

- *Azotobacter* numbers in rhizosphere soil of wheat

Figure (4) illustrated that the numbers of *Azotobacter* in the non-polluted and polluted soils irrigated with the different water sources and cultivated with wheat, significantly increased in all treatments up to the 30th day, thereafter, the bacterial numbers had bean reduced until the end of the experiment. The treatments of the Nile (control) and sewage waters attained the highest numbers of such associative N_2- fixers, followed by the industrial waste water, descendingly. It was evident that the presence of high concentrations of heavy metals in the industrial waste water, was behind its least figures.

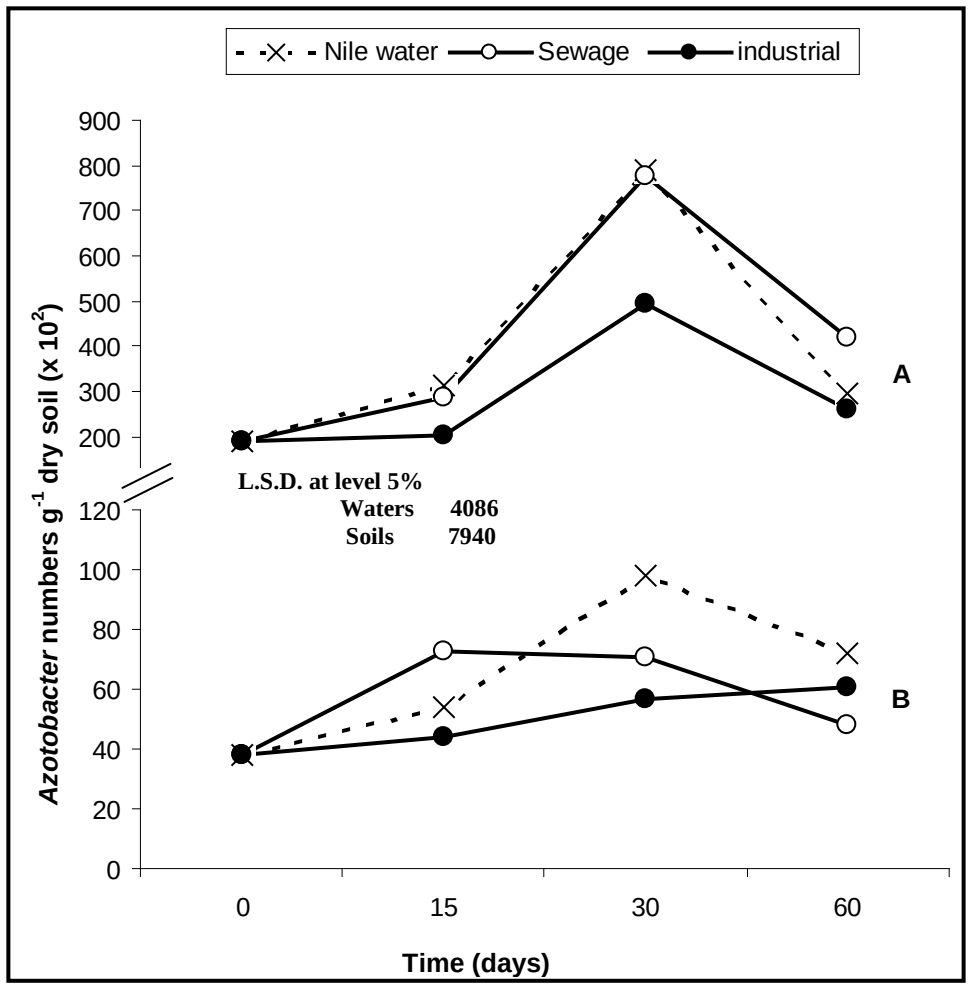

Fig. (4) *Azotobacter* numbers as affected by the different irrigation water sources in the non-polluted soil (A) and polluted soil (B), cultivated with wheat.

In the polluted soil, *Azotobacter* numbers were lower in comparison to the non-polluted soil. In both soils, the Nile water was of the highest *Azotobacter* numbers, followed by the sewage and industrial waters, respectively. This diminution is logically, due to presence of high levels of heavy metals in such polluted waste waters and soil. Earlier works declared the detrimental effects of different heavy metals in irrigation water or in polluted soil having high concentrations of heavy metals above the permissible limits in soil, according to the commission of European communities (see Table 3) (Sabine *et al.,* 1992, Jacek, 1995, Martensson and Torstensson 1996, Ken *et al.,* 1998, Banger 2003, Wilke *et al.,* 2005 and Vasquez *et al.,* 2006).

Extent of suppression, resulted by the effect of heavy metals on *Azotobacter*, was higher in the polluted soil than that in the non-polluted one, was also detected among the water sources. Such action handicaped the growth and augmentation of *Azotobacter* cells, (Wu *et al.,* 2006).

Table (3): Permissible limits of heavy metals (mg kg^{-1}) in soils receiving sewage sludge (Amar *et al.,* 1993)

Metal	CEC (1986)*	U.K. (1989)†			Germany (1992)‡	
		pH 6-7	pH 5.5-6	pH 5-5.5	pH > 6	pH 5-6
Zn	150-300	300	250	200	200	150
Cu	50-140	135	100	80	60	60
Ni	30-75	75	60	50	50	50
Cd	1-3	3	3	3	1.5	1.0
Cr	100-150ş	400ş	400ş	400ş	100	100
Pb	50-300	300	300	300	100	100

*** Commission for the European Communities (1986) Directive.**
† Department of the Environment (1989) limits, U.K.

‡ German Federal Ministry of the Environment (1992) limits.
Ş Provisional.

b2. Symbiotic Bacteria (with legumes)
- Rhizobia (faba bean root nodulation)

Results in Table (4) revealed that the numbers of faba bean root nodules in the non-polluted soil irrigated with the Nile or sewage waters were large and pink in colour (indicating the presence of leghaemoglobin, participating in diazotrophy), few in number and largely confined to the upper part of the root system, and score of nodule characteristics ranged from 8.00 to 7.25 per a root. The treatment of industrial waste water in the non-polluted soil, as well as, all treatments of the polluted soil irrigated with the Nile, sewage or industrial water, gained small, colourless, and numerous nodules, which were evenly spread throughout the entire root system. The latter nodulation pattern is a characteristic of uneffective nodules unable to fix atmospheric nitrogen. The scores of these treatments ranged from 5.85 to 3.53 . This was attributed to the high concentrations of heavy metals in the polluted soil or in the industrial waste water (see Tables 1 and 2). Data of the dinitrogen biologically fixed (Table 4), quantitavely reflected such differences in the capacity of effective nodule formation. These findings agree closely with those obtained by McGrath *et al.* (1988), Amar *et al.* (1992), McGrath *et al.* (1995), Guo *et al.* (2002) and Chen *et al.* (2003), who demonstrated that nodulation process was greatly affected by the addition of Cd, especially at the level of 10 and 20 mg kg^{-1}. The inhibition of plant growth especially the root system, increased as the Cd concentration increased, with deleterious effects on the roots, the weight ratio of root/leaf and on the ultrastructure of root nodule, in which the effective N_2-fixing area was reduced and the N_2-fixing cell in the area was also reduced. In addition, the content of Cd in different parts of the plant was as follows: roots > stems > seeds, indicating that the accumulation of Cd by roots was much larger than that by any other part and might cause a deleterious effect to the root systems. The activities of bacteria led to an increase of water dissolved organic carbon concentration and a decrease of pH value, which enhanced metal mobility and biovailability (e.g. an increase of solubility of heavy metals, such as Zn, Cu, Ni, Cd and Pb) (Ken E. Giller *et al.,* 1998 and Wu *et al.,* 2006).

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الأنشطة الميكروبية فى الأراضى الملوثة كيميائياً والمنزرعة بمحاصيل الحبوب والبقول

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المخلص العربى

درس تأثير التلوث الكيماوى للتربة بالعناصر الثقيلة (بمنطقة حلوان الصناعية) وكذلك مصدر مياه الرى (مياه النيل ، مياه الصرف الصحى "المجارى" المعالجة ، المخلفات السائلة للمصانع بعد معالجتها) على الأنشطة الميكروبية فى تربتين (غير ملوثة وأخرى ملوثة كيماوياً) ومزروعتين قمح وفول بلدى (كل على حدة). وصممت تجربة أصص لدراسة الأنشطة الميكروبية وهى تحديداً تنفس التربة (انطلاق ثانى أكسيد الكربون) ، نشاط انزيم الديهيدروجينيز، نشاط انزيم النيتروجينيز ، أعداد بكتريا الأزوتوباكتر فى ريزوسفير نباتات القمح ، وذلك عند فترات نمو خضرى مختلفة ، وكذلك عملية تكوين العقد الجذرية على جذور نباتات الفول البلدى (عند عمر ٦٠ يوم من الزراعة) .

وكانت النتائج المتحصل عليها هى كما يلى :

[١] سجل أعلى معدل لانطلاق ك أ٢ من التربة الغير ملوثة والمروية بمياه النيل والمنزرعة بأى من القمح أو الفول ، وكان أقل معدل هو من التربة الملوثة والمروية بمياه صرف المصانع والمنزرعة بكل من المحصولين ، وكانت مياة الصرف الصحى ذات نتائج وسطية .

[٢] أدى الرى بمياه الصرف الصحى الى زيادة نشاط أنزيم الديهيدروجينيز بمعدل معنوى فى التربة الغير ملوثة المنزرعة بالقمح أو الفول ، أما الرى بمياه صرف المصانع فقد أدى الى تقليل أو خفض نشاط الانزيم نفسه مع أى من المحصولين . أما فى التربة الملوثة فقد

تفوقت مياه النيل على مياه الصرف الصحى لكلا المحصولين ، وأظهرت بقية المعاملات نتائج وسطية متباينة القيم .

[٣] كان نشاط انزيم النيتروجينيز الأعلى معنوياً فى التربة الغير ملوثة والمروية بمياه النيل بالمقارنة بمصادر الرى الأخرى مع محصول القمح (فى الريزوبلان) . وكان ترتيب مصادر مياه الرى تنازليا هو مياه النيل ثم مياه الصرف الصحى وأخيراً مياه صرف المصانع ، فيما عدا بعض التداخلات فى القيم بين مياهى الصرف الصحى والصرف الصناعى فى حالة الأرض الملوثة.

[٤] أوضحت النتائج أن نشاط انزيم النيتروجينيز لجذور الفول فى التربة الغير ملوثة ، بصرف النظر عن مصدر مياه الرى ، كان أعلى بكثير عنه فى التربة الملوثة . وقد سجل الرى بمياه الصرف الصحى أعلى معدلات لنشاط الانزيم فى التربة الغير ملوثة ، تلتها مياه النيل وأخيرا مخلفات صرف المصانع . أما فى التربة الملوثة فقد جاء ترتيب مياه النيل أولاً ثم مياه الصرف الصحى وأخيراً مياه صرف المصانع .

[٥] سجلت اعداد الأزوتوباكتر فى ريزوسفير نباتات القمح زيادة مستمرة فى كل معاملات التجربة حتى اليوم الثلاثين ، ثم بدأت فى التناقص حتى نهاية التجربة ، وسجلتا معاملتى الرى بمياه النيل ومياه الصرف الصحى أعلى الأعداد للأزوتوباكتر ، وجاءت مياه صرف المصانع أخيراً ، فى كلا التربتين .

[٦] أدى رى التربة الغير ملوثة بمياه النيل أو مياه الصرف الصحى الى تكوين عقد جذرية كبيرة وردية اللون (فعاله) وقليلة العدد نسبياً وموزعة على الجزء العلوى لجذور نباتات الفول .

[٧] ظهرت العقد الجذرية لنباتات الفول ، فى جميع معاملات التربة الملوثة والمروية بمصادر مياه الرى المختلفة بالاضافة الى معاملة واحدة من التربة الغير ملوثة وهى المروية بمياه صرف المصانع ، صغيرة الحجم ومعظمها عديم اللون وأعدادها كثيرة ومنتشرة على سطح جذور نباتات الفول بالتساوى تقريباً ، وهو ما يدل على عدم فعاليتها فى تثبيت النيتروجين الجوى .

Time intervals (days)

Time intervals (days)

Time intervals (days)

Time intervals (days)

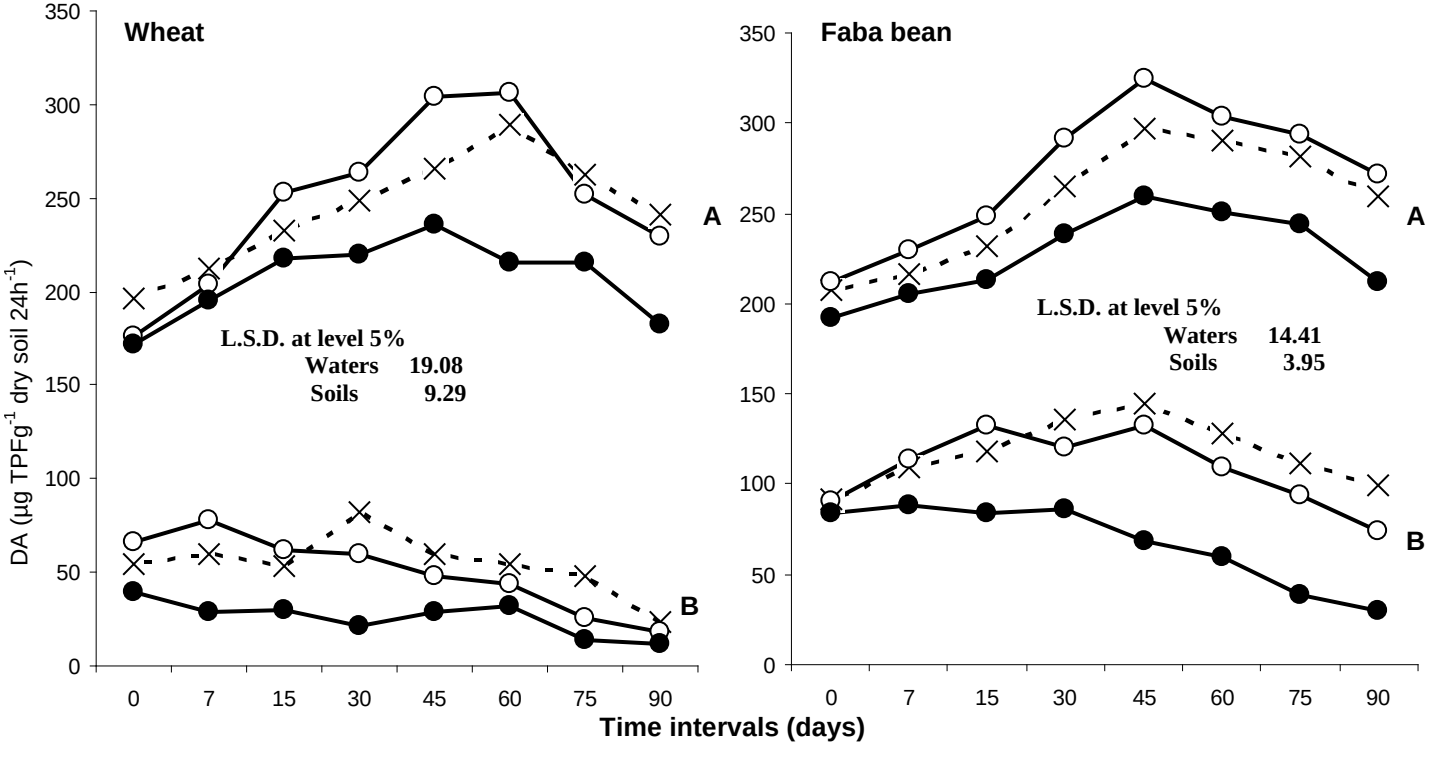

Fig. (2) Effect of irrigation water sources on dehydrogenase activity (DA) in the non-polluted (A) and polluted soils (B), cultivated with wheat and faba bean.

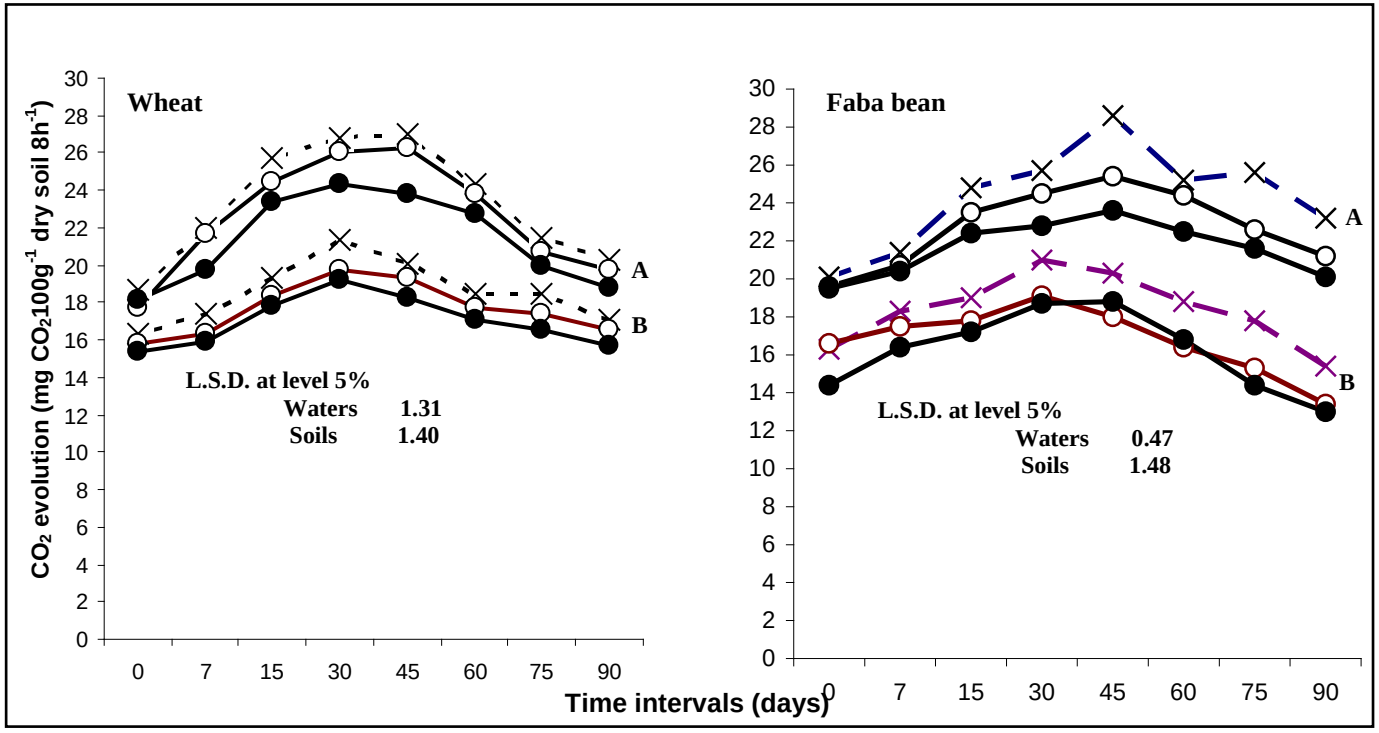

Fig. (1) Effect of irrigation water sources on carbon dioxide evolution from the non-polluted (A) and polluted (B) soils, cultivated with wheat and faba bean.

Table (1): Physical and chemical analyses of the experimental soils.

(A) Physical properties

Soil used	$CaCO_3$ %	Organic matter %	Particle fraction, %			Texture class
			Clay	Silt	Sand	
Non-polluted	5.2	3.0	32.4	38.8	28.8	Clay loam
Polluted	5.5	1.65	31.5	43.5	25.0	Clay loam

(B) Chemical properties

Soil used	pH*	EC** $dS.m^{-1}$ at 25°C	C.E.C*** meq/100 g soil	Soluble ions, meq 100 g^{-1} soil								Total N ppm	Available P ppm
				Cations				Anions					
				Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}		
Non-polluted	7.6	0.36	39.5	0.57	0.25	0.76	0.19	0.00	0.43	0.76	0.58	760	12.3
Polluted	7.8	30.90	22.1	24.80	20.00	117.00	1.50	0.00	2.40	116.00	44.90	1685	108.2

*** In 1:2.5 soil: water suspension. **Electerical conductivity of soil paste extract. ***Cation exchange capacity.**

(C) Heavy metal contents

Soil used	Element, ppm													
	Fe		Mn		Zn		Cu		Pb		Co		Cd	
	T*	S**	T	S	T	S	T	S	T	S	T	S	T	S
Non-polluted	1121	100	499	6	220	0.60	30	0	50	0	18	0	1	0
Polluted	4193	485	2200	160	970	80.00	290	54	392	96	152	10	18	5

*** T = Total ** S = Soluble**

Table (2): Chemical analysis of waters used for irrigation

(a) Cations and anions

Water source	pH	EC $dS.m^{-1}$ at 25°C	Ion, meq L^{-1}								Nitrogen ppm		Phosphorus ppm
			Cations				Anions						
			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	NH_4^{+}	NO_3^{-}	Available
Nile	7.5	0.38	1.28	0.71	1.81	0.10	0.00	2.63	0.67	0.55	0.00	0.02	0.01
Sewage	7.3	0.82	3.30	0.44	4.33	0.27	0.00	2.67	2.78	2.89	17.60	15.70	0.36
Industrial	7.8	1.45	4.84	3.45	5.64	0.25	0.00	0.35	3.80	7.03	0.66	1.29	0.14

(b) Heavy metals

Water source	Element, ppm							O_2 , ppm		
	Fe	Mn	Zn	Cu	Pb	Co	Cd	DO	BOD	COD
Nile	0.78	0.17	0.09	0.01	0.10	0.00	0.00	2.7	2.9	3.7
Sewage	3.01	0.50	0.48	0.23	0.48	0.10	0.18	1.2	149.0	169.0
Industrial	38.60	2.50	0.89	0.48	4.08	1.50	1.00	3.2	75.6	230.8

Table (4): Assessment of nodulation of faba bean roots and N_2-fixed induced by indigenous strains of *Rhizobium leguminosarum,* in the different treatments, after 60 days of planting *.

Nodules Features	Nodulation Rating	
	1	3
Size (mm)	Small (<2 mm)	Large (>2 mm)
Colour	Colourless	Pink
Number (per pot)	< 250	> 250

Treatments		Degrees of nodule specifications				Nodules number per plant	Dry weight of nodules mg/plant	N_2 –Fixing capacity	
Soil	Irrigation water	Size	Colour	Number	Score **			mg N_2 per*** plant for 900 h light	Kg N_2 per feddan (4200 m^2)
Non polluted	Nile	2.99	3.00	1.25	7.25	59.0	242	67.4	16.0
	Sewage	2.85	2.90	2.25	8.00	82.0	394	96.6	23.0
	Industrial	1.39	1.46	3.00	5.85	106.0	267	39.8	9.5
polluted	Nile	1.28	1.27	2.10	4.65	83.0	224	19.2	4.6
	Sewage	1.18	1.12	1.50	3.80	107.0	234	15.9	4.5
	Industrial	1.00	1.03	1.50	3.53	119.0	261	11.5	2.7

* Each replicate was investigated by three research – workers; each figure is therefore a mean of 12 individual assessments per treatment.

** The score was estimated on the basis of size, colour, and number of nodules and summation of every individual assessment.

*** Amounts of fixed dinitrogen were calculated from the data obtained for nitrogenase activity (See Fig. 3b).