MICROORGANISMS AS A TOOL FOR DIAGNOSIS OF SOME SOIL POLLUTANTS

By

WISAM MOHAMMED ABD AWAD
B.Sc. Agric. Sci. (Soil Sciences and Water Resources), Fac. Agric., Baghdad Univ., 2005.

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DEDICATION

To my home country IRAQ
To Agriculture College / Baghdad University
To Agriculture Faculty / Cairo University
To my Father.. who's I haven't seen ever
To my beloved family:

Mom,
Brother "Husam"
Sister "Zina" & sweetly her daughter "Farah"
Ante "Najah"
All my uncles and friends
Thanks for your
unconditional love,
constant support,
and encouragement.

I love you all dearly

Name of Candidate: Wisam Mohammed Abd **Degree:** M.Sc.
Title of Thesis: Microorganisms as a Tool for Diagnosis of Some Soil Pollutants

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ABSTRACT

The objectives of the present work were to determine the diversity of microorganisms in a heavily contaminated soils with heavy metals or pesticides and to identify their most resistant species . Three soil types were selected to represent clay alluvial (FFS), calcareous (WNS) and sewage farm (ARS) soils and subjected to two incubation experiments, one to study the effect of heavy metals (Cd, Zn, Pb and Ni) and the other to study the effect of two pesticides (Harness and Rugby) on the diversity of their microorganisms (bacteria, fungi and actinomycetes). The data of the first experiment showed that Alluvial (FFS) and Wadi-Natrun (WNS) had the highest values of maximum adsorption and fixation capacity of Cd, Zn, Pb and Ni metals. Abu Rawash soil (ARS) recorded the highest numbers of culturable bacterial even with the presence of increasing concentrations of cadmium, lead and zinc followed by alluvial soil (FFS) and calcareous soil (WNS). The bacterial population present in Abu-Rawash (ARS) was relatively more tolerant to Cd toxicity than that for alluvial (FFS) one while Ni toxicity order was: (FFS) >(ARS) > (WNS). A significant positive correlation coefficients were recorded between viable bacterial numbers and activity of microbial enzyme dehydrogenase in all heavy metal contaminated soils. The morphological examination showed that heavy metal resistant bacterial isolates for were <u>*Bacillus*</u> *sp* .mostly isolated from Abu-Rawash with one Isolate No. BM+64 was <u>Cocci</u> sp. Isolate BM+48 was the most resistant to heavy metals and was identified as *Bacillus cereus/ or B.thuringiensis* which was obtained from Abu-Rawash soil (ARS). The highest counts of fungi were noticed in the calcareous soil (MNS) and alluvial one (FFS) treated with Ni. Isolates No. FM01 and FM14 were the most tolerant for the highest concentrations of the applied Zn, Pb and Cd. Three isolates (FM01, FM10 and FM15) were classified as *Aspergillus nidulans*, while three isolates (FM04, FM08 and FM28) were recognized as *Aspergillus fumigates,* two isolates (FM09 and FM14) were recognized as *Aspergillus flavus.* Fungi isolate FM04 (*Aspergillus fumigates*) was the most resistant to Pb, while isolates FM10 and FM15 both (*Aspergillus nidulans*) were the most tolerant to Zn. Abu-Rawash (ARS) soils (sewage farm) showed the highest population of actinomycetes at all the highest levels of Cd, Zn and Pb followed by the calcareous (WNS) and normal alluvial (FFS) soils. AM10 was the only one that tolerated the stresses of Zn, Ni, Pb, and Cd at concentrations of 75, 75, 100 and 12 mg/L.

The second incubation experiment showed that The initial concentration of soluble carbon for Harness treatment was higher than that for Rugby. Abu-Rawash sewage farm soil (ARS) had the highest bacterial counts under the stresses of both Rugby and Harness with relatively lower numbers in Harness. The bacterial isolates dominated by *Bacillus spp.* with 60% of short rods bacteria. isolates BP01, BP07 and BP15 tolerated upto 2mg/l of Harness while isolates BP01, BP15 and BP18 tolerated the same level as Rugby. Isolate BP01 was the most tolerant one to both harness and Rugby and it was identified as *Bacillus subtilis.* Abu-Rawash soil (ARS) had the highest fungal counts under the stresses of both Rugby and Harness pesticides. Harness was more toxic to fungi than Rugby in all the investigated isolates. The pesticides resistant fungi isolates were identified as *Aspergillus spp.*, and *Cladosporium spp.* which were found in Abu-Rawash (ARS). The FP03 isolate was identified morphologically as *Aspergillus flavus* which showed the highest tolerance to both Rugby herbicide and Harness nematocide. The counts of actinomycetes in Harness treatment were, generally, lower than that in Rugby in both alluvial (FFS) while the reverse was true in the sewage farm soil (ARS). Harness was more toxic to actinomycetes than Rugby in all the investigated isolates. Actinomycetes isolate AP07 was the most tolerant one to 2.0 mg/l of both Rugby and Harness while AP06 had tolerance to 2 mg/l of Harness and it was identified as *Nocardia spp.*

Key words: Bacterial resistance, Fungi, Actinomycetes, Heavy metals, pesticides, dehydrogenase

CONTENTS

INTRODUCTION

Heavy metals contamination of soil is widespread due to metal processing industries, tannery, combustion of wood, coal and mineral oil, traffic, and plant protection. Metals can exist in the soil solution as free cations (e.g. Cu^{2+}, Cd^{2+}, Zn^{2+}), as soluble complexes with inorganic or organic ligands (e.g. $ZnCl^{+}$, $CdCl^{3-}$, metal citrates) and associated with colloidal material. However, metal toxicity is greatly affected by the physico–chemical nature of the environment and the chemical behavior of the particular metal species in question. The residence of heavy metals (*e.g.* Pb and Hg) in many soil systems is considered to be hundreds or even thousands of years (Hissler and Probst, 2006; Klaminder *et al.*, 2006). Pesticides also have long residence time in the soil which has toxic effects on soil life.

Soil microorganisms, as an essential component of most terrestrial ecosystems, play a critical role in the environment due to their role in cycling mineral compounds and in the decomposition of organic material. Environmental stress caused by heavy metals or pesticides generally decreases the diversity and activity of soil microbial populations, and upsets the ecological balance of population interactions within the soil community. The toxic effects of heavy metals result mainly from the interaction of metals with proteins (enzymes) and inhibition of metabolic processes. Therefore, the use of microbial parameters has been proposed for monitoring soil pollution by heavy metals. Effects of heavy metals on soil microbial communities may be assessed by a variety of methods including the measurement of microbial biomass and activity or changes in soil microbial community structure. However, microbial communities are influenced not only by heavy metals but also by other environmental factors such as soil pH, temperature, moisture and organic matter quality, e.g., C/N ratio.

So far, many researchers have measured the soil microbial activities as indicators of soil degradation by heavy metals and pesticides. The knowledge about heavy metal resistance capacity generally broadens the insights into the ability of bacteria to inhabit polluted environments, but can also be applied in biotechnology for example as a bioreporter (bio-indicator) or bioremediation purposes. Bacterial bioreporters, which are living microorganisms that produce a specific, quantifiable output in response to target chemicals, take advantage of heavy metal inducible transcription systems and sense bioavailable metals depending on metal flux through the soil. Thus, the bioreporter technology is closely related to the research about heavy metal resistance mechanisms and will be introduced alongside the actual resistance mechanisms.

Therefore, the present study was undertaken, with the soil collected from the agricultural field with different technological activities and amended with a graded, known concentration of metal ions and its combinations and some pesticides in the lab conditions. The survival pattern of the common major groups of indigenous microbial population (bacteria, fungi and actinomycetes) was studied.

The specific aims of the research in this thesis were:

1. determining the types and amounts of pollutants prevailing in the soil used in three types of technological activities (intensive agriculture, industrial activities, sewage water disposal).
2. identifying the numbers and types of microorganisms (Bacteria - Actinomycetes - Fungi) in the examined soils from each type of technological activities.
3. studying the effect of increasing concentration of soil heavy metals or pesticides on the microbial structure in the studied soils.
4. determining the minimum inhibitory concentration from each type of pollutants for Bacteria, Actinomycetes and Fungi isolated from artificially polluted soils.
5. isolation and characterizing the heavy metal or pesticide tolerant sp. of bacteria and fungi as bio-indicators.

REVIEW OF LITERATURE

1. Sources of Heavy Metals in Contaminated Soils

Heavy metals occur naturally in the soil environment from the pedogenetic processes of weathering of parent materials at levels that are regarded as trace and rarely toxic (Pierzynski *et al.*, 2000 and Kabata-Pendias and Pendias, 2001). Due to the disturbance and acceleration of nature's slowly occurring geochemical cycle of metals by man, most soils of rural and urban environments may accumulate one or more of the heavy metals above defined background values high enough to cause risks to human health, plants, animals, ecosystems, or other media.

D'Amore *et al.* (2005) stated that heavy metals essentially become contaminants in the soil environments because (i) their rates of generation via man-made cycles are more rapid relative to natural ones, (ii) they become transferred from mines to random environmental locations where higher potentials of direct exposure occur, (iii) the concentrations of the metals in discarded products are relatively high compared to those in the receiving environment, and (iv) the chemical form (species) in which a metal is found in the receiving environmental system may render it more bioavailable. Alloway (1995) and Lombi and Gerzabek (1998) suggested a simple mass balance of the heavy metals in the soil can be expressed as follows:

$$M_{\mathrm{total}} = (M_p + M_a + M_f + M_{\mathrm{ag}} + M_{\mathrm{ow}} + M_{\mathrm{ip}}) - (M_{\mathrm{cr}} + M_l), \qquad (1)$$

where "*M*" is the heavy metal, "*p*" is the parent material, "*a*" is the atmospheric deposition, " *f* " is the fertilizer sources, "ag" are the agrochemical sources, "ow" are the organic waste sources, "ip" are other inorganic pollutants, "cr" is crop removal, and "*l*" is the losses by leaching and volatilization.

It is projected that the anthropogenic emission into the atmosphere, for several heavy metals, is one-to-three orders of magnitude higher than natural fluxes (Lombi and Gerzabek, 1998). Heavy metals in the soil from anthropogenic sources tend to be more mobile, hence bioavailable than pedogenic, or lithogenic ones (Kuo *et al.*, 1983 and Sposito and Page, 1984). Metal-bearing solids *at* contaminated sites can originate from a wide variety of anthropogenic sources in the form of metal mine tailings, disposal of high metal wastes in improperly protected landfills, leaded gasoline and lead-based paints, land application of fertilizer, animal manures, biosolids (sewage sludge), compost, pesticides, coal combustion residues, petrochemicals, and atmospheric deposition (Khan *et al.*, 2008 and Zhang *et al.*, 2010).

Large quantities of fertilizers are regularly added to soils in intensive farming systems to provide adequate N, P, and K for crop growth. The compounds used to supply these elements contain trace amounts of heavy metals (e.g., Cd and Pb) as impurities, which, after continued fertilizer, application may significantly increase their content in the soil (Jones and Jarvis, 1981). Metals, such as Cd and Pb, have unknown physiological activity. Application of certain phosphatic fertilizers inadvertently adds Cd and other potentially toxic elements to the soil, including F, Hg, and Pb (Raven *et al.*, 1998).

Ling-Yu *et al.* (2010) stated that the accumulation of heavy metals, such as Cr, Ni, Cu, As, Cd, and Zn in soils was significantly affected by land use patterns. The accumulation of heavy metals in greenhouse soils was higher than others may be due to the heavy applications of chemical fertilizer, organic fertilizer, and pesticides with high contents of heavy metals.

Several common pesticides used fairly extensively in agriculture and horticulture in the past contained substantial concentrations of metals. For instance in the recent past, about 10% of the chemicals have approved for use as insecticides and fungicides in UK were based on compounds which contain Cu, Hg, Mn, Pb, or Zn. Jones and Jarvis, (1981) reported examples of such pesticides are copper-containing fungicidal sprays such as Bordeaux mixture (copper sulphate) and copper oxychloride.

The application of numerous biosolids (e.g., livestock manures, composts, and municipal sewage sludge) to land inadvertently leads to the accumulation of heavy metals such as As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Mo, Zn, Tl, Sb, and so forth, in the soil (DeVolder *et al.*, 2003 and Kaasalainen and Yli-Halla, 2003).

Malarkodi *et al.* (2007) showed that the soils irrigated with the sewage water contained almost all the heavy metals (Pb, Ni, Cd, Cr, Cu and Zn) exceeded the critical limit while those contaminated with electroplating and textile effluents were rich in Ni and Cr.