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Full Length Research Paper

Biological characterization of sandy soils Irrigated with sewage effluent for extended periods

Saber, M., Azza, SH. Turky, Fatma, H*. Abd-El-Zaher and Dalia, M. Abd-El-Mowla

National Research Center Cairo, Egypt

****Corresponding author: Fatma, H; E-mail: fatimaom_nrc@yahoo.com***

ABSTRACT
Thirty four sewaged and un-sewaged soil samples from the surface and sub-surface layers were collected from both El-Gabal El-Asfer and Abu-Rawash sewage farms representing soils under different landscapes irrigated with sewage effluent for extended periods ranging between 0 and 82 years. Results confirmed remarkable changes in the biological characteristics of soil. Faecal coliform were found in all sewaged soils irrespective of the period of sewage farming or landscape. Faecal coliform intensities ranged from158 to $15x10^2$ viable cell/gram dry soil after 25 and 82 years of sewage farming. Sewage farming tended to build the microbial biomass in soil, as bacteria, streptomyces and fungi counts increased markedly during the first 25 years then tended to stabilize. The dehydrogenase activity increased, parallel to CO_2 evolution, from 0.18 in un-sewaged soil to 17.86 µl H_2/gm soil /24 hours in soils sewage for 25 years and reached a maximum of 69.72 µl H_2/gm soil /24 hours after 82 years. A polynomial relationship existed between both CO_2 evolution & Dehydrogenase activity and soil biomass at the surface and sub-surface soils. *[ISSN-]*

Key words: *Biological characterization, sandy soils, sewage effluent, extended periods.*

INTRODUCTION
It is now well fulfilled that soil is embodying an irregular puzzling mixture where physical, chemical and biological phenomena bow towards stability. Worthy to mention that whereas higher plants can grow in a sterile ecosystem retaining only organic and inorganic salts, yet the function of microorganisms in farming is unarguable, hence, bio-properties are of major concern in soil characterization. The biological indicators should be those easy to measure, highly sensitive and anticipative. In this regard, however, two major parameters were cared about, the size of microbial biomass mainly enteric pathogens, bacteria, streptomyces, fungi as well as their activities.

Masto et al (2009) arranged soil bio-indicators in descending order of worth as follows, microbial biomass, CO_2 evolution, and dehydrogenase activity. Abd-el-Malek et al (1976) biologically characterized sewaged soils at El-Gabal-El-Aster farm using total bacterial counts, dehydrogenase activity, oxidation of organic carbon, and CO_2 evolution and reached a conclusion that dehydrogenase activity test was the most reliable parameter. The present work aims at biologically characterizing the effect of prolonged sewage farming on soil using dehydrogenase activity, CO_2 evolution, bacterial, streptomyces and fungi counts as well as the existence of enteric pathogens as effectual parameters.

MATERIALS AND METHODS
Soil Sampling
Thirty two sewaged soil samples from the surface (0-30 cm) and sub-surface (30-60 cm) layers were collected from both El-Gabal El-Asfer (16 samples) and Abu-Rawash (16 sample) sewage farms in addition to two un-sewaged soils.

Collected soil samples represented soils under different landscapes irrigated with sewage effluent for varied extended periods ranging between 25 and 82 years as well as un-sewaged sandy soil. Soil samples were grinded into fine particles with one mm average size and cold stored in their fresh state until analyses.

Methods of Soil Biological Characterization

The key components that biologically characterize sewaged soils and affect their quality in terms of agronomic value and environmental impacts were determined including enteric pathogens, microbial biomass, dehydrogenase activity and CO_2 evolution. The total bacterial, streptomyces and fungi were enumerated using serial dilution method on Topping, Starch Sasein and Martin media respectively. Biomass was counted after five days incubation period at 30°C, and all counts were related to oven dry weight. Fecal Coliform bacteria were grown in MacConky broth, the presence of gas and acid after 24 hours, incubation at 44ºC indicated positive tubes (Atlas 2005) and all counts were related to oven dry weight. Dehydrogenase activity in the sewaged soils was estimated according the method given by Skujins (1973). Carbon dioxide evolution from the sewaged soils was measured according to the method given by Kabble (1966) and modified by Monib et al. (1981). Statistical analyses were carried out using Origin Pro 8.0 software

RESULTS

Changes in soil biological characteristics are judicious indicators for its quality, since they are more dynamic and often more sensitive than physical or chemical soil properties. Visser and Parkinson (1992) stated that any change in entire or definite soil biomass could be considered as a sensitive indicator of a change in the soil biological quality status. As far as sewage farming is carefully thought about, it was noticed that its prolonged use promoted the soil biological activities as represented by basal respiration, microbial biomass and enzyme activities (Antolin et al. 2005 and Fernandes et al. 2005). In this context, our concern had been copiously directed towards the effects of sewage effluent on soil biomass intensities as well as on their action.

Although sewage farming increases the entire microbial biomass in soil, it is important to state that despite some of these biomass components are constructive, others are hazardous and considered as bio-contaminants leading to major hygienic problems.

For sure great potential for the soil biological contamination comes from irrigation with raw sewage effluent impregnated with intensive densities of enteric pathogens that represent a major threat to public health, food safety, and environmental quality. It is mostly known that it is not practical to check the presence or absence of all enteric pathogenic microorganisms in sewaged soils in a timely fashion. For this reason, the indicator microorganism concept was established many years ago to allow monitoring of a limited number of microbiological constituents. Regulatory agencies generally rely on tests for faecal coliform bacteria to indicate the biological contamination of sewaged soils. Although faecal coliforms themselves are not pathogenic, they confirm the existence of enteric pathogens in soil.

No doubt, the technologies applied in sewage effluent treatment do not remove all enteric pathogens, and in many cases their re-growth in soil might be significant. Data given in graphically presented in Figure(1) indicate that faecal coliform bacteria were mainly originated from sewage effluent as the un-sewaged sandy soil was free from them. Results showed that faecal coliform counts in all sewaged soils were higher in the top soil layer compared to the sub-soil layer, irrespective of the period of sewage farming or landscape.

In the top soil layer ranged from 154- 158 to 1540-2240 viable cell/gram dry soil after 25 and 82 years of sewage farming at El-Gabal El-Asfer farm. On the other hand, faecal coliform intensities in the sub-soil layer ranged from 56-106 t0 394-640 viable cell/gram dry soil after 25 and 82 years of sewage farming at El-Gabal El-Asfer farm. The counts of faecal coliform in the soils irrigated with sewage effluent for extended periods between 25 and 82 years both at El-Gabal El-Asfer and Abu-Rewash farms were in between the above mentioned counts and were certainly linked to some extent with the time of the last sewage irrigation before sampling. In harmony with that Saber (1986) found that faecal coliforms increased to $35x10^3$ after 60 years of irrigation with decanted sewage effluent at El-Gabal El-Asfer farm.

It was noticed that the general behavior of enteric pathogens in sewaged soils was to stabilize in association with prolonged sewage farming, the longer the time of exposure, the higher the counts. From a kinetic point of view, which is a time dependent phenomena, such stabilization represents an adverse effect antagonizing the safe food production besides disseminating diseases between labors. This finding calls for a strict application of decontaminating protocols to remove enteric pathogens from sewaged soil to ensure sustainable farming, safe food production and better environment. Sewage farming for extended periods associated with farming ameliorated the soil characteristics through enriching the soil with extra organic matter which accelerated the proliferation and activates of the soil biomass (Saber 1986). Parallel to counting soil micro-flora, their activities should be also measured through various criteria such as CO_2 evolution, heat output, rate of nucleic acid analyses and enzyme activities. Two main criteria were investigated in the current study to measure biomass activities in sewaged soils, i.e., dehydrogenase activity and carbon dioxide evolution.

It is obvious in Figure (1) that the un-sewaged sandy soil was meager in its microbial population, thereafter sewage farming, besides supplied the soil with extra microorganisms supported the proliferation of the endogenous ones. The total bacterial counts jumped from $108x10^5$ in the un-sewaged soils to $2548\text{-}3320x10^5$ in soils sewaged for 25 years at El-Gabal El-Asfar farm. Afterwards, the length of the time of irrigation with sewage effluent did not seem to significantly influence the bacterial counts as evidenced from their fluctuations between $3320x10^5$ and $42000x10^5$ within 25-82 years of sewage farming both at El-Gabal El-Asfer and Abu-Rawash farms.

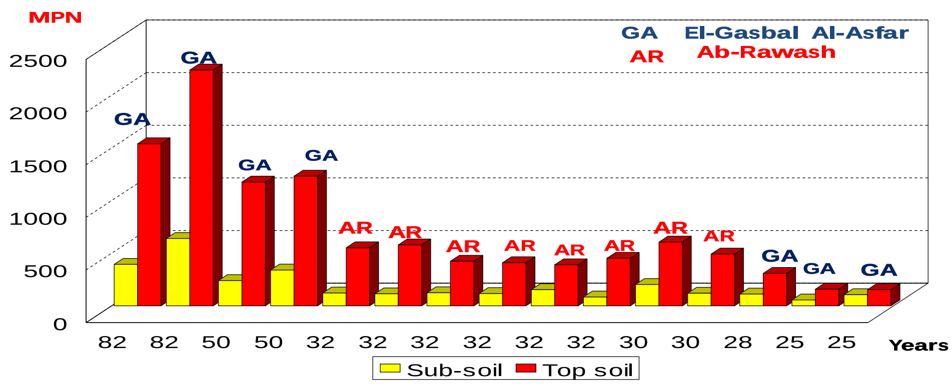

Figure 1. Existence of faecal coliforms in top and sub soil sewaged for extended periods under different landscapes

The changes recorded in the total bacterial counts in both surface and sub-surface soil samples as a result of prolonged sewage farming (Figure 2) followed a similar polynomial behavior with the different equation and regressions. The equations of the trends for surface and subsurface soil samples were $Y=206.58x^2\text{-}6477x+533.94$ and $Y=0.2635x^2\text{-}8.0306x+81.191$ respectively. The regression values for the bacterial counts in relation to the period of sewage farming in the surface and subsurface soil samples were $R^2 = 0.9731$ and 0.7666 respectively.

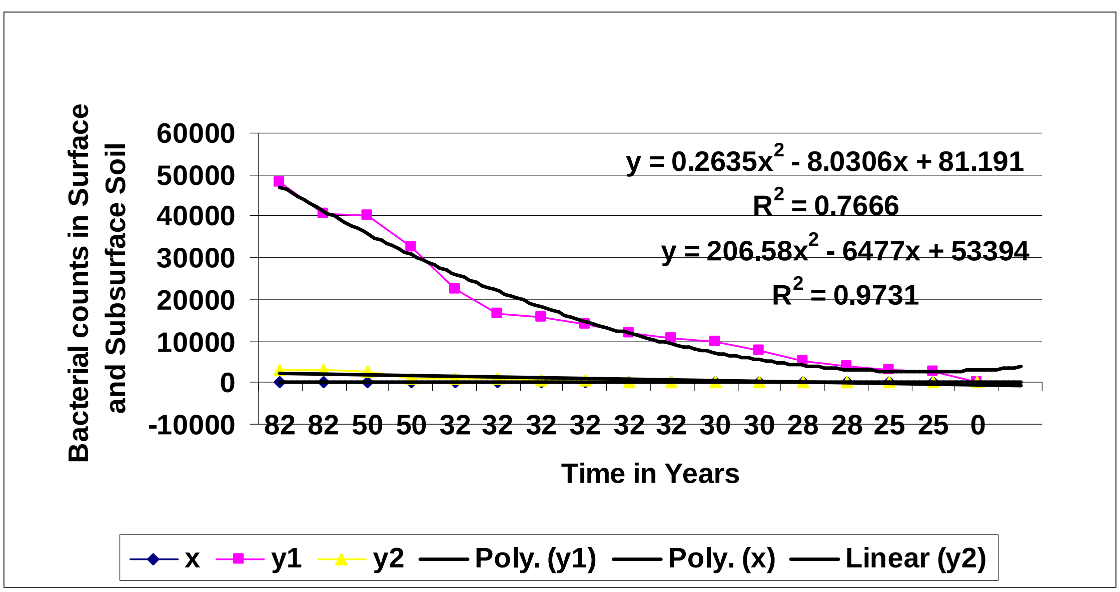

Figure 2. Changes in total bacterial counts in relation to the period of sewage farming

The same trend was noticed by Kelly and Tate (1998) who confirmed that sewage farming increased the microbial community size and heterotrophic activity in soil**,** and by Abd-El-Malek et al (1976) who recorded significant changes in the intensities of bacteria in El-Gabal Al-Asfer sewaged soils, the higher intensities the longer period of sewage farming.

Streptomyces behaved similarly as bacteria, their counts increased from $10x10^2$ in un-sewaged soils to $4600x10^3$ in soil irrigated for 25 year with sewage effluent at El-Gabal El-Asfer farm. As regarded fungi, they behaved almost the same as bacteria and streptomyces with no obvious changes in their counts. These results, however, go hand in hand with those previously found by Saber (1986) who found after 60 years of sewage farming that total bacteria increased from 5 to 620 $x10^5$, sreptomyces from 1 to $591x10^3$, and fungi from 8 to$127x10^3$.

Many researches suggested the activity of soil enzymes as a diagnostic index of soil contamination status. Casida (1977) and Pankhurst et al (1997) recommended the use of dehydrogenase technique as appropriate indicator in characterizing soil biological status because of its intimate relationship to soil micro-flora, ease to measure, and rapid response to changes in soil besides being similar to using Warburg respirometer. But, since soil enzymes differ in origin, function and location within the soil matrix and respond to different key environmental signals (Burns, 1982), it would be useful to condense the information they provide into a single numerical value.

Dehydrogenase values in different soils containing different biomass do not always reflect the total numbers of viable biomass isolated on a particular medium. It just could be regarded as indicative of the overall biomass activities in soil. Numerous studies confirmed correlations between dehydrogenase activity and several biomass parameters such as microbial numbers, soil respiratory activity, ATP concentration, other enzyme activities and soil organic matter content (Malkomes 1991).

Kinetic changes in dehydrogenase are a time dependent phenomena that describes the direction of change weather ameliorating or devastating soil ecosystem. Dadenko, (2006) mentioned that despite that the activity of all biological processes has seasonal dynamics; dehydrogenase appeared as the most dynamical one.

Data given in Figure (3) present the values of dehydrogenase activity in the different sewaged soil collected from both El-Gabal El-Asfer and Abu-Rawash sewage farm.

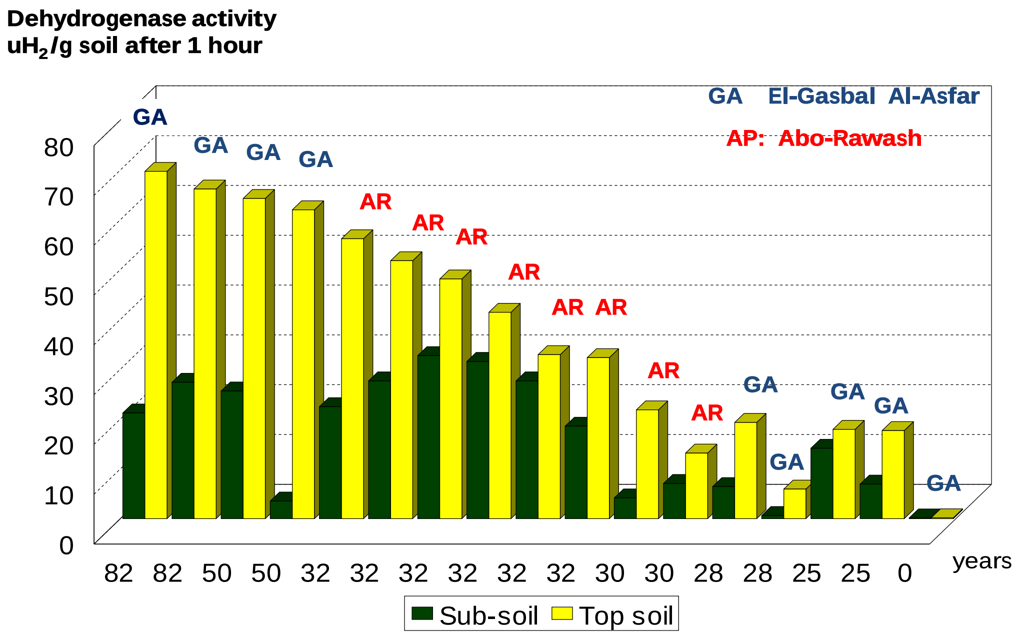

Figure 3. Changes in dehydrogenase activity in top and sub soil sewaged for extended periods under different landscapes

Sewage farming seemed to significantly enhance the biomass activity in soil, and hence their activities represented by dehydrogenase. The dehydrogenase activity increased from 0.18 in un-sewaged soil to 17.86 µl H_2 /gm soil /24 hours in soils sewaged for 25 years at El-Gabal El-Asfer farm. Dehydrogenase activity continued to increase, parallel to CO_2 evolution, as time of sewage faming goes on to a maximum level, in the investigated sewaged soils, reaching 69.72 µl H_2 /gm soil /24 hours after 82 years of sewage farming at El-Gabal El-Asfer farm **.**

In accordance with these findings**,** Dehydrogenase activity showed a strong relation with the changes recorded in bacterial counts in of the surface soil samples where R^2 equaled to 0.956, and followed a clear polynomial regression with an equation y=0.0245x2-4.7448x+77.536 (Figure 4). The same trend holds true for the dehydrogenase activity in the sub-surface soil samples where a significant relation with their bacterial counts were noticed, however, at smaller R^2 value equals to 0.3825 compared to that of the surface soil samples. The trend of the relation between dehydrogenase activity and bacterial counts in the subsurface soil samples followed the polynomial trend with the equation: Y=0.1669x2+1.587x +20.347 (Figure 5). The significant regression between the bacterial counts and dehydrogenase activity in surface soil might be attributed to the both relative higher organic matter content.

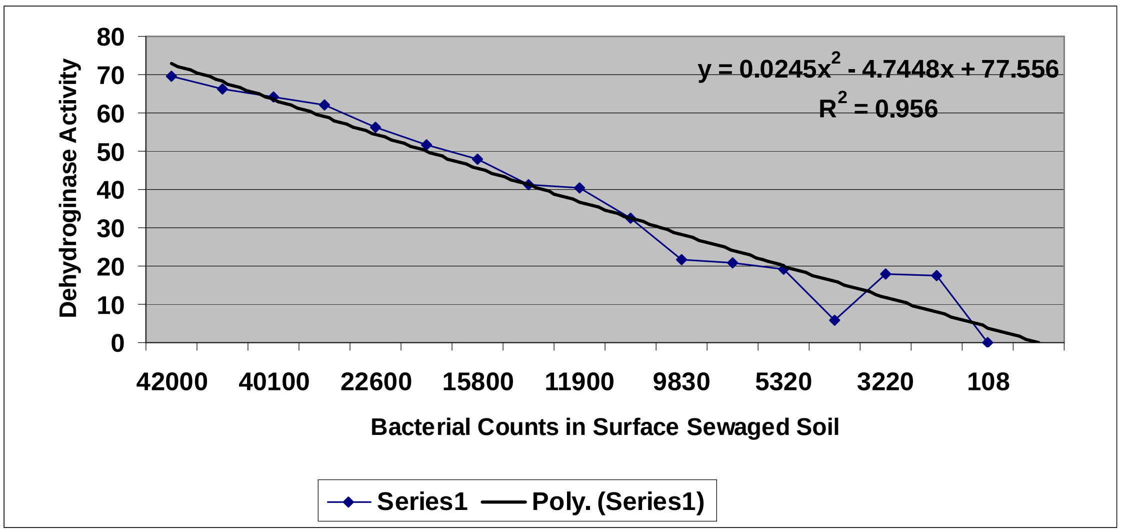

Figure 4. Relation between dehydrogense activity and bacterial counts in surface soil

Abd-El-Malek et al. (1976) noticed that prolonged sewage farming at El-Gabal El-Asfar farm, raised the dehydrogenase activity, which was found to be the most reliable parameter to reveal this fact**.** Batra and Manna (1997) measured the dehydrogenase activity in sewaged soils and found it to be negatively correlated with pH (r = -0.96) and EC_e (r= -0.767), while it was positively correlated with organic C(r = 0.812).

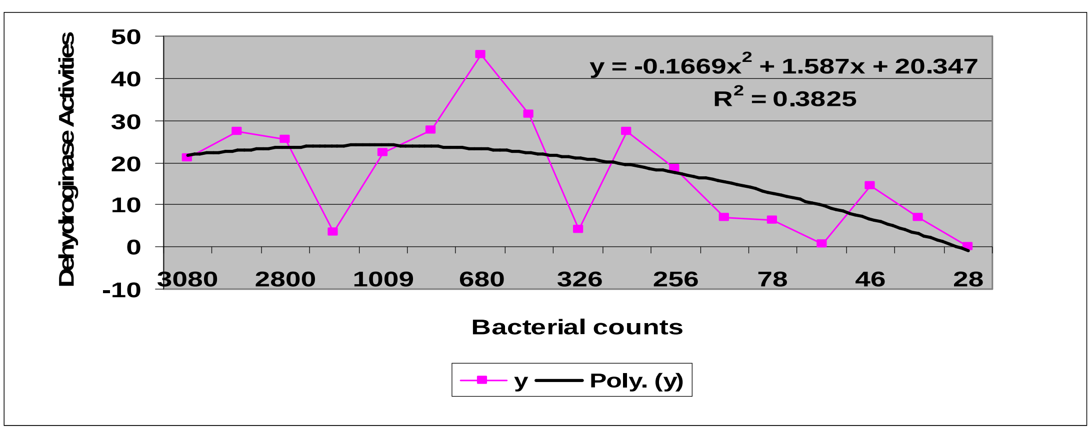

Figure 5. Relation between dehydrogense activity and bacterial counts in subsoil

The second parameter used to biologically characterize sewaged soil was carbon dioxide evolution as recommended by Masto et al (2009) who stated that it was found to be the most sensitive indicator. Results presented in Figure (6) present the values of CO_2 evolution from the different sewaged soil collected from both El-Gabal El-Asfer and Abu-Rawash sewage farms. Sewage farming seemed to significantly enhance the biomass activity in soil, and hence their activities represented by CO_2 evolution. The CO_2 evolution increased from 0.15 mg/day oven dry basis in un-sewaged soil to 0.23 mg/day in soils sewaged for 25 years at El-Gabal El-Asfer farm. The amounts of CO_2 evoluted from sewaged soils continued to increase, parallel to dehydrogenase activity, as time of sewage faming goes on from 0.15 mg/day oven dry basis to a maximum level, in the investigated sewaged soils, reaching 1.0 mg/day oven dry basis after 82 years of sewage farming at El-Gabal El-Asfer farm. Abd-El-Malek et al. (1976) found that prolonged sewage farming at El-Gabal el-Asfar farm raised the organic carbon, and the production of CO_2.

Evolution of CO_2 from surface and subsurface soil samples was strongly related with their recorded bacterial counts. The R^2 values in both surface and subsurface soil samples were 0.1968 and 0.7570 respectively after 20 days incubation period. In the surface soil samples, the relation between CO_2 evolution and total bacterial counts followed a polynomial regression at the different sampling intervals 10,20,30 days with the equations $Y=0.0153x^2+0.5679x+8.6065$, $Y=0.0114x^2-0.5374x+6.8562$ and $Y=0.0084x^2-0.5336x+7.9447$ respectively (Figures 6 & 7).

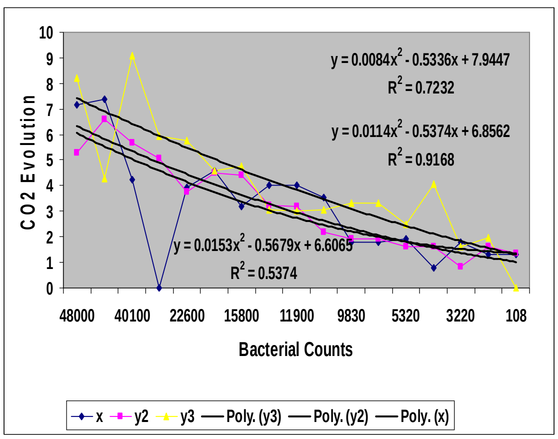

Figure 6. Relation between CO_2 evolution and bacterial **counts in surface soil**

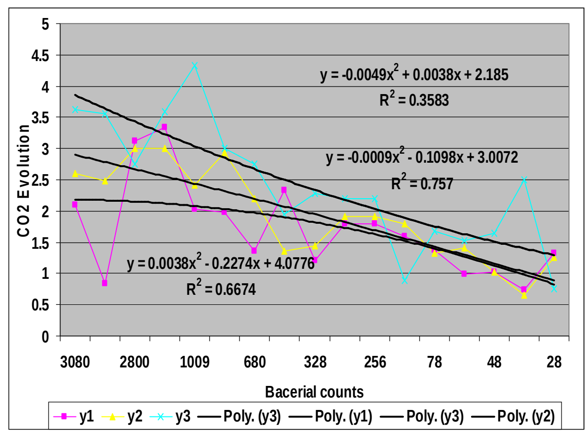

Figure 7. Relationship between CO_2 evolution and bacterial counts in subsoil

The relation between CO_2 evolution and total bacterial counts in the subsurface soil samples followed the same trend with the different equations $Y=0.0049x^2+0.0038x+ 2.185$ for CO_2 evolution after 10 days, $Y=0.0009x^2-0.1098x+3.0072$ for CO_2 evolution after 20 days and $Y=0.0038x^2-0.2274x+4.0776$ for CO_2 evolution after days 30 days.

DISCUSSION

Although sewage farming acclaimed us to accomplish vast paces towards evoking agricultural production, it heightened bio-antagonism, disfigured biomass and broadened contaminants in the soil ecosystem. These obstacles led to a severe unfriendly environmental aftermath, as well as to unsustainable agriculture.

One of the main targets of sustainable management of sewaged soil is improving their ecosystem for the sake of safe food production. This would never be achieved farewell as agricultural practices continue to exaggerate the level of biological and/or chemical contaminants which jeopardizes both wealthy agricultural and sustainability and necessitates evaluating their biological characteristics to install a new farming system committed to following environmental and sustainable approaches, and producing healthy food.

Despite being in the decade of biological credibility, several constraints are surrounding the notoriety of sustainable sewage farming. Sustainability means economic feasibility, appropriate technology and social commitment. Within this context, however, environmental merit is vital for sustainable sewage farming to survive. Sewage farming routines should be developed within the frame of the recent achievements in environmental biotechnology, particularly bioremediation which is increasingly acknowledged as a potential solution to copious problems overlaying present situation (Saber 2001). Intonations, in the present report, are being guided to perceive the effect of prolonged sewage farming on the biological characteristics of soil.

A viable and diverse microbial community had been considered as essential in sustainable agriculture (Kennedy & Smith 1995) as it acts as an early indicator of changes in soil processes (Islam & Weil 2000). The microbial biomass showed consistent intensities under the different landscapes. On the whole, biological indicators seem to be sensitive and detectable rather early in assessing the soil quality in comparable to the soil chemical and physical indicators (Karlen *et al.* 1999).

As far as our main concern in the present work is the bioremediation of both biological and chemical contaminants in sewaged soils, the action of tried bioremediation treatments on the soil biological characteristics should be assessed. For sure, bioremediation should not adversely affect the biological features of the sewaged soil. This, however could be followed through comparing the main biological features of the sewaged soil initially and after bioremediation

Gained results confirmed the presence of enteric pathogens in all sewaged soils. It seems reasonable to conclude that the potential transfer of enteric pathogens from sewaged soils to humans is of real concern under Egyptian conditions due to the existence of a broad range of pathogens in sewaged soils as showed in the results and the widespread use of manual labor on the land, having close contact with the sewaged soils, and relatively low standards of hygiene.

Since only a small portion of soil physiological biomass is culturable (Ward et al. 1990)**,** their counts were not used as a bio-indicator in the present work. On the other hand, the microbial biomass as represented by the total counts of bacteria, streptomyces and fungi was considered a highly sensitive bio-indicator for the biological characteristics of soil, within the frame that the higher the intensities the higher the bioactivity. In the present work, microbial biomass was markedly influenced with sewage farming, the longer the period of sewage farming the higher the counts of bacteria, streptomyces and fungi counted in sewaged soils.

Respiration is one of the essential roles dehydrogenase plays in the metabolism of soil biomass to gain their energy; hence it is largely linked with the rate of CO_2 evoluted from a given soil as evidenced in the present work and previously mentioned by Kelly et al (2003). The role of microbial biomass in formatting the soil biological characteristics might be confirmed with their functions as represented in the current work with both dehydrogenase activity and evolution of CO_2.

Soil enzymes are protein substances biologically secreted by biota having key biochemical functions, and are powerful tools applied in the assessment of short- or long-term changes in soil (Shukla and Varma 2011)**.**

There is growing interest in soil enzymes as early indicators of soil quality. However, despite there being an abundant literature on this subject, most comparative assessments are not as robust as desired. Garcı´a-Ruiza et al (2008) stated that soil properties based on biological and biochemical activities, especially those involved in energy flow and nutrient cycling, had been shown to respond to small changes in soil conditions, thus providing information sensitive to subtle alterations of soil quality.

From a kinetic perspective, measuring enzymes activities in soil provide a unique opportunity for an integrated biological assessment as sensitive indicators of soil ecological quality (Nannipieri et al. 2002). For that Yakovchenko et al. 1996 suggested that using soil enzyme activities as indicators of soil contamination would be acceptable together with other biochemical properties like CO_2 evolution to develop more complex expressions minimizing limitation of soil enzyme as a sole contamination indicator. In the present work both dehydrogenase activity and CO_2 evolution exhibited the same trends, i.e., the longer the sewage farming period the higher their values.

It seems reasonable to state that safe use of sewaged soils in agriculture necessitates continuous evaluation of their biological, hygienic, chemical and physical as well as aesthetical characteristic. It is well known that microorganisms respond quickly to varied environmental stresses, e.g., contamination as they have intimate relations with their surroundings due to their high surface to volume ratio. In most instances, changes in microbial populations or their activity could precede detectable changes in the soil's characteristics, thereby providing an early sign of soil contamination.

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