

MONITORING THE EFFECT OF COMPOSTED SEWAGE SLUDGE APPLICATION ON SOIL QUALITY AND SUNFLOWER BIOMASS PRODUCTION

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Abstract:

Soil is an important natural resource that needs to be preserved and its quality and productive capacity improved. Soil fertility decline is a major ecobiophysical problem confronting crop production in the world. Microbial activities are considered as early indicator of changes in soil properties resulting from soil amendment. This study was done for 15 weeks to evaluate the effects of composted municipal wastewater sludge (MWS) application on change in the dynamics of organic matter, soil microbiomes, enzymatic activities and sunflower growth in relation to organic matter content and nutrient release from MWS in chernozem meadow soil (Szeged, Hungary). Mineralized N in nitrate form was observed at high concentration in rhizospheric soil compared to non-rhizospheric soil, which indicate higher microbial and enzymatic and high mineralization processes. The results indicate that composted MWS improved soil properties such as pH, moisture, organic C, N, P and S content, and CO₂-C release. Soil amended with composted MWS rates of 30 and 60% exhibit higher in sunflower dry matter, microbial contents and enzymatic activities than the non-rhizosphere and virgin soil control. Also, application of composted MWS to agricultural soil as organic fertilizer is an environmentally sound technique.

Keywords:

Biological and physicochemical changes, municipal wastewater sludge, soil quality, sunflower, mineralization, rhizosphere

1 INTRODUCTION

Conversion of organic nitrogen (N), phosphorus (P) and sulphur (S) to available mineral forms of ammonium, phosphate and sulphate occurs through the microbial activity and the rate of conversion is influenced by factors affecting microbial activity such as temperature, moisture as well as pH by the N, P and S content of soil and decaying plant and animal residues. Microorganisms play a dual role in soil as agents of organic matter degradation with concurrent of nutrients and CO₂-formation as well as a labile nutrients pool. Roots may have a greater influence on soil processes than their pool size suggests due to microorganisms that live in rhizosphere. Microbial bioprocesses are important and essential for soil fertility and plant growth and development. Soil microorganisms mineralize, oxidize, reduce and immobilize mineral and organic matters (OM) in soil. Any changes in the soil may alter the number or the activity of soil microorganisms, which can affect soil biochemical processes and ultimately influence soil fertility and plant growth [1]. Soil microbial biomass is the living part of soil organic matter (SOM) and acts as an important ecological indicator as well as is responsible for the decomposition and mineralization of plant and animal residues in the soil. Thus, changes in soil microbial biomass may lead to the changes in important functions, such as OM decomposition and nutrient cycling. Because microbes are living, they respond more quickly to the changes in soil conditions than SOM [2].

Current interest in examine the soil quality has been triggered by increasing awareness of soil as a component of the biosphere. Crop responses to sludge application vary by source, application rate, plant species, soil type, climatic conditions, and management practices [3]. Soil quality is defined as “the capacity of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality and promote plant and animal health [4]. Several factors make soil quality very difficult to define, because soils are inherently variable [5]. There is growing recognition for the need to develop sensitive indicators of soil quality in promoting appropriate soil management strategies for long-term sustainability of terrestrial ecosystems. Soil quality influences agricultural sustainability, environmental quality and consequently plant, animal and human health. Historically, chemical and physical properties have been used as a crude measure of soil productivity. The evaluation of soil quality is quite complex and requires the consideration of physical, chemical and biological variables [6]. Nutrient cycling is a key ecosystem function and essential for the conversion of nutrients to plant available forms. Cultivation and grazing affect C, N, P, and S cycling in soils differently [7]. Relatively, little attention has been paid to S. Availability of organic and inorganic soil S to plants and microbes can both be controlled through enzyme activities. Aryl-sulphatase is commonly detected S-transforming enzymes in soil. Aryl-sulphatase catalyzes the mineralization of organic sulfur, which leads to the release of plant available inorganic S. Soil enzymes have been suggested as potential indicator or monitoring tools to assess soil quality and health also can effectively reflect the biological status of the soil. Soil dehydrogenases is a group of intracellular enzymes present in living soil microbes, regulating the metabolic reactions involved in oxidative energy transfer [8]. Many reports e.g., GARCÍA et al. [9] had found that soil enzymatic activities are enhanced by the addition of organic amendments. DE CAIRE et al. [10] mentioned that there is evidence that urease activity in soils can be increased by the addition of OM that promotes microbial activity.

2 MATERIALS AND METHODS

2.1 Soil characterization, sampling and amendment

The soil samples were collected from the top 20 cm of chernozem meadow from the virgin (not cultivated or treated) sampling area from Szeged, Hungary. The soil samples were divided into three sub-samples: virgin soil (control), virgin soil amended with MWS originated from the plant of wastewater treatment in Hódmezővásárhely, Hungary at 15, 30, 45 and 60% (w/w) rates and the third sub-sample is virgin soil samples amended with various MWS rates and cultivated with sunflower (*Helianthus annuus* L.) of 10 days seedlings old in plastic pots of 3 kg capacity. Table (1) represents some physicochemical properties of used soil and municipal wastewater sludge samples. Fifteen weeks after soil amendment and cultivation in greenhouse, plants were harvested and the following evaluations were carried out to study the effects of MWS on plant growth and soil quality.

Table (1) The physicochemical properties of used soil and municipal wastewater sludge samples

Parameters	Virgin sample of chernozem meadow soil	Municipal wastewater sludge
Clay and slit content (Li), %	51.7	n.d.
Soil plasticity (K _A)	53.07	n.d.
pH _(KCl)	6.02	7.8
Dry matter content, %	n.d.	42.9
Organic carbon content, %	2.87	20.4
Humus content, %	3.55	n.d.
Salt content, (%)	0.081	n.d.
CaCO ₃ , (%)	2	n.d.
Total N, mg/kg	334.7	43311
NO ₃ -N, mg/kg	39	n.d.
NH ₄ -N, mg/kg	4.5	n.d.
Mg, mg/kg	257	11860
Na, mg/kg	53	1441
AL-P ₂ O ₅ , mg/kg	378	20104
AL-K ₂ O, mg/kg	428	2908
Zn, mg/kg	1.1	1068
Cu, mg/kg	2.4	182.3
Mn, mg/kg	61	351.2
Fe, mg/kg	1094	13610
Cd, mg/kg	1.02	4.168
Pb, mg/kg	0.96	540.7

n.d.: Not determined,

AL: Ammonium lactate soluble P and K

The plants were collected, excess soil particles were removed and the roots were washed with sterile distilled water. For the following investigations, plants shoot and roots were carefully cut and the rhizospheric soil collected.

2.2 Plant biomass and total pigments content

The shoot and root dry weights of sunflower plants were measured after dried in a hot air oven at 70°C to fix weight. The Root:Shoot ratios (RSR) were calculated. Pigments were extracted by pounding fresh leaves of sunflower plants in 80% acetone, and absorbance of the resulting extracts was measured at 662 nm, 645 nm and 470 nm. Levels of chlorophylls and total carotenoids (xanthophylls and carotenes) were determined from the following equations given by LICHTENTHALER & WELLBURN [11] using UV-VIS spectrophotometer.

$$\text{Chl a} = (11.75 \times A_{662}) - (2.35 \times A_{645}), \text{Chl b} = (18.61 \times A_{645}) - (5.03 \times A_{662})$$

$$\text{Car} = [(1000 \times A_{470}) - (2.27 \times \text{Chl a}) - (81.4 \times \text{Chl b})]/227$$

2.3 Physicochemical properties

Soil pH was measured in 1 M KCl suspensions (1:2.5 w/v) with a glass electrode. Soil moisture was measured gravimetrically from drying soil sub-samples in an oven to constant weight at 105°C. The percentage of water holding capacity (WHC, %) was measured with known amount of soil and the

volume of water relationship, to give a condition of 45% of the maximum WHC following the protocol proposed by MISHRA [12].

Determination of soluble nutrient element content: According to the Hungarian Standard MSZ 20135/1999 [13], the method describes the procedure of determination of Na^+ , K^+ , Ca^{2+} and Mg^{2+} in soil using the atomic spectroscopic techniques.

Determination of total soluble heavy metal content: According to the Hungarian Standard MSZ 21470-50/1998 [14], the standard describes the procedure for determination of Fe^{2+} , Mn^{2+} and Zn^{2+} content in soil by atomic spectroscopic methods after $\text{HNO}_3/\text{H}_2\text{O}_2$ extraction/digestion in microwave.

Soil mineralization of organic C, N, P and S

Because the soil sub-samples had been stored at 4°C, soil sub-samples were pre-incubated as previously described to allow the microbial activity to restore and stabilize. Total organic carbon (TOC) content was determined by oxidation with potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) in a concentrated sulphuric (H_2SO_4) medium and excess dichromate evaluated using Mohr's salt [$(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$] according to WALKLEY & BLACK [15] and YEOMANS & BREMNER [16]. Organic carbon mineralization (C_{\min}) was evaluated by measurement of soil CO_2 respiration. The C_{\min} was expressed as mg $\text{CO}_2\text{-C}$ released per kg soil weight and incubation time.

Determination of N mineralization and nitrification rates: Total N content in soil was determined by Kjeldahl digestion–distillation procedure [17]. Potential net N mineralization and net nitrification rates were determined by aerobic incubation of the soils. Nitrogen mineralization (Available N: AN), which includes $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, was determined in 20 g of soil, which were extracted with 100 ml 2 M KCl for 1 h and filtered through Whatman quantitative filter paper. The soil clear filtrate was analyzed for $\text{NH}_4^+\text{-N}$ at 660 nm and for $\text{NO}_3^-\text{-N}$ at 540 nm using alkaline phenol according to CATALDO et al. [18] and DORICH & NELSON [19], respectively. The organic N mineralization rate was estimated by the sum of ammonification and nitrification rates. The C/N ration was computed by dividing the OC concentration by the TNC. The TOC, TNC, and AN records are presented on a soil dry weight basis. The net ammonification and nitrification rate was calculated as the difference of N-NH_4^+ and N-NO_3^- contents before and after incubation.

Phosphorus mineralization was determined according to methods for P extraction and analysis in soils as detailed by OLSEN and SOMMERS [20]. Sub-soil samples were extracted in dilute $\text{NH}_4\text{F-HCl}$ at the beginning and end of incubation period and the extracted P was estimated colorimetrically using the ammonium molybdate-stannous chloride blue colour method. Mineralized inorganic P was extracted with 0.5 M NaHCO_3 and was analyzed by the ammonium molybdate–ascorbic acid method described in biomass P measurement. Available extractable S was measured after the extraction with 0.01 M CaCl_2 and analyzed according to SUBBA RAO [21]. The $\text{SO}_4\text{-S}$ extracts were measured by converting them to barium sulfate by the addition $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ crystals; and the turbidity was measured at 340 nm on a UV-VIS spectrophotometer. Available S in soil sub-sample is expressed as $\text{SO}_4\text{-S}$ in mg/kg.

2.4 Basal soil respiration

To determine the soil respiration rates, 50 g of soil samples were placed in hermetically sealed glass bottle, moistened at 45% and incubated in the dark at 28°C for 10 days. The amount of OC released as CO_2 and absorbed in vials containing 10 ml of 0.5 M NaOH placed inside bottle. The CO_2 emitted was measured as the Na_2CO_3 formed by titration with 0.1 M HCl.

2.5 Soil microbial activities

Microbial characterization: Quantitative enumerations of mesophilic culturable populations of aerobic heterotrophs bacteria, fungi and actinomycetes were expressed by colony forming units (CFU). The populations were measured in 10 g of sieved soil was added to 90 ml of sterile saline solution (0.85%) in a 250-ml flask, and the suspension was shaken at 150 rpm for 30 min. Ten-fold serial dilutions were made and 1 ml of 10^{-4} , 10^{-5} , 10^{-6} and 10^{-7} dilution was used to inoculate Petri dishes of different cultural media. Plates were incubated at 28°C for 2, 7 days for bacterial and fungal cultivation, respectively. Bacteria were determined on nutrient agar medium supplemented by filter-sterilized cycloheximide (100 mg/ml final concentration) after autoclaving to prevent fungal growth. Fungal population was estimated on Rose Bengal-Streptomycin Agar [22] and modified potato dextrose agar (MPDA) supplemented with streptomycin (30 µg/ml) to inhibit bacterial growth according to ALEF & NANNIPIERI [23]. Actinomycetes were counted on Starch Casein Agar medium of KÜSTER & WILLIAMS (1964) supplemented by cycloheximide (100 µg/ml) and modified by addition of Nystatin and nalidixic acid which were used as antifungal and antimicrobial agent respectively in plates (WILLIAMS & DAVIES 1965). The plates were inverted and incubated for 7 days at 28°C. The results are reported as \log_{10} of bacterial, fungal or actinomycetes CFU/g of dry soil.

Determination of soil microbial biomass: The most common technique used to estimate microbial biomass C, N, P and S were measured by the chloroform fumigation-extraction method. The MBC (C_{mic}), MBN (N_{mic}), MBP (P_{mic}) and MBS (S_{mic}) were measured by ethanol-free chloroform fumigation-extraction method. Briefly, 30 g soil for each sample was fumigated with ethanol-free chloroform for 24 h at 28°C after one week incubation at 45% WHC. Simultaneously, another unfumigated set was prepared and incubated under the similar conditions. After complete removal of $CHCl_3$, organic C and N from fumigated and non-fumigated soil sub-samples were extracted with 0.5 M K_2SO_4 with a soil: extractant ratio of 1:5 (w/v), inorganic P was extracted with 0.5 M $NaHCO_3$ (pH 8.5) with a soil:extractant ratio of 1:20 (w/v). Phosphate was measured by photospectrometry at 882 nm as described by JØERGENSON et al. [26]. Also, S was extracted with 0.01 M $CaCl_2$ for 30 min on a rotating shaker [21]. MBC was calculated as: $MBC = E_C/k_{EC}$, where E_C = (organic C extracted from fumigated soils) – (organic C extracted from non-fumigated soils) and $k_{EC} = 0.38$ [27]. MBN was calculated as: $MBN = E_N/k_{EN}$, where E_N = (total N extracted from fumigated soils) – (total N extracted from non-fumigated soils) and $k_{EN} = 0.54$ [28]. MBP was calculated as: $MBP = E_P/k_{EP}$, where E_P = (total P extracted from fumigated soils) – (total P extracted from non-fumigated soils) and $k_{EP} = 0.40$ [29]. MBS was calculated as: $MBS = E_S/k_{ES}$, where E_S = (total S extracted from fumigated soils) – (total S extracted from non-fumigated soils) and $k_{ES} = 0.35$ [30]

Detection of phosphate-solubilizing microorganisms: One ml of homogenous soil sub-sample suspension of low dilution (10^5 , 10^6 and 10^7) was plated on the surface of the agar plate containing a medium described by PIKOVSKAYA [31] and according to the procedure of GOLDSTEIN [32]. After incubation for 5 days at 28°C, colonies surrounding with clear zones were counted. Colonies showing solubilization zones over 0.5 mm in diameter were counted.

Detection of cellulose-decomposing microorganisms: Microbial populations utilize cellulose as C source were detected by spread plate technique with 1 ml of soil suspension of low dilution (10^5 , 10^6 and 10^7) on carboxymethylcellulose (CMC) medium according to HENDRICKS et al. [33]. Plates were incubated at 28°C for 2 and 5 days. The CMC plates were flooded with Gram's iodine which formed a bluish-black complex with cellulose but not with hydrolyzed cellulose, giving a sharp and distinct zone around the cellulase-producing microbial colonies within 3 to 5 minutes. According to KASANA

et al. [34] this is more rapid and efficient method than Congo red. The clear zone formed by isolates is used as indicator for cellulase activity.

2.6 Determination of enzymatic potential activities

Hydrolysis of FDA was evaluated according to the methods of SCHNÜRER & ROSSWALL [35]. The enzyme potential activity is expressed as mg fluorescein /kg dry soil/h. **Dehydrogenase** was determined according to GARCÍA et al. [36]. **Urease** and N- α -benzoyl-L-argininamide (BAA) hydrolyzing **protease** were determined following the method of NANNIPIERI et al. [37]. Urease and protease activities are expressed as mg NH₄/kg dry soil/h. **Acid phosphatase** was determined by spectrophotometry at 398 nm [38]. The enzyme activity is expressed as mg PNP/kg dry soil/h. **β -glucosidase** was determined using p-nitrophenyl- β -D-glucopyranoside as substrate. The amount of PNP was determined in a spectrophotometer at 398 nm [38]. **Aryl-sulphatase activity** was measured colorimetrically according to at 420 nm [39] and is expressed as mg PNP/kg dry soil/h.

3 RESULTS AND DISCUSSION

The application of MSW to soil can increase OM [40], CEC [41] soil WHC [42], pH of acidic soils [40], and soil microbial [43] and enzymatic activities [42] in the soil and can decrease soil bulk density [44]. The present investigations confirm the recent results mentioned above. The importance of the reuse of sewage sludge in agriculture is derived from its high nutrient content that can improve the soil characteristics crop production. Accordingly, it is important to characterize the ecotoxicological potential of sewage sludge amended soil with such valuable properties. Greenhouse experiment was conducted to evaluate the effect of application of sewage sludge used as biofertilizer on the growth of sunflower plants with different sludge rates (0, 15, 30, 45, and 60%).

3.1 Changes in physicochemical properties

Our results clarified that MWS enhanced the OM and biological characteristics e.g., the OC content and soil respiration were found to be higher in the cultivated and uncultivated soil sub-samples in comparison with the biodynamically properties of the control virgin soil. Table 2 shows that the application of MWS to the virgin chernozem meadow soil sample provided a good environmental medium for plant growth by increasing the pH and moisture content over the virgin control samples. The increases in soil moisture content will reduce the amount of water used in irrigation and the pH ranged between 6.19 and 6.71 creating a favourable medium for promoting plant growth.

Table 2 Application of MWS changes the pH and moisture content of chernozem meadow virgin unfertilized soil samples.

Soil system	Various MWS rates (%)	pH _(KCl)	Moisture content (%)
Virgin unfertilized soil	0	6.02	100
Virgin soil amended with MWS rates (Non-rhizosphere)	15	6.19	118.1
	30	6.37	132.76
	45	6.50	152.5
	60	6.71	165.4

Our results are in an agreement with PRASANNA et al. [45] who pointed out those organic amendments are benefit for plant growth by increasing soil moisture holding capacity, improving soil texture, and providing plant nutrients such as nitrogen and phosphorus. Plant available macronutrients were affected by organic fertilizer application as the significantly higher of Na, K, Ca and Mg contents were found in soil under organic farming (Figure 1a and b). The addition of MWS to soil acts as nutrient reservoir. These nutrients released to the soil medium throughout the mineralization. The results showed higher nutrient contents of Na, K, Ca and Mg and essential elements (Fe, Mn and Zn) in MWS treated rhizospheric and non-rhizospheric soil sub-samples in comparison with untreated virgin soil (Figure 1a and b). The higher content of C, N, P, S and K indicates that OM can maintain the nutrients supply into MWS amended soil better than the untreated virgin soil.

In general, soil treated with MWS had better soil properties than untreated soil sub-sample. The MWS amended soil had OC, total N, available P and S content higher concentration than in the virgin soil sample. Organic fertilizer is also known as a slow release nutrient source, so the nutrients can be effectively used for plant uptake, preventing nutrient losses from soil. Our results indicated that the significantly higher of Fe, Mn and Zn, in the investigated soil samples may also due to the higher content and type of MWS use.

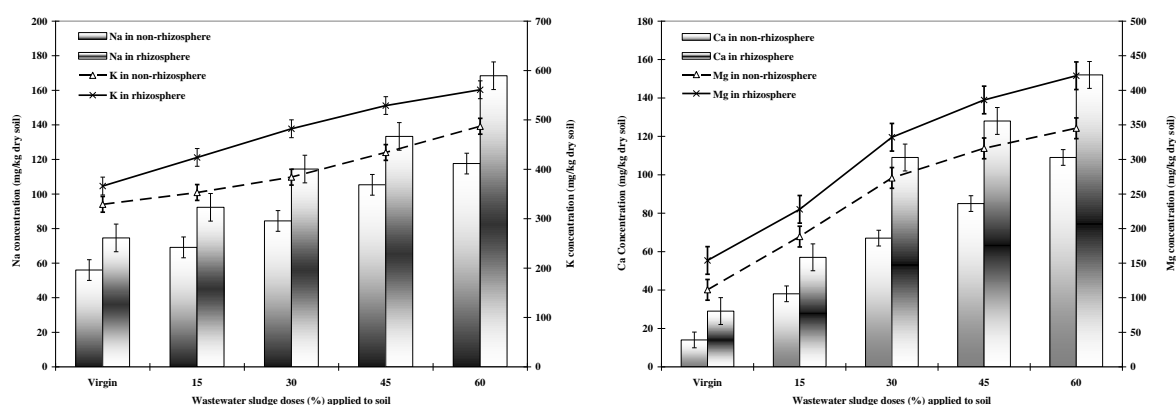


Figure 1a Effect of wastewater sludge on the soluble metal content in soil sub-samples

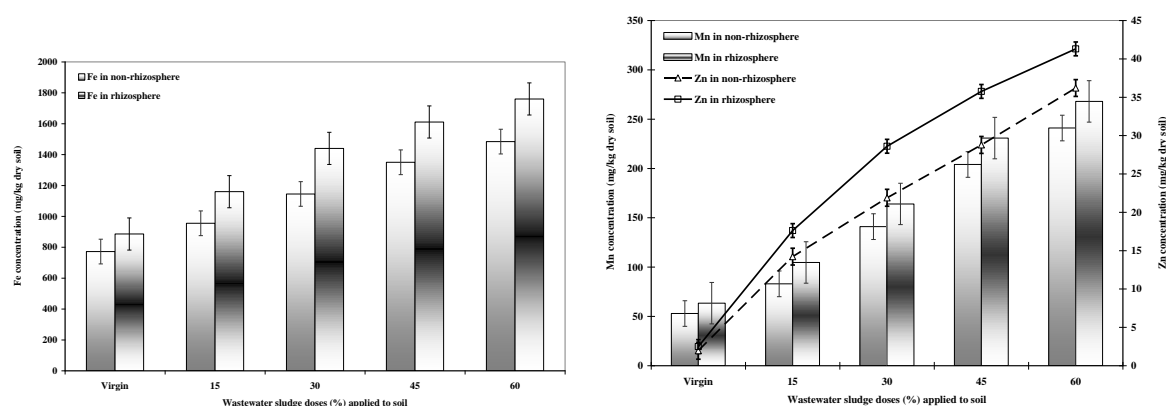


Figure 1b Effect of wastewater sludge on the soluble metal content in soil sub-samples

Soil pH decreased whereas electrical conductance, OC, total N, available P and exchangeable Na, K and Ca increased in soil amended with sewage sludge in comparison to unamended soil. Sewage sludge amendment led to significant increase in Pb, Cr, Cd, Cu, Zn and Ni concentrations of soil [46]

Due to our experimental work, we agreed with these results, but we found soil samples received MWS had increased their pH values of the soil sub-samples from 6.02 in virgin control sample to 6.19 (15%), and the highest was in soil samples treated with 60% MWS.

3.2 Plant growth

Figure 2. demonstrates that the growth of sunflower plants were influenced by the application of MWS doses which caused a marked increases in the plant dry weight, suggesting that the MWS contains a plant growth promoting elements. However, there was a tendency of R:S ratio to decrease as rates of MWS application increased (Figure 2a).

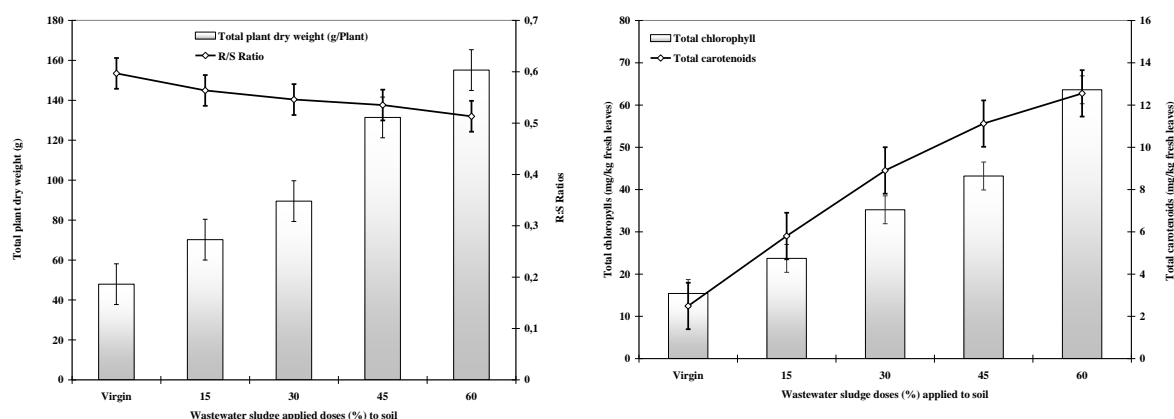


Figure 2 Effect of wastewater sludge on dry weight and pigmentation of sunflower grown in various soil sub-samples

Changes in chlorophyll content in leaves of sunflower plants were used as an early indicator to predict the effectively of application of different rates of MWS treatments. The obtained results indicate that the MWS soil amendment with different rates was significantly affected the chl a, chl b and carotenoid contents in the sunflower leaves (Figure 2). In spite of that, the carotenoids content significantly increased by 16 % in soil treated with MWS in comparison with control soil. Furthermore, under the highest MWS rate, a significant increase in the content of chlorophylls a and b and of carotenoids was noted.

3.3 Relationship between the microbial content and soil fertility

Microorganisms play a major role on decomposition of several organic compounds frequently used in agriculture, which directly affect the synthesis and decomposition of SOM [47]. In general, our results showed that higher microbial activity and potential activities of FDA and dehydrogenase occurred with organic fertilization throughout the application of MWS (Figure 3), these indicating the effects on the content and activity of soil microorganisms, which results in high nutrient cycling in the agroecosystem. The main microbial enzymes involved in the mineralization of SOM are cellulases, proteases, ureases and phosphatases [48]. Cellulase decomposers hydrolysed cellulose compounds present in fresh plant residues that are continuously deposited above soil [49]. Also, our results demonstrated that the quantitative counting of cellulose-decomposers in MWS amended soil was higher than those present in virgin soil samples (Figure 4).

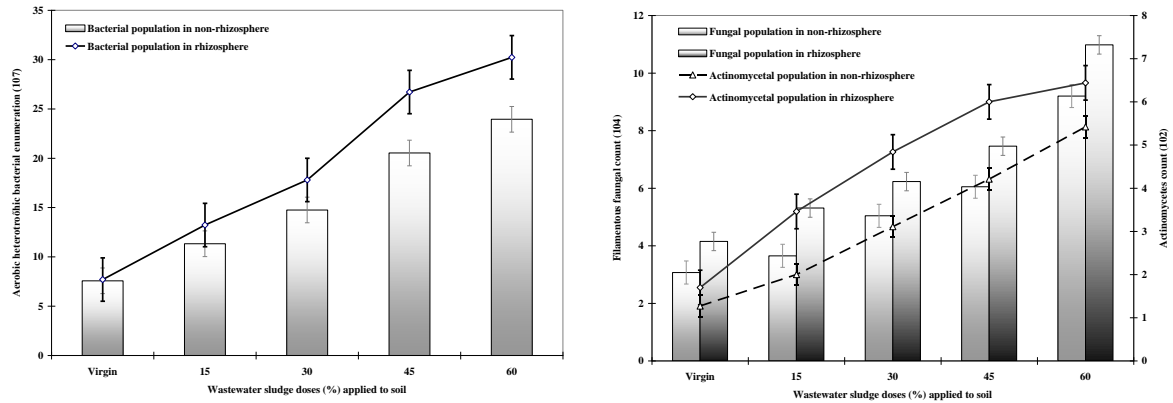


Figure 3 Effect of wastewater sludge on microbial content in soil sub-samples

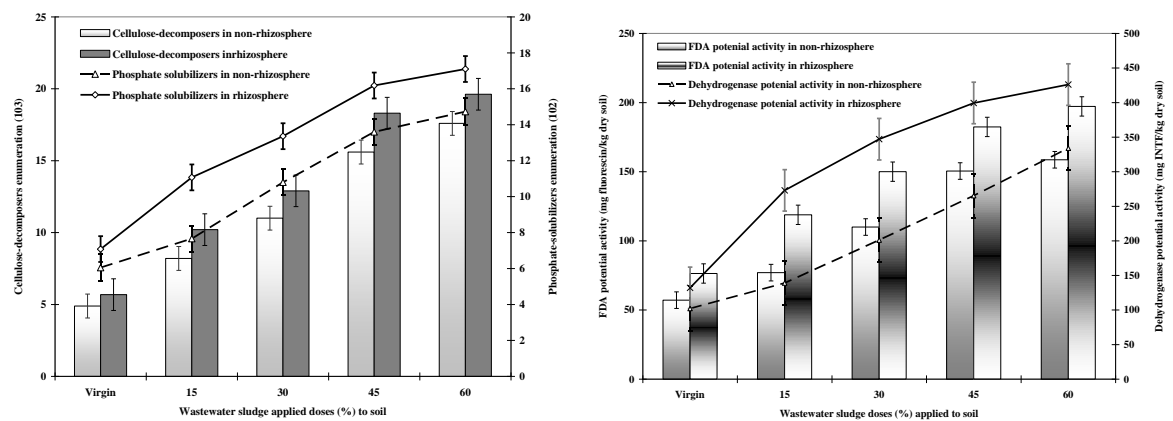


Figure 4 Effect of wastewater sludge on cellulose-decomposers, phosphate-solubilizers microbial content as well as the potential activities of FDA and dehydrogenase in soil sub-samples

Our results are agreed with SHENTU et al. [50] that soil biota is a significant component of soil quality as microorganisms play a vital role in soil ecosystem functioning related to soil fertility and primary production through OM decomposition and nutrient cycling.

3.4 Changes in microbial biomass C, N, P and S

Usually, larger differences were observed in the soil of higher MWS rate, for soil MBC and enzyme activities. Higher microbial activity was observed in the MWS soil cultivated by sunflower plants than in the uncultivated system (Figure 5). Dehydrogenase activity is directly linked with living cells associated with microbial oxido-reduction processes [51], which are important for OM degradation and transformation. Since, dehydrogenase activity is not active as extracellular enzymes in soil, it is considered to be a good indicator of overall microbial activity [36, 52]. Physiological and biochemical methods for estimating soil microbial biomass are usually calibrated against other methods and parameters. The estimation of microbial biomass can provide useful information on the changes in soil biological properties [53]. The strongest and most widely used calibration equations for quantifying microbial biomass are based on soil samples with a very wide range of biomass values [27, 54].

The potential activity of β -glucosidase in MWS managed (rhizospheric and non-rhizospheric) soil was higher than the virgin soil. MOESKOPS et al. [55] reported significant correlation of β -glucosidase

activity and SOC content with significantly higher β -glucosidase activity in organically managed soils. It has been shown that microbial activity and biomass is higher in fields with organic amendments than fields with conventional fertilizers [56]. Furthermore, the microbial biomass reflects the contribution of soil microorganisms as both a source and a sink of C in soil ecosystem [57]. Soil microbial biomass, both source and sink of available nutrients plays a critical role in nutrient transformation. As a consequence, our results are agreed with that the microbiological properties, such as soil enzymatic activities have been suggested as potential indicators of soil quality because of their essential role in soil biology, ease of measurement and rapid response to changes in soil management [58].

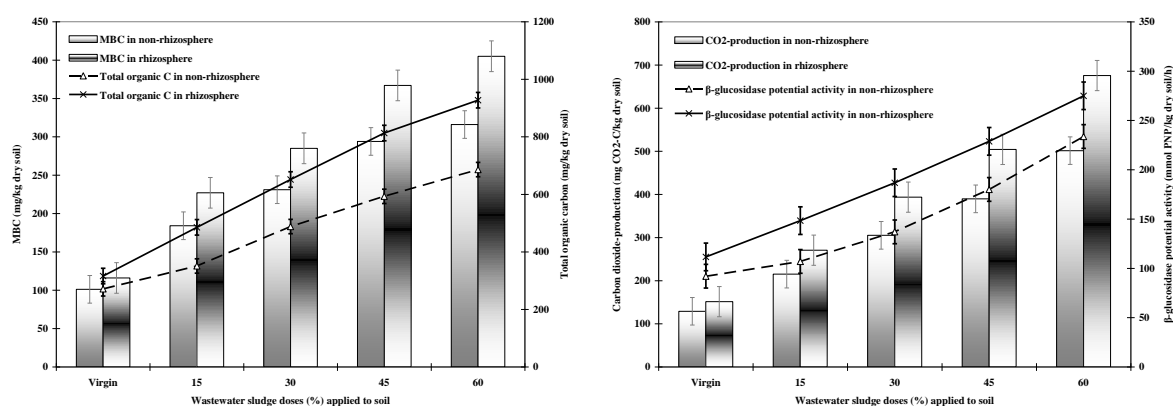


Figure 5 Effect of wastewater sludge on MBC, TOC as well as the potential activities of β -glucosidase and CO₂-production in soil sub-samples

Our results showed that the application of wastewater treatment increases the MBN, TNC and the potential activities of urease and protease, and these stimulation effects were increased by increasing the application dose of MWS (Figure 6) in comparison with virgin soil samples.

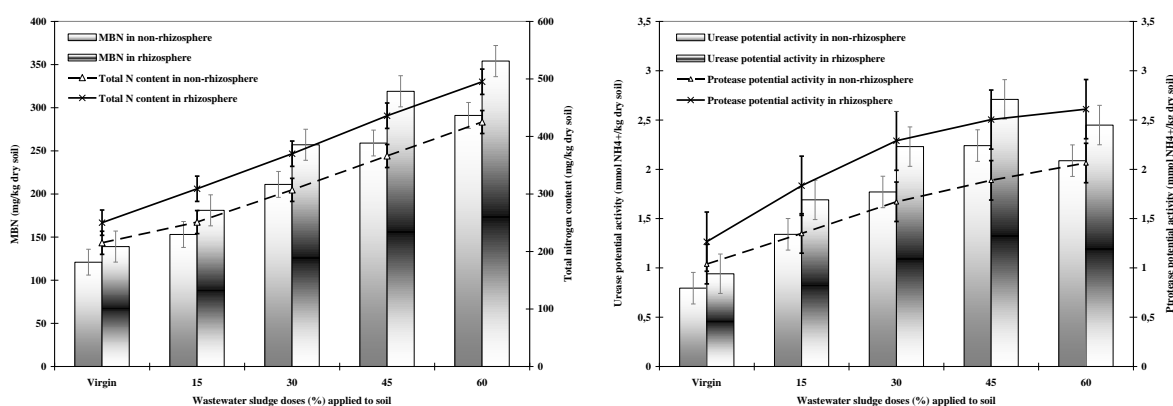


Figure 6 Effect of wastewater sludge on MBN, TNC as well as the potential activities of urease and protease in soil sub-samples

Our results are in an agreement with the recent studies [59, 60], that soil is a highly complex biological system which is subject to dynamic changes under the effect of biotic and abiotic factors. The use of microbiological and biochemical properties of soil for the estimation of changes taking place in soil environment as a result of e.g., application MWS is fully justified. Nitrogen fertilization is the most important management strategy for the improvement of agricultural crops. Urea is the most widely

used source of organic N fertilizer in the world, which is easily hydrolyzed to NH_4^+ and CO_2 by urease enzyme [61]. Organic N also affects directly the distribution and action of proteolytic enzymes in soils [62].

Figure 7 illustrates the effects of MWS on the MBP, TP, $\text{PO}_4\text{-P}$ and potential activity of phosphatase under different treatments. It is clear that the addition of MWS increases the P content in rhizospheric more the non-rhizospheric and virgin soil had the lowest content. The stimulation of the P content was influenced by the application dose, too.

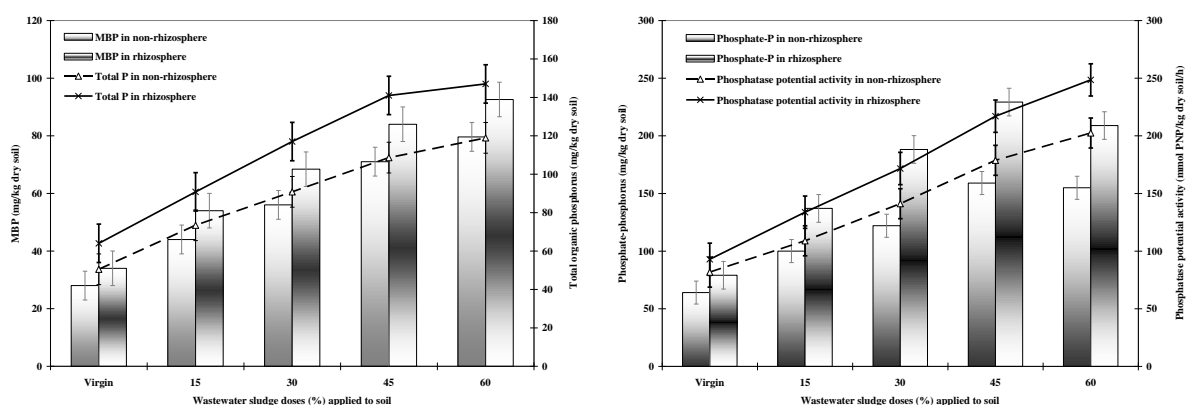


Figure 7 Effects of MWS on the MBP, TP, $\text{PO}_4\text{-P}$ and potential activity of phosphatase under different treatments.

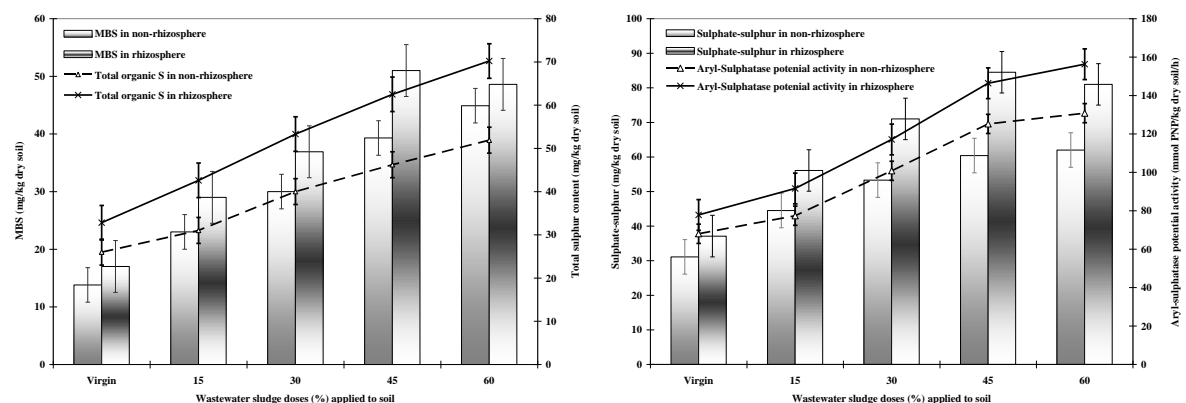


Figure 7 Effects of MWS on the MBS, TS, $\text{SO}_4\text{-S}$ and potential activity of aryl-sulphatase under different treatments.

It is well established that the bulk of the S present in surface soils occurs mainly in organic forms and that it becomes available for plant uptake only if it is mineralised to inorganic sulphate by soil microorganisms. Microbial S values showed direct relationships with both microbial C and with total soil organic S. Again, there were significant differences between non-rhizospheric and rhizospheric soil systems. This result is in agreed with BANERJEE and CHAPMAN [30].

The results indicated that the enzymatic activities tended to be higher in soil treated with composted MWS than in samples without application of organic amendment. Although, our conclusion is in agreement with VEPSÄLÄINEN [63] in which soil enzyme activities are commonly correlated with each other, it is advisable in soil quality studies to measure a pattern of several enzyme activities

simultaneously. Also, we are agreed with ROLDÁN et al. [64] that soil enzyme activity can be used as an indicator of soil quality for assessing the sustainability of agricultural ecosystems.

3.5 Net N mineralization and nitrification

The concentration of NO₃-N in virgin soil amended with MWS was significantly affected by the interaction of rate of MWS application. On the other hand, the control (virgin unfertilized soil) treatment produced the lowest concentration of soil NO₃-N, which was, however, not significantly different from that produced by the application of 15% of MWS (Figure 8). Soil NO₃-N concentrations measured at the end of the experiment, which can be considered residual concentrations, increased as MWS rates increased.

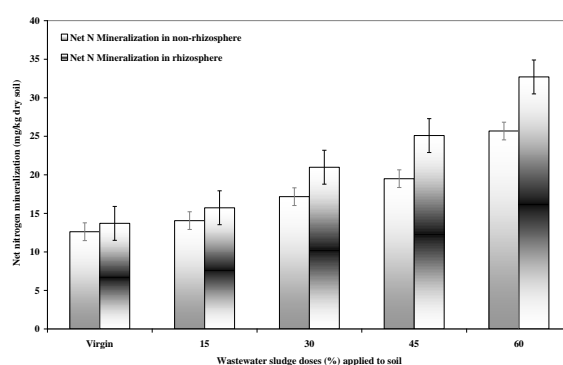


Figure 8 Effects of MWS on the net N mineralization under different treatments.

In this study, the increase in MWS rates produced increases in soil NO₃-N concentration in both uncultivated and cultivated virgin soil amended with different rates of MWS.

CONCLUSION

A substantial increase in the quantities of soil microbial biomasses and enzymatic activities take place in the mineralization of organic matter present in the wastewater sludge applied to the soil samples; however, there was a recovery of soil microbial biomass and enzymatic activities followed the use of soil organic fertilization practices. The biochemical indices selected in the research can provide a reliable and useful indication of soil biological quality of soil fertility after application of wastewater sludge.

A greenhouse pot experiment is described for studying the changes in soil biodynamics after the application of MWS. The result demonstrated that MWS have a great potential to enhance soil organic matter, nutrient availability, microbial activity, microbial biomass and enzyme production. Soil chemical and biological indicators of soil quality were generally higher in MWS systems compared to virgin soil system. High of soil enzymes activity and microbial biomass C as affected by organic matter input in MWS systems emphasize the important role of element cycling processes supported by an abundant and active soil biological community. The research study highlights that a portion of C, N, P and S were increased in soil amended with higher rates of MWS. In the rhizosphere of sunflowers a closed relationship between colonization densities of bacteria, fungi and actinomycetes and the activities of cellulose-decomposers and phosphate-solubilizers as well as enzymatic activities were observed. The increase in dehydrogenase activities in rhizospheric soil can be attributed to increase in microbial CFU. Microbial interactions in the MWS treated soil are the determinants of soil fertility

and environmental health, our understanding of these interactions therefore has implications for sustainable soil management. However, the subject of agroecosystem is very complex and need more research to understand the interaction between the plant, the rhizosphere environment, and nutrient availability.

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