

CHANGING IN ORGANIC CARBON DYNAMICS AND BIOLOGICAL ACTIVITIES IN CLAY LOAM BROWN FOREST SOIL BY APPLICATION OF MUNICIPAL SEWAGE SLUDGE

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Summary: A greenhouse pot experiment was conducted to assess the effects of two municipal sewage sludges (MSS) application on change in the dynamics of organic carbon, microbial population, enzymatic activities and alfalfa growth in relation to heavy metal content and nutrient release from sewage sludge in clay loam brown forest soil. The treatment of MSS increases soil organic matter and improves soil structure. Municipal sewage sludge of high heavy metal content (HHM) significantly decreased soil bioproductivity, reduced its biochemical properties, and lowered the microbial contents in comparison with MSS of low heavy metal (LHM) content. Total alfalfa dry matter yield and nitrogen content increases linearly with increasing MSS application rates. Soil treated with MSS rates of 40 and 60 % exhibit higher in crop dry matter, microbial contents and enzymatic activities than the control. Overall, our results demonstrate that soil treated with LHM content can improve soil quality and soil biological and biochemical properties.

Introduction

Soil organic matter (SOM) and specifically soil organic carbon (SOC) are known to play important roles in the maintenance and improve many physical, chemical and biological properties of soil such as soil structure, cation exchange capacity, buffer capacity and the water holding capacity. Since SOM consists of carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P) and sulphur (S), it is difficult to actually measure the SOM content and most analytical methods determine the SOC content and estimate SOM through a conversion factor. The amount of SOC that exists in any given soil is determined by the balance between the rates of organic carbon (OC) input and output. However, soil type, climate, management, mineral composition, topography, soil biota and the interactions between each of these factors are modifying factors that will affect the total amount of SOC in a profile as well as the distribution of SOC contents with depth. It is important to note that any changes made to the natural status of the soil systems will result in different conditions under which SOC enters and exits the system. Therefore, perturbed systems may still be in the process of attaining a new equilibrium C content and any measurements of SOC have to take into account that the soil is in the process of re-establishing equilibrium, which could take >50 years (BALDOCK and SKJEMSTAD 2002). Municipal sewage sludges (MSSs) increased organic matter (OM), total N, and available P in the soil and reduced soil acidity, with more evident effects at the highest rate, as well as it caused the most pronounced OM topsoil accumulation. Nevertheless, the disposal of MSS can often contain significant concentrations of organic and inorganic nutrients including N and P and offer a biologically valuable fertilizer. There is potential for these nutrients present in MSS to be used as a fertilizer source to increase the agricultural

productivity. Municipal SS is an environmentally sound practice sanctioned by many scientific research works (e.g., KRANERT et al. 2008; BARRAL et al. 2009, ROCA-PEREZ et al. 2009; BUNDELA et al. 2010; PAZ-FERREIRO et al. 2012; HERNANDEZ-SORIANO et al. 2013). Municipal SSs are not only the sinks for nutrients but also for hazardous substances. When MSS is used in agricultural practices these hazardous substances may have deleterious effects in soil environment depending on their concentration. An increase in microbial activity has been reported in soils amended with MSS. Within this system, microorganisms perform an important task in the decomposition and transformation of soil materials, and are involved in the biogeochemical cycles of C, N, S, P, etc. However, the interest in microbial functionality has grown in recent years to understand the relationship between microbial communities and their surrounding environment. Application of MSS in agricultural soils can directly improve soil physico-chemical properties and crop production. In relation to soil biological properties, numerous researchers have reported different effects of MSS on soil microbial biomass and activity (e.g., BARRAL et al. 2009, ROCA-PEREZ et al. 2009). The potential benefits of SS addition to soils are balanced by the risk of increasing soil heavy metals content (PAZ-FERREIRO et al. 2012). Depending upon the MSS source, it usually contains variable amounts of heavy metals (HMs) e.g., cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), zinc (Zn), etc. The low concentrations of these metals are essential for plant growth but repeated applications of metal concentrated MSS may lead to increase the level of these metals in soil. Also, MSSs often contain significant amounts of sorbents (e.g., oxides, organic matter, phosphates) or have favourable properties (e.g. pH) that reduce HM solubility and phytoavailability. BASTA et al. (2005) mentioned that SSs often contain up to 50% natural organic matter (NOM) and 50% inorganic mineral forms (e.g., iron, manganese, and aluminium oxides; silicates; phosphates; and carbonates). The sorbent phase responsible for reduction in HM bioavailability in SSs has been a matter of debate. Some authors suggested that HMs are sequestered in SSs mainly by chelation with OM, whereas others point out the importance of the inorganic surfaces in SSs on sequestering HMs (HETTIARACHCHI et al. 2003). According to ZUHAIRI (2003) batch sorption provides a quick method of estimating the maximal contaminant retention capacity of soil. Sorption of metals by soils is strongly influenced by various soil characteristics. For example, some studies have shown that the sorption of metals by soils tends to increase with increasing pH (VIOLANTE et al. 2010), OM (LAIR et al. 2007), cation exchange capacity (KWON et al. 2010), and the contents of iron (KARPUKHIN and LADONIN 2008) and manganese (BROWN and PARKS 2001) oxides. MANTOVI et al. (2005) stated that significant accumulations of total Zn and Cu were detected in amended topsoil, but no other heavy metals (Cd, Cr, Ni, Pb), whose total concentration remained well below the hazard limits.

Soil fertility fundamentally, it is determining the productivity of all farming systems, which is mostly defined in terms of the ability of a soil to supply nutrients to crops. WILD (1993) defined it as the ability of soil to produce crops. Similarly, SWIFT and PALM (2000) suggested that it is helpful to view soil fertility as an ecosystem concept integrating the diverse soil functions, including nutrient supply, which promote plant production. This definition is appropriated to organic farming, as organic farming recognises the complex relationships that exist between different system components and that the sustainability of the system is dependent upon the functioning of a whole integrated and inter-related system (ATKINSON and WATSON 2000). DORAN and SAFLEY (1997) initially distinguished

between “soil quality” and “soil health” before inclusively using the term “soil health” and defining it as “the continued capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain biological productivity, promote the quality of air and water environments, and maintain plant, animal and human health”.

Currently, the use of MSS to improve the nutrient contents of the soil is a common practice. Contamination of soils by potentially toxic elements (e.g., Cd, Ni, Cr, Pb) from amendments of MSS is subject to strict controls in relationship to total permissible metal concentrations, soil properties, and intended use within the European Community. The biosolids incorporation has modified the soil composition, leading to the increment of HMs (JORDÁN et al. 2009). MANTOVI et al. (2005) mentioned that the considerable amount of data on the use of MSS in agriculture that was collected at the end of the 20th century (PAGE and CHANG, 1994) has led to strict American and European regulations, USEPA 40CFR 503 and 86/278 EEC, respectively, aimed at the safe recycling of this material and an enhancement of its characteristics (McGRATH et al. 1994).

The concentration of HMs and other pollutants in the MSS determines the acceptability for soil application and the appropriate loading rates to protect crops and soils environment. It has been demonstrated that HMs adversely affect biological functions in soil, including the size, activity and diversity of the soil microbial community (CHANDER et al. 2001), and the activity of enzymes involved in biotransformation (BELYAEVA et al. 2005). Heavy metals in MSS seemed interesting to determine their total effect on organic matter (OM) mineralization and microbial activities in the soil.

Microbial biomass carbon (MBC) is a standard technique used to determine the effect of toxic substances on the soil microbial community (STOCKDALE and BROOKES 2006). Assay of soil respiration also helps to quantify the effects of metals on the total biological activity of soils. Additions of HMs to soils usually cause an immediate decrease in respiration rates, but long-term responses are determined by the properties of both the metal and the soil (NWUCHE and UGOJI 2008). The increase in microbial activity is ascribed to the soil enrichment of sources of C and nutrients stimulating the soil microbial populations (DIACONO and MONTEMURRO 2010). The activity of soil microorganisms is measured either by conventional plate count technique or by soil respiration (CO₂) and microbial biomass (JENKINSON 1988). Various measurements have been used as indices of microbiological activity in soils (NACHIMUTHU et al. 2007). These include basal respiration, substrate induced respiration, metabolic quotient, microbial biomass, inhibition of substrate-induced respiration by streptomycin sulphate (bacterial inhibition) and actidione (fungal inhibition) and enzyme activities.

ARSHAD et al. (2011) established that crop rotations and reduction in tillage are commonly recommended for sustained crop production and enhancing soil quality. The MBC followed the trend among treatments in whole and light fraction OM, and total extracted sugars. BHATTACHARYA et al. (2001) studied the dynamics of soil quality indicators, such as MBC, soil respiration, urease and acid phosphatase activities in soil amended with different doses of Calcutta municipal solid waste compost (MSWC) over 120 days of incubation at 30 °C under 60 % soil water holding capacity. These parameters were found to increase with the increase in doses of MSWC. Authors indicated that soil MBC and soil respiration activity reached peak values at 30 days of incubation and then gradually decreased up to 120 days of incubation. Also, urease and acid phosphatase activities showed peak values at 60 and 90 days of incubation, respectively. The increase

in soil enzyme activities with the fall in MBC suggested that the release of enzymes was associated with lysis of microbial cells at the end of their life cycle. There was no indication of any detrimental effect on soil quality indicators of application of MSWC to soil. The objective of this study was to examine the effect of various MSS application on change in the dynamics of organic carbon, microbial population, enzymatic activities and alfalfa growth in relation to heavy metal content and nutrient release from MSS in clay loam brown forest soil environment.

Materials and methods

Soil characterization, sampling, and amendment

The soil used in pot experiment was clay loam brown forest collected from farmland surface layer (0–200 mm) of an agricultural area of Gödöllő, Hungary in May 2007. Two different MSSs were selected depending on their HMs content. One is characterized as low (L) and the second is high (H) HMs content. The main physico-chemical parameters of soil and MSSs are shown in Table 1. Fresh soil was sieved through a 4 mm sieve and mixed with MSSs to form 0, 20, 40, 60 and 100% (soil : sludge; w/w), and then placed into pots with 42 cm in height and 23 cm in diameter. All treatments were designed in triplicates and submitted for statistical analysis.

Table. 1 The physico-chemical properties of used soil and municipal sewage sludge samples
1. táblázat A vizsgált talajok és települési szennyvizek fiziko-kémiai tulajdonságai

Parameters	Clay loam brown forest soil, Gödöllő	Municipal sewage sludge, Nyíregyháza	
		LHM - NySzv III.	HHM - NySzv IV.
pH _(H2O)	4.72	7.99	7.17
Dry matter content %	–	74	70
Organic matter %	–	25.6	48.2
Humus content %	1.24	–	–
Total N content mg·kg ⁻¹	8,411	75,700	98,900
NO ₃ -N, mg·kg ⁻¹	133.08	–	–
NH ₄ -N, mg·kg ⁻¹	410.69	–	–
Ca, mg·kg ⁻¹	856	5707	29724
Mg, mg·kg ⁻¹	203	2810	5072
Na, mg·kg ⁻¹	21	1290	1349
P ₂ O ₅ , mg·kg ⁻¹	121.31	9700	9100
K ₂ O, mg·kg ⁻¹	107	3120	3596
Zn, mg·kg ⁻¹	38.1	453	134
Cu, mg·kg ⁻¹	22.9	100	161
Mn, mg·kg ⁻¹	136	309	497
Fe, mg·kg ⁻¹	1187	11153	14802
Cd, mg·kg ⁻¹	0.18	1	2.4
Cr, mg·kg ⁻¹	–	34	46.4
Ni, mg·kg ⁻¹	–	15	39.2
Pb, mg·kg ⁻¹	15.1	30	83
As, mg·kg ⁻¹	7.4	0.6	6.8
Hg, mg·kg ⁻¹	–	0.04	2.8

Pots were incubated in greenhouse at Szent István University (Gödöllő), with 22–25°C average temperature. Distilled water was added to each pot to bring the moisture content of 45% water holding capacity throughout the plantation period. Pots were seeded with 3 alfalfa (*Medicago sativa* L.) seedlings of 15 days old. After 84 days of plantation, soil sub-samples were assayed for the following investigations:

Dynamics of organic carbon

Total organic carbon (TOC) was analyzed by dichromate ($K_2Cr_2O_7$) oxidation and titration with ferrous ammonium sulphate (WALKLEY and BLACK 1934). The water soluble C (WSC) contents of the liquid fractions were determined spectrophotometrically at 590 nm after addition of $K_2Cr_2O_7$ and H_2SO_4 (digestion at 150 °C for 15 min) according to SIMS and HABY'S (1971) method. The hot-water extractable C (HWEC) fraction of SOM can be determined quickly by simple analytical method. The HW extraction technique delivers a fractionation according to turnover rates of SOM by keeping a soil:water mixture (1:5 w/v) for 60 min under reflux (SCHULZ and KÖRCHENS 1998).

Evaluation of soil respiration

To determine the soil respiration rates, 500 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 organic C 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 in the presence of phenolphthalein indicator until a colourless end point was reached.

Measurement of microbial biomass carbon

Microbial biomass carbon content was determined by chloroform fumigation extraction of the sample with ethanol-free chloroform and C concentration in the extract was measured by the extraction of both fumigated and unfumigated samples with 0.5 M K_2SO_4 , according to VANCE et al. (1987), and a K_{EC} value of 0.45 was used to convert the measured flush of C to MBC (YAO et al. 2003). So, MBC was calculated as follows: $MBC = EC/K_{EC}$, where $EC = (OC \text{ extracted from fumigated soils}) - (OC \text{ extracted from non-fumigated soils})$ and $k_{EC} = 0.45$.

Determination of enzymatic activities

Fluorescein diacetate (FDA) hydrolyzing activity of the control and amended soil sub-samples were determined by measuring the released fluorescein at 490 nm according to ALEF (1995). Dehydrogenase activity was determined by the method of GARCÍA et al. (1993). Urease activity was determined in 0.1 M phosphate buffer at pH 7; 1 M urea and 0.03 M N α -benzoylargininamide was used as substrate. The activity was determined by the NH_4^+ released (NANNIPIERI et al. 1980). Phosphatase and β -glucosidase activities were determined using *p*-nitrophenyl phosphate disodium (PNPD, 0.115 M) or *p*-nitrophenyl- β -D-glucopyranoside (PNG, 0.05 M) as substrates, respectively (MASCIANDARO et al. 1994). Similarly, aryl-sulphatase activity was determined as proposed by TABATABAI and BREMMER (1970), after the soil incubation with *p*-nitrophenyl sulphate and measured at 400 nm.

Microbial population in rhizosphere of alfalfa

The enumeration of microbial population in rhizosphere of alfalfa was done using the serial dilution plate method. The total colony forming units (CFU) of bacteria and fungi were recorded on Ken Knight and Munaier's agar (ALLEN 1959) and Martin's Rose Bengal agar (MARTIN 1950) media, respectively. Enumeration of cellulose decomposers was determined according to HENDRICKS et al. (1995). For phosphate solubilized microorganisms, method of GOLDSTEIN (1986) was applied. The plates were incubated at 28 °C and microbial population densities were calculated and expressed as \log_{10} of CFU $\times 10^n$ g⁻¹ air dried soil, where 10^n was dilution factor.

Plant biomass and total nitrogen content

At harvest, plants were dried at 75°C and ground in a stainless steel Wiley mill. Total nitrogen content in soil was determined by Kjeldahl digestion–distillation procedure (KEENEY and NELSON 1982).

Results and discussion

Continuous application of MSS in agriculture increases the concentration of HMs in soil and HMs-rich MSS drastically reduced the yield of some crops after a critical amount of MSS applied to the soil. These critical limits depend on the MSS source, application rate and frequency. Phyto- and rhizobioremediation using plants and related microorganisms are the promising approach to clean up the HMs contaminated environment. The results of pot experiment illustrated the followings:

Dynamics of TOC, CO₂-C and MBC

There were great variations within the soil treated with different MSS types and concentrations regarding to the dynamics of TOC, CO₂-C and MBC. Due to these, the stability of OC in soil sub-samples was differed. The results of the present study showed that CO₂-C and TOC content of the soil increased with the addition of MSS (Figure 1a); especially at higher rates of low heavy metals content of used MSS (LHMs-MSS). The CO₂-C values ranged from 1.45 to 9.45 mg CO₂-C kg⁻¹ soil. So, soil respiration (expressed as mg CO₂-C released kg⁻¹ soil) is a useful index for measuring soil microbial activity. The WSC ranged from 99 to 743 and HWSC ranged from 111 to 507 mg kg⁻¹ soil (Figure 1b). The TOC values were suffered great variations during the plantation period. The addition of the MSS, regardless of their HMs concentrations, increased the WSC and HWSC content in the soil (Figure 1b). A higher WSC fractional value was detected in the soil amended with higher rates (Figure 1b). The C content in the HWS fraction of SOM is a simple determinable and suitable parameter for estimating the supply of soil with decomposable SOM. Immediate and significant increases upon TOC and CO₂ release, due to the presence of SOM in the amendments. However, these parameters return quickly to background level, as soil microorganisms rapidly mineralize the added OM in the form of MSS. The results indicated that LHMs-MSS application to soil stimulates the growth of soil microbial content, probably due to the OC and other nutrients comparing with the control of MSS unamended soil. The present investigation observed significant increases in microbial activity as measured by CO₂ release as a function of increased doses of

applied MSS and agreed with SAHA et al. (2010). In case of applying MSS with high HMs (HHMs) content, our results are in agreement with many authors e.g., PECKENHAM et al. (2008), HAYNES et al. (2009) and LI and ZHOU (2010) who reported that DOC as an important contributor to the elevated mobility of HMs in soils amended with MSSs. Due to a close, linear and positive relationship exists between the OC and MBC contents, the results indicated that MBC (values ranged from 156.1 to 430.1 mg kg⁻¹) was lower in HHMs-MSS content than in LHMs-MSS content (Figure 2). Soil microbial diversity is a crucial measure of sustainable soil ecosystems.

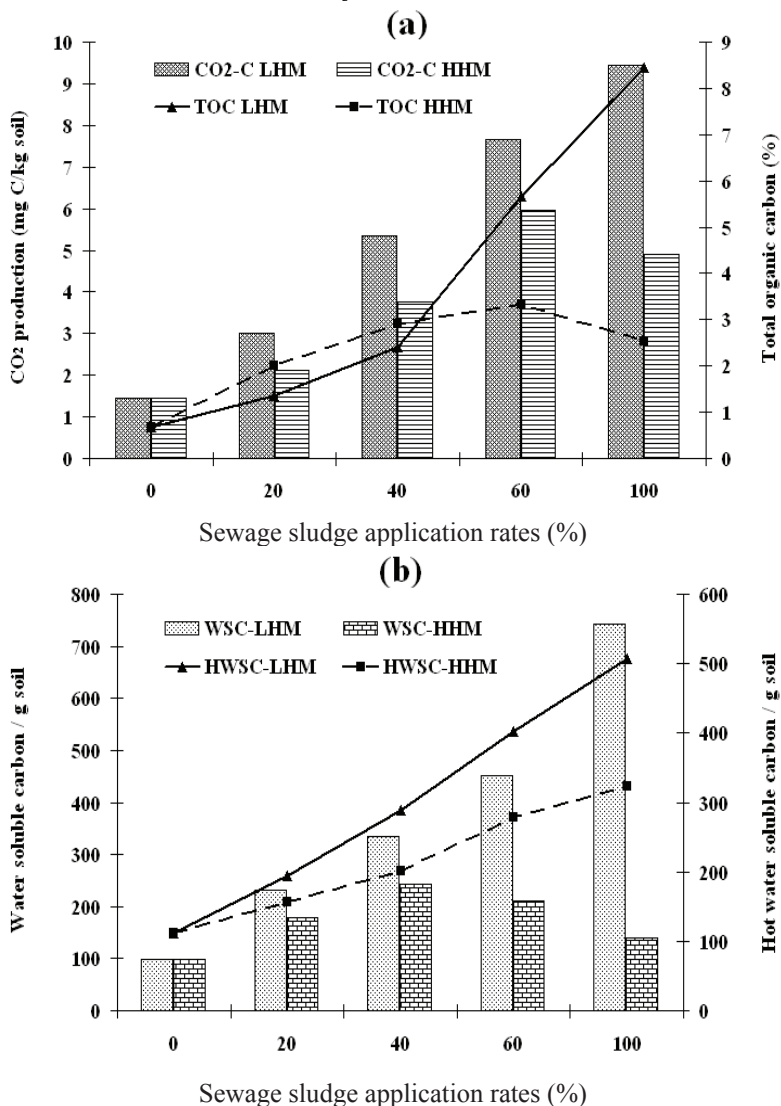


Figure 1. Sewage sludge applications influence soil respiration (a) and total organic carbon (b) using water and hot water soluble carbon methods

1. ábra Az alkalmazott szennyvíziszapok hatása a talajlégzésre (a) és az összes szerves szénre (b) vízben és a forró vízben oldódó szénmeghatározás módszerével

Microorganisms in a soil form part of the biomass and contribute to the reserve of soil nutrients and are generally referred to as the microbial biomass (INSAM 1990). Soil pollution by HMs is a serious worldwide problem and can be potentially harmful to human health via the food chain. Heavy metals reach the soil environment through both pedogenic and anthropogenic processes. Heavy metals mostly occur naturally in soil parent materials, chiefly in forms that are not available for plant uptake (BOLAN et al. 2008).

Soil OC plays an important role in soil biological, chemical and physical properties. In fact, these properties are considered critical indicators for the health and quality of the soil. The results obtained in the present study are similar to those by BANERJEE et al. (1997), who verified that the application of MSS favoured an increase in microbial biomass, as well as the activity of soil microbiota, probably by the presence of OM in MSS. Soil respiration mainly comes from microbial respiration, which is the major product of aerobic catabolic processes in the C cycle and is used as an indicator of total soil microbial activity. Results indicated that application of MSS increased the soil microbial biomass and the activity, probably because the activity of soil microorganisms was stimulated by the presence of OM in the MSS. The MBC and FDA (Figure 2) illustrated more accurate indicators of variations in soil quality than other soil C fractions. The MSS application to the soil of increased soil MBC, which can be attributed to the incorporation of easily increase the biological activities indicated by the high values of FDA and then increases the biodegradable OM and to the MB contained in the MSS.

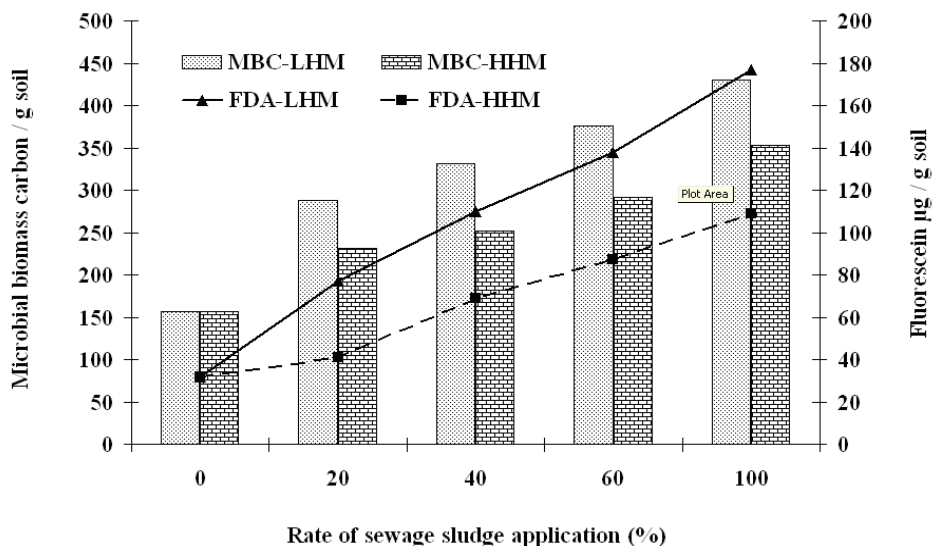


Figure 2. Effects of sewage sludge applications on the microbial biomass carbon (MBC) and the total biological activity detected by FDA method

2. ábra Szennyvíziszap alkalmazások FDA módszerrel kimutatható hatása a mikrobiális szén biomassza mennyiségére (MBC) és a összes biológiai aktivitásra

Our results are in agreement with CHANDER et al. (1995) when the values of MBC in the soil amended with the high mixing rate of MSS were lower than those in the soil

amended higher, indicating that the high level of HMs content which affected MBC. It seems that at a low rate, the positive effects of the OM added did not counterbalance the significant effect produced by the higher MSS rates. The subsequent slight recovery in MBC values was probably due to the microorganisms (mainly bacteria) becoming adapted to the high OM concentrations. In comparatively, soil amended with HHM-MSS reduced significantly the values of MBC. Our results are confirmed that the increase in microbial activity is ascribed to the soil enrichment of C sources and nutrients stimulating the soil microbial populations (DIACONO and MONTEMURRO 2010) and EMMERLING et al. (2000) and LEITA et al. (1999) were explained that an increase in the content of SOM and nutrients stimulates microbial activity and also produces greater biomass, thus leading to more respiration.

Changes in soil enzymatic activities

Increasing the enzymatic activities in the alfalfa rhizosphere with the MSS application rate is probably due to the addition of labile C supplied by MSS, indicating that the microbial biomass is active and potentially mineralized OM. The activity of soil enzymes are used as indicators to reflect the microbial activity and react quickly to environment change. Figures 3a and 3b show the evolution of five enzymatic activities measured during the experimental period.

The results showed that elevated MSS mixing rate differently impacted on soil enzyme activities. Increases of FDA (Figure 2), phosphatase, β -glucosidase and aryl-sulphatase activities (Figure 3a), urease and dehydrogenase (Figure 3b) under addition of MSS occurred with increasing the application rate. These increases were more significant in soil amended with LHMs than with HHMs content compared to control.

Dehydrogenase activity has been proposed as an indicator of the total metabolic activity of soil microorganisms (SKUJINS 1976). In our experiment, this enzymatic activity increased by the addition of MSS to the soil and remained stable throughout the subsequent incubation time (Figure 3b). It is important to be pointed out that the stimulation of the dehydrogenase activity generated by the addition of more MSS and was lower by MSS of HHM content, suggesting that MSS had an effect on the synthesis of this endocellular enzyme, similar to its effect on the MBC depending up on the levels of toxic elements. The high level of MSS significantly increased, soil urease activity and the values of which remained higher in the amended soils than in the control throughout the plantation period (Figure 3b), probably due to the formation of urease-humus complexes that stabilize this enzyme in the soil. This result is in agreed with the observation of NANNIPIERI et al. (1996). The fact that the activity of urease was always higher in the soil amended with MSS suggests that higher rates of LHM content have a positive effect on this enzymes activity. The activity of different hydrolases related with the C (β -glucosidase), N (urease), and P (alkaline phosphatase) cycles and with the formation of humic substances (O-diphenol oxidase) were increased significantly as well as positively and significantly correlated with TOC and OC in all determined in SS amended soil sub-samples (BASTIDA et al. 2008).

All treatments led to a significant increase in soil phosphatase activity with respect to the control (Figure 3a). At the end of the plantation time, higher phosphatase values than those of the control soil were detected in all the soils amended with a high rate of either MSS.

Our results are confirmed by COOK and ALLAN (1992) when β -glucosidase is an enzyme involved in the C cycle and hydrolyses β -glucosidic bonds of the carbohydrate chains. The activity of this enzyme reflects variations in the more biodegradable C fraction, and is therefore very useful for following the mineralization of OM. However, the activity detected at the end of the plantation period was significantly higher in amended soils with LHM content than in amended soils with HHM content and the control soil, particularly when MSS had been applied at the higher rate. CARBONELL et al. (2009) mentioned that application of MSS increased the activities of dehydrogenase, phosphatase, respiration rate and soil microbial content.

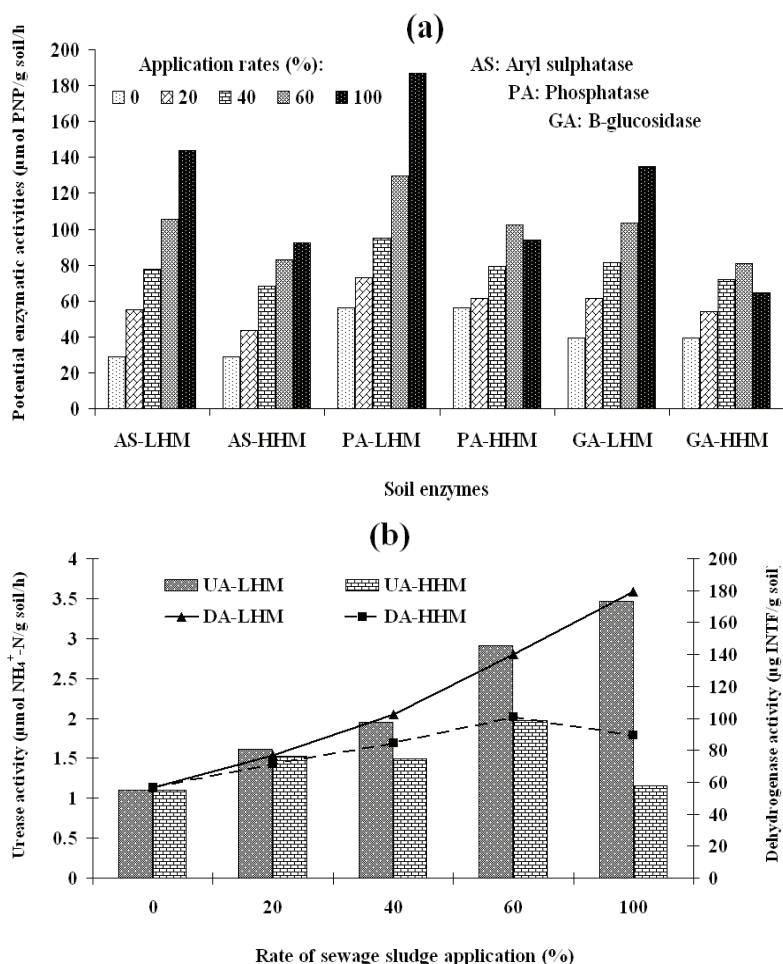


Figure 3. Effects of low and high heavy metal concentrations in sewage sludge treated soil on enzymatic activities (a) aryl-sulphatase, phosphatase, β -glucosidase, and (b) urease and dehydrogenase activities

3. ábra Az alacsony és a magas nehézfém koncentrációja hatása az enzimaktivitásra (a) aril-szulfatáz, foszfatáz, β -glükózidáz és (b) ureáz és dehidrogenáz tevékenységekre szennyvíziszappal kezelt talajon

These results are in agreement with our results as it is present in Figs 3a and 3b. From this study it can be concluded that the addition of MSS, independently of their degree of HMs contamination increased the TOC. In this respect, the enzymatic activities studied helped us to follow the changes occurring in the OM during the experiment. In general, the OM added as MSS had an effect on the enzymatic activities. More studies are needed to deeper our knowledge of the effect of HMs contamination on enzymatic activities. However, our results indicated that there are positively related correlation between the investigated enzymes and OM in the applied MSS. This indicates that an aggregate of multi-enzymatic activities may be better correlated with soil fertility than a single enzyme. Particularly, the enzymatic activities in soil amended with MSS-LHM content were markedly higher than those in the soil amended with MSS-HHM content.

Density of Soil microbial population

It was found that the population size and community structure of soil microorganisms of the investigated soil sub-samples are sensitive to changes in soil contaminated MSS with high concentration of HMs. The results of quantitative analysis of soil microbial (bacterial and fungal) populations were shown in Figure 4. The soil microbial populations were far higher under MSS application than under control. The results indicated that the most metal-resistant filamentous fungal isolates isolated from MSS amended soil belonged to genera *Aspergillus* (10.3%), *Penicillium* (9.2%), *Alternaria* (3.7%), *Geotrichum* (9.7%), *Fusarium* (13.2%), *Rhizopus* (12.3%), *Monilia* (2.5%) and *Trichoderma* (39.1%). These results are in agreement with ZAFAR et al. (2007).

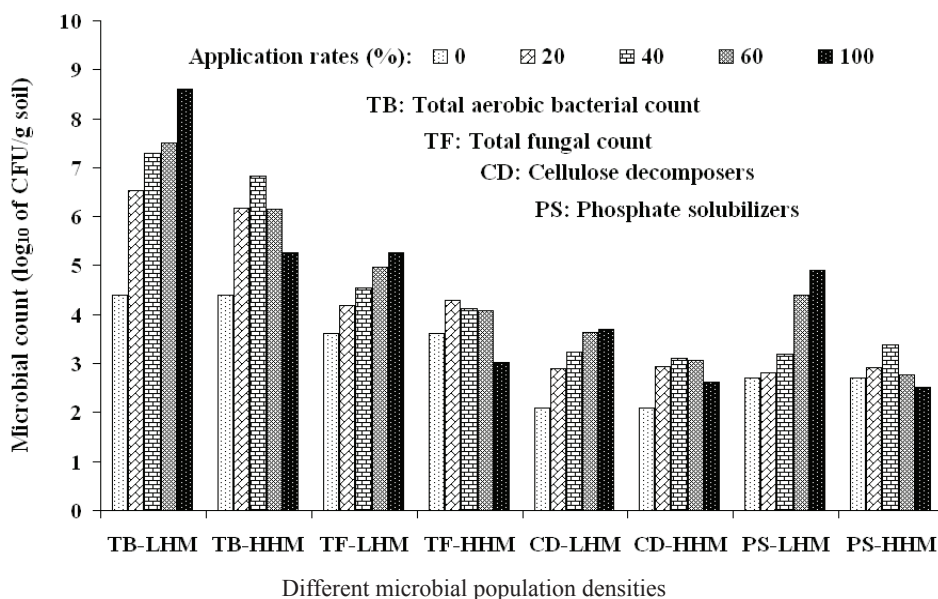


Figure 4. Some soil microbial contents influenced by low (LHM) and high (HHM) heavy metal concentrations in sewage sludge treated soil

4. ábra A kis (LHM) és a nagy (HHM) nehézfém koncentráció hatása a talaj mikrobiális tartalmára szennyvíziszappal kezelt talajon

Bacteria showed a marked increased in population size with increasing MSS mixing rates, other soil microorganisms; fungi in population size responded similarly with bacteria, but the variance was big discrepancy on population size, although all treatments showed significant difference on population size in comparison between the MSSs of LHM and HHM contents compared to control except the fungal population and phosphate solubilizers at MSS of HHM. Our results are similar to RENATO et al. (2012) who stated that the organic matter from the sewage sludge had a significant influence on the soil microbial biomass; nevertheless, at the end of the experiment the equilibrium of the soil microbial biomass (defined as microbial metabolic quotient, qCO_2) was recovered. Soil urease, acid and alkaline phosphatase activity were strongly influenced by sewage sludge applications.

According to our observation, we are in agreement with BOSATTA and L GREN (1993) on the basis of soil microbial biomass (C_{mic}) is both a labile nutrient pool and an agent of transformation and cycling of OM and plant nutrients in soils; so, it is one of the most important microbiological properties. Several studies (e.g., IQBAL et al. 2010; S NCH Z-MONEDERO et al. 2004) indicated that the C_{mic} responds more rapidly to changes resulting from soil management activities than SOM and, consequently, may be an early and sensitive indicator of soil quality change.

Plant biomass and total nitrogen content

The present investigation showed that the dry matter of alfalfa yield and total N increased linearly with increasing MSS application rates (Fig 5).

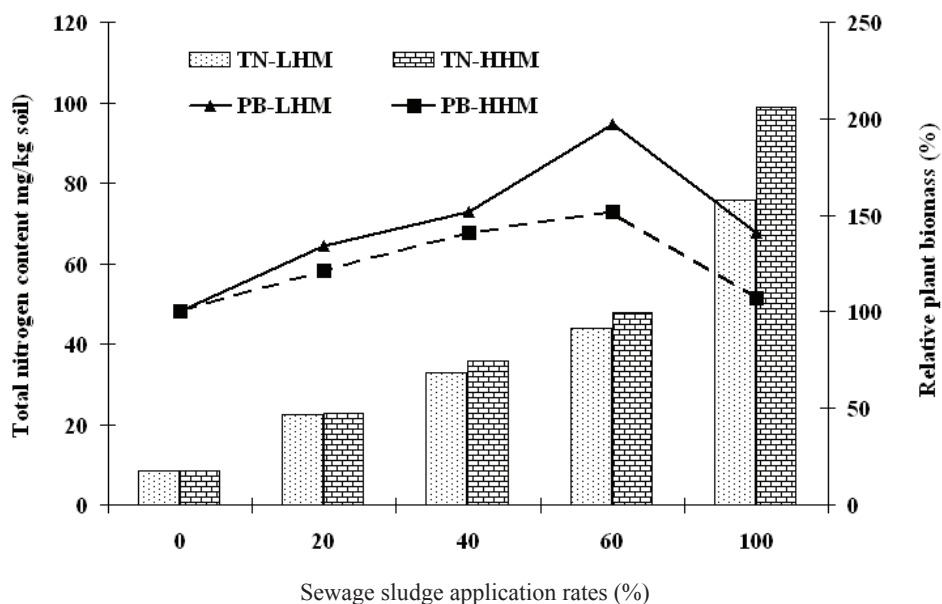


Figure 5. Sewage sludge applications influence the relative plant biomass and total nitrogen content
5.  bra A szennyv ziszap alkalmaz sok befoly solj k a relat v n v ny i biomassza  s  sszes n v g n tartalmat

Soils amended with MSS rates of 40 and 60% exhibit higher in plant dry matter than the control soil. The increase in dry matter and N content of alfalfa growing in MSS-amended soils may be attributed to high OM content and high macro- and micro-nutrient concentrations. This enhancement confirms the increase in shoot weight of legumes grown in MSS-amended soil, as previously reported by IBEKWE et al. (1995). The tendency observed of alfalfa cultivated in soils amended with MSS rates from 40 to 60% show maximal growth, dry weight. The increase of both shoot dry weight and the N content in alfalfa was mainly due to the plant establishment. The result illustrated that the beneficial effect of MSS increased by increasing the application rate to be at maximum at 60% and then decreased but also, they were higher than the controls. Because of low bioavailability of HMs, the HMs present in the parent materials are often not available for plant uptake and cause minimum impact to soil organisms. Unlike pedogenic inputs, Addition of HMs through anthropogenic activities typically has high bioavailability (LAMB et al. 2009). Due to this, we are full agreeing with the results of SINGH and AGRAWAL (2008) established that the mature municipal solid waste compost might be used as conditioner for clay soil, but not for sandy soil, also we agreed with ABUSUWAR and EL ZILAL (2010) who found that chicken manure application significantly improved forage quality in terms of crude protein and crude fiber, with highest protein and lowest fiber recorded for the highest dose of chicken manure applied compared to the control. Also, our results are confirmed by AHMED et al. (2010) who mentioned that the plant macro- and micro-nutrients as well as OM make sludge disposal in soil an attractive option. Nitrogen has received most attention and it is normally the most abundant sludge nutrient. One of the best alternatives to waste disposal is through the soil-plant system as a fertilizer. Based on properties different wastes can be co-recycled in order to take simultaneously the best profit and minimize environmental pollution. Our results are in agreement with the results of BEN REBAH et al. (2002) who established that applying SS as an amendment enhanced the rhizobial number in soils from 10^3 to 10^4 cells/g and improved significantly the shoot dry weights and N contents of alfalfa plant. This improvement increased with sludge rate and with the cut. Our results are in agreement with CELIS et al. (2011) who indicated that the amount of C-CO₂ produced by soil microorganisms was directly proportional to the dose of amended sludge. Similarly, greater β -glucosidase, urease, and acid phosphatase were more active at 60 t sludge/ha. However, both respiratory and enzymatic activities were greater in sludge-amended soil cropped with *O. compressus*. This greater activity was notorious when the legumes achieved greater phytomass development, thus highlighting the root's stimulating effect on soil biological activity. ROIG et al. (2012) showed that the input of SS enhances soil properties proportionally to the application doses and/or frequency. The organic amendments increased the OM content, the soil N, and the microbial activity, improving C and N mineralization processes and some enzymatic functions. However, a maximum dose was identified (40 Mg/ha/year), beyond which soil properties do not improve, and may even worsen. Regarding environmental risks, although the bioluminescent bacteria test showed no toxicity on soil extracts, potential adverse effects such as some potentially toxic elements accumulation, phytotoxicity and the likelihood of groundwater pollution by nitrates or dissolved organic matter should be taken into account.

Elevated metal concentrations can be toxic to plants and soil biota responsible for major ecological functions. Municipal SSs can be used under controlled conditions for

many beneficial purposes. However, MSS can act both as a source and sink for HMs. When soils are amended with MWS contaminated by HMs, dissolved organic carbon (DOC) present in MSS forms soluble complexes with the metals and can reduce sorption in soils. Therefore, metal-DOC complexes can increase the mobility of metals. While DOC has the ability to mobilize metals, metal-DOC complexes can still reduce the bioavailability of metals to plants and soil biota. Furthermore, biological and biochemical parameters, e.g., enzymatic activities, are considered early and sensitive bioindicators of soil quality. Monitoring soil quality by means of bioindicators can be of help for the management and sustainability of soils that received MSS application. Our results indicated that MSS of HHMs content decreased soil bioproductivity, reduced its the biochemical properties, and lowed the microbial contents in the clay loam brown forest soil in comparison with MSS of LHMs content. These results suggest that the amount of MSS applied has to be calculated based on the biochemical properties, and annual applications must be avoided to prevent over-applications. All biochemical indicators showed significant variation and directly proportional to the MSS rates. The current study showed that none of the parameters examined was negatively affected by MSS application of LHM content.

SINGH et al. (2007) showed that use of sewage sludge, a biological residue produced from sewage treatment processes in agriculture is an alternative disposal technique of waste. Meanwhile, soil pH decreased whereas electrical conductance, organic carbon, 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. MUDGAL et al. (2010) estimated that in current time the environment is heavily polluted by various toxic metals, which create a danger for all living beings. These metals are retarding farming efficiency and destructing the health of the plants and animals. Now a day "green technology" minimized this problem to some extent. In this technology to clean up the contamination, such plants grow which can tolerant metal. The application of genetic manipulation and the use of naturally occurring metal tolerant plants should accelerate the process of transmitting this technology from experimental place to field. Thus careful investigation of the mechanism of tolerance of heavy metal at physiological and genetic level is essential. According to our opinion, the problem is still there because of the accumulation of HMs in the plant dry matter. To reduce the negative effects due to the agricultural use of sewage sludge, specific actions should promote the achievement and utilization of sewage sludge with low heavy metal content and the identification of soils more sensitive to heavy metal accumulation, which may possibly lead to ecotoxicity or increased heavy metal content in crop products.

Careful management of MSS can minimize these risks and objections. It should be concluded that the accumulative concentration of HMs in MSS amended soil should be calculated after every application of MSS. The results suggest that MSS addition induces a reactivation of soil quality and activity, as indicated by plant dry matter, microbial content and enzymatic activities.

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AGYAGOS VÁLYOG BARNA ERDŐTALAJ VÁLTOZÓ SZERVES SZÉN DINAMIKÁJA ÉS A
BIOLÓGIAI AKTIVITÁSA KOMMUNÁLIS SZENNYVÍZISZAP ALKALMAZÁSÁVAL

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Kulcsszavak: lucerna növekedés, biokémiai és mikrobiális tevékenység, települési szennyvíz, talajminőség

Összefoglalás: Üvegházás tenyészedény kísérlet történt két települési szennyvíziszap hatásainak vizsgálatára, a szervesszén-dinamika, a mikrobiális populáció, az enzim-aktivitás és a lucerna növekedés változására vonatkozóan a szennyvíziszap nehézfém- és tápanyag-kibocsátásával kapcsolatban agyagos vályog barna erdőtalajon. A települési szennyvíziszap-kezelés növeli a talaj szervesanyag-tartalmát, és javítja a talaj szerkezetét. A nagy nehézfém tartalmú kommunális szennyvíziszap jelentősen csökkentette a talaj biológiai produktivitását, rontotta a biokémiai tulajdonságait, és csökkentette a mikrobiális tartalmát szemben az alacsony nehézfém-tartalmú települési szennyvíziszappal. Az összes lucerna szárazanyag-hozam és nitrogén-tartalom lineárisan nő a növekvő kommunális szennyvíziszap kijuttatással. A 40 és 60% települési szennyvíziszappal kezelt talajon a termés szárazanyag-tartalma és a mikrobiális enzimaktivitása nagyobb volt, mint a kontrollé. Összességében, az eredmények azt mutatják, hogy az alacsony nehézfém-tartalmú települési szennyvízzel történő kezelés javíthatja a talaj minőségét és a talaj biológiai és biokémiai tulajdonságait.