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# Enzymatic activity in soil treated with exogenous organic matter

*[1]Sylwia Siebielec, [2]Grzegorz Siebielec, [2]Aleksandra Ukalska-Jaruga, [3]Magdalena Urbaniak*

[1]Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation – State Research Institute
Czartoryskich 8, 24-100 Puławy, POLAND
[2]Department of Soil Science Erosion and Land Protection, Institute of Soil Science and Plant Cultivation – State Research Institute
Czartoryskich 8, 24-100 Puławy, POLAND
[3]European Regional Centre for Ecohydrology PAN, Tylna 3, 90-364 Łódź, POLAND

**Summary**. The content of organic matter affects the quality and productivity of agricultural soils. Due to the shortage of manure as the basic source of organic matter for agricultural soils, alternative and commonly available exogenous sources of organic matter, such as sewage sludge and bottom sediments, are taken into account. The aim of the study was to evaluate the effect of the addition of various types of manure, municipal sewage sludge and bottom sediments on the enzymatic activity of soils (dehydrogenases, acidic and alkaline phosphatases). Manure and sludge were applied to the soil in two doses, corresponding to 20 and 40 tons of dry matter of material per ha. The control was soil fertilized with ammonium nitrate in the amount corresponding to 170 kg N per ha. The experiment included 2 different variants of the same fertilization combinations: without plants and with a test plant (*Sinapis alba* L.). The greenhouse experiment showed the stimulating effect of one of the municipal sewage sludge and poultry manure. On the other hand, the introduction of bottom sediments to the soil limited its enzymatic activity.

**Key words:** dehydrogenases, acidic phosphatase, alkaline phosphatase, manure, sewage sludge, bottom sediment.

## INTRODUCTION

Maintaining adequate levels of soil organic matter (SOM) is one of the most important elements of sustainable soil management (Carter, 2002; Garrigues et al., 2012). Organic matter has a key role in maintaining the soil's capacity to perform production and ecological functions. It determines properties such as the sorption and buffering capacity of the soil and processes referred to as biological activity. It stabilises soil structure and increases soil resistance to compaction and degradation by water and wind erosion. The preservation of SOM resources is also important for the role of soils in fixing carbon dioxide from the atmosphere. Organic matter plays an important role both in the water cycle and in shaping soil biodiversity and the landscape (Darwish et al., 1995; Stratton et al., 1995; Raviv, 2005; Leroy et al., 2008). The input of organic matter influences biological activity directly (Raviv, 2005) or indirectly by affecting the stability of soil structure and water retention (Fujino et al., 2008). The main source of organic matter in agriculturally used soils is crop residues and the primary source of so-called exogenous organic matter (EOM) is manure. Due to the scarcity of manure as a primary source of organic matter in many regions, alternative and widely available exogenous sources of organic matter such as compost, digestate, sewage sludge, bottom sediments, organic industrial waste, etc. are being considered (Darwish et al., 1995; Stratton et al., 1995; Leroy et al., 2008). The world currently generates 1.3 billion tonnes of municipal solid waste each year. By 2025, the world could generate 2.2 billion tonnes of solid waste annually (Moya et al., 2017). Recycling solid waste and animal manure for use as fertiliser would reduce dependence on synthetic fertilisers. EOM can come from a wide range of bio-waste from a variety of sources, it can be also present in different forms (solid, liquid, semi-liquid) and it can be subjected to different chemical or physical treatments before being applied to the soil. Depending on these factors, EOM will have diverse physicochemical properties which, when introduced into soil, can affect soil properties and biological life, which is key to soil function and to building resistance to degradation.

Manure is a traditional natural fertiliser composed of the excrement (faeces and urine) of livestock and litter. The chemical composition of manure can vary, depending on the species and age of the animals, the feeding regime and quality of the feedstuff used, as well as the condition of the animal, the amount of litter used and the degree of digestion of the material. In the conducted experiment, sewage sludge and bottom sludge were also used as alternatives to

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Corresponding author:
Sylwia Siebielec
e-mail: ssiebielec@iung.pulawy.pl
phone +48 81 4786952

manure. So far, studies on the effects of applying sewage sludge to the soil have mainly concerned the transformation of nutrients (nitrogen, phosphorus) and the impact on yield (productive function of the soil), with much less attention paid to their influence on biological issues, such as e.g. the enzymatic activity of soils. Scientific studies report that on average, municipal sludge produced in Poland contains 2.6% nitrogen (N) and 1.83% phosphorus (P) in dry matter (Siebielec, Stuczyński, 2008). On the other hand, due to the fact that bottom sediments are disposed of only when lakes, reservoirs or rivers are cleaned from the sediments, the problem of safe sediment management is underestimated. As a result, research on ways to manage bottom sediments is scarce and there is no legal regulation dedicated to this issue (Siebielec et al., 2015). Available scientific studies indicate a high variability of trace element content in lake sediments, also spatial and seasonal variability within the same site. In the case of rivers, the quality of bottom sediments largely depends on the size of the river, the area through which it flows, and the nature of the catchment (Siebielec et al., 2015). The bottom sediments and sewage sludge take part in the carbon, nutrient and pollutant cycles in the environment. There is therefore a rationale for the recycling of sludge and sediment; however, the difficulty may be associated with the presence of pollutants in these materials the unknown consequences of their introduction on soil biological life.

Biological soil parameters are early and sensitive indicators of soil ecological stress (Garcia et al., 2000). One of the key parameters in studies of soil health is enzymatic activities (Bielińska et al., 2000; Gostkowska et al., 1998; Siebielec et al., 2020a; Siebielec et al., 2020b). Soil enzymes are involved in many important soil processes, including the decomposition of organic matter, the transformation of soil humus and the release of nutrients during plant vegetation. In addition, they are involved in detoxification, nitrification, and denitrification processes (Bielińska et al., 2013; Kucharski, 1997). The research hypothesis was that the type of organic fertilization can significantly affect the biological activity of soil, depending on the chemical composition and origin of EOM. Therefore, the aim of this study was to evaluate the effect of manure from different sources and of sewage sludge and bottom sediment on the enzymatic activity of soils.

## MATERIALS AND METHODS

In 2020, a greenhouse pot experiment was conducted at IUNG-PIB during the summer. The experiment was carried out in 3 L plastic pots filled with loamy sand soil, containing 0.69% of organic carbon and with neutral pH (6.4 water slurry). The soil had an average level of available phosphorus (12,1 mg 100 $g^{-1}$) according to the Egner-Riehm method.

The following fertilisation options were tested in the study:

– without EOM
1. control (ammonium nitrate),
– with EOM
2. cattle manure,
3. pig manure,
4. goat manure,
5. poultry manure,
6. rabbit manure,
7. horse manure,
8. urban bottom sediment,
9. rural bottom sediment,
10. sewage sludge I,
11. sewage sludge II.

The tested batches of manure came from production farms located in the Lublin Province. Bottom sediments were taken from ponds located in urban and rural areas and sewage sludge was brought from two municipal sewage treatment plants located in Wielkopolskie and Lubelskie voivodeships in cities with populations of 8,000 (sewage sludge I) and 47,000 (sewage sludge II) respectively. Sludge was collected after being dewatered in a press. The basic properties of the organic materials tested are presented in Table 1. Cattle and rabbit manure were the richest in carbon. On the other hand, sewage sludge and rabbit manure contained the most nitrogen per dry matter. Among manures, poultry manure was the least rich in nitrogen. The additives differed considerably in phosphorus content – sewage sludge I and pig manure contained the most phosphorus. Most of the manures were very rich in potassium. Except for poultry manure, all other manures contained 4.18–7.17% potassium in relation to its dry matter. Bottom sediments and sewage sludges were much less rich in this nutrient. The mineral fraction predominated in bottom sediments, so they contained much less carbon and nitrogen than the other materials. Table 1 shows the contents of basic trace elements. Cadmium and zinc were the highest in sewage sludge II, but these were the contents allowed for the use of municipal sewage sludge in agriculture, according to the Regulation of the Minister of the Environment of 6 February 2015.

Soil portions (1300 g) were transferred into the pots and then EOM materials were added and thoroughly mixed with soil. The pots were then watered to a moisture content close to 60% of the field water holding capacity. After 2 weeks, plants were seeded.

EOM materials were applied to the soil at two rates, corresponding to 20 and 40 tonnes of dry matter per ha. The control was soil fertilised with ammonium nitrate at a rate corresponding to 170 kg N per ha. The experiment included 2 different variants of the same fertilisation combinations: with and without plants. The latter variant was tested to evaluate the effect of fertilisation alone on soil

Table 1. Chemical characteristics of the soil additives tested in the pot experiment.

| Soil additive | Carbon content (C) | Nutrient content | | | Trace metal content | | | |
|---|---|---|---|---|---|---|---|---|
| | | Nitrogen | Phosphorus | Potassium | Zinc (Zn) | Lead (Pb) | Cadmium (Cd) | Chromium (Cr) |
| | [%] | [%] | [%] | [%] | [$\text{mg kg}^{-1}$] | [$\text{mg kg}^{-1}$] | [$\text{mg kg}^{-1}$] | [$\text{mg kg}^{-1}$] |
| Cattle manure | 41.8 | 2.06 | 0.62 | 5.37 | 147 | 1.6 | 0.35 | 4.7 |
| Pig manure | 30.1 | 2.26 | 1.62 | 7.18 | 195 | 5.6 | 0.37 | 21.4 |
| Goat manure | 38.5 | 2.35 | 0.99 | 5.75 | 93 | 2.1 | 0.25 | 9.9 |
| Poultry manure | 17.6 | 1.75 | 0.74 | 1.04 | 342 | 86.6 | 0.63 | 63.1 |
| Rabbit manure | 37.1 | 3.25 | 1.36 | 7.62 | 206 | 2.4 | 0.45 | 10.3 |
| Horse manure | 35.9 | 2.43 | 1.29 | 4.18 | 121 | 2.3 | 0.32 | 10.2 |
| Rural bottom sediment | 1.2 | 0.07 | 0.03 | 0.09 | 7 | 4.8 | 0.07 | 5.7 |
| Urban bottom sediment | 1.9 | 0.10 | 0.03 | 0.03 | 65 | 17.7 | 0.22 | 21.2 |
| Sewage sludge I | 27.3 | 3.55 | 2.74 | 0.36 | 344 | 11.1 | 0.53 | 96.4 |
| Sewage sludge II | 32.7 | 5.24 | 1.28 | 0.23 | 1309 | 16.8 | 1.06 | 65.4 |

biological activity. The plant tested in the experiment was mustard (*Sinapis alba* L.), a member of Brassicaceae family. Mustard, due to its fast emergence and biomass growth, is a plant willingly cultivated by farmers (Harasimowicz-Hermann, Hermann 2006). Each of the tested variants was represented by 3 replications (pots).

After 2 months, when the plants had sufficient biomass, the plants were cut and soil samples were collected (Fig. 1). The samples were then sieved through a 2 mm mesh to remove plant debris and thoroughly mixed. For quality control of the results obtained and for reliable statistical processing, analyses for enzymatic activity (dehydrogenases, acid and alkaline phosphatase) were performed in 3 replicates. Soil samples intended for enzymatic analyses were stored in closed plastic bags at temperature of 4±1 °C. The dehydrogenase activity assay was performed according to Casida et al. (1964) by the colorimetric method using 3% TTC (triphenyltetrazolium chloride) as substrate, after 24 h incubation at 37 °C, at 485 nm wavelength. Alkaline and acid phosphatase activities were performed by a colorimetric method using PNP (sodium p-nitrophenylphosphate), after a 1 h incubation at 37 °C, at 410 nm (Tabatabai, Bremner, 1969). Results were processed using analysis of variance with Scheffe's test ($P < 0.05$) using Statistica v.13.0 software.

## RESULTS AND DISCUSSION

Organic matter is a key parameter for the proper functioning of the soil environment. High soil organic matter (SOM) can increase the supply of nutrients and improve the physical and biological status of the soil, so maintaining SOM is important to maintain the productivity of agroecosystems. Tillage and fertilisation practices significantly affect soil organic matter (Gong et al., 2009). Fertilisation is mainly used for plant nutrition and quality, but it also affects the soil environment – e.g. the chemical composition of organic compounds and nitrogen in the soil, which in turn, can change the activity and diversity of soil microorganisms (Ding et al., 2016; Jangid et al., 2008). It is not only the amount of SOM in the soil that is important, but also its quality, as organic matter is a source of energy for microbial growth and enzyme production (Fontaine et al., 2003).

Agriculturally used soils have different microbiological and physicochemical characteristics that can influence soil microbial activity (Acosta-Martinez et al., 2008). Enzymatic activity, which can be expressed through the parameters of dehydrogenase activity and acid and alkaline phosphatase activities, is one of the main biological indicators for the direct determination of the biological quality

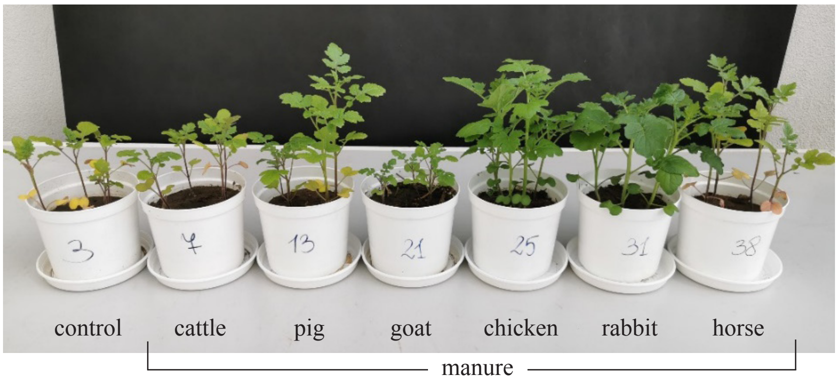

Figure 1. Comparison of plant biomass with manure addition – dose 40 t ha$^{-1}$. Photo: own source.

of soils. Soil extracellular enzymes are synthesized and secreted by soil microorganisms (Burns et al., 2013). Enzymes reflect the intensity and direction of various biochemical processes occurring in the soil environment (Wang et al., 2015). Soil enzymes include hydrolases, which help to extract carbon, nitrogen and phosphorus to support primary metabolism; or oxidoreductases that contribute to the breakdown of organic compounds (Tiemann, Billings, 2011).

Dehydrogenases, classified as oxidoreductases, accelerate dehydrogenation reactions of specific substrates in biochemical oxidation processes of organic compounds. They are present in all living microbial cells and are therefore often considered as indicators of overall soil biological activity (Moeskops et al., 2010; Kaczyńska et al., 2015). The role of dehydrogenases is the biological oxidation of soil organic matter through the transfer of hydrogen from the organic substrate to inorganic acceptors. Thus, dehydrogenases activity reflects the rate of transformation occurring in the soil (Stręk et al., 2015; Brzezińska et al., 2001). According to the literature, dehydrogenases are an important determinant of the intensity of soil respiration, mainly of bacteria and radiators (Zhang et al., 2010). Phosphatases, on the other hand, catalyse the hydrolysis of organic phosphorus compounds and are a determinant of the potential rate of mineralisation of these compounds in the soil environment. It is generally accepted that phosphatases are ubiquitous in soil and are produced by microorganisms and plant roots in response to low levels of inorganic phosphate (Januszek, 1999; Nannipieri et al., 1990; Dick, Tabatabai 1993). Recent research by Margalef et al. (2017), on the other hand, has shown that phosphatase activity in soil is most dependent on organic phosphorus content rather than on the availability of mineral forms of the element. It should therefore be considered that these enzymes are important for the use of alternative sources of phosphorus in the form of EOM from waste recycling and the expected reduction in the use of mineral phosphorus from fossil sources.

Table 2 shows the enzymatic activity of the soil in the planted pots with the addition of EOM materials at a dose equal to 20 t ha$^{-1}$. The highest enzymatic activity for acid phosphatase was obtained in the soil with the addition of sewage sludge II (93.5 μg PNP g d.m.$^{-1}$ h$^{-1}$). There were no significant differences in acid phosphatase activity for soils with the other additives as well as for the control fertilized with mineral nitrogen. In contrast, alkaline phosphatase activity was significantly lowest in the soil with the addition of urban bottom sediments (69.7 μg PNP g d.m.$^{-1}$ h$^{-1}$), and its activity was stimulated to the greatest extent by poultry and rabbit manure and sewage sludge II. This observation does not confirm the reports on the increase of phosphatase activity at lower phosphorus supply because the bottom sediments contributed the least phosphorus among all soil additives. Also in the case of dehydrogenase activity, the lowest value was observed in the soil with the addition of urban bottom sediments (20.6 μg TTC g d.m.$^{-1}$ h$^{-1}$). The soil with the addition of rural bottom sediments had slightly higher activity. Dehydrogenases were most active after application of 20 t of sewage sludge II and poultry manure.

Table 2. Enzymatic activity in the planted variant as an effect of exogenous organic matter addition – rate 20 t ha$^{-1}$.

| Soil additive | Acid phosphatase activity | Alkaline phosphatase activity | Dehydrogenases activity |
|---|---|---|---|
| | [μg PNP g d.m.$^{-1}$ h$^{-1}$] | [μg PNP g d.m.$^{-1}$ h$^{-1}$] | [TTC g d.m.$^{-1}$ h$^{-1}$] |
| Fertilized control | 60.1 b | 89.3 ac | 41.8 abcd |
| Cattle manure | 55.3 b | 95.3 ab | 62.3 ab |
| Pig manure | 52.1 b | 88.8 ac | 51.8 abcd |
| Goat manure | 51.3 b | 96.6 ab | 69.4 a |
| Poultry manure | 59.3 b | 118.8 b | 73.9 a |
| Rabbit manure | 49.3 b | 100.1 ab | 57.3 abd |
| Horse manure | 54.8 b | 84.1 ac | 62.8 ab |
| Rural bottom sediment | 49.0 b | 80.1 ac | 27.1 cd |
| Urban bottom sediment | 53.7 b | 69.7 c | 20.6 c |
| Sewage sludge I | 45.9 b | 94.9 ab | 33.9 bcd |
| Sewage sludge II | 93.5 a | 98.9 ab | 73.5 a |

Means marked with the same letter did not differ significantly across the stress level within soil ($p < 0.05$, $n = 3$) according to the Scheffe test.

The effect of municipal sewage sludge application on enzyme activity at a rate of 20 t was not conclusive, as it was variable depending on the sludge applied and the activity parameter measured. However, the results indicate that some sewage sludge can stimulate enzyme activity to a greater extent than manure. Bottom sediment, on the other hand, inhibited enzymatic activity in soil, regardless of its origin. A study by Urra et al. (2019) indicated that the microbial quality of soils was higher in soils enriched with sewage sludge. The stimulating effect of sewage sludge application on the enzymatic activity was also observed in a long-term field experiment conducted on a contaminated smelter waste dump. Specifically, the application of sewage sludge and waste lime increased the activity of dehydrogenases, acid phosphatase, and alkaline phosphatase compared to the control treatment where no organic material was added (Siebielec S. et al., 2018). The study by Siebielec G. et al. (2018) evaluated the long-term effects of sewage sludge, digestate, and mineral fertilizers on plant yield and soil biological activity. The study was conducted in 1 m$^2$ lysimeter plots. In that study, sewage sludge stimulated both plant biomass, to a greater extent than mineral nitrogen fertilization, and enzymatic activity of soils (Siebielec G. et al., 2018).

The agricultural use of sewage sludge is one of the possible ways of its management. The characteristics of sewage sludge depends on the quality of the wastewater and the type of treatment processes used. Being rich in organic matter and inorganic nutrients, sewage sludge can be an alternative to manure as long as the level of potentially toxic pollutants is low. Literature data show that enzymatic activities as well as crop yields in sludge-enriched soil, respectively, are generally higher compared to control treatments (Singh, Agrawal, 2008; Siebielec G. et al., 2018). In this study, for sludge I, the lower enzymatic activity of the soil may have been due to lower amounts of organic carbon introduced with the sludge, less favorable organic matter characteristics of the sludge, or the presence of contaminants such as chromium (96,4 mg kg$^{-1}$ sludge). The low enzymatic activity after the introduction of bottom sediments was probably due to the lower amounts of organic carbon and the presence of various types of contaminants in these sediments other than those determined in our study.

Table 3. Enzymatic activity in the planted variant as an effect of exogenous organic matter addition – rate 40 t ha$^{-1}$.

| Soil additive | Acid phosphatase activity | Alkaline phosphatase activity | Dehydrogenases activity |
|---|---|---|---|
| | [μg PNP g d.m.$^{-1}$ h$^{-1}$] | [μg PNP g d.m.$^{-1}$ h$^{-1}$] | [TTC g d.m.$^{-1}$ h$^{-1}$] |
| Fertilized control | 60.1 bc | 89.3 cd | 41.8 bcd |
| Cattle manure | 59.9 bc | 108.7 bcd | 92.2 a |
| Pig manure | 50.9 c | 101.3 cd | 85.6 ad |
| Goat manure | 48.95 c | 115.1 bcd | 92.4 a |
| Poultry manure | 81.1 b | 173.9 a | 108.9 a |
| Rabbit manure | 65.1 bc | 153.5 ab | 76.2 acd |
| Horse manure | 59.9 bc | 104.1 cd | 105.7 a |
| Rural bottom sediment | 51.7 c | 74.5 d | 21.5 b |
| Urban bottom sediment | 50.2 c | 74.3 d | 22.4 b |
| Sewage sludge I | 46. 7 c | 109.4 bcd | 32.4 bc |
| Sewage sludge II | 156.6 a | 124.6 bc | 93.9 a |

Means marked with the same letter did not differ significantly across the stress level within soil ($p < 0.05$, n = 3) according to the Scheffe test.

Table 4. Enzymatic activity in the planted variant as an effect of exogenous organic matter addition – rate 20 t ha$^{-1}$.

| Soil additive | Acid phosphatase activity | Alkaline phosphatase activity | Dehydrogenases activity |
|---|---|---|---|
| | [μg PNP g d.m$^{-1}$ h$^{-1}$] | [μg PNP g d.m$^{-1}$ h$^{-1}$] | [TTC g d.m$^{-1}$ h$^{-1}$] |
| Fertilized control | 44.7 b | 56.0 ac | 9.8 b |
| Cattle manure | 49.9 b | 80.6 abc | 28.3 bc |
| Pig manure | 43.9 b | 60.4 abc | 23.9 bc |
| Goat manure | 40.6 b | 78.3 abc | 30.6 abc |
| Poultry manure | 50.6 b | 94.8 b | 22.4 bc |
| Rabbit manure | 41.1 b | 88.0 ab | 30.9 abc |
| Horse manure | 50.7 b | 62.9 abc | 37.3 ac |
| Rural bottom sediment | 48.1 b | 45.5 c | 18.8 bc |
| Urban bottom sediment | 49.3 b | 43.9 c | 17.5 bc |
| Sewage sludge I | 48.6 b | 96.2 b | 36.4 ac |
| Sewage sludge II | 74.8 a | 58.8 abc | 50.9 a |

Means marked with the same letter did not differ significantly across the stress level within soil ($p < 0.05$, n = 3) according to the Scheffe test.

Table 3 contains enzyme activity data for the plant combination after application of the rate of 40 t ha$^{-1}$. Similarly as in the case of the lower rate, the highest acid phosphatase activity was recorded in soil with sludge II. Alkaline phosphatase activity was again lowest in the soil with the addition of bottom sediments. Similar trends were observed for dehydrogenase activity, which was lowest at this dose after application of both sediments. For manures, generally higher activities of all enzymes tested were observed after application of the rate of 40 t ha$^{-1}$ than after a lower application rate. Some authors indicate that high phosphatase activity is observed in soils with low availability of phosphorus (Kowalska et al., 2017). Such an increase in activity was not observed in the case of the bottom sediments that contributed by far the least phosphorus and, moreover, phosphatase activity increased slightly with increasing application rate and thus phosphorus supply. Additionally, the soil used in the experiment had an average initial phosphorus availability. This fact indicates that the amount of organic matter introduced to the soil and the presence of compounds inhibiting enzymatic activity in some materials may have had a decisive effect on phosphatase activity.

The highest alkaline phosphatase activity for the rate of 40 t ha$^{-1}$ was measured in the soil with the addition of poultry manure (173.9 μg PNP g d.m.$^{-1}$ h$^{-1}$). Poultry manure contains all the necessary nutrients required for crop production (Hanč et al., 2008). Factors influencing the composition of poultry manure are: type of birds, type and amount of nutrients in the feed, type of litter and other factors related to production. There are few data in the literature on the effect of poultry manure on the microbial activity of soils. As emphasized by the authors, the determination of biochemical properties gives the possibility of a more complex assessment of the changes occurring in the soil as a result of fertilization with manure.

Table 4 shows the enzymatic activity values in plant-free soil at a rate of EOM materials equivalent to 20 t ha$^{-1}$. Similar to the variant with plants, the highest acidic phosphatase activity was obtained in soil with the addition of sewage sludge II (74.8 µg PNP g d.m.$^{-1}$ h$^{-1}$). The results of acidic phosphatase were the same at the rate of 40 t ha$^{-1}$ without plants (Table 5), where also the same sediment stimulated acid phosphatase activity to the greatest extent. For the no-plant variant, no significant differences in acidic phosphatase activity were found for soils with other EOM additions, as well as for the control. The lowest alkaline phosphatase activity was found in soil with the addition of bottom sediments. Values for dehydrogenases were highest in the no-plant variant in soil with the addition of sewage sludge II. The lowest dehydrogenase activity was found in the control soil (9.8 µg TTC g d.m.$^{-1}$ h$^{-1}$). Horse manure also had a strong stimulating effect on dehydrogenase activity in soil without plants, which was observed for both tested rates.

Table 5 shows the enzymatic activity in the soil in the no-plant variant with the addition of EOM at a rate of 40 t ha$^{-1}$. The highest acidic phosphatase activity, as in the other variants, was obtained in soil with the addition of sewage sludge II. There were no significant differences in phosphatase activity for the other combinations. The highest alkaline phosphatase activity was in the response to addition of poultry manure for both rates. The control had the lowest dehydrogenase activity in the soil without plants (9.8 µg TTC g d.m.$^{-1}$ h$^{-1}$), similarly as after applying a lower rate of EOM.

The presence of plants did not clearly modify the enzymatic activity in the EOM-supplemented soil, both in terms of the effect of individual materials and the values of enzymatic parameters. Only in the case of dehydrogenases in the control soil there was a significant decrease in the activity in the variants without plants compared to the planted soil. From this, it can be concluded that in EOM treated soil the activity is strongly stimulated by the introduction of organic matter but without organic addition the interaction between plants and microorganisms is fundamental for the biological activity of the soil.

An increase in the amount of sewage sludge produced in Poland and a decrease in the availability of manure in some regions encourages for the use of a part of sludge in agriculture, and in reclamation. The prerequisite for such an agricultural use of sludge must be its positive effect on soil quality, low level of contamination, and high efficiency of nutrients with no negative effects, e.g. on soil biological activity. In our study, the effect of the application of municipal sewage sludge varied and was probably dependent on the presence of microbial activity inhibitors in the sludge , which may come from both wastewater and the technological process used in the wastewater treatment plant. This aspect requires detailed studies.

Table 5. Enzymatic activity in the no-plant variant as an effect of EOM addition - rate 40 t ha$^{-1}$

| Soil additive | Acid phosphatase activity | Alkaline phosphatase activity | Dehydrogenases activity |
|---|---|---|---|
| | [µg PNP g d.m.$^{-1}$ h$^{-1}$] | [µg PNP g d.m.$^{-1}$ h$^{-1}$] | [TTC g d.m.$^{-1}$ h$^{-1}$] |
| Fertilized control | 44.7 b | 56.0 b | 9.8 c |
| Cattle manure | 47.9 b | 91.3 ab | 36.7 abc |
| Pig manure | 35.2 b | 73.6 ab | 30.9 bc |
| Goat manure | 48.9 b | 91.8 ab | 53.3 ab |
| Poultry manure | 46.5 b | 127.1 a | 24.3 bc |
| Rabbit manure | 42.2 b | 87.3 ab | 44.7 abc |
| Horse manure | 52.8 b | 73.4 ab | 76.5 a |
| Rural bottom sediment | 50.3 b | 47.6 b | 15.4 bc |
| Urban bottom sediment | 51.2 b | 54.2 b | 16.3 bc |
| Sewage sludge I | 44.2 b | 86.7 ab | 28.9 bc |
| Sewage sludge II | 77.5 a | 66.7 b | 34.0 bc |

Means marked with the same letter did not differ significantly across the stress level within soil ($p < 0.05$, n = 3) according to the Scheffe test.

## CONCLUSIONS

1. Comparison of different manure types showed a strong stimulation effect on enzymatic activity of soils after applying poultry manure. There were no clear and repeatable differences between the other manure types. Only in the no-plant variant did horse manure significantly increase dehydrogenase activity, which characterizes the overall biological activity of soils. In many cases, the enzymatic activity of the soil after the introduction of manure remained at a level similar to that of the control soil.
2. The enzymatic activity of soil was stimulated to the greatest extent by one of the municipal sewage sludge. However, the effect of sludge application varied. It should therefore be assumed that the effect of sewage sludge on soil biological activity will depend on the chemical characteristics of the individual sludge. This issue requires further research aimed at demonstrating the characteristics of sewage sludge that stimulate or inhibit soil biochemical activity.
3. Bottom sediments inhibited the enzymatic activity of soils, regardless of their origin. It was most probably connected with the low content of organic matter and with the presence of a wide range of chemical pollutants, including those not determined in the described study. It can be assumed that the usefulness of bottom sediments as a soil amendment may be therefore limited.
4. In soil fertilized with EOM, the presence of plants did not clearly modify enzymatic activity, whereas in soil without organic fertilization, the interaction of plants with microorganisms is fundamental for soil biological activity.

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| Author | ORCID |
|---|---|
| Sylwia Siebielec | 0000-0001-9516-1939 |
| Grzegorz Siebielec | 0000-0001-8089-6123 |
| Aleksandra Ukalska-Jaruga | 0000-0002-9253-2383 |
| Magdalena Urbaniak | 0000-0001-5118-4530 |

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