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Biochemical Parameters of Fallow Light Soil Enriched with Sewage Sludge

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Abstract: One way to manage sewage sludge, which is consistent with the assumptions of the European Green Deal, is to use it in agriculture. The study focused on the possibility of using soil enzyme activity and the GMea index (the geometric mean of enzyme activities) in connection with the total organic carbon (TOC) and the total nitrogen (TN) content to assess the quality of fallow light soil after exogenous organic matter (EOM) fertilization. Exogenous organic matter in the form of stabilized municipal sewage sludge was introduced into the soil. The experiment included five variants: one control site and four sites with 30, 75, 150, and 300 Mg ha^{-1} of sewage sludge added to the soil. The contents of TOC, TN and heavy metals (Zn, Cu, Pb, Cd) in the soil material were assayed. In addition, the activity of soil enzymes, i.e., neutral phosphatase, urease, protease and dehydrogenase, was examined, and the geometric mean of the enzyme activities (GMea index) was calculated. Fertilization of light soil with sewage sludge resulted in an increase in TOC and TN proportionally to the EOM dose. The addition of sewage sludge increased the content of tested heavy metals in the soil and did not exceed the levels considered acceptable. The introduction of sewage sludge contributed to the stimulation of biological life in the soil. This was evidenced by an intensification of soil enzyme activity. However, individual enzymes showed a different response to EOM fertilization, while GMea showed a significant increase in the quality of the fallowed soils as the EOM rate increased to 150 Mg ha^{-1}.

Keywords: fallowing soil; exogenous organic matter; heavy metals; enzyme activity; GMea index

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1. Introduction

The short-term exclusion of parts of land from cultivation is a method of improving the soil properties known since the early days of agriculture. The development of rural areas and agriculture has changed the approach to land exclusion from cultivation. Currently, the main factors influencing the exclusion of soils from agricultural production are economic reasons resulting from the quality of soils and thus the unprofitability of crop production [1–3] and political reasons aimed at protecting agricultural markets and mitigating climate change [4–6].

The development of rural areas and agriculture has changed the approach to land exclusion from cultivation. Currently, the main factors influencing the exclusion of soils from agricultural production are economic reasons resulting from the quality of soils and thus the unprofitability of crop production [1–3] and political reasons aimed at protecting agricultural markets and mitigating climate change [4–6]. Most of this is marginal land, fallow land or abandoned agricultural land [7–9]. The abandonment of agricultural land is a process observed in most European countries. In Poland, it began with the political transformation of the 1990s and currently affects more than 2 million ha of arable land [10].

The basis for the functioning of terrestrial ecosystems is soils, which provide and regulate key ecosystem services. These include the production of biomass, including food and animal feed and energy crops; the protection of water resources and biodiversity, and making terrestrial ecosystems more resistant to climate change [11]. Rehabilitated land represents a land resource that can be directly returned to agricultural production or can provide environmental services. The development of long-term fallow land for agriculture is in accordance with the European Union's Soil Strategy 2030, which aims to address soil and land issues in a comprehensive manner and to step up efforts to better protect soils and soil biodiversity and to reduce soil sealing [12].

Currently, there are several concepts for the management of land unsuitable for agriculture in Europe and worldwide. Depending on the type of soil and the reason for its fallowing, it can be used in different ways [13], including for agricultural production purposes [14].

The latest idea of fallow land management, recommended by scientists and practitioners, is to implement solutions that maintain fallow fields in a state of viability and enable them to fulfill three or even four functions. These include CO_2 sequestration, energy production, increasing biodiversity and, in the event of a food crisis, resuming agricultural production [15,16].

When restoring fallow land to use, it is crucial to improve its deficient properties, especially to increase organic matter reserves. Soil organic matter (SOM) is a recognized and important reservoir of C and is usually associated with other soil functions such as soil structure, plant nutrition, and a source of energy for soil biota [17]. As SOM is crucial to soil function and agro-ecosystem productivity, various management strategies have been developed to increase SOM in cultivated soils [18], one of which is the introduction of exogenous organic matter (EOM) into the soil. EOMs are organic by-products and wastes that, when introduced into soils, promote soil carbon storage, increasing soil fertility and crop production, while at the same time, through their use as fertilizers, they reduce agriculture's dependence on non-renewable resources such as P or K mineral fertilizers [19].

Sewage sludge (SS) is a good source of EMO, and using it as a source of organic carbon for soils provides a solution to close the nutrient cycle between developing cities and rural agricultural areas [20,21]. Sludge is used as a source of nitrogen (N), phosphorus (P), and organic matter [22]. Moreover, the organic carbon (OC) content of sludge-enriched soil can be up to three times that of soil enriched with inorganic fertilizers [23], making it a good material for soil conditioning. Sewage sludge is a nutrient-rich organic material that contains elements important for plant growth, i.e., NPK and, to a lesser extent, Ca, S and Mg [24]. Combining sewage sludge with agricultural soils can improve soil fertility by promoting microbial activity, improving the physical [25,26] and physicochemical properties of the soil as well as the recycling of plant nutrients [27]. The practice of applying such organic waste to agricultural land as a soil conditioner has become a very attractive management strategy, as it can partially replace the use of chemical fertilizers such as phosphate and nitrogen fertilizers, reducing costs and energy expenditure [28]. Assessment of the suitability of sewage sludge for use on agricultural soils should take into account potential environmental hazards, including the possibility of water contamination with nitrogen or phosphorus compounds and soil and water contamination with heavy metals or epidemiological hazards from pathogenic bacteria and pathogens [29]. When introducing sewage sludge to soils, special attention should be paid to the content of heavy metals in the sludge and the soil to which it will be introduced [30]. Previous studies provide divergent information on the effect of sewage sludge fertilization on the accumulation of heavy metals in soils [31]. Studies by Cheng et al. [32] did not show an increase in the content of heavy metals and their soluble salts under the influence of sewage sludge used in fertilizer doses. Other research shows that fertilization with sewage sludge affects environmental pollution and indirectly affects human health [30].

Soil biological properties, including the activity of enzymes secreted by microorganisms, mesofauna and plant roots, can be used together with physicochemical parameters as indicators of soil health [33]. Soil enzymes play a key role in soil function as they catalyze the mineralization of soil organic matter (SOM), bind C, process nutrients (N, P, S), and are involved in waste management and trace gas emissions [34,35]. These catalysts regulate essential soil ecosystem services [36] and respond rapidly to changes in soil management [37]. Indicators using soil enzyme activity provide information on environmental quality and health, directions of environmental change and can be a useful index for the sustainable management of soil and environmental stability [38,39]. In addition, they are associated with various agricultural practices, such as irrigation, the use of inorganic fertilizers and organic additives, and soil cultivation [40,41]. As a result, soil enzymes are widely used as indicators of soil health due to their strong correlation with soil quality [41]. They present several advantages as indicators, such as operational practicality, sensitivity, integrability, measurability, practicality and cost-effectiveness [42]. Due to the simplicity and speed of measurement, as well as the correlation with most soil properties, the measurement of soil enzyme activity is becoming increasingly attractive for use as indicators of soil health [43]. However, due to the diverse soil properties and functions that can affect soil fertility and health, a credible assessment of soil health can be offered by simultaneous testing of the activity of a number of soil enzymes in combination with analysis of biological, physicochemical and chemical parameters [38,44].

The aim of this study was to assess the influence of sewage sludge on the biochemical parameters of fallow soil. The hypothesis was examined that sewage sludge as a source of exogenous organic matter introduced into fallow soil influences soil enzyme activity through changes in carbon and nitrogen content and selected heavy metals.

2. Materials and Methods

2.1. Study Area and Field Experiment

The experiment was carried out on soil excluded from use (fallow) for 6 years in south-eastern Poland (Modliszewice in the Końskie municipality, Świętokrzyskie Voivodeship). According to the international classification of WRB [45], the soil on which the experiment was carried out was Podzols with a granulometric composition of weakly glycolic sand. The basic parameters of soil properties are presented in Table 1.

Plots of 3 m × 5 m were randomly determined, and EOM in the form of stabilized municipal sewage sludge was introduced into the soil (Table 1). Sewage sludge was used only once. The stabilization of sewage sludge was carried out by the autothermal oxygen stabilization method according to the technology of FUCHS (Germany). The sludge was integrated into the soil at the topsoil level, and willow (*Salix viminalis*) was planted. The experiment included 5 variants: S0—the control object, without the addition of sewage sludge to the soil, and objects with the addition of 30, 75, 150 and 300 Mg ha^{-1} of sewage sludge to the soil.

Table 1. Basic parameters of the properties of soil and sewage sludge (average values, dry matter) [46].

Properties	Units		Fallow Soil	Sewage Sludge
Particle size composition	% (weight) fraction	Sand	86.0	-
		Silt	7.0	-
		Clay	7.0	-
Reaction (pH unit)	H_2O		5.3	6.2
	KCl		4.3	6.0
Hydrolytic acidity	cmol(+) kg^{-1}		4.7	4.7
Exchangeable cations			1.3	50.0
Cation exchange capacity (CEC) of the soil			6.0	54.7
Degree of saturation of the sorption complex	%		21.7	91.4

Table 1. *Cont.*

Properties	Units	Fallow Soil	Sewage Sludge
Available phosphorus (P)	mg kg^{-1}	47	645.0
Available potassium (K)		35	192.0
Available magnesium (Mg)		43	120.0
Total organic carbon (TOC)	g kg^{-1}	11.2	210.0
Total nitrogen (TN)		1.4	17.8
TOC/NT		7.9	11.8
Copper (Cu)	mg kg^{-1}	3.5	86.0
Zinc (Zn)		33.0	2300.0
Lead (Pb)		8.4	125.0
Cadmium (Cd)		0.5	5.0

2.2. Sampling and Analyses

Soil samples for laboratory analyses were taken four times: after sludge introduction and willow planting (I) and after the end of the growing season in the first (II), second (III) and third (IV) year of the study.

Soil samples were taken at a depth of 0–20 cm at 8 randomly selected sites from each experimental plot. Soil material from each plot was averaged into one composite sample and analyzed in triplicate. Soil samples for biochemical analyses were handled according to ISO 18400 [47].

Chemical analyses consisted of determining the following parameters: total organic carbon (TOC), total nitrogen (TN), and total heavy metals Zn, Cu, Pb and Cd. The TOC content was determined with a TOC analyzer [48], using the TOC-VCSH SSM-5000A (Shimadzu Corp., Kyoto, Japan). The TN content was determined using the modified Kjeldahl method [49] with a Kjeltech TM 8100 distilling apparatus. The calculated C:N is the ratio of TOC to TN. The general forms of four heavy metals—cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn)—were determined in accordance with EN ISO 16170:2016-09 [50].

The activity of four soil enzymes, i.e., neutral phosphatase, urease, proteases and dehydrogenases, was determined by Schinner et al. [51]. Neutral phosphatase is involved in the decomposition of phosphorus compounds, while urease and proteases participate in the nitrogen metabolism cycle. Dehydrogenases participate in the biogeochemical carbon cycle in the environment. The classification of the soil enzymes tested included their abbreviations, units used to present the analytical data as well as substrates and products used in the assays, which are presented in Table 2. The activities of the enzymes were determined using a CECIL CE 2011 (Cecil Instrumentation Ltd., Cambridge, UK) spectrophotometer at the following wavelengths: λ = 410 nm for neutral phosphatase and urease, λ = 470 nm for proteases and λ = 485 nm for dehydrogenases (Table 2).

The list of reagents with their type and manufacturer used in laboratory analyses is presented in Table S1 (in the Supplementary Materials).

Table 2. Determination of the activity of soil enzymes.

Enzymes	EC	Acronym	Substrate Name	Product Name	Unit Name
Neutral Phosphatase	3.1.3	APh	*p*-nitrophenyl phosphate disodium	*p*-nitrophenol (PNP)	mmol PNP kg^{-1} h^{-1}
Urease	3.5.1.5	AU	urea	N-NH_4^+	mg N-NH_4^+ kg^{-1} h^{-1}

Table 2. *Cont.*

Enzymes	EC	Acronym	Substrate Name	Product Name	Unit Name
Proteases	3.4.4	APr	sodium caseinate	tyrosine	mg tyrosine kg^{-1} h^{-1}
Dehydrogenses	1.1	ADh	2.3.5-triphenyltetrazolium chloride (TTC)	triphenyl formazane (TPF)	mg TPF kg^{-1} 24 h^{-1}

Explanations: EC—the Enzyme Commission number (a numerical classification scheme for enzymes based on the chemical reactions they catalyze).

2.3. Statistical Analysis

Analysis of variance (ANOVA) was used to statistically analyze the results. The differences between the means for the main factors (dose of sewage sludge and date of sampling) were checked with Tukey's multiple comparison test. A significance level of $p < 0.05$ was assumed, which indicated the presence of statistically significant differences. Linear correlation analysis (r) and principal component analysis (PCA) were performed to illustrate the relationships between the main factors. The statistical analysis of the study results was performed using Microsoft Office Excel 2019 and Statistica PL 13.3 (TIBCO Software Inc., Tulsa, OK, USA).

3. Results and Discussion

Insufficient fallowing and land deposition can lead to soil degradation, including increased water and wind erosion, humus loss and nutrient leaching [52]. Changes occurring in soils excluded from agricultural production are diverse in terms of speed and nature. Therefore, it is not easy to clearly determine the changes occurring in degrading fallow agrocenoses. The pace and direction of changes depend on factors such as soil genesis and type, soil condition, agricultural practices, crop rotation, and the purpose of land exclusion [16,53].

The podzolic soil, which had been set fallowed for 6 years and was made of poorly loamy sand, was characterized by unfavorable properties, including low contents of TOC and NT and available forms of P, K and Mg (Table 1). Bünemann et al. [17] indicate that the integration of fallow land into agricultural use requires the improvement of its defective properties. SOM is important in shaping soil fertility and its ability to provide ecosystem services [54]. Numerous studies indicate that an effective management strategy to increase SOM in cultivated soils is to introduce exogenous organic matter (EOM) into the soil [55].

3.1. Content of TOC, TN in the Soil and C/N

The increase in organic matter (TOC) under the influence of sewage sludge was confirmed under different soil–climatic conditions. The results showed that the fallowed light soil fertilized with EOM in the form of sewage sludge, irrespective of the application rate, was characterized by a significantly higher average total organic carbon content than the soil without organic fertilizer (Table 3). Compared to soil without EOM fertilization (control object S0), the average increase in soil TOC content of the object with the lowest EOM dose (S30) was 24% and with the highest (S300) was 192%. The results obtained are confirmed in studies by numerous authors, which have shown significant correlations between the TOC content in soil and TOC content in sludge [56,57] and the applied dose [58]. Achkir et al. [59] showed that the application of sewage sludge improved total organic carbon content in a dose-dependent manner; in particular, the addition of 30% dry sludge resulted in significant increases in TOC content. The results presented in Table 3 showed that the average TOC content in soil increased from year to year of the study. With the increase in the dose of sewage sludge, the TOC content increased. The results obtained are in line with those of the authors Myszura-Dymek and Żukowska [60], who indicate that the increase in soil TOC content in the following 2–4 years after sludge application is due to its direct and indirect effects. By improving soil properties, sewage sludge increases the net biomass production of cultivated crops, and its residues provide the starting material for the formation of soil organic matter [61].

Table 3. Content of total organic carbon in the soil.

Date of Sampling	Dose of EOM g kg^{-1}					Average for the Term
	S0	S30	S75	S150	S300	
I	10.65 ± 0.02	12.95 ± 0.02	13.42 ± 0.02	23.00 ± 0.08	33.40 ± 0.22	18.68 A
II	10.90 ± 0.08	13.91 ± 0.01	15.82 ± 0.02	26.17 ± 0.17	30.30 ± 0.50	19.42 B
III	11.17 ± 0.02	14.01 ± 0.02	21.82 ± 0.02	29.27 ± 0.26	37.40 ± 0.22	22.57 C
IV	12.67 ± 0.24	15.70 ± 0.24	22.17 ± 0.25	20.30 ± 0.50	42.03 ± 0.12	22.74 C
Average for the variant	11.35 a	14.14 ab	18.31 b	24.68 c	35.78 d	

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil; A–C—different uppercase letters indicate significant differences for the research term; a–d—different lowercase letters indicate significant differences in the dose of sewage sludge.

Nitrogen is one of the most important components in plant nutrition, and its content mainly determines plant growth and yields [62]. The nitrogen content in sewage sludge ranges from approximately 20 to 40 g kg^{-1}. It occurs mainly in organic forms (70–90%) and as a result of organic matter transformations in the soil, it is transformed into mineral forms [63]. The TN content in the sludge used in the study was 17.8 g kg^{-1}. The analysis of the nitrogen content showed that in all study dates, the TN content in the soil fertilized with sewage sludge was significantly higher than in the unfertilized soil (Table 4). The exception was the TN content in the soil of variant S30 in the fourth study date. Compared to the soil without EOM fertilization (control object S0), the average increase in TN content in the soil of the object with the lowest EOM dose (S30) was 11.8%, and that with the highest (S300) 163%. Variance analysis showed that a significant increase in TN content was recorded in the soil of variants S75, S150 and S300. The obtained research results are consistent with the results of other authors, who indicated that the introduction of sewage sludge to the soil increases the TN content, and the extent of this increase depends on the TN content in the sludge, the degree of sludge processing, the level of fertilization and the soil texture [56,57,64,65]. In the subsequent study dates, an increase in the TN content in the soil was observed: from 24.5% in the second date to 80% in the fourth date. The increase in the TN content in the soil in the subsequent dates is caused by the increased mineralization of the organic matter of the sediments and the biomass of plant residues [66].

The changes in TOC and TN content discussed above were reflected in changes in the C/N ratio, which were not clearly directed (Table 5). Regardless of the applied dose of sewage sludge, the C/N ratio was low and close to the optimal one for microbiological transformations [65]. In the soil of variants S30 and S75 in the first term of the study, the C/N ratio was lower, and in the remaining terms, it was close to that found in the control soil (S0). In the soil of variants S150 and S300 in the first term, the C/N ratio was similar to that in soil S0. In the subsequent terms, a decrease in the C/N ratio was noted in the soil of these variants, which was particularly visible in the first term of the study. Similar results were obtained by Mañas et al. [67] and Egiarte et al. [68] who explain the narrowing of the C/N ratio with the passage of time after the introduction of sewage sludge into the soil by the increased mineralization of organic matter and enrichment of the soil with mineral N.

Table 4. Content of total nitrogen in the soil.

Date of Sampling	Dose of EOM g kg^{-1}					Average for the Term
	S0	**S30**	**S75**	**S150**	**S300**	
I	0.92 ± 0.02	1.37 ± 0.00	2.07 ± 0.12	1.96 ± 0.02	2.26 ± 0.01	1.71 A
II	1.25 ± 0.02	1.60 ± 0.01	2.17 ± 0.02	2.44 ± 0.04	3.14 ± 0.02	2.13 A
III	1.35 ± 0.03	1.70 ± 0.02	2.27 ± 0.02	2.63 ± 0.02	3.43 ± 0.01	2.28 AB
IV	2.22 ± 0.02	1.74 ± 0.02	2.62 ± 0.01	2.72 ± 0.01	6.09 ± 0.02	3.08 B
Average for the variant	1.44 a	1.61 a	2.28 b	2.44 b	3.79 c	

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil; A,B—different uppercase letters indicate significant differences for the research term; a–c—different lowercase letters indicate significant differences in the dose of sewage sludge.

Table 5. The influence of the interaction of experimental factors on the C:N ratio in the soil.

Date of Sampling	Dose of Sewage Sludge				
	S0	**S30**	**S75**	**S150**	**S300**
I	11.6	9.5	6.5	11.8	10.4
II	8.7	8.7	7.3	10.7	9.7
III	8.3	8.3	9.6	11.1	10.9
IV	5.7	9.0	8.5	7.5	6.9

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil.

3.2. Heavy Metal Content in Soil

The study evaluated changes in the content of selected heavy metals (Zn, Cu, Pb and Cd) in fallow soil fertilized with sewage sludge. The choice of the metals was dictated by the fact that the Zn content of the sludge was high and close to the permissible content of sludge used in agriculture [69], Cu is a deficient component in Polish soils [70], while Pb and Zn are highly ecotoxic.

The heavy metal contents of the sludge used in the study (Table 1) were below those permitted by EU [71] and Polish [69] regulations. The average content of Zn, Cu, Pb and Cd in the studied fallow light soil increased, generally significantly, as the proportion of EOM in the soil increased (Figure 1, Tables S2–S5 in Supplementary Materials). Compared to the control soil (S0), the average increase in the content of these elements in the soil was, at the lowest sludge dose (S30), from about 30% (for Zn and Cu) to 152% (for Cd), and in the combination with the highest dose (S300), it was from 162 (for Pb) to 607% (for Cu). The magnitude of the observed differences depended on the test date and the element analyzed. The content of Zn, Cu and Pb, regardless of the date of testing, was below the permissible levels in agricultural soils [72]. The Cd content exceeded the permissible content for very light agricultural soils in the variants S150 in the 1st and 2nd study dates and S300 in all study dates, but it was within the permissible content for light and medium agricultural soils as well as forest and industrial soils [72]. In the 3rd and 4th study dates (after a period of rapid biomass growth of *Salix viminali* L.), heavy metal contents were significantly lower than in date I (Figure 1, Tables S2–S5 in Supplementary Materials). This demonstrates the phytoremediation abilities of willow. *Salix viminalis* L. has characteristics beneficial for phytoremediation [73], including high biomass productivity, adaptability to new environmental conditions, resistance to contaminants present in the soil and the selective accumulation of contaminants [74–76]. In our study, the contents representing the potential risk of contamination concerned Cd. Cadmium is one of the most toxic metals and tends to adsorb in the topsoil [77]. Cosio et al. [75] indicated that Salix species were promising for Cd phytoextraction, as on soil with high cadmium concentrations, there was little reduction in biomass yield and the biomass was characterized by significant increases in Cd content, mainly in shoots and roots.

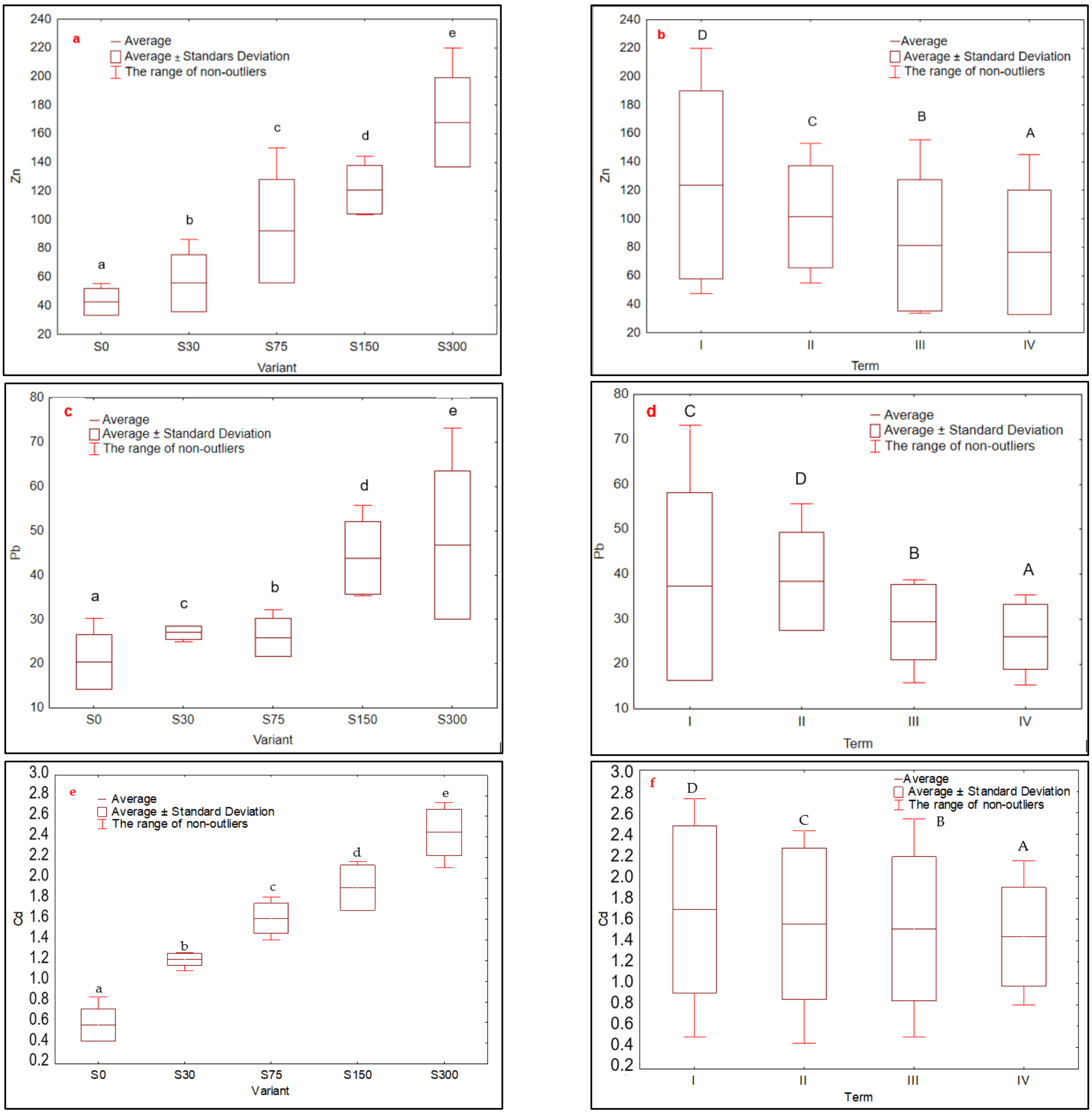

Figure 1. *Cont.*

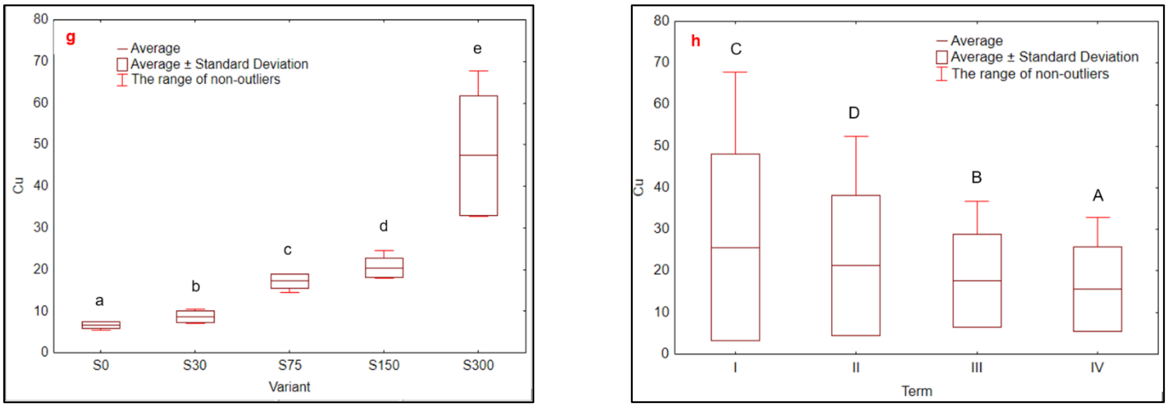

Figure 1. Box and whiskers plot: (**a**) average Zn content for the variant, (**b**) average Zn content for the term, (**c**) average Pb content for the variant, (**d**) average Pb content for the term, (**e**) average Cd content for the variant, (**f**) average Cd content for the term, (**g**) average Cu content for the variant, (**h**) average Cu content for the term. A–D—different uppercase letters indicate significant differences for the research term; a–e—different lowercase letters indicate significant differences in the dose of sewage sludge.

The overall enzymatic activity of the soil, which can be expressed by, among other things, the activities of neutral phosphatase, urease, proteases and dehydrogenases, is one of the main biological indicators for directly determining the quality and health of soils [78]. Soil enzymes include hydrolases, which help to extract nitrogen (urease and proteases) and phosphorus (phosphatases) to support primary metabolism; or oxidoreductases (e.g., dehydrogenases) that contribute to the breakdown of organic compounds [79]. The intensity of the enzymatic processes studied in the light fallow soil depended on the EOM dose applied, the timing of the study and the individual properties of the soil enzyme (Tables 6–9).

The mean activity of neutral phosphatase (Table 6) and urease (Table 7) generally increased significantly with increasing the proportion of sewage sludge in the light soil tested. The introduction of EOM into the soil also stimulated protease activity (Table 8) with no statistically significant differences between the S75, S150 and S300 sites. Studies by other authors have shown a significant correlation between the sludge application rate and the activity of phosphatases [80], urease [81,82] and soil proteases [81]. Many authors have shown that fertilization of soil with sewage sludge stimulates enzyme activity, which has been attributed to significant amounts of nutrients, organic matter, and the additional pool of microorganisms introduced with EOM [83–85]. Some micro-organisms contained in sewage sludge can colonize the soil to some extent and introduce extracellular enzymes into the soil, thus contributing to a periodic increase in the enzymatic activity of the soil [84]. Studies show that enhancing fallow light soil with sewage sludge stimulates the activity of enzymes responsible for the environmental cycling of P and N. The results of the correlation analysis show a significant relationship between AU and the content of TOC and TN in the soil and between APh and TOC (Table 10). Such correlations were not found for proteolytic activity, which may indicate that APr is not dependent on the soil C and N content. The higher protease activity of EOM-modified soils than of control soils probably depended on the proteins added with the sludge, which stimulated both microbial growth and the microbial synthesis of proteases [86].

Table 6. Neutral phosphatase activity in soil.

Date of Sampling	Dose of EOM mmol PNP kg^{-1} h^{-1}					Average for the Term
	S0	**S30**	**S75**	**S150**	**S300**	
I	14.96 ± 0.32	19.76 ± 0.14	31.28 ± 1.06	82.83 ± 0.07	94.68 ± 0.70	48.70 A
II	17.91 ± 0.70	37.82 ± 0.40	47.03 ± 0.11	78.50 ± 0.04	78.76 ± 1.06	52.01 B
III	16.12 ±0.07	70.94 ± 0.14	79.90 ± 1.06	77.98 ± 0.45	77.62 ± 0.70	55.34 C
IV	22.26 ± 0.07	58.54 ± 0.38	59.40 ± 0.38	64.26 ± 1.06	72.25 ± 0.78	64.52 C
Average for the variant	17.81 a	46.77 b	54.40 c	75.90 d	80.83 e	

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil; A–C—different uppercase letters indicate significant differences for the research term; a–e—different lowercase letters indicate significant differences in the dose of sewage sludge.

Table 7. Urease activity in soil.

Date of Sampling	Dose of EOM mg N-NH_4^+ kg^{-1} h^{-1}					Average for the Term
	S0	**S30**	**S75**	**S150**	**S300**	
I	18.44 ± 0.04	36.48 ± 0.06	41.33 ± 0.40	51.80 ± 0.08	56.41 ± 0.30	40.89 D
II	17.96 ± 0.04	26.35 ± 0.25	27.68 ± 0.09	29.57 ± 0.29	33.36 ± 0.26	26.99 C
III	13.70 ± 0.01	14.49 ± 0.13	15.53 ± 0.05	15.10 ± 0.08	26.06 ± 0.04	16.98 B
IV	11.05 ± 0.00	11.09 ± 0.08	12.86 ± 0.04	13.40 ± 0.02	16.90 ± 0.07	13.06 A
Average for the variant	15.29 a	22.10 b	24.35 b	27.47 c	33.18 d	

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil; A–D—different uppercase letters indicate significant differences for the research term; a–d—different lowercase letters indicate significant differences in the dose of sewage sludge.

Table 8. Proteases activity in soil.

Date of Sampling	Dose of EOM mg Tyrosine kg^{-1} h^{-1}					Average for the Term
	S0	**S30**	**S75**	**S150**	**S300**	
I	15.89 ± 0.01	23.12 ± 0.02	26.17 ± 0.05	26.30 ± 0.01	32.09 ± 0.07	24.71 A
II	16.88 ± 0.03	24.40 ± 0.01	26.43 ± 0.09	38.22 ± 0.02	41.52 ± 0.06	29.49 B
III	20.85 ± 0.18	33.16 ± 0.20	40.73 ± 0.02	39.19 ± 0.02	48.12 ± 0.06	36.42 C

Table 8. *Cont.*

Date of Sampling	Dose of EOM mg Tyrosine kg^{-1} h^{-1}					Average for the Term
	S0	S30	S75	S150	S300	
IV	38.64 ± 0.18	48.70 ± 0.02	67.28 ± 0.02	64.26 ± 0.04	5120 ± 0.02	54.02 D
Average for the variant	23.06 a	32.34 b	40.15 c	41.99 c	43.24 c	

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil; A–D—different uppercase letters indicate significant differences for the research term; a–c—different lowercase letters indicate significant differences in the dose of sewage sludge.

Table 9. Dehydrogenase activity in soil.

Date of Sampling	Dose of EOM mg TPF kg^{-1} 24 h^{-1}					Average for the Term
	S0	S30	S75	S150	S300	
I	1.86 ± 0.01	1.88 ± 0.00	3.62 ± 0.01	8.67 ± 0.03	2.49 ± 0.00	3.62 D
II	1.25 ± 0.00	1.41 ± 0.01	2.88 ± 0.02	3.62 ± 0.03	2.35 ± 0.00	2.30 C
III	1.28 ± 0.01	1.40 ± 0.01	2.09 ± 0.02	2.20 ± 0.01	2.25 ± 0.02	1.80 B
IV	1.22 ± 0.01	1.26 ± 0.01	1.97 ± 0.01	1.97 ± 0.02	2.13 ± 0.04	1.71 A
Average for the variant	1.40 a	1.49 a	2.64 b	4.12 c	2.31 b	

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil; A–D—different uppercase letters indicate significant differences for the research term; a–c—different lowercase letters indicate significant differences in the dose of sewage sludge.

Table 10. Significant correlation coefficients between the examined parameters of soil.

	TOC	TN	Zn	Cu	Pb	Cd	APh	AU	APr	GMea
TOC		0.945	0.989	0.924	0.969	0.955	0.932	0.952	ns	0.921
TN	*		0.978	0.993	ns	0.934	ns	0.934	ns	ns
Zn	**	**		0.955	0.924	0.968	0.916	0.96	ns	0.908
Cu	*	**	*		ns	0.896	ns	0.903	ns	ns
Pb	**	-	*	-		0.906	0.931	0.912	ns	0.907
Cd	*	*	**	*	*		0.974	0.996	0.951	0.950
APh	*	-	*	-	*	**		0.971	0.96	0.981
AU	*	*	*	*	*	**	**		0.93	0.93
APr	-	-	-	-	-	*	*	*		0.969
GMea	*	-	*	-	*	*	**	*	**	

Explanations: ** significant at $\alpha = 0.001$; * significant at $\alpha = 0.01$; ns—not statistically significant.

Amino acids resulting from protein proteolysis can be directly taken up by microorganisms and higher plants, but most of these compounds are further converted by ammonification. This process is mainly carried out by ammonification microorganisms, which produce urease. Its activity indicates the intensity of the transformation of nitrogenous compounds in the soil and thus can indicate the availability of nitrogen to plants [87]. Urease is also used to assess the ecological status of soils exposed to organic waste [81].

Phosphatases are assumed to be ubiquitous in soil and are produced by soil microorganisms and plant roots in response to low levels of inorganic P [88]. However, a recent study by Margalef et al. [89] showed that the phosphatase activity in soil depended on the organic phosphorus content rather than the availability of mineral forms of this element. In the context of the present study, APh indicates that EOM can be an important source of phosphorus. Sewage sludge, being rich in organic matter and inorganic nutrients, can be an alternative to manure as long as the level of potentially toxic contaminants is low [80]. This treatment improved soil microbiological and biochemical properties, such as some soil enzyme activities (neutral phosphatase, urease, protease, and dehydrogenases), which promote the recycling of nutrients for crops.

Based on the activity of dehydrogenases, which occur only in living cells, one can assess the total range of oxidative activity of soil microflora. ADh can be a good indicator of microbial activity. This was confirmed by studies by Joniec et al. [84,90], which indicate that sewage sludge stimulated dehydrogenase activity and increased the abundance of many groups of bacteria and fungi. In this study, the highest average dehydrogenase activity was observed in soil to which 150 Mg ha^{-1} of sludge was introduced. The activity of dehydrogenases in the soil of the S150 object was 2 times higher compared to the activity of this group of enzymes in the soil of the S0 object (Table 9). In contrast, a weakening of ADh activity was observed after the largest sludge application (S300) compared to the variant with the addition of 150 Mg ha^{-1} of sludge (S150). The weakening of ADh under S300 conditions may have been associated with a several-fold increase in the heavy metal content of the soil of this variant (Figure 1). The influx of heavy metals into the soil causes quantitative and qualitative changes in the composition of the soil microflora, resulting in changes in enzymatic activity [91,92]. However, our own studies did not show statistically significant correlations between ADh and heavy metals (Zn, Cu, Pb, Cu).

The contents of Zn, Pb, and Cd in the soil were significantly positively correlated with APh (respectively, r = 0.916, r = 0.931 and r = 0.974) and AU (r = 0.960, r = 0.912 and r = 0.996). The Cu content was positively correlated only with AU (r = 0.903) (Table 10). Heavy metals can negatively affect biological processes in soil. The inhibition of soil enzyme activity depends on the concentration and type of heavy metal, with levels of inhibition varying with enzyme type. However, it has been observed that at certain concentrations, some heavy metals can stimulate enzyme activity [93]. Reduced enzyme activity may result from heavy metal interactions within the enzyme–substrate complex and cause a denaturation of the enzyme protein. Many metal ions are enzyme activators, e.g., Fe^{2+} ions activate the catalytic activity of peroxidases, while Mn^{2+} activates phosphotransferases, Mg^{2+} activates phosphatases, and Zn^{2+} activates dehydrogenases [94]. TOC and TN were also significantly positively correlated with the assimilable content of Zn (r = 0.989, r = 0.978), Cu (r = 0.924, r = 0.993) and Cd (r = 0.955, r = 0.934). The Pb was positively correlated only with TOC (r = 0.969). Kwiatkowska-Malina [95] observed that organic matter in acidic soils is the main adsorbent of trace metals.

The soil quality index was determined based on the geometric mean activity of the enzymes tested (GMea) [96]. Individual enzymes showed a different response to EOM fertilization, while GMea showed a significant increase in the quality of the fallowed soils as the EOM rate increased to 150 Mg ha^{-1} (Table 11). GMea was a suitable index to integrate the whole set of soil enzyme values into a single numerical value, which was sensitive to the enrichment of soils with EOM [97].

Table 11. GMea index values.

Date of Sampling	Dose of EOM					Average for the Term
	S0	**S30**	**S75**	**S150**	**S300**	
I	8.88	13.31	18.70	31.45	25.55	19.58 B
II	9.08	13.61	17.74	23.80	22.50	17.35 A
III	8.77	14.78	17.77	17.85	21.34	16.10 A
IV	11.53	14.13	18.10	18.17	19.37	16.26 A
Average for the variant	9.56 a	13.96 b	18.08 c	22.82 d	22.19 d	

Explanations: EOM—exogenous organic matter; date of sampling: I—after the introduction of sediment and planting willow in spring in the first field of research, II—after the end of the vegetation period in the first year of research, III—after the end of the vegetation period in the second year of research, IV—after the end of the vegetation period in the third year of research year of research; Dose of sewage sludge: S0—control object, without the addition of sewage sludge to the soil, S30—object with the addition of 30 Mg ha^{-1} of sewage sludge to the soil, S75—object with the addition of 75 Mg ha^{-1} of sewage sludge to the soil, S150—object with the addition of sewage sludge 150 Mg ha^{-1} of sewage sludge to the soil, S300—object with the addition of 300 Mg ha^{-1} of sewage sludge to the soil; A,B—different uppercase letters indicate significant differences for the research term; a–d—different lowercase letters indicate significant differences in the dose of sewage sludge.

The term of the observations had a statistically significant effect on the enzymatic activity of the soil with no unidirectional changes in the enzymes studied. The highest activity of APh and APr was found in the 4th study term, and the lowest was found in the 1st study term. Opposite results were obtained for AU, ADh and the GMea index. The decrease in AU observed during the study period, of an enzyme with a very narrow substrate spectrum, may indicate depletion of the substrate necessary for the enzyme's activity. Enzyme activity is variable over time and limited by substrate availability [98]. Joniec [84], on the other hand, showed that a single application of a higher amount of sewage sludge (100 Mg ha^{-1}) to the soil resulted in increased enzymatic activity that persisted for up to several years.

Principal component analysis (PCA) (Figure 2) was used to estimate the causal relationships between the parameters analyzed. Factors 1 and 2 together describe 93.16% of the variance of the examined chemical and biochemical properties of soils. Factor 1 explains 83.29% of the variance of the examined parameters and is significantly correlated with all indices except CN and ADh. Factor 2 explains 9.87% of the variability of the examined traits and is most significantly correlated with CN and ADH. The contents of TN, CU, AU, Cd, Zn, APr and APh are positively correlated with each other. Thus, if the content of one indicator increases, the content of the other also increases. Among the indicators studied, there are no negatively correlated ones. Considering both dimensions (factors), it can be seen that variant S0 and S30 had the lowest values of all indicators, while variants S150 and S300 had the highest values, of which S150 stood out in terms of the amount of CN and Adh compared to the other variants. The S300 variant had significantly higher TOC, TN, AU, Zn, Cu and Cd contents than the other variants. In the opinion of Ghaemi et al. [99], PCA is a method for selecting the most effective parameters that play an important role in the sustainable development of soil. On the basis of the PCA analysis results, Lemanowicz et al. [94] confirmed the influence of agricultural practices on the biochemical properties of Phaeozem soils. Makó et al. [100], on the other hand, using PCA, showed that chemical properties can be used to identify the Chernozems and Luvisols, whereas physical properties can be used to identify Arenosols and sandy Cambisols.

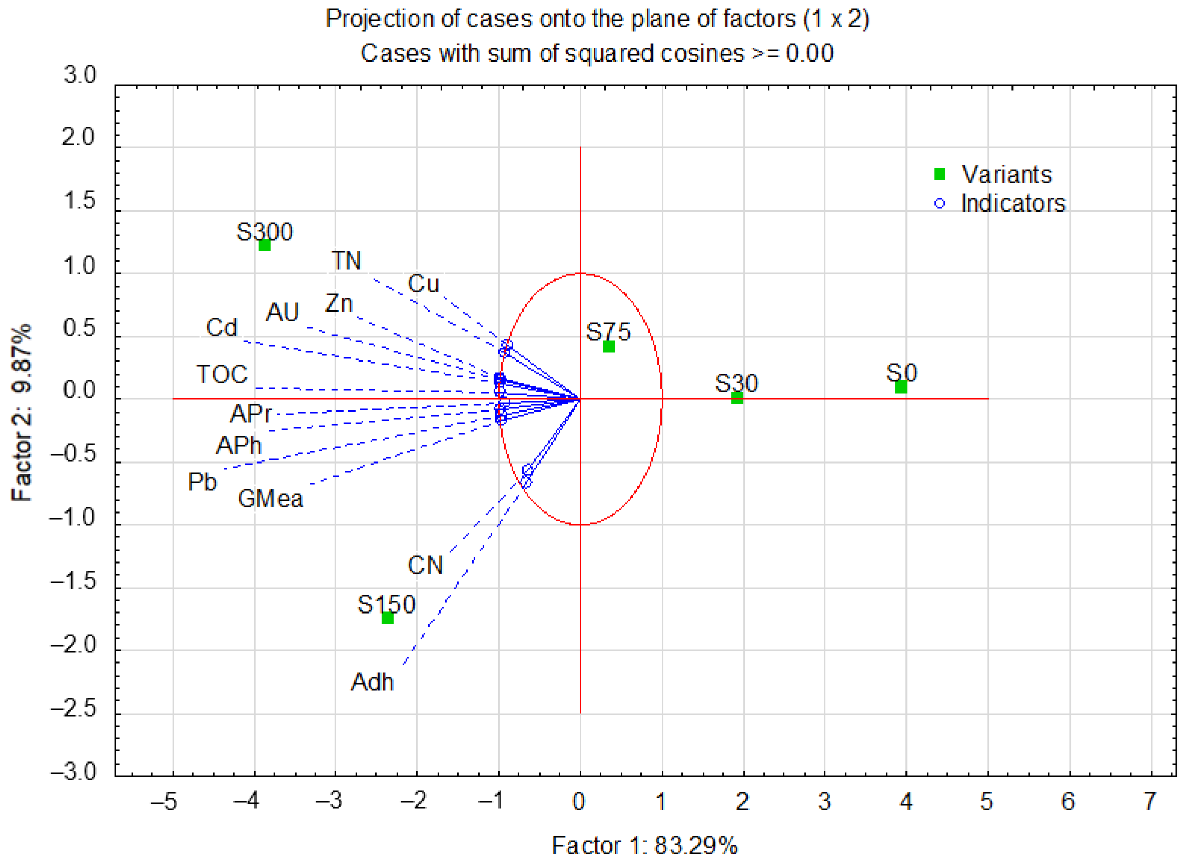

Figure 2. Biplot—combination of bivariate factor plot for EOM doses as cases (S0, S30, S150, S300) with bivariate factor plot for variables such as TOC, TN, C/N, Zn, Cu, Pb, Cd, Aph, AU, APr, ADh, GMea.

4. Conclusions

One of the possible ways to manage sewage sludge is to use it in agriculture. The study focused on the possibility of using soil enzyme activity and the GMea index in connection with TOC and TN content to assess the quality of fallow light soil after EOM fertilization. The application of sewage sludge increased TOC in the fallow soil in a dose-dependent manner, especially in variants S75 to S300. The addition of sewage sludge and subsequent planting of willow (*Salix viminalis*) had a beneficial effect on the TOC balance in the subsequent years of the study. A significant increase in TN content was recorded in the soil of variants S75, S150 and S300. The higher TN content in the subsequent years of the study, which was significant in the fourth year of the study, resulted from the increased mineralization of the organic matter of the sediments and the biomass of plant residues. Regardless of the applied dose of sewage sludge, the C/N ratio was low and close to the optimal one for microbiological transformations. The addition of sewage sludge increased the content of assessed heavy metals in the soil. The extent of this increase depended on the sludge dose and the specificity of the assessed metal. A significant decrease in the content of heavy metals in the soil was noted after 4 years, which indicates the phytological meliorative abilities of willow (*Salix viminalis*). The introduction of sewage sludge has helped to stimulate biological life in the soil. This is evidenced by the intensification of soil enzyme activity. However, individual enzymes showed a different response to EOM fertilization, while GMea showed a significant increase in the quality of the fallowed soils as the EOM rate increased to 150 Mg ha^{-1}. GMea was a suitable index to integrate the whole set of soil enzyme values into a single numerical value, which was sensitive to the enrichment of soils with EOM. Enzymatic activity was also shown to be time-variable and limited by substrate availability. The natural use of EOM to improve the quality of fallow soils allows accumulated and immobilized nutrients, mainly carbon and nitrogen, to be incorporated into the cycle. This is in line with the assumptions of the European Green Deal, including actions to increase the content of organic matter, improve the ecological condition of soils and limit the use of mineral fertilizers. However, we believe that in order to obtain a more complete picture of the changes taking place in organically amended soils, especially with sewage sludge, it is advisable to continue monitoring enzymatic activity and chemical parameters in the subsequent years.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14101810/s1, Table S1: List of reagents with type and manufacturers used during laboratory analyses, Table S2: The influence of the interaction of experimental factors on the zinc content in the soil, Table S3: The influence of the interaction of experimental factors on the copper content in the soil, Table S4: The influence of the interaction of experimental factors on the lead content in the soil, Table S5: The influence of the interaction of experimental factors on the cadmium content in the soil.

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