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The Usefulness of Ozone-Stabilized Municipal Sewage Sludge for Fertilization of Maize (*Zea mays* L.)

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Abstract: Sewage sludge generated in the wastewater treatment process is a waste material and a serious environmental nuisance. Due to its specific properties, the management and final disposal of sewage sludge is a considerable problem also in Poland. Ozonation of sewage sludge is the most commonly used process based on the use of oxidizing agents for stabilization of the waste. This process results in substantial reduction of the sludge volume and simultaneous production of small amounts of toxic by-products. Despite the effectiveness of ozone in sanitation and reduction of sludge amounts and in improvement of many parameters, still little is known about the use of ozonated sewage sludge for agricultural purposes, e.g., fertilization of arable crops. Therefore, the present study was an attempt to evaluate the effect of ozone-stabilized sewage sludge on maize development in initial stages of growth in pot experiment conditions. We analyzed the effect of ozone-stabilized sewage sludge in soil on dry matter yields of aboveground parts of maize. We also conducted physiological measurements of chlorophyll content, fluorescence, and exchange. Additionally, the content of macro- and microelements and toxic heavy metals in aboveground maize biomass was determined. The ozone-stabilized sewage sludge exerted a positive impact on all maize parameters in the initial stage of growth. Compared to the control, plants fertilized with this type of sludge were characterized by a 50% higher yield of aboveground biomass and over 80% higher content of chlorophyll. Furthermore, the content of most macro- and microelements in the aboveground biomass was generally higher in plants fertilized with the ozonated sludge than in plants from the other experimental variants. The chlorophyll fluorescence and gas exchange parameters in plants fertilized with ozonated sludge were improved. No excessive accumulation of Pb and Cd was detected. The present results have confirmed that ozone-stabilized sewage sludge can be used for cultivation of agricultural plants, as it improves utilization of deposited nutrients. The improved bioavailability of nutrients was associated with ozonation-induced initial degradation of organic matter and release of deposited plant nutrients.

Keywords: maize; municipal sewage sludge; sewage sludge stabilization; ozonation process; gas exchange; relative chlorophyll content; chlorophyll fluorescence

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1. Introduction

Sewage sludge is an insoluble residue from the wastewater treatment process. With its properties, sewage sludge is a waste material that must be properly managed and finally disposed of due to restrictive legal regulations regarding environmental protection [1,2].

The amount of sewage sludge generated every year is gradually increasing, which is associated with social and economic development. Millions of tons of this waste are generated annually mainly in industrialized and developed countries, e.g., Japan (70 million), China (30 million), and the USA (6 million) [3]. In Poland, nearly 1 million tons of sewage sludge dry matter was produced in 2020 [4]. Due to the large amounts of sewage sludge produced every year and its specific properties, many countries, including Poland, face a huge problem of proper management and final disposal of this waste. Reduction of the amounts and neutralization of sewage sludge as well as enhancement of the recovery of resources deposited therein are essential for elimination of environmental pollution caused by the accumulation of the waste [5]. Therefore, there are attempts to reduce the generation of sewage sludge with the use of various physical and chemical processes, e.g., thermal hydrolysis, mechanical disintegration, ultrasonic treatment, hydrolysis and acidification, microwave irradiation, alkaline treatment, and ozonation [6–13]. Ozonation of sewage sludge, i.e., an oxidation process, is most widely used as one of the methods for stabilization of sludge. This process results in a large decrease in the sludge volume, simultaneously yielding a small amount of toxic by-products [5,13–15]. It has been shown that oxidation-based treatments in combination with biological degradation are highly effective in reducing the amounts of generated sludge, but economic viability is their main limitation [16]. The use of ozonation to reduce the amounts of sewage sludge was proposed in the mid-1990s and has already been used in both full-scale industrial and municipal wastewater treatment plants [16]. At present, sludge ozonation is economically feasible for large-capacity treatment plants, in areas where the costs of sludge treatment are very high, or in the case of operational problems such as sludge foaming and swelling [16].

Sewage sludge has certain physical, chemical, and microbiological properties that considerably impede or even prevent its management and final disposal. Moreover, its chemical composition is highly heterogeneous and depends on, e.g., the wastewater type, treatment technologies used, seasons of the year, etc. [17].

The ozonation process leads to changes in sewage sludge properties. As a strong oxidant, ozone inactivates microorganisms via destruction of their cell walls and disruption of viral and bacterial metabolism, thereby contributing to sanitization of sludge [13,18]. Ozonation results in disintegration of sewage sludge and increases the content of soluble organic matter [19]. Hence, ozonated sludge is characterized by a much better dewatering and settling ability, which reduces the amounts of this waste material [13,20].

One of the methods for sewage sludge management is its use in agricultural applications, including fertilization of crops, which is one of the most rational and cost-effective solutions [3,15]. The potential use of sewage sludge for agriculture purposes is determined by its physical, chemical, and biological properties, e.g., dry matter content, pH, macro- and microelement content, and concentrations of inorganic, organic, and microbiological pollutants [21]. Sewage sludge contains large amounts of organic matter and plant nutrients, such as N or P, which improve soil physical, chemical, and biological properties, as widely documented in the literature [22–24]. With its high content of organic matter, N, P, and other elements that are important for fertilization purposes, sewage sludge should be included in plant fertilization schemes, for example, in the case of crops intended for food and feed production, provided that the allowable content of pollutants specified by legal regulations is not exceeded [17,25,26]. Municipal sewage sludge from small wastewater treatment plants is especially suitable for agricultural use [25]. In Poland, over 16% of the total pool of sewage sludge dry matter was used for agricultural purposes in 2020 [4]. In Poland, there has been a systematic increase in the area of farmland cultivated in maize, which is one of the highest yielding cereal plants. Due to the large mass of its vegetative and generative organs, maize requires high doses of all nutrients [27].

The ozonation of sewage sludge has been shown to be effective in reducing its amounts and sanitary contamination [10,13,16]. However, little is known of the use of sewage sludge after the ozonation process, e.g., in the production of plants intended for consumption. Therefore, this study was an attempt to assess the effect of ozone-stabilized sewage sludge on maize development in the initial stages of growth in pot experiments. Of note, the sludge organic matter subjected to the ozonation process undergoes considerable conversion [13]; therefore, it was assumed that the ozonated sewage sludge would be characterized by greater bioavailability of deposited nutrients, which would be directly reflected in the growth and development of maize plants.

The aim of the study was to evaluate the effect of maize fertilization with ozone-stabilized sewage sludge. The impact of ozonated sewage sludge on chlorophyll fluorescence and gas exchange parameters as well as macro- and microelement content in aboveground maize biomass in the initial growth stage was assessed.

2. Materials and Methods

2.1. Municipal Sewage Sludge Preparation

The experiment was based on the use of municipal sewage sludge from a mechanical and biological wastewater treatment plant in Ropczyce (southeastern part of Poland, Podkarpackie Province, GPS coordinates 50°04′50.0″ N 21°35′26.8″ E). The sewage flowing into the treatment plant is a mixture of domestic wastewater with rainfall or snowmelt water. Raw sewage is fed into the main pumping station and then transferred onto gratings. After separation of solids, the sewage is transferred to a horizontal sand separator and then to a bioreactor equipped with predenitrification, dephosphatation, denitrification, and nitrification chambers. The surplus activated sludge is collected in the secondary settling tank and partially returned to the predenitrification chamber. The remaining part of the sludge is thickened in a gravity thickener and dewatered on a filter press. The maximum load of the activated-sludge wastewater treatment plant expressed as the population equivalent is 9247. The Wielopolka River is the recipient of the sewage.

The sewage sludge collected from the thickening chamber of the mechanical-biological wastewater treatment plant in Ropczyce was stabilized using an originally designed reactor, whose operation was described in an earlier study [13].

After the ozonation process, the sludge was thickened by gravity filtration using a sludge bagger (DRAIMAD® type, EKOFINN-POL, Banino, Poland). The 24 h filtration resulted in a 95% dewatering level.

In accordance with the results presented in [13], sewage sludge stabilized with ozone for 60 min was selected for further pot experiments due to the optimal properties of the process.

2.2. Pot Experiment Design

The effect of ozone-stabilized sewage sludge on the physiological parameters of maize plants in the initial growth stages was assessed in a pot experiment. The pot experiment was carried out in a completely randomized system, in 3 independent series, with sewage sludge collected in different terms: spring, summer, and autumn—series I, II, and III, respectively. Each variant of the experiment was represented by 3 pots. Approximately 6 kg of dry soil material with sand (S) texture (89% sand, 9% silt, 2% clay) was placed in 20 × 20 × 23 cm plastic pots (6 L volume). The properties of the soil material used in the experiment are shown in Table 1.

Table 1. Selected properties of soil used in the pot experiments (mean ± SE).

pH	SOC	Nt	Available Forms of Nutrients				Total Concentrations of Elements				
			P	K	Mg	Zn	Cu	Ni	Cr	Pb	Cd
H_2O	%		mg kg^{-1}								
6.67 ± 0.07	0.06 ± 0.01	0.01 ± 0.0	45.8 ± 8.7	11.5 ± 0.2	17.4 ± 1.1	28.9 ± 0.6	4.89 ± 0.3	0.23 ± 0.04	0.15 ± 0.01	1.45 ± 0.2	0.06 ± 0.00

Explanation: SOC—soil organic carbon; Nt—total nitrogen.

Soil parameters used in the experiments were determined as follows: particle size distribution was determined by the laser diffraction method using a Laser Particle Sizer ANALYSETTE 22 (Fritsch, Idar-Oberstein, Germany); pH was analyzed by pH-meter by Hanna Instruments HI 4221 (Nusfalaucity, Romania) in a 1:2.5 soil–water suspension; soil organic carbon (SOC) was determined using the Walkley–Black procedure; total nitrogen (Nt) was determined by the dry combustion method using the auto analyzer Vario El CUBE (Elementar Analysensysteme GmbH, Langenselbold, Germany); available forms of nutrients (P, K, Mg) were determined by Mehlich 3 method; and total concentrations of elements (Zn, Cu, Ni, Cr, Pb, Cd) were determined by atomic absorption spectrometry technique (HITACHI Z-2000 Tokyo, Japan) after mineralization of 2 g dry soil samples in 70% $HClO_4$.

The pots were saturated to 55% of the maximum water capacity and left for 14 days. Next, portions of non-ozonated sewage sludge SS_N and ozonated (for 60 min) sewage sludge SS_O were added to the pots and mixed with the surface layer of the soil. Soil without the addition of the sewage sludge was the control sample, hereinafter referred to as the Control.

The pots were again left for 21 days at the temperature of 21 °C, maintaining 55% maximum water capacity. After this time, 6 seeds of maize (variety MAS 29.T) were sown per pot, assuming a density of 280–320 m^{-2} of plants. The pots were then placed in a growth chamber (Model GC-300/1000, JEIO Tech Co., Ltd., South Korea) at 22 ± 2 °C, humidity 60 ± 3% RH, a photoperiod of 16/8 (L/D) h, and an approximately 300 $\mu E\ m^{-2}\ s^{-1}$ light intensity maximum. The pot positions in the growth chamber were randomized every week.

Each experimental series was run until the plants reached the 6-leaf stage (BBCH16 Code). In our conditions, this phase was achieved by the plants after ca. 2 months of the experiment. During the experiment, each pot was maintained at 55% maximum water capacity.

The sewage sludge doses used in the experiment were selected in accordance with the Regulation of the Minister of Environment in force in Poland on the use of sewage sludge for agricultural purposes, including production of plants intended for human and animal consumption [24]. The following doses of sewage sludge were used: 3, 6, and 9 Mg ha^{-1} d.m., i.e., D1, D2, and D3, respectively. The doses corresponded to the amounts of sewage sludge allowed to be used in one application once a year, a cumulative dose for 2 years, and a cumulative dose for 3 years, respectively [28]. The sewage sludge doses used in the experiment per pot in each series of experiments are presented in Table 2. No supplementary fertilization with NPK mineral fertilizers was used throughout the study period. The properties of sewage sludge used in each series of the pot experiment are shown in Table 3 in the Results and discussion section.

Table 2. Doses of sewage sludge dry matter per pot in each experiment series.

Sewage Sludge Dose (g pot^{-1})	Experiment Series					
	SS_N			SS_O		
	I	II	III	I	II	III
D1	11.58	11.54	11.56	11.49	11.44	11.45
D2	23.16	23.08	23.12	22.98	22.88	22.90
D3	37.74	34.62	34.68	34.48	34.32	34.36

Explanation: D1, D2, D3—doses of sewage sludge used in the experiment; SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; I, II, III—experiment series.

Table 3. Selected properties of sewage sludge used in the pot experiments (mean ± SE). Identical superscripts denote no significant ($p > 0.05$) differences between the experimental variants according to the post hoc Tukey HSD test.

Parameters	Units	Experiment Series					
		SS_N			SS_O		
		I	II	III	I	II	III
dry matter	%	6.92 ^{a} ± 0.26	7.52 ^{ab} ± 0.18	7.39 ^{ab} ± 026	8.54 ^{c} ± 0.11	9.44 ^{d} ± 0.19	9.11 ^{d} ± 0.15
pH	-	6.89 ^{a} ± 0.07	6.82 ^{a} ± 0.11	6.92 ^{a} ± 0.09	6.83 ^{a} ± 0.10	6.75 ^{a} ± 0.06	6.80 ^{a} ± 0.08
TOC	% d.m.	42.3 ^{ab} ± 0.12	40.7 ^{a} ± 0.25	44.2 ^{b} ± 0.69	42.0 ^{ab} ± 0.18	40.6 ^{a} ± 0.21	43.9 ^{b} ± 0.19
Nt	% d.m.	7.36 ^{b} ± 0.03	7.06 ^{ab} ± 0.04	7.02 ^{ab} ± 0.05	7.13 ^{ab} ± 0.03	6.93 ^{a} ± 0.01	6.85 ^{a} ± 0.06
P	g kg^{-1} d.m.	12.31 ^{a} ± 1.49	17.23 ^{c} ± 2.04	13.73 ^{b} ± 1.76	13.33 ^{ab} ± 1.65	17.87 ^{c} ± 2.03	13.07 ^{ab} ± 1.21
Ca	g kg^{-1} d.m.	29.72 ^{a} ± 2.56	48.38 ^{c} ± 11.2	27.25 ^{a} ± 5.21	29.61 ^{a} ± 3.43	57.55 ^{cd} ± 8.23	31.49 ^{ab} ± 5.24
Mg	g kg^{-1} d.m.	1.70 ^{a} ± 0.98	2.48 ^{ab} ± 0.78	2.23 ^{b} ± 0.85	1.94 ^{ab} ± 0.67	2.33 ^{ab} ± 0.43	2.12 ^{ab} ± 0.33
K	g kg^{-1} d.m.	5.62 ^{a} ± 1.06	8.12 ^{d} ± 1.23	7.49 ^{c} ± 1.24	5.21 ^{a} ± 0.78	5.90 ^{ab} ± 0.96	5.54 ^{a} ± 0.42
Fe	g kg^{-1} d.m.	6.20 ^{b} ± 0.57	8.39 ^{cd} ± 0.87	3.94 ^{a} ± 0.46	7.61 ^{c} ± 0.69	9.65 ^{d} ± 0.83	4.51 ^{a} ± 0.54
Mn	mg kg^{-1} d.m.	82.3 ^{a} ± 9.9	97.4 ^{ab} ± 11.7	87.8 ^{a} ± 10.5	97.3 ^{ab} ± 11.7	111.7 ^{b} ± 13.4	84.8 ^{a} ± 11.4
Zn	mg kg^{-1} d.m.	467 ^{a} ± 33	648 ^{b} ± 55	565 ^{ab} ± 48	512 ^{a} ± 46	667 ^{b} ± 59	634 ^{b} ± 51
Cu	mg kg^{-1} d.m.	155 ^{a} ± 20	163 ^{a} ± 13	163 ^{a} ± 12	168 ^{a} ± 11	178 ^{a} ± 14	175 ^{a} ± 17
Ni	mg kg^{-1} d.m.	6.81 ^{a} ± 0.61	8.13 ^{b} ± 0.89	7.89 ^{ab} ± 0.73	7.96 ^{ab} ± 0.93	8.70 ^{b} ± 0.88	7.85 ^{ab} ± 0.75
Cr	mg kg^{-1} d.m.	9.86 ^{a} ± 0.92	13.13 ^{b} ± 1.52	14.16 ^{c} ± 1.23	10.79 ^{ab} ± 1.21	13.72 ^{b} ± 1.43	13.56 ^{b} ± 1.30
Pb	mg kg^{-1} d.m.	11.37 ^{a} ± 1.11	13.36 ^{b} ± 1.71	11.80 ^{a} ± 1.29	12.77 ^{ab} ± 1.32	14.91 ^{c} ± 1.24	13.29 ^{b} ± 1.19
Cd	mg kg^{-1} d.m.	0.52 ^{a} ± 0.10	0.58 ^{ab} ± 0.12	0.53 ^{a} ± 0.11	0.57 ^{ab} ± 0.10	0.64 ^{bc} ± 0.15	0.61 ^{b} ± 0.18

Identical superscripts denote no significant ($p > 0.05$) differences between the experimental variants according to the post hoc Tukey HSD test. Explanation: TOC—total organic carbon; Nt—total nitrogen; SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; I–III—main experiment series.

2.3. Plant Sample Analysis

2.3.1. Relative Chlorophyll Content (CCl)

The measurements were performed using a Chlorophyll Content Meter CCM-200 plus (Opti-Sciences, Hudson, NH, USA) calculating an index in CCl units based on absorbance at 650 and 940 nm. Relative chlorophyll content was measured on fully expanded maize leaves (five leaves per pot), twice during each experiment series.

2.3.2. Chlorophyll Fluorescence

Chlorophyll fluorescence was measured using a manual chlorophyll fluorescence meter (Pocket PEA, Hansatech Instruments, King's Lynn, Norfolk, UK). The measurements were made on leaves after previous dark adaptation with the use of a leaf clip. Four leaves per pot were analyzed. The maximal available intensity was 3500 µmol, which was applied for 1 s with light with a peak wavelength of 627 nm. The following parameters were determined: Fv/Fm—maximal photochemical efficiency; Fv/F0—maximum quantum yield of primary photochemistry; PI—performance index. Chlorophyll fluorescence was measured twice during each experiment series.

2.3.3. Gas Exchange

A Portable Photosynthesis Measurement System LCpro-SD (ADC BioScientific Ltd., Hoddesdon, UK) was used to determine the net photosynthetic rate (PN), transpiration rate (E), stomatal conductance (gs), and intercellular CO_2 concentration (Ci) on fully expanded leaves. In the determination process, the light intensity was 300 mol m^{-2} s^{-1}, and the leaf chamber temperature was 22 °C. Two leaves per pot were analyzed. The following parameters were measured: net photosynthetic rate (PN), transpiration rate (E), and stomatal conductance (gs). Water use efficiency (WUE) was calculated by dividing PN by E. Gas exchange parameters were measured twice during each experiment series.

2.3.4. Mineral Composition of Plant Materials

At the end of the experiment, the plants were carefully removed from the pots, and their aboveground parts were separated from the underground parts. Dry matter content in aboveground parts was determined with the dryer-weight method by drying the plants at 45 °C. The dried material was homogenized in a laboratory mill and mineralized in 60% HNO_3. The contents of basic macro- and microelements (Ca, Mg, K, Na, Fe, Mn, Zn, Cu) and toxic heavy metals (Pb and Cd) were determined in the mineralizates with the atomic absorption spectrometry technique (HITACHI Z-2000 Tokyo, Japan). P content was determined with the colorimetric method. N in the aboveground and underground parts of the plants was determined with the dry combustion method using a Vario El Cube elemental analyzer (GmbH, Germany).

2.4. Statistical Analysis

All statistical analyses were performed using STATISTICA 13.3 software (StatSoft, Tulsa, OK, USA). To show the existence of uniform groups of objects (α = 0.05), the Tukey multiple comparison HSD test was performed following a two-dimensional analysis of variance (ANOVA).

3. Results

3.1. Selected Properties of Sewage Sludge Used in the Pot Experiment

The properties of the sewage sludge used in each series of the experiment are presented in Table 3. The mean dry matter content in the sludge, ranging from 8.54 to 9.44%, was significantly higher in SS_O than in SS_N. Regardless of the stabilization method, the sewage sludge used in each series was slightly acidic, and the pH values in both variants did not show any significant differences. Moreover, the sludge used in the experiment was characterized by high contents of TOC and macro- and microelements. In general, the sludge used in series II was characterized by the highest content of macro- and microelements. In terms of the chemical composition, there were significant differences only in the K level between the sludge variants used in the experiment. The content of this element in the ozonated sludge used in series II and III was significantly lower than in the non-ozonated sludge (Table 3).

3.2. Relative Chlorophyll Content (CCl)

The application of the sewage sludge for maize fertilization significantly differentiated the relative chlorophyll content (CCl). As in the case of the dry matter yield, the CCl values in the SS_N and SS_O-fertilized plants were significantly higher than in the control and generally increased relative to the applied sewage sludge dose (Figure 1). The CCl values ranged from 29.5 to 32.7 in the SS_N plant samples and from 32.0 to 33.3 in the SS_O plant samples. However, the mean values of CCl determined in the SS_O-fertilized plants did not differ significantly from those noted in the SS_N-fertilized maize. Moreover, a large variability of results between the experiment series was observed, in the case of both SS_N and SS_O, as evidenced by the large differences between the minimum and maximum values (Figure 1). In contrast, the mean CCl values in the control maize leaves were relatively stable in the three experiment series (Figure 1).

3.3. Chlorophyll Fluorescence

In contrast to the content of chlorophyll, the sewage sludge dose and type were not associated with significant differences in the values of chlorophyll fluorescence parameters (F_v/F_m, F_v/F_0, and PI). However, in comparison with the control, these parameters exhibited an upward trend after sewage sludge fertilization (Table 4). The increasing sludge doses (from D1 to D3) did not produce a significant increase in these parameters, except for PI in the ozone-stabilized sludge, whose value increased in D2 and D3 compared to D1. The ozonation process did not increase F_v/F_0 and F_v/F_m in the sewage sludge dose groups, while PI increased significantly within these groups (D1, D2, D3).

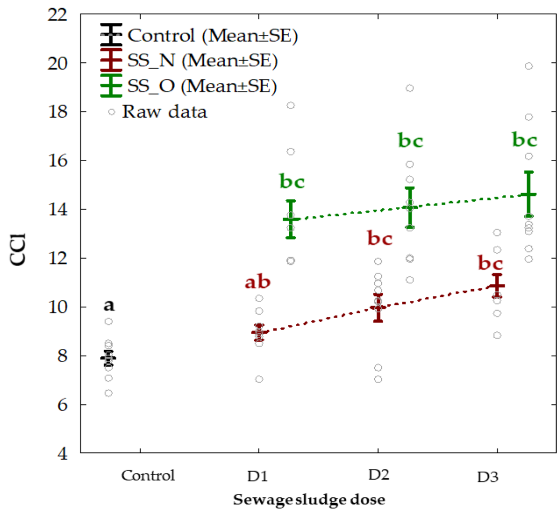

Figure 1. Changes in the relative chlorophyll content in leaves depending on the type and dose of sewage sludge (mean values from the experiment series). Mean values (n = 9) ± standard error. Identical superscripts denote no significant (p > 0.05) differences between the experimental variants according to the post hoc Tukey HSD test. Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha $^{-1}$, respectively.

Table 4. Chlorophyll fluorescence parameters in maize leaves depending on the sewage sludge dose and type. Mean values (n = 9) ± standard error. Identical superscripts denote no significant (p > 0.05) differences between the experimental variants according to the post hoc Tukey HSD test.

Parameters	Parameters	Control	D1		D2		D3	
			SS_N	SS_O	SS_N	SS_O	SS_N	SS_O
Fv/Fm	Mean	0.74 ^{a}	0.76 ^{ab}	0.77 ^{ab}	0.76 ^{ab}	0.77 ^{ab}	0.75 ^{ab}	0.78 ^{b}
	SE	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Min	0.70	0.69	0.73	0.73	0.70	0.70	0.77
	Max	0.77	0.78	0.79	0.78	0.79	0.78	0.80
Fv/F0	Mean	2.89 ^{a}	3.17 ^{ab}	3.40 ^{ab}	3.20 ^{ab}	3.45 ^{b}	3.15 ^{ab}	3.60 ^{b}
	SE	0.16	0.14	0.12	0.10	0.07	0.14	0.09
	Min	2.18	2.47	2.73	2.73	3.15	2.40	3.02
	Max	3.37	3.47	3.70	3.51	3.67	3.45	3.88
PI	Mean	1.37 ^{a}	1.92 ^{b}	2.67 ^{c}	2.06 ^{bc}	2.77 ^{d}	2.07 ^{bc}	3.07 ^{d}
	SE	0.09	0.14	0.14	0.14	0.13	0.13	0.19
	Min	1.03	1.29	1.93	1.50	2.16	2.40	2.39
	Max	1.91	2.51	3.36	2.51	3.53	3.45	4.43

Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha $^{-1}$, respectively.

3.4. Gas Exchange

The application of the different sludge fertilization forms resulted in an increase in gas exchange parameters (Pn, E, gs), compared to the control. However, not all sewage sludge doses and types used in the experiment produced significant differences in these values (Table 5). The Ci value was lower than in the control variant, whereas no significant differences in WUE were found between the experiment variants. In comparison with SS_N, Pn significantly increased after the D1, D2, and D3 SS_O fertilization, whereas there were no differences between samples treated with different doses in the same fertilization scheme. The largest fluctuations in the Pn values, which ranged from 6.21 to 17.49 µmol $(CO_2)m^{-2}s^{-1}$, were recorded in the D2 SS_N samples. The lowest values, i.e., from 6.59 to 10.54 µmol $(CO_2)m^{-2}s^{-1}$, were noted in the D3 SS_N samples. There were no differences in parameters E and gs relative to the type of sludge, except for the D3 variants, where a significant increase was noted in the SS_O samples compared to those of SS_N.

Table 5. Gas exchange parameters in maize plants depending on the sewage sludge dose and type. Mean values ($n = 9$) ± standard error. Identical superscripts denote no significant ($p > 0.05$) differences between the experimental variants according to the post hoc Tukey HSD test.

Parameters	Units	Parameters	Control	D1		D2		D3	
				SS_N	SS_O	SS_N	SS_O	SS_N	SS_O
Pn	μmol(CO_2)$m^{-2}s^{-1}$	Mean	8.54 ^{a}	10.68 ^{ab}	14.79 ^{b}	11.85 ^{ab}	15.71 ^{b}	9.31 ^{a}	15.46 ^{b}
		Min	5.26	6.34	9.02	6.21	9.35	6.59	9.86
		Max	13.75	17.80	16.83	17.49	21.35	10.64	20.78
		SE	1.04	1.18	0.78	1.47	1.45	0.58	1.42
E	mmol(H_2O)$m^{-2}s^{-1}$	Mean	1.47 ^{a}	1.88 ^{ab}	2.10 ^{ab}	1.80 ^{ab}	2.41^{b}	1.68 ^{a}	2.47 ^{b}
		Min	0.96	1.27	1.49	1.18	1.68	1.29	1.76
		Max	2.21	2.53	2.33	2.55	3.03	2.02	3.92
		SE	0.16	0.18	0.11	0.16	0.17	0.07	0.22
gs	mmol $m^{-2}s^{-1}$	Mean	0.06 ^{a}	0.08 ^{ab}	0.10 ^{bc}	0.08 ^{ab}	0.10^{bc}	0.06^{a}	0.11^{c}
		Min	0.04	0.06	0.06	0.04	0.07	0.04	0.09
		Max	0.07	0.10	0.14	0.11	0.14	0.07	0.13
		SE	0.00	0.01	0.01	0.01	0.01	0.01	0.01
C_i	mmol L^{-1}	Mean	188 ^{b}	144 ^{ab}	108 ^{b}	152 ^{ab}	122^{b}	134 ^{ab}	115 ^{b}
		Min	144	109	61	115	80	105	76
		Max	236	194	183	204	178	195	166
		SE	12.6	11.6	14.2	12.8	12.4	14.0	10.6
WUE	μM CO_2/mM H_2O	Mean	6.46 ^{a}	6.34 ^{a}	6.15 ^{a}	6.91 ^{a}	6.12 ^{a}	5.85 ^{a}	5.57 ^{a}
		Min	4.98	5.28	4.44	5.18	4.72	4.60	3.09
		Max	7.72	8.31	7.48	9.03	7.21	6.35	6.83
		SE	0.35	0.32	0.36	0.46	0.32	0.23	0.35

Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha $^{-1}$, respectively. Pn—net photosynthetic rate; E—transpiration rate; gs—stomatal conductance; C_i—intercellular CO_2 concentration; WUE—water use efficiency.

3.5. Dry Matter in Aboveground Plant Parts

Both the dose and the type of sewage sludge exerted a statistically significant effect on the yield of the aboveground maize biomass (Figure 2). The lowest dry matter yield was recorded in the control samples (with no sewage sludge application) and generally increased relative to the applied sewage sludge dose. The mean dry matter yield of plants in the samples fertilized with the non-ozonated sewage sludge (SS_N) ranged from 5.04 to 5.50 g pot^{-1}, and its highest values were recorded in the D2-treated samples. The mean dry matter yield in the SS_O-fertilized plants (ranging from 5.29 to 6.24 g pot^{-1}) differed significantly from values recorded in the SS_N-fertilized plants and increased in a dose-dependent manner (Figure 2). The dry matter yield in each experiment series exhibited reproducible dependencies, which was evidenced by the small dispersion of the values (Figure 2).

3.6. Chemical Composition of the Aboveground Biomass of Maize Plants

The content of all analyzed macronutrients (N, K, Ca, Mg, and P) in the aboveground plant parts was significantly correlated with the sewage sludge dose and type used in the experiment (Figure 3). The mean N content in the aboveground parts ranged from 26.41 to 36.99 g kg^{-1}, and the lowest values were noted in the control (Figure 3A). In general, the highest mean N content was found in the SS_O-fertilized plants, and their N contents differed significantly from those recorded in the other experiment samples. Such relationships were observed in the case of doses D1 and D2. In the case of dose D3, the SS_N-fertilized plants had higher mean content of N, but the differences from the SS_O values were not significant (Figure 3A). The highest mean K content was found in plants fertilized with the highest dose of SS_O (41.9 g kg^{-1}), and the results in this variant were significantly different from those obtained in the other variants (Figure 3). A significantly higher accumulation of K in the aboveground biomass was also detected in the lowest dose variant (D1) of SS_N (Figure 3B). In turn, similar mean K content was determined in the aboveground maize biomass in the other experimental variants. The mean content of Ca in the aboveground biomass of the sewage sludge-fertilized maize was significantly higher than in the control, and higher values were recorded in the SS_O-fertilized plants (Figure 3C). In turn, a higher concentration of Mg was found in the SS_N-fertilized plants than in the SS_O fertilization variant, and the mean content of this element was significantly higher in all the sewage sludge-fertilized plants than in the control (Figure 3D). The mean content of P in the aboveground maize biomass increased linearly with the increasing sewage sludge doses and was substantially higher than in the control. Higher contents of this element were found in SS_O than in SS_N treated plants (Figure 3E).

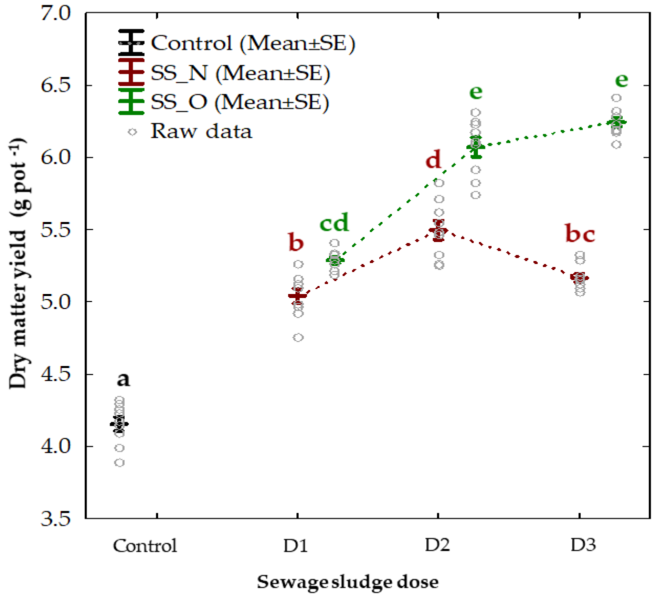

Figure 2. Dry matter yield of aboveground parts of maize plants relative to the dose and type of sewage sludge. Mean values (n = 9) ± standard error. Identical superscripts denote no significant ($p > 0.05$) differences between the experimental variants according to the post hoc Tukey HSD test. Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha $^{-1}$, respectively.

As in the case of macronutrients, the mean content of micronutrients in the aboveground maize biomass was significantly correlated with the sewage sludge dose and type (Figure 4). The aboveground biomass of SS_O-fertilized maize exhibited substantially higher contents of Fe, Mn, Zn, and Cu than in the analogous variants of SS_N fertilization (Figure 4). The mean content of Fe ranged from 311.8 mg kg^{-1} in the control to 407.7 mg kg^{-1} in D2 SS_O. In general, the highest content of this element was exhibited by the D2 SS_N- and D2 SS_O-fertilized plants (Figure 4A). The mean Mn content was lower in the SS_N and SS_O variants (doses D1 and D2) than in the control. The content of this element was significantly higher only in the case of dose D3 in SS_N and SS_O (Figure 4B). The mean Zn content ranged from 38.18 to 60.09 mg kg^{-1}. Compared to the SS_N variant, the SS_O-fertilized maize exhibited higher Zn content. Similar relationships were also observed in the case of Cu, where higher contents of this element were observed in the SS_N-fertilized plants, except for dose D1 (Figure 4C,D).

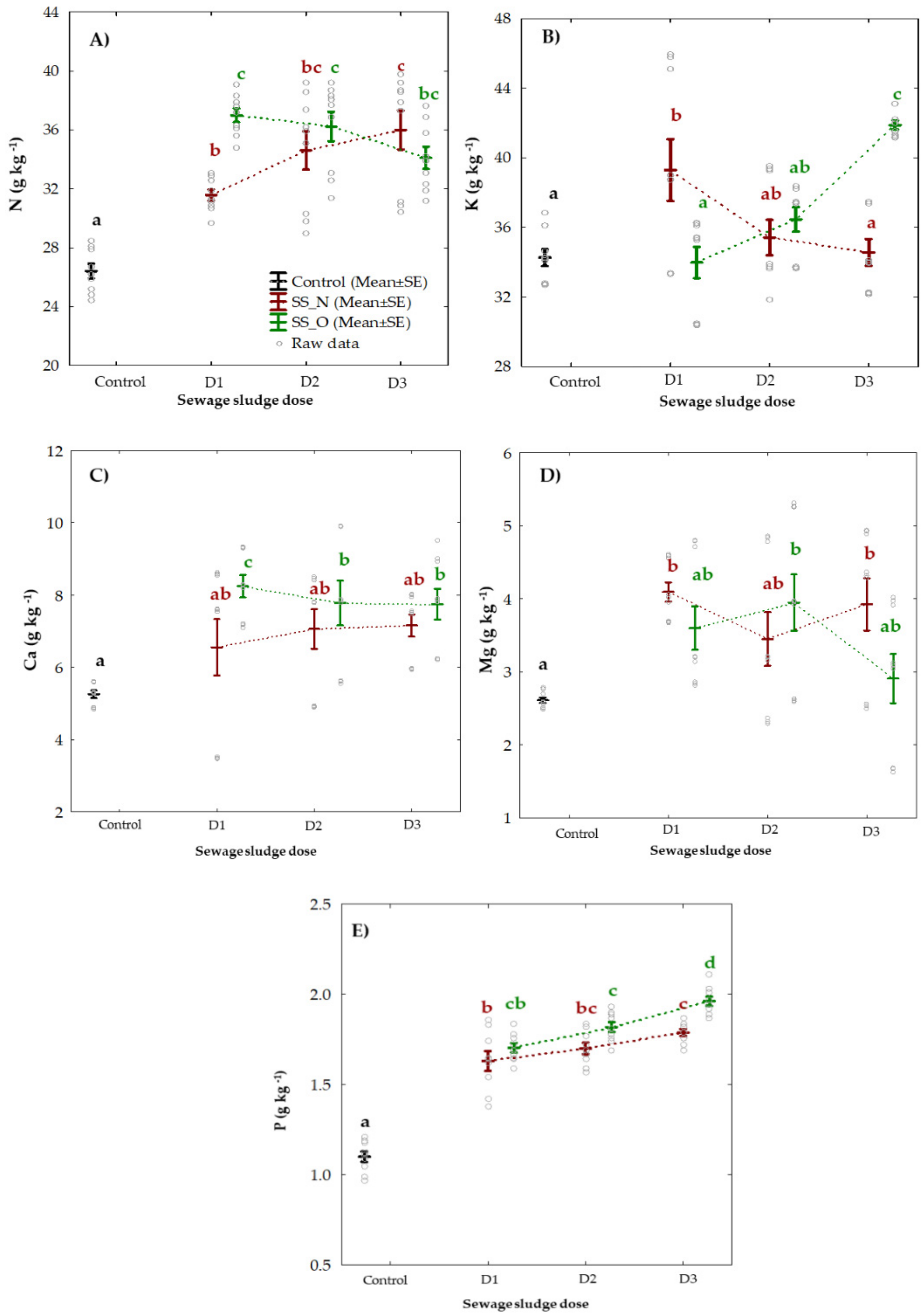

Figure 3. (**A**–**E**). Content of macronutrients (N, K, Ca, Mg, P) in the aboveground biomass of maize depending on the type and dose of sewage sludge (mean ± SE values from experiment series). Mean values (n = 9) ± standard error. Identical superscripts denote no significant ($p > 0.05$) differences between the experimental variants according to the post hoc Tukey HSD test. Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha $^{-1}$, respectively.

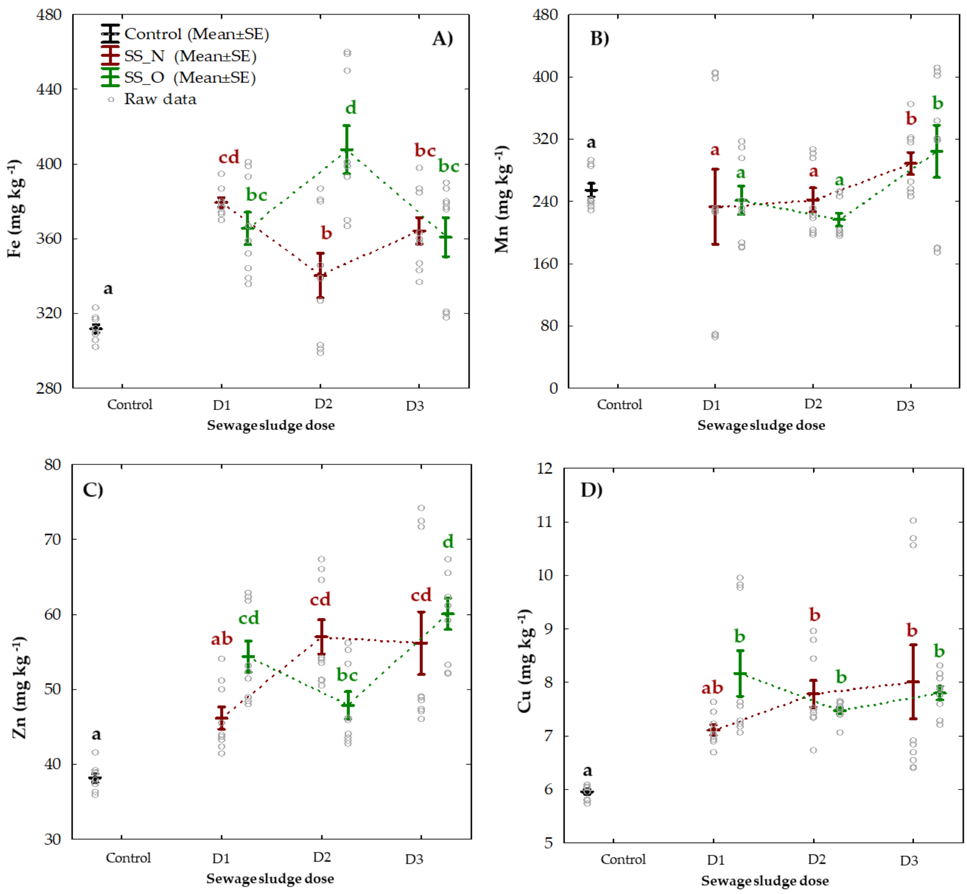

Figure 4. (**A**–**D**). Content of micronutrients (Fe, Mn, Zn, Cu) in the aboveground biomass of maize depending on the type and dose of sewage sludge (mean values from experiment series). Mean values (n = 9) ± standard error. Identical superscripts denote no significant ($p > 0.05$) differences between the experimental variants according to the post hoc Tukey HSD test. Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha^{-1}, respectively.

In the present study, higher levels of toxic heavy metals (Pb and Cd) were detected in the sewage sludge-fertilized objects than in the control (Figure 5). The mean Pb content in the aboveground maize biomass ranged from 0.83 to 1.80 mg kg^{-1}, with the lowest value in the control and the highest level in D1 SS_O. With the exception of D1 SS_O, the differences in the Pb content in the aboveground maize biomass between the experimental variants were statistically insignificant, which indicates that the application of sewage sludge, regardless of the stabilization method, does not induce major changes in Pb accumulation in the aboveground maize parts (Figure 5A). Different relationships were observed in the case of Cd. Its content differed significantly between the control and the other variants. The method of sludge stabilization had no significant impact on the Cd content in the plants (Figure 5B). Its mean values ranged from 0.21 to 0.50 mg kg^{-1} (Figure 5B). The lowest mean content of this element in the aboveground biomass was recorded in the control, while the highest level was determined in the D1 SS_O-fertilized plants. There was also a downward tendency in the Cd content accompanying the increasing doses of both non-ozonated and ozonated sewage sludge. Despite the differences, the method of sewage sludge stabilization did not have a significant effect on the Cd content in the aboveground biomass (Figure 5B).

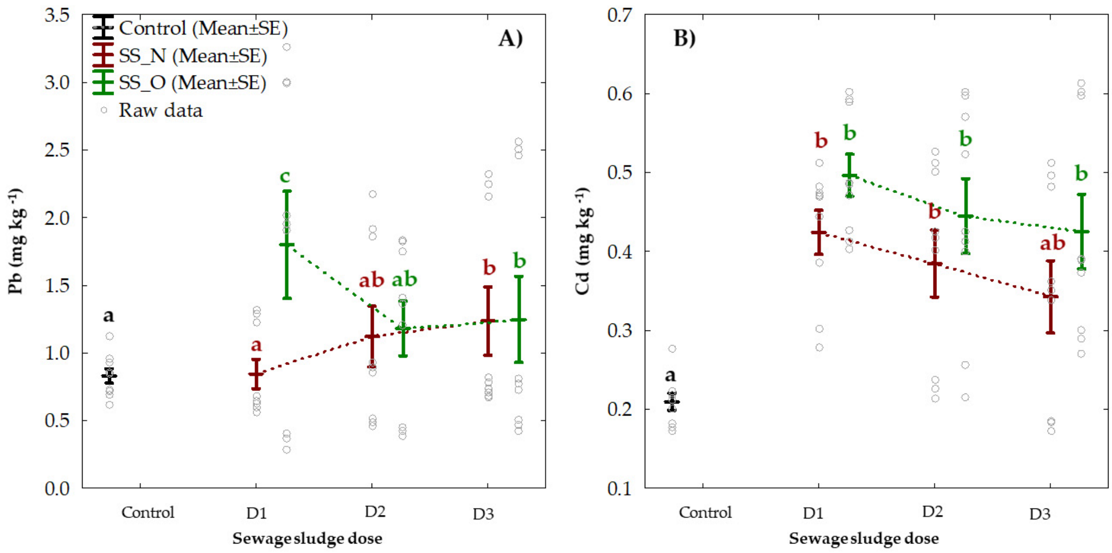

Figure 5. (**A**,**B**). Content of toxic heavy metals (Pb, Cd) in the aboveground biomass of maize depending on the type and dose of sewage sludge (mean values from experiment series). Mean values (n = 9) ± standard error. Identical superscripts denote no significant (p > 0.05) differences between the experimental variants according to the post hoc Tukey HSD test. Explanation: SS_N—non-ozonated sewage sludge; SS_O—ozonated sewage sludge; D1, D2, D3—dose of sewage sludge—3, 6, 9 Mg ha^{-1}, respectively.

4. Discussion

The sewage sludge used in the present study was characterized by a diverse chemical composition. In general, the sludge used in series II was characterized by the highest content of organic matter and macro- and microelements (Table 3). It was found that the sludge stabilization method did not significantly differentiate the content of these elements; nevertheless, it significantly modified the bioavailability of sludge components, as evidenced by the results. The availability of macro- and microelements such as Ca, S, Mg, K, N, P, and Fe is crucial for the proper course of plant life processes that directly contribute to plant growth and yield [29]. It is believed that, in the soil and climatic conditions of Poland, N is the main determinant of crop yield size and quality; hence, balanced fertilization with this element is necessary [30,31]. Maize has a high potential yield, which requires incurring fertilization costs and suitable weather conditions during the growing season. A deficiency of nutrients in early growth stages is the main factor limiting its yield [31]. Macro- and microelements present in sewage sludge introduced into soils ensure a better supply of these nutrients for plants, thus reducing the need for mineral fertilizers [25,32]. Potassium, present in sewage sludge in low amounts, is most intensively absorbed by maize from the phase of 5–6 leaves to flowering. This element increases plant resistance to lodging and improves water use efficiency, which is especially important in the case of light soils [33]. Given the potassium deficiency in sewage sludge used as fertilizers, additional mineral K fertilization is recommended. Although no supplementary K fertilization was applied in the present experiment, the use of SS_O contributed not only to the higher dry matter yield but also to better physiological parameters, which indicated proper development of the plants. Similarly, mean content of N, P, K, Ca, and Mg in aboveground maize biomass was higher in the SS_O-fertilized plants, which may indicate improved bioavailability of these elements from sewage sludge after the ozonation process.

The better nutritional status of plants undoubtedly contributed to the higher dry matter yield, which was significantly correlated with the sewage sludge type and dose used in the experiment, as there was a generally linear upward tendency with the increasing sewage sludge dose. The dry matter yield of the aboveground plant parts in the maize plants fertilized with the highest dose of non-ozonated and ozonated sewage sludge (D3) was approximately 50% higher than in the control (Figure 1). Proper plant growth and development ensuring satisfactory yields depend on physiological processes, in particular on the efficiency of photosynthesis. A deficiency of plant nutrients, in particular N, P, Mg, and K, leads not only to a decline in photosynthesis efficiency but also to a reduction in the transpiration level, stomatal conductance, and content of chlorophyll and carotenoids. In conditions of an insufficient N supply in plants, the number of electron acceptors in PSII decreases. This process leads to a decrease in the activity of RuBisCo and phosphoenolpyruvate (PEP) carboxylase [34,35]. Chlorophyll content in leaves can be used as a parameter in the assessment of the impact of various factors on plants, including proper plant nutrition [31]. For example, the chlorophyll content in plant leaves directly indicates estimated N uptake from soil in various environmental conditions [36]. Similarly to P and Mg, N deficiency significantly reduces the relative chlorophyll content in leaves [37]. In the present study, the mean CCl values in the plants fertilized with the highest dose of SS_ N and SS_O were 78 and 85% higher, respectively, than in the control (Figure 2). This proves the efficiency of sewage sludge fertilization, as reflected in increased yields. Investigations conducted by Peng et al. (2018) showed a positive effect of treatment with ozonated sewage sludge on chlorophyll content in lettuce [24]. In a study on the effect of sewage sludge, Khaliq et al. (2017) reported an increase in chlorophyll content in green bean and white radish plants [38].

Another method for assessment of plant nutritional status, especially N content, is based on measurements of chlorophyll fluorescence [39]. The present study showed an upward trend in chlorophyll fluorescence values associated with the sewage sludge fertilization. Similarly, Lakhdar et al. (2012) reported a stimulating effect of sludge fertilization on physiological processes in maize plants [40]. As suggested by Wang et al. (2016), the supply of N in fertilizers can increase photosynthetic efficiency by maintenance of appropriately high levels of parameters F_v/F_m and F_v/F_0 [41]. The more efficient utilization of radiation absorbed via photochemical processes, which was reflected in the higher F_v/F_m value, can be explained by the effective release of macro- and micronutrients contained in sewage sludge, which are required for the biosynthesis of enzymes involved in photochemical processes. An indisputable effect of the better supply of nutrients to the sewage sludge-fertilized maize plants in early development stages was the improvement in gas exchange parameters. A similar relationship was reported by Lakhdar et al. 2012 and Chen et al. 2005; in their studies, sewage sludge stimulated CO_2 assimilation, which indicates a strong relationship between photosynthetic activity and adequate amounts of nutrients provided by sludge [40,42]. Organic matter contained in sewage sludge ensures long-lasting release of macro- and micronutrients necessary for the biosynthesis of enzymes involved in photochemical processes [43]. Increased N content in the substrate may, therefore, enhance Rubisco enzyme synthesis, which significantly improves photosynthesis [44].

Despite the economical water use (low transpiration ratio 1:256), maize has high water requirements. The amount of rainfall during the maize growing season from April to May is the basic determinant of maize yield size in Poland, as it can reduce yields to a considerably greater extent than thermal conditions. Therefore, water shortage is a serious factor limiting maize yields especially in light soils. Water content, especially in light soils, is largely determined by organic matter content. It was calculated that if the humus content in Polish soils increased by 1%, approximately 30 mm more precipitation water could be stored annually [45]. Sewage sludge contains from 50 to 70% organic matter; hence, fertilization with sludge increases its resources in soils, especially in lighter soils [46]. Higher amounts of organic matter in soils improve their physical properties, thereby indirectly ensuring better water supply for plants, which is highly important in plant production [47,48]. In this study, the better water supply in addition to the better supply of essential nutrients may have contributed substantially to the results, i.e., the yield and specific physiological parameters of the sewage sludge-fertilized maize plants. Water deficiency reduces nutrient uptake from soils, which results in reduced N and P concentrations in plant tissues. This is especially important in the case of the light soil with low water holding capacity used in the experiment. Water deficiency in soils also leads to lower release of nutrients in the mineralization process and reduced nutrient diffusion and flow, which impede their uptake by plant roots [49].

The photosynthetic water use efficiency (WUE) index was determined based on the ratio of the net photosynthetic rate to the transpiration rate (Pn/E). This index provides valuable information about plant adaptation to prevailing conditions. Leaf stomata control CO_2 incorporation in the photosynthesis process and transpiration-related water loss. The degree of stomatal opening regulates WUE; therefore, stomatal conductance is necessary to stimulate the efficiency of crop yields in agricultural ecosystems [47]. The present experiment showed only an upward trend in the WUE value in the control in comparison with the sewage sludge-fertilized plants. In their experiment on wheat, Wang et al. (2016) showed an increase in the WUE value accompanying lower levels of N fertilization. This may indicate a more economical water use in plants subjected to lower N doses. Sewage sludge increases the supply of this element, thus contributing to better growth and development of plants [41].

One of the factors limiting the use of sewage sludge for plant fertilization is the high content of contaminants, including toxic heavy metals. Micronutrients present in sewage sludge (especially Zn and Cu) may become toxic if they are introduced in excessive doses. It is estimated that mean Zn contents in aboveground parts of contamination-unaffected plants range from 20 to 100 mg kg^{-1}. Zn deficiency in plants is usually detected at content levels below 20 mg kg^{-1}, whereas the toxic effect of this element is exerted at a level exceeding 300–400 mg kg^{-1} [45]. The mean Zn content in the present study ranged from 38.18 to 60.09 mg kg^{-1}, with a tendency to increase with the increasing sewage sludge doses. Most frequently, sewage sludge fertilization of soils results in a significant increase in Zn and Cu contents and excessive accumulation of these elements in plants. Therefore, it is important not to exceed the recommended levels of heavy metals in sludge [46].

The mean Pb and Cd content in the sewage sludge used in the present experiment was low and did not exceed the limits specified by legal provisions; hence, the analyzed sludge could be used as a plant fertilizer. Absorption of heavy metals contained in sewage sludge varies and depends on many factors. As shown by Zhang et al. (2017), the total contents of Cu, Zn, Ni, Pb, Cr, Cd, Mn, and Mg fluctuated slightly after the ozonation process [14]. In turn, He et al. (2021) reported that ozonation of sewage sludge induced an increase in the total concentration of metals corresponding to increasing ozone doses. As demonstrated in their study, the heavy metal content increased from 4.79 mg L^{-1} to 11.49 mg L^{-1} after one ozonation pre-treatment cycle, which confirms the effectiveness of ozone in destruction of flocs and microbial cells [5]. Despite the slight fluctuations in the total content of heavy metals in sewage sludge subjected to ozonation, the mobility, bioavailability, and environmental hazards posed by these metals deposited in soils are associated with their chemical speciation [50]. Metals deposited in sewage sludge have various chemical forms—exchangeable and acid-soluble, reducible (bound to Fe-Mn oxides), oxidizable (sulfide portion and bound to organic matter), and residual forms [51,52]. Ozonation may lead to the release of heavy metals present in the fraction associated with organic matter, which may have an impact on their bioavailability for plants [15]. Excess concentrations of bioavailable forms of heavy metals may have a toxic effect on plants. The present study revealed higher accumulation of Cd in the SS_O-fertilization variant, which may indicate that ozonation increases the levels of bioavailable fractions of these elements. Cd accumulation in the SS_N- and SS_O-fertilized plants declined with the increasing sewage sludge dose. This is probably related to the higher content of other elements, which impeded the Cd uptake. Due to the chemical similarity between Ca and Cd, Ca can mediate physiological or metabolic changes induced by Cd in plants. Calcium, which is present in significant amounts in sewage sludge, can protect plants against Cd stress by alleviation of growth inhibition, regulation of metal uptake and translocation, mitigation of oxidative damage, etc. [53]. It is also worth noting that, despite the higher Cd levels in the sewage sludge-fertilized plants, the mean Cd and Pb contents determined in the sludge used in the experiment were far below the lower limit, making it suitable for production of food and feed crops. Importantly, despite the increased accumulation of toxic heavy metals in the sewage sludge-fertilized plants, there were no disturbances in the plant physiological status parameters, i.e., CCl, gas exchange, or chlorophyll fluorescence indices.

Sewage sludge is a valuable source of macro- and microelements; therefore, this soil fertilizer provides plants with biogenic elements necessary for proper growth and development. The present study showed a significant effect of the sewage sludge type and dose on accumulation of macro- and microelements in aboveground parts of maize plants. In general, increased accumulation of these elements was observed in the SS_O-fertilized plants. This is the result of the higher bioavailability of nutrients deposited in ozonated sewage sludge and initial degradation of the hard-to-decompose organic matter contained in sewage sludge. Ozonation yields sewage sludge with better technological parameters, as described in our previous study [13]. Additionally, such sludge can be successfully used for crop fertilization. The bioavailability of nutrients contained in ozone-stabilized sludge is better than in traditional sludge and has an impact on plant growth and development, which may improve the quality of crop yields. Our study shows that ozone-stabilized sewage sludge can be used for agricultural purposes. This research should be confirmed in field-scale experiments. Despite the stabilization process, sewage sludges are waste materials. The influence of ozone-stabilized sewage sludge on the growth and development of other plant species on different soil types should be analyzed as well. In addition, further research in this area is necessary, taking into account the impact of sewage sludge ozonation on the decomposition of pharmaceuticals, organic pollutants, or microplastics.

5. Conclusions

The present study assessed the potential of using ozone-stabilized sewage sludge in the cultivation of maize. The results revealed the following findings:

1. Due to the high content of organic matter and macro- and microelements, the sewage sludge used in the experiment had a significant effect on maize growth and development in early stages of development.
2. Substantially better results were achieved by the application of ozone-stabilized sewage sludge. The better absorption of nutrients resulted in higher CCl content and parameters of chlorophyll fluorescence and gas exchange in plants fertilized with ozonated sewage sludge.
3. The present study showed higher contents of Pb and Cd in plants fertilized with ozonated sewage sludge; however, these metals did not exert a harmful effect, as evidenced by the yield and physiological parameters.
4. The analyses carried out in the experimental conditions confirmed the effectiveness of the use of ozone-stabilized sewage sludge for fertilization of maize. Nevertheless, these results should also be supported by studies of other plants to confirm the effectiveness of this type of sewage sludge in the fertilization of crops.

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