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INFLUENCE OF SEWAGE SLUDGE ON THE CONTENT OF SELECTED METALS AND CHLOROPHYLL IN LAWN GRASS MIXTURES

WPŁYW OSADU ŚCIEKOWEGO NA ZAWARTOŚĆ WYBRANYCH METALI I CHLOROFILU W MIESZANKACH TRAW GAZONOWYCH

Abstract: The aim of this study was to analyze the influence of municipal sewage sludge from the Municipal Wastewater Treatment Plant in Sokolka on the content of magnesium and selected micronutrients such as zinc, manganese, copper and iron in the soil and lawn grasses and on the content of chlorophyll *a* and *b* in the analyzed plants. These elements are especially important in the process of photosynthesis. In addition, the paper discusses the relationship between the studied elements and the content of assimilation pigments.

The experimental plots were sown with two lawn grass mixtures: Eko and Roadside, and three doses of sewage sludge 0.0 (control), 7.5 kg $\cdot$ m^{-2} and 15.0 kg $\cdot$ m^{-2} were used. Chlorophyll *a* and *b* content was determined using spectrophotometer by measuring absorbance at λ = 663 and 645 nm. Metals concentrations in soils with sewage sludge and in plant material were determined using Atomic Absorption Spectrometry. It was found that the sludge dose had a significant effect on the manganese content in the soil taken from the experiment in Piastowska Street. In the experiment in Popieluszki Street the application of sewage sludge had a significant impact on the content of zinc and copper in the studied mixtures of grasses and in Raginisa Street – on the contents of zinc, copper and manganese.

The analysis of correlations for the results obtained from experiment in Hetmanska Street revealed strong positive correlations between the content of Zn and Mn in the soil and the content of chlorophyll *a* in plants (r = 0.84 and r = 0.83, respectively) and strong negative correlations between the content of Zn in the soil and the content of chlorophyll *b* in lawn grasses collected in June and August (r = –0.81 and r = –0.85, respectively). Strong correlations between the concentration of zinc and the content of chlorophyll *b* in grass samples collected in Hetmanska Street, Piastowska Street and Popieluszki Street (r = 0.85, r = 0.86 and r = 0.98, respectively) and the Cu content in plants and chlorophyll *b* for plants collected in Hetmanska Street (r = 0.97), Piastowska Street (r = 0.86), Popieluszki Street (r = 0.90) and Raginisa Street (r = 0.82).

The content of chlorophyll *b* was differentiated by dose of sludge and sampling time. The highest average chlorophyll *b* content in the grass samples collected in June and July was found (1.10 mg $\cdot$ g^{-1} of f.m.), and the lowest in samples collected in October (0.39 mg $\cdot$ g^{-1} of f.m.). The content of chlorophyll *a* in the analyzed

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samples of grasses decreased depending on the sampling time. As with the chlorophyll *b* content the lowest average content of chlorophyll *a* was in samples collected in October (0.45 mg · g^{-1} of f.m.). The average content of chlorophyll *a* in samples collected in June and July were similar independently of the fertilization of sewage sludge (0.64 mg · g^{-1} of f.m.).

Keywords: chlorophyll, grasses, magnesium, micronutrients, sewage sludge

A side effect of the rapid development of civilization is the emergence of ever-increasing amounts of sewage sludge and its management is a serious problem [1]. According to Central Statistical Office [2] 519.2 thousand Mg d.m. of municipal sewage sludge were produced in 2011 in Poland, and it is estimated that by 2018 over 700 thousand Mg d.m. of sewage sludge will be produced. Generated in wastewater treatment sludges require management not only for legal reasons, but also practical and aesthetic and, if possible, sludges after processing should return to the environment [3]. Many authors [4–6] report that sludge contains valuable nutrients, therefore, can be used as fertilizer. It contains also organic matter, which improves soil structure. It is confirmed by Szwedziak's studies [6] in which sludge placed on the upper layers of the soil were the source of many nutrients for plants, influenced soil formation processes and contributed to increased soil biological activity. However, as noted by Bien et al [3] for large sewage treatments plant the pathway for agricultural use is virtually closed. This is due to inadequate physico-chemical properties of sediments, mainly abnormal concentrations of heavy metals. In Poland the limits of heavy metals in terms of the natural use of sewage sludge are regulated by the Regulation of the Minister of the Environment of 13 July 2010 on municipal sewage sludge [7].

The lawns in parks and in passageways meet a number of key ecological safety and aesthetic functions [8] and may also influence the well-being of man. They are usually established on the poor soils having defective physical and chemical properties and poor trophic character, what contributes to the bad conditions for plants [9]. According to Kalembasa and Malinowska [10], one of the ways of use of sludge is to use it in perennial crops, which gradually releases the nutrients for providing plant growth at a satisfactory level. Therefore, sludge can be a good base of nutrients for growing grass in urban areas.

In order to effectively clean soil environment of metals, the plants should be of high resistance to harsh environmental conditions, have the capacity to accumulate xenobiotics from soil, as well as to grow fast and to produce a great quantity of biomass. Such great phytoremediative potential is characteristic for grasses [11], which collect metals mainly from soil solution through the root system [10], but they also absorb them from the atmosphere (*eg* in the area of traffic pathways) through stomata and leaf surfaces in gaseous form or as dissolved metal ions in rainwater [12]. These heavy metals can negatively affect physiological processes such as photosynthesis, respiration and transpiration. Metals such as Fe, Cu, Mn, Zn, etc. are essential for normal growth and development of the plant as they are constituents of many proteins, including enzymes. Both excess and deficiency of these elements have a negative impact on the development of plants. According to Prasad [13], the occurrence of Fe deficiency in soil contributes to the development of leaf chlorosis, i.e. the inability to produce chlorophyll and therefore reduces the photosynthetic rate and the consequences of the scarcity of assimilates is inhibition of shoot growth. Iron deficiency causes anatomical deformities of roots. The most important physiological function of manganese is its participation in the oxygen-evolving comlex in light reactions of photosynthesis [14]. Toxicity of trace metals also consists of substituting the appropriate metal in the structures of metalloproteins and other substances (*eg* Mg in chlorophyll, Ca in calmodulin), for connecting to the preferential oxygen, nitrogen and sulphur of many different molecules, their interaction with the functional groups, for example, phosphate and ATP or ADP with carboxyl groups [13].

Chlorophylls are essential molecules for light-harvesting and energy transduction in oxygenic photosynthesis, which comprises a porphyrin ring and hydrocarbon tail, in most cases phytol [15]. The major functions performed by chlorophylls in photosynthetic reactions are: absorbing light efficiently in the light-harvesting complexes; transferring the excitation energy with high quantum efficiency to the reaction centers; and performing the primary charge separation across the photosynthetic membranes and generating membrane potential that leads to ATP and strong reductants (NADPH) production [16].

The aim of this study was to analyse the influence of municipal sewage sludge from the Municipal Wastewater Treatment Plant in Sokolka on the content of magnesium and selected micronutrients such as zinc, manganese, copper and iron in the soil and lawn grasses and on the content of chlorophyll *a* and *b* in the analyzed plants**.**

Materials and methods

The study was conducted on four specially prepared test areas along main streets of Bialystok (Hetmanska Str., Piastowska Str., Popieluszki Str. and Raginisa Str.). Each test area was divided into 3 blocks (30 m^2 each) and each of them was divided into 6 plots of 5 m^2 area. In Fall of 2010 test areas were fertilized with stabilized municipal sewage sludge with three doses 0.0 (control), 7.5 and 15.0 $kg \cdot m^{-2}$ (sewage sludge contains 19.3 % of dry matter) from the Municipal Wastewater Treatment Plant in Sokolka. The doses of sewage sludge were established according to Kiryluk [17] who found in several years study that the most effective doses for turfing of municipal waste disposal areas were those above 40 $Mg \cdot ha^{-1}$.

Before the establishment of the experiment both sewage sludge and soil from each combination were analyzed according to the Regulation of the Ministry of the Environment of July 13th, 2010 concerning municipal sewage sludges [7]. The analyses were done by the Regional Chemical and Agricultural Station in Bialystok (Tables 1 and 2).

In the experiment two grass mixtures were used: Eko from Nieznanice Plant Breeding Station which included 30.0 % of *Lolium perenne* Niga cv., 15.0 % of *Poa pratensis* Amason cv., 22.6 % of *Festuca rubra* Adio cv. and 32.4 % of *Festuca rubra* Nimba cv., and Roadside from Barenbrug which included 32.0 % of *Lolium perenne* Barmedia cv., 5.0 % of *Poa pratensis* Baron cv., 52.0 % of *Festuca rubra rubra* Barustic cv., 5.0 % of *Festuca rubra commutata* Bardiva (BE) cv. and 6.0 % of *Festuca rubra commutata* Bardiva (NL) cv.

Table 1

Selected properties of municipal sewage sludge

Properties	Magnitude
pH	6.7
Dry weight [%]	19.3
Organic matter [$g \cdot kg^{-1}$ d.m.]	584.0
Total P [$g \cdot kg^{-1}$ d.m.]	27.0
Total N [$g \cdot kg^{-1}$ d.m.]	40.0
Ammonium N [$g \cdot kg^{-1}$ d.m.]	1.0
Mg [$g \cdot kg^{-1}$ d.m.]	7.0
Cu [$mg \cdot kg^{-1}$ d.m.]	194.0
Zn [$mg \cdot kg^{-1}$ d.m.]	1459.0

Table 2

Selected physical and chemical properties of soils at four studied locations

Properties	Hetmanska Str.	Piastowska Str.	Popieluszki Str.	Raginisa Str.
pH	7.9	7.7	7.6	7.4
Sand [%]	75.9	71.9	75.7	84.4
Silt [%]	22.0	25.4	22.3	14.7
Clay [%]	2.1	2.7	2.0	1.0
Textural class	loamy sand	sandy loam	loamy sand	sand
Cu [$mg \cdot kg^{-1}$ d.m.]	9.5	16.8	17.9	8.8
Zn [$mg \cdot kg^{-1}$ d.m.]	40.9	195.0	82.9	36.6

In October 2011 samples of soil (0–20 cm) were collected. Heavy metals concentrations in soils with sewage sludge and in plant material were determined using Atomic Absorption Spectrometry Varian Spectra AA-100. The samples of soil were mineralized in temperature at about 450 ^{o}C and remains were dissolved in *aqua regia* (HCl and HNO_3 mixture, 3:1 *v/v*) in 80 °C according to PN-ISO 11047: 2001 [18]. The samples of mixtures of grasses were mineralized in temperature at about 450 °C and remains were dissolved in concentrated HNO_3 [19].

For chlorophyll determination fresh plant material was homogenized in a mortar with addition of $CaCO_3$ and quartz sand. Chlorophyll was extracted with 80 % acetone. Chlorophyll *a* and *b* content was determined using HACH DR5000 spectrophotometer by measuring absorbance at λ = 663 and 645 nm.

$$\text{Chlorophyll } a = (12.7 \cdot D_{663} - 2.7 \cdot D_{645}) \cdot V \cdot (1000\ W)^{-1}$$
$$\text{Chlorophyll } b = (22.9 \cdot D_{645} - 4.7 \cdot D_{663}) \cdot V \cdot (1000\ W)^{-1}$$

where: D_{645} and D_{663} – optical density at λ = 645 and 663 nm, respectively,
V – volume of the solution [cm^3],
W – fresh weight of the leaves sample [g].

The results were statistically analyzed using analysis of variance with Tukey test at significance level at a = 0.05. The correlations between analysed parameters were calculated using Statistica 9.0.

Results and discussion

It seems reasonable the use of sludge in remediation urban soils as they require remediation treatment because of their progressive chemical degradation that leads to a sustainable and progressive deterioration of their properties [20]. The use of sludge has a double benefit; on the one hand, sewage sludge is utilized, on the other hand, one returns to use land transformed by human activity or not used at all [21]. According to Kalembasa and Malinowska [10], such organic substance introduced into the contaminated soil may increase the mobility of heavy metals as a result their complexation with the low molecular organic compounds. It may lead to the situation that one will not be able to determine the actual risk they pose to the environment or their availability to living organisms, the risk to human health, of water pollution and uptake by plants [22]. Plants absorb metals from the soil solution mainly through the root system [23], but they also absorb them from the atmosphere (*eg*. in the area of transportation pathways) through stomata and leaf surfaces in gaseous form or as metal compounds dissolved in rainwater [12]. In this study, the dose of sewage sludge differentiated the content of the analyzed elements in soils and plants, but in most cases the differences were not statistically significant. Only in the case of Piastowska Str. sludge dose had a significant impact on the content of manganese in the soil, while in the case of Raginisa Str. affected the content of zinc in plants. In both cases, with increasing doses of sewage sludge the contents of the studied elements in soil and grass mixtures increased. The study of Wisniewska and Kalembasa [24] suggests that increasing doses of sewage sludge application resulted in increased accumulation of zinc in Italian ryegrass. Authors obtained similar results for the copper content. According to Kalembasa and Malinowska [25], uptake and use of ions by plants from sewage sludge is dependent on many factors *ie*. holding water capacity, redox potential, soil temperature as well as, microbial activity in the rhizosphere. Each of these factors (either alone or in combination with the others) can stimulate or inhibit mineral nutrient uptake by the plant and can influence plant chemical composition.

The highest coefficient of variation of heavy metals in soil was found for zinc (64.5 %), its content in the soil ranged from 27.5 to 207.3 mg $\cdot$ kg^{-1} d.m., while the lowest coefficient of variation in the soil were calculated for magnesium and zinc (3.6 and 3.5 %, respectively), which content in the soil ranged from 14652.0 to 16395.0 for magnesium and from 9.3 to 23.5 mg $\cdot$ kg^{-1} d.m. for zinc. In plants the greatest variability was found for manganese (24.8 %), its content in the above ground parts of grass mixtures ranged from 32.3 to 73.7 mg $\cdot$ kg^{-1} d.m. The lowest variability was observed in the case of plant magnesium content (2.0 %), its content in the test plants ranged from 3419.4 to 3712.1 mg $\cdot$ kg^{-1} d.m. Heavy metals leaching from leaf litter penetrate back into the soil, and thus they re-enrich soil. They become more available and more mobile than the metals present in the soil solution, and they can be re-absorbed by the plant [26]. Toxicity of trace metals also consists of substituting the appropriate metal in the structures of proteins and other substances (*eg* chlorophyll Mg, Ca in calmodulinie), for connecting to the preferential oxygen, nitrogen and sulphur of many different molecules, their interaction with the functional groups, for example, phosphate and ATP or ADP with carboxyl groups [13].

Zinc, copper, manganese and iron are considered necessary for the proper growth and development of plants. Both deficiency and excess of these elements in plant can have a negative impact on the plant. Zinc activates many plant enzymes. For example zinc ions activate carbonic anhydrase, alcohol dehydrogenase and dehydrogenases depending on NADH and NADPH. Zinc also regulates the proportions of components, which affect the permeability of cell membranes. Zinc ions also determine the processes of formation of functional ribosomes. Deficiency of this element impairs the synthesis of tryptophan, which directly affects the production of auxin, and indirectly leads to reduced growth rate of the plant. Zinc ions have a high capacity to translocate to the above ground of plant [27].

In our study the concentration of zinc in soils enriched with sewage sludge was in the range of limits values according to the Regulation of the Ministry of the Environment of September 9th, 2002 [28] on standards for soil quality and earth quality for urban soils (Table 3). The zinc content in the tested mixtures of lawn grasses ranged from 45.5 in the control plants to 68.9 mg $\cdot$ kg^{-1} d.m. in plants grown on plots with the highest addition of sewage sludge at Raginisa Str. According to Wilk and Gworek [1], the average content concentration of zinc in crop plant is 10–100 mg $\cdot$ kg^{-1} d.m. To cover plant demand for zinc, its adequate content concentration is 15–30 mg Zn $\cdot$ kg^{-1} d.m. However, the zinc can be accumulated in the plants even in large amounts (more than 1 %) with no apparent toxicity symptoms. It is also worth noting that the most sensitive to the deficiency of this nutrient are herbaceous grasses.

Many authors [1, 27, 29] point out that the solubility of zinc compounds and related with this bioavailability to plants decreases with increasing pH. In the present study it is not confirmed. However, Bjelkova et al [30] suggest that zinc content in the grasses could be also affected by the location of the experimental plots. The source of zinc in plants and soils near the routes are also dust resulting from wear of tires and other parts of vehicles that get into the soil and plants along with rainfall and as a dry precipitation.

Another essential trace element for plants is copper, which activates a number of enzymes in plants and is a component including catechol and ascorbate oxidases and copper flavoproteids. This element is involved in major processes of photosynthesis and respiration, in the processes of synthesis of proteins, in the metabolism of nitrogen compounds, in the transport and metabolism of carbohydrates and in cell membranes metabolism (copper affects their permeability). Furthermore, copper regulates synthesis of DNA and RNA as well as, affects the sexual reproduction of plants. However, as microelement it is absorbed by plants in trace amounts. Accumulates mailny in roots, but under conditions of severe pollution of the environment its concentration increases in the above ground parts of the plant [27].

The concentration of copper in soils amended with sewage sludge was in the limits values according to the Regulation of the Ministry of the Environment 2002 [28]. The

Table 3

Mean metals concentration in the soil amended with sewage sludge and in grasses

Street	Degree of sewage sludge [$kg \cdot m^{-2}$]	pH_{KCl}	Cu	Fe	Mg	Mn	Zn	Cu	Fe	Mg	Mn	Zn
			Soil [$mg \cdot kg^{-1}$ d.m.]					Plant [$mg \cdot kg^{-1}$ d.m.]				
Hetmanska	0	7.3	13.1	8746.7	15271.8	261.2	59.8	9.8	347.0	3673.1	49.9	49.8
	7.5	7.4	14.3	8582.3	15601.8	235.7	54.0	10.3	339.8	3632.7	45.0	61.0
	15	7.3	13.4	9418.5	15378.0	254.3	51.5	10.8	368.2	3712.1	50.0	63.7
Piastowska	0	7.3	18.7	12245.7	16395.0	304.9	123.8	9.6	364.3	3596.4	38.3	55.2
	7.5	7.2	23.3	13656.3	16329.0	323.3	188.0	13.5	413.5	3575.6	39.1	65.2
	15	7.0	23.5	14330.7	16251.0	353.4	207.3	10.7	326.5	3599.0	32.3	60.3
Popieluszki	0	7.4	18.7	11970.0	15964.8	400.0	87.8	9.6	312.5	3556.9	43.6	49.5
	7.5	7.2	18.2	10744.9	15660.0	395.6	80.7	11.1	330.3	3590.4	40.3	57.8
	15	7.2	19.1	11668.1	15691.8	379.9	89.5	10.6	426.9	3601.0	38.4	64.2
Raginisa	0	7.6	9.3	7328.4	14652.0	232.8	27.5	7.1	245.3	3419.4	37.7	45.5
	7.5	7.5	11.7	7657.4	15033.0	200.4	44.8	8.7	351.8	3561.5	58.2	58.6
	15	7.3	12.1	7287.8	14947.8	203.0	48.7	9.6	298.0	3639.3	73.7	68.1
Mean		7.3	16.3	10303.1	15598.0	295.4	88.6	10.1	343.7	3596.5	45.6	58.2
Standard deviation		0.2	4.6	2471.3	566.8	74.0	57.1	1.5	48.7	72.0	11.3	7.0
Relative standard deviation [%]		2.1	3.5	23.9	3.6	25.1	64.5	15.1	14.2	2.0	24.8	12.1

copper content in plants can be highly differentiated depending on the part of the plant and stage of development, on the variety and species. Its average physiological content in the above ground parts is from 5 to 30 mg $\cdot$ kg^{-1} [27]. The toxic content of copper in these plants is estimated to be 20–100 mg $\cdot$ kg^{-1}, but in plants from highly contaminated soils, the concentration of copper may be several thousand mg $\cdot$ kg^{-1} [30]. In our study the average content of this element in the above ground parts of lawn grasses was in the range 7.1–13.5 mg $\cdot$ kg^{-1} of d.m. (Table 3), and therefore it is within the range of content recognized as physiological.

Correlation analysis showed a negative correlation between soil pH and copper content in the plant at Popieluszki and Raginisa Streets ($r = -1.0$, $r = -0.8$, respectively), significant at $p < 0.05$. Generally, the copper solubility decreases with increasing pH of the soil, as reported by several authors [1, 31].

Manganese activates many enzymes, forming chelate bonds between the proteins and their substrates. It activates decarboxylases and dehydrogenases in the process of mitochondrial respiration. Manganese takes part in the light phase of photosynthesis (in oxygen-evolving complex) and enzymes involved in the reduction of nitrates. Level of manganese in plants is in the range 3.6–15.0 mg $\cdot$ kg^{-1} d.m. [31], while Ostrowska et al [32] reported that its content in the grass can be from 50.0 to 147.0 mg $\cdot$ kg^{-1} d.m. Our findings indicate rather low and moderate level of accumulation of this element in the mixtures of grasses (Table 3), despite fertilization with sewage sludge and location of plots along the transport routes.

Iron is involved in the process of photosynthesis and metabolism of nucleic acids, stimulates the synthesis of chlorophyll, is involved in nitrate reduction and fixation of free nitrogen and regulates oxidative-reductive reactions. The iron content in plants usually ranges from several mg Fe $\cdot$ kg^{-1} of d.m. to several hundred or even several thousand mg Fe $\cdot$ kg^{-1} of d.m. An excess of iron in the environment is toxic for plants by interaction with other compounds. Iron can be accumulated in large amounts by plants without apparent adverse effects [31]. Each metal has a specific pattern of uptake, transport and accumulation in the plant, whereas in the presence of other ions in the soil interactions between the ions can change this pattern. The same combinations of metals may interact synergistically with each other in one plant species, but antagonistically in other one [33].

Correlation analysis of the results from the Hetmanska Str. showed positive correlation between the content of manganese in the soil and the copper and iron in plants ($r = 0.6$, $r = 0.7$) and content of iron in the soil and zinc in plants ($r = 0.6$), while the negative between content of manganese in the soil and magnesium in plants ($r = -0.8$). In the case of Piastowska Str. there were obtained strong correlations between soil copper content and zinc content in plants ($r = 0.8$) and content of manganese in the soil and iron in plants ($r = -0.7$). For Popieluszki and Piastowska Streets there was correlation between content of copper in soil and zinc in plants ($r = 0.6$) with $\alpha = 0.05$. In the case of Raginisa Str. there was positive correlation between the contents of copper and zinc in the soil and of iron in plants ($r = 0.7$ and $r = 0.6$, respectively), and the content of copper in the soil and magnesium in plants ($r = 0.6$).

Macronutrients in the plant play a building role. Magnesium is an important component of chlorophyll. About half of the magnesium contained in the leaves occurs in chloroplasts. Magnesium is also a component of protopectin in the cell walls. Magnesium ions are activators of many enzymes involved in the synthesis of DNA and RNA, kinases transfering phosphate groups, enzymes involved in photosynthesis and respiration. This element is very mobile and is involved in the regulation of acidity (pH) in the cell.

Each of the analyzed elements had an impact on the content of chlorophyll *a* and *b* in the above ground parts of lawn grass mixtures. In most cases, the ratio of chlorophyll *a* to *b* was relatively low (usually less than 1). The reason for this may be the growth conditions of mixtures of grasses sown along the traffic routes exposed to pollution caused by heavy traffic, which can be an additional stress factor. This may explain the particularly low ratio of chlorophyll *a* to *b* in the case of plants collected from control plots in the first months of the study. According to Pinto et al [34], some heavy metals are essential to the metabolism of living organisms at very low content, they may become toxic at higher levels. Some heavy metals, such as Cu^{2+}, Cd^{2+}, Zn^{2+} and Ni^{2+}, are known to substitute the central Mg^{2+} atom in the chlorophyll molecule, a process that lowers the fluorescence quantum yield and results in a shift in the fluorescence spectrum. Moreover, metal excess can lead to binding of metals to proteins thus disturbing protein structures [35].

Our study shows that municipal soil amendment sewage sludge influenced chlorophyll *a* and *b* content and their ratio in lawn grass mixtures. The content of chlorophyll *a* in aboveground parts of grasses was dependent on the used grass mixture, harvest time, location and to a lesser extent on the dose of sewage sludge (Fig. 1).

Toxicity can also be related to oxidative stress induced in living systems either by increasing concentrations of reactive oxygen species or by reducing cellular antioxidant capacity [34]. In primary producers these effects may result in inhibition of chlorophyll production, photosynthesis and growth [36]. They may cause physiological and anatomical changes in plants. The same reaction took into account Prasad [13], discussing the negative impact of metals present in the cell for biochemical processes, such as photosynthesis, respiration, and transpiration. The presence of heavy metals influences the cell nucleus and cell division, causing a decrease in mitotic activity and abnormal cytokinesis, damage to DNA, RNA, decrease in transcriptional activity, chromatin condensation, chromosomal aberrations and destruction of nuclear envelope [37].

The content of chlorophyll *b* was differentiated by dose of sludge and sampling time. The highest average chlorophyll *b* content in the grass samples collected in June and July was found (1.10 mg $\cdot$ g^{-1} of f.m.), and the lowest in a samples collected in October (0.39 mg $\cdot$ g^{-1} of f.m.). The increase in content of chlorophyll *b* was proportional to the dose of sewage sludge. The content of chlorophyll *a* in the analyzed samples of grasses decreased depending on the sampling time (Fig. 1). As the chlorophyll *b* content, the lowest average content of chlorophyll *a* was in samples collected in October (0.45 mg $\cdot$ g^{-1} of f.m.). The average content of chlorophyll *a* in samples collected in June and

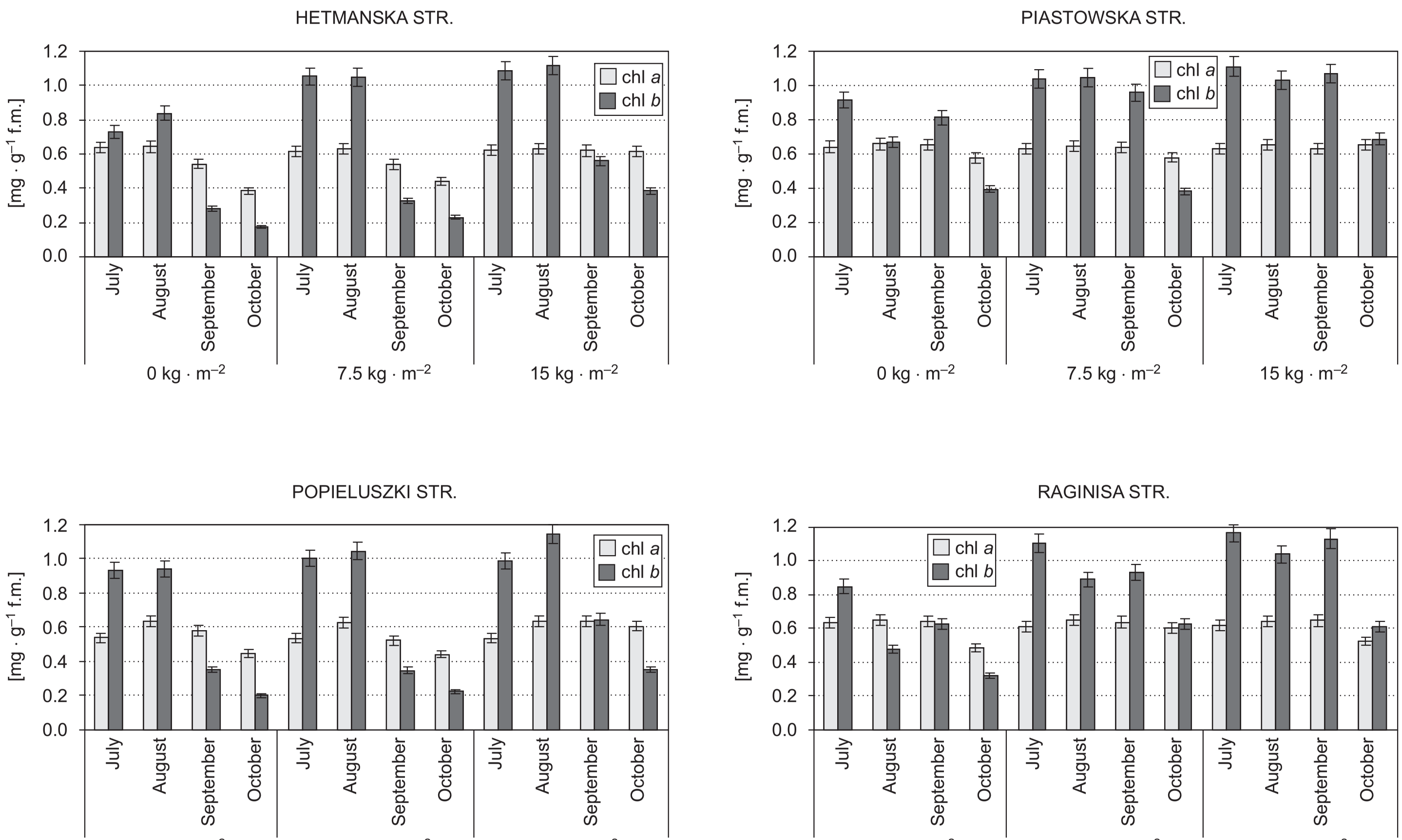

Fig. 1. Influence of doses of sewage sludge, time of sampling and localization on leaves' chlorophyll *a* and *b* content [mg · g^{-1} f.m.]

July were similar independently of addition of sewage sludge (0.64 mg · g^{-1} of f.m.) (Fig. 1).

The analysis of correlations for the results obtained from Hetmanska Street revealed strong positive correlations between the content of Zn and Mn in the soil and the content of chlorophyll *a* in plants (r = 0.84 and r = 0.8, respectively) and strong negative correlations between the content of Zn in the soil and the content of chlorophyll *b* in lawn grasses collected in June and August (r = –0.8 and r = –0.8, respectively). Strong correlations between the concentration of zinc and the content of chlorophyll *b* in grass samples collected in Hetmanska Street, Piastowska Street and Popieluszki Street (r = 0.8, r = 0.8 and r = 0.9, respectively) and the Cu content in plants and chlorophyll *b* for plants collected in Hetmanska Street (r = 0.9), Piastowska Street (r = 0.9), Popieluszki Street (r = 0.90) and Raginisa Street (r = 0.8). Moreover, it was found positive correlations between the content of Fe in the soil and the content of chlorophyll *a* and *b* in lawn grasses collected at Piastowska Street (r = 0.8 and r = 0.7, respectively). According to Hänsch and Mendel [37] iron, like copper is also of great importance for life plants. As redox-active metal it is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis (ethylene, gibberellic acid, jasmonic acid), production and scavenging of reactive oxygen species, osmoprotection, and pathogen defense. Up to 80 % of the cellular iron is found in the chloroplasts that is consistent with its major function in photosynthesis.

Conclusions

1. Amendment with sewage sludge has contributed to an increase in the content of manganese in the soil at Piastowska Str. and zinc in plants at Raginisa Str. In other cases, the municipal sewage sludge did not affect the accumulation of the analyzed elements in soil and grass mixtures.
2. The content of the analyzed elements in plants was at a moderate level, and was far from toxic levels.
3. Alkaline pH of soils caused the limited availability of Cu, Mn and Fe by lawn grass mixtures.
4. Soil manganese in Piastowska and Hetmanska Streets caused lower uptake of iron and magnesium by studied plants.
5. The sewage sludge amendment significantly influenced chlorophyll *b* content in lawn grass mixtures in the first month of the growing season, which resulted in a low ratio of chlorophyll *a* to *b.* This may indicate that the applied dose of sludge caused some kind of stress in the studied plants.
6. The availability of metals for plants should be concidered taking into account not only dose of applied sewage sludge but also the correlations between different metals and environmental factors.

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WPŁYW OSADU ŚCIEKOWEGO NA ZAWARTOŚĆ WYBRANYCH METALI I CHLOROFILU W MIESZANKACH TRAW GAZONOWYCH

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Abstrakt: Celem badań było określenie wpływu komunalnego osadu ściekowego, pochodzącego z Miejskiej Oczyszczalni Ścieków w Sokółce, na zawartość magnezu i wybranych mikroskładników, tj. cynku, manganu, miedzi i żelaza w glebie i mieszankach traw gazonowych oraz na zawartość chlorofilu *a* i *b* w analizowanych roślinach. Wybrane składniki mineralne mają ważne znaczenie w procesie fotosyntezy. Ponadto w pracy określono zależności pomiędzy badanymi pierwiastkami a zawartością barwników asymilacyjnych.

Na poletkach wysiano dwie mieszanki traw gazonowych: Eko i Roadside oraz zastosowano trzy dawki osadu ściekowego: 0 (kontrola), 7.5 kg $\cdot$ m^{-2} i 15 kg $\cdot$ m^{-2}. Całkowitą zawartość metali w glebie i w roślinach określono metodą absorpcyjnej spektrometrii atomowej, natomiast zawartość chlorofilu *a* i *b* za pomocą spektrofotometru, mierząc absorbancję przy długościach fali λ = 663 i 645 nm.

Stwierdzono, że dawka osadu ściekowego miała istotny wpływ na zawartość manganu w glebie w doświadczeniu przy ul Piastowskiej. W doświadczeniu przy ul. Popiełuszki dawka osadu ściekowego wpłynęła istotnie na zawartość cynku i miedzi w badanych mieszankach traw, natomiast przy ul. Raginisa na zawartość cynku, miedzi i manganu.

Analiza korelacji wykazała silną współzależność pomiędzy stężeniem cynku a zawartością chlorofilu *b* w próbkach traw pobranych przy ul. Hetmańskiej, Piastowskiej i Popiełuszki, (r = 0,85, r = 0,86 i r = 0,98) oraz zawartością miedzi i chlorofilu *b* w roślinach pobranych przy ul Hetmańskiej (r = 0,97), Piastowskiej (r = 0,86), Popiełuszki (0,90) i Raginisa (r = 0,82).

Termin poboru próbek oraz zastosowany dodatek osadu ściekowego istotnie różnicowały zawartość chlorofilu *b*. Największą zawartość chlorofilu *b* stwierdzono w próbkach traw pobranych w czerwcu i w lipcu (1,10 mg $\cdot$ g^{-1} ś.m.), natomiast najmniejsze w próbkach z października (0,39 mg $\cdot$ g^{-1} ś.m.). Z kolei zawartość chlorofilu *a* w analizowanych próbkach traw zmniejszała się w zależności od terminu poboru. Podobnie jak w przypadku chlorofilu *b* najmniejszą zawartość chlorofilu *a* stwierdzono w próbach zebranych w październiku, średnio ok. 0,45 mg $\cdot$ g^{-1} ś.m, natomiast od lipca do czerwca zawartość chlorofilu *a* utrzymywała się na podobnym poziomie średnio ok. 0,64 mg $\cdot$ g^{-1} ś.m. bez względu na zastosowany dodatek osadu.

Słowa kluczowe: chlorofil, trawy, magnez, mikroelementy, osad ściekowy