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USING TALL WHEATGRASS *AGROPYRON ELONGATUM* L.'BAMAR' ON GROWING SUBSTRATES WITH SEWAGE SLUDGE AND HALLOYSITE FOR DEGRADED LAND RECLAMATION – POT EXPERIMENT

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Abstract: The pot experiment was to determine the possibility of using tall wheatgrass *Agropyron elongatum* L. 'Bamar' on the growing substrates with sewage sludge and halloysite for degraded land reclamation. This article analyzes the impact of differentiated growing substrates on the phytostabilisation/phytoremediation processes of heavy metals: Cr, Cu, Cd, Zn, and Pb. They involved experimental variants of growing substrates with differentiated addition of halloysite, i.e. 10%, 30%, 50%, and 25%. The reference material was constituted by two control cultivations with growing substrates consisting of sewage sludge and river sand with sewage sludge. The pot experiment results indicated that halloysite had, by improving the growing substrate mechanical parameters, a positive impact on the test plant's growth rate, which was characterized with lesser variability in the germination level and faster growth rate. Halloysite led to the reduction of soil acidity in relation to the control cultivation substrates, which allows for using this clay mineral as an addition to the growing substrates based on sewage sludge. After two experimental cycles, the studies showed that the growing substrates were characterized with lower heavy metal values than those initial ones recorded for sewage sludge. The addition of halloysite differentiated the phytochemical accumulation of heavy metals in biomass. This gives vast possibilities to shape directions of land reclamation processes and provides the base for field works allowing for the full recognition of the impact of halloysite on the phytostabilisation and phytoextraction of heavy metals.

Keywords: *Agropyron elongatum* L. 'Bamar'; heavy metals; phytostabilisation; phytoremediation

1. INTRODUCTION

Sewage sludge from sewage treatment plants belongs to hazardous waste. Due to the presence of heavy metals, toxic organic compounds, as well as pathogenic bacteria and viruses, it usually undergoes thermal utilization or deposition in landfill sites (Adriano et al., 2004). Sewage sludge also has high water content and unpleasant odor due to the presence of volatile organic compounds (Fytili & Zabaniotou, 2008). The above-mentioned factors limit the possibility of its effective use. An alternative for management of stabilized sewage sludge seems to be a conception of its natural use. Nowadays, management of sewage sludge for agricultural purposes is gaining popularity, and its aspects are being analyzed in professional literature (e.g. Ahmed et al., 2010; Usman et al., 2012). Sewage sludge, because of high concentrations of biogenic nutrients, facilitates the growth and development of plants, which is especially useful for conducting agrotechnical treatments on degraded lands (Grobelak et al., 2013; Bauman-Kaszubska & Sikorski, 2014; Liphadzi & Kirkham, 2006). The use of stabilized sewage sludge in a land reclamation process may foster the minimization of financial outlays and, at the same time, maximization of effects for previously assumed goals. One of the advantages of using sewage sludge is that traditional land reclamation methods are related to high energy output, while conducting plant cultivation allows the process to be more optimized as well as improves water retention in soils (Adriano et al., 2004). A common problem with using sewage sludge for land reclamation purposes is that it has high (excessive) content of heavy metals. Heavy metals, due to their proven toxicity, may cause restrictions on germination and biomass growth, and consequently, worsen the parameters of the conducted land reclamation processes (Wang et al., 2012; Chibuike & Obiora, 2014; Sewalem et al., 2014). This imposes the search for new solutions aimed at both stabilizing heavy metals in soils and preserving the availability of biogenic elements for plants.

Nowadays, such additions to growing substrates, which allow for the optimal conduct of land reclamation processes with using plants, are gaining in importance (Burlakovs et al., 2013). Available research studies indicate the possibility of using halloysite [$Al_2Si_2O_5(OH)_4 \cdot (H_2O)$] in the processes of phytostabilisation of heavy metals in soils (Słomkiewicz & Świercz, 2011; Świercz et al., 2016). Phytostabilisation is one of these techniques which are recommended in remediation treatments of soils contaminated with heavy metals (Favas et al., 2014; Oh et al., 2014). This technique may seem to reduce the risk of incorporating heavy metals into the trophic chain by limiting both the availability of heavy metals for plants and their leaching due to soil erosion as well as overland flow (Grobelak et al., 2013).

The article constitutes a contribution to a series of studies aimed at selecting grass species and appropriate doses of sludge and halloysite for the phytoremediation process of soils contaminated with heavy metals. The aim of these studies was to assess the possibility of using *Agropyron elongatum* L. 'Bamar' on the growing substrates with sewage sludge and differentiated addition of halloysite. On this basis, the following three study questions were formulated: (1) Does the addition of halloysite to the growing substrates have an impact on the substrate parameters and level of heavy metal absorption by the test plant?; (2) Is tall wheatgrass *Agropyron elongatum* L. 'Bamar' a species characterized with the rapid plant growth and biomass growth on differentiated growing substrates with sewage sludge?; (3) Does the addition of halloysite to the growing substrates have an impact on the rate of test plant's germination and growth, and if yes, which variant is the most favorable for the crop yield efficiency?

2. MATERIALS AND METHODS

2.1. Pot experiment: cultivar selection and characteristics

One of the key factors determining an efficient phytoremediation process is choosing an appropriate plant species. Grass species are the most recommendable as they have a well-developed and fibrous root system which allows for maximizing its rhizosphere volume. Moreover, a rapid grass growth prevents chemical compounds from leaching back into soils (Adriano et al., 2004).

The experiment was based on the cultivation of tall wheatgrass *Agropyron elongatum* L. 'Bamar'. It has low habitat requirements and is characterized by a rapid biomass growth rate under natural conditions; and therefore, it has qualities of plants recommendable for land reclamation processes (Martyniak et al., 2011; Raymond et al., 2011). This cultivar is widely used as energetic crops; however, so far, the experimental studies involving this plant, sewage sludge and zeolites for the purpose of land reclamation have not been popularized (Lalak et al., 2016).

2.2. Test plant's cultivation parameters

The experiment was being conducted in the form of pot cultivations maintained in a KK 350/FIT 700S phytotrone chamber by the POL-EKO-APARATURA GP. Its use ensured constant growth parameters for plants (Świercz & Słomkiewicz, 2015). The experiment was conducted in two cycles – each of them consisted of six variants. Each variant, in turn, consisted of three observation plant pots (the control variants involved two plant pots because of the phytotrone chamber capacity). Each cycle lasted 8 weeks during which identical circadian parameters of the plant growth, i.e. light, temperature and humidity were being preserved (Table 1). The cycles followed each other in the same sequence, i.e. after completing the first cycle (I cycle), the second one (II cycle) was established on the same growing substrate.

2.3. Cultivation substrate parameters

The experiment was conducted with the use of various growing substrates with differentiated percentage of sewage sludge and halloysite (Table 2).

The stabilized sewage sludge was collected from a sewage treatment plant in the city of Ostrowiec Świętokrzyski (Poland). Halloysite, in turn, was taken from the Dunino open-pit mine located near the city of Legnica (Poland), which is recognized as its worldwide producer. Halloysite is a natural aluminosilicate mineral – it is characterized by high porosity, ion exchange and specific surface area (Table 3) (Świercz & Słomkiewicz, 2015; Cholewa & Kozakiewicz, 2012).

2.3.1. Growing substrate preparation and conducted physicochemical analyses

Halloysite was initially ground in amortar, sieved through a 2 mm mesh sieve, washed with distilled water, and dried to the air-dry state. A mineral base for substrates was made of washed contamination-free river sand. Before filling the pots up, the mineral base was sieved through a 1 mm mesh sieve and mixed mechanically with sewage sludge and halloysite. The substrates were brought to moisture content of 50–60% maximum water holding capacity and then left for 48 hours. After establishing the geochemical balance, all pots were sown with 1 g of tall wheatgrass *Agropyron elongatum* L. 'Bamar' seeds (approx. 150 seeds). Seeding was done according to the producer's recommendations. The experiment included two control cultivation pots – one with sewage sludge without additions and one consisting of mineral substrate with sewage sludge in a ratio of 1:1.

Table 1. Growth parameters for tall wheatgrass *Agropyron elongatum* L. 'Bamar' pot cultivation – circadian rhythm

Section/parameter	phase S1 – morning	phase S2 – afternoon	phase S3 – evening	phase S4 – night
Time [hour]	3	9	3	9
Temperature [°C]	18	24	20	16
Air humidity [%]	70	75	60	60
Light intensity [%]	70	100	70	10
Substrate humidity [% FWC]	60	60	60	60

Table 2. Substrate variants in tall wheatgrass *Agropyron elongatum* L. 'Bamar' pot cultivation

Symbol	Percentage of halloysite [%]	Soil substrate composition [total weight 2000 g]		
		sewage sludge	halloysite	mineral substrate – river sand
Variant 1 – V1	10	1800	200	0
Variant 2 – V2	30	1400	600	0
Variant 3 – V3	50	1000	1000	0
Variant 4 – V4	25	1000	500	500
Control variant A	0	2000	0	0
Control variant B	0	1000	0	1000

Table 3. Parameters of cultivation substrate components

Stabilized sewage sludge of different origins – random sample analysis*		Halloysite $Al_2Si_2O_5(OH)_4{\cdot}H_2O$
I analysis	II analysis	
Reaction [pH] –7.60	Reaction [pH] – 7.30	Al – 19.57 [%]
Dry mass [%] –2.05	Dry mass [%] – 10.7	Si – 18.51 [%]
Organic matter [% d.m.] –60.7	Organic matter [% d.m.] – 82.0	Fe – 11.38 [%]
P [% d.m.] – 3.02	P [% d.m.] – 2.46	Ti – 1.37 [%]
Ca [% d.m.] – trophic chain 4.51	Ca [% d.m.] – 7.20	Ca – 0.51 [%]
Mg [% d.m.] – 0.95	Mg [% d.m.] – 5.00	Mn – 0.22 [%]
N_{NH4} [% d.m.] – 3.85	N_{NH4} [% d.m.] – 0.48	P – 0.23 [%]
N_{og}[% d.m.] – 4.11	N_{og}[% d.m.] – 8.70	Cr – 0.04 [%]
Cd [mg kg^{-1}/d.m.] – 3.39	Cd [mg kg^{-1}/d.m.] – 0.50	Cu – 0.01 [%]
Cu [mg kg^{-1}/d.m.] – 302	Cu [mg kg^{-1}/d.m.] – 101	Zn – 0.01 [%]
Ni [mg kg^{-1}/d.m.] – 26.8	Ni [mg kg^{-1}/d.m.] – 14.1	Ni – 0.05 [%]
Pb [mg kg^{-1}/d.m.] – 39.6	Pb [mg kg^{-1}/d.m.] – 80.0	
Zn [mg kg^{-1}/d.m.] – 3085	Zn [mg kg^{-1}/d.m.] – 598	
Hg [mg kg^{-1}/d.m.] – 1.76	Cr [mg kg^{-1}/d.m.] – 0.25	
Cr [mg kg^{-1}/d.m.] – 1.76	Cr [mg kg^{-1}/d.m.] – 22.7	
Salmonella sp. – present	*Salmonella* sp. – 0	
Ascaris sp. living eggs – 0	*Ascaris* sp. living eggs – 0	
Trichuris sp. living eggs – 0	*Trichuris* sp. living eggs – 0	
Toxocara sp. living eggs – 0	*Toxocara* sp. living eggs – 0	

***** Results of independent analyses conducted by accredited environmental laboratories and delivered by the sewage sludge provider; the scope of sewage sludge analyses is determined by the Ordinance of the Minister of Environment of 6 February 2015 on municipal sewage sludge (Journal of Laws of 2015, Item 257).

During the vegetation period, no additional fertilizers were applied, only watering with deionized water. During the experiment, such cultivation parameters as germination level and rate were read at constant intervals. The plants were harvested after 56 days. After completing the experiment, the samples consisting of growing substrates and aerial parts of plants were submitted to a laboratory analysis. The pots were refilled with the substrate mixture up to 2 dm^3 and a similar experimental cycle was established. The soil substrates, after being dried, ground the mortar and sieved through the mesh sieve, were analyzed following three repetitions in terms of (Ostrowska et al., 1991):

- pH_{KCl} by the potentiometric method with using a pH meter and Elmetron electrode in a ratio of 1:2.5, 1M KCl soil;
- total content of heavy metals: Cr, Cu, Cd, Zn, and Pb by treating1g of material with 10 ml HCl and then 0.5 ml H_2O_2 and 1% HNO_3 for 24 hours. The obtained solution was afterwards filtered and the content of heavy metals was determined by the flame atomic absorption spectroscopy (FAAS).

The total content of heavy metals: Cr, Cu, Cd, Zn, and Pb was determined in the biomass, after being dried and ground in a Fritsch planetary mill, by the flame atomic absorption spectroscopy (FAAS).

2.3.2. Statistical analysis

The obtained results were analyzed statistically. One-way analysis of variance (ANOVA) and nonparametric tests were performed. The analyses of the significance of differences among the individual experimental variants and the measured parameters were carried out at the significance level of $p<0.05$. Statistical and graphical analyses were performed with the use of Statistica v. 13 program.

In order to analyze the content of heavy metals in the soil and biomass, the measurement uncertainty was determined, consisting of: volumetric vessel uncertainty, uncertainty of standards, relative standard deviation of repeatability, as well as relative standard deviation of recovery. Two independent reference curves were designated using the Merck and Sigma's certified reference materials. Measurement uncertainty was 25%.

In order to determine the accuracy for indications of the pH meter and electrode, the electromotive force was defined –the linearity of pH meter indications in the analyzed range was checked. Standard buffers with pH: 4.01, 7.00 and 9.21 were used. Uncertainty in pH_{KCl} was 5%.

3. RESULTS AND DISCUSSION

3.1. Tall wheatgrass germinationandgrowth

The level of test plant's germination was variable (Fig. 1). The calculated coefficients of variation indicated that the highest variation in the amount of germinated seeds in the first cycle was recorded for the control cultivations A and B (Table 4).

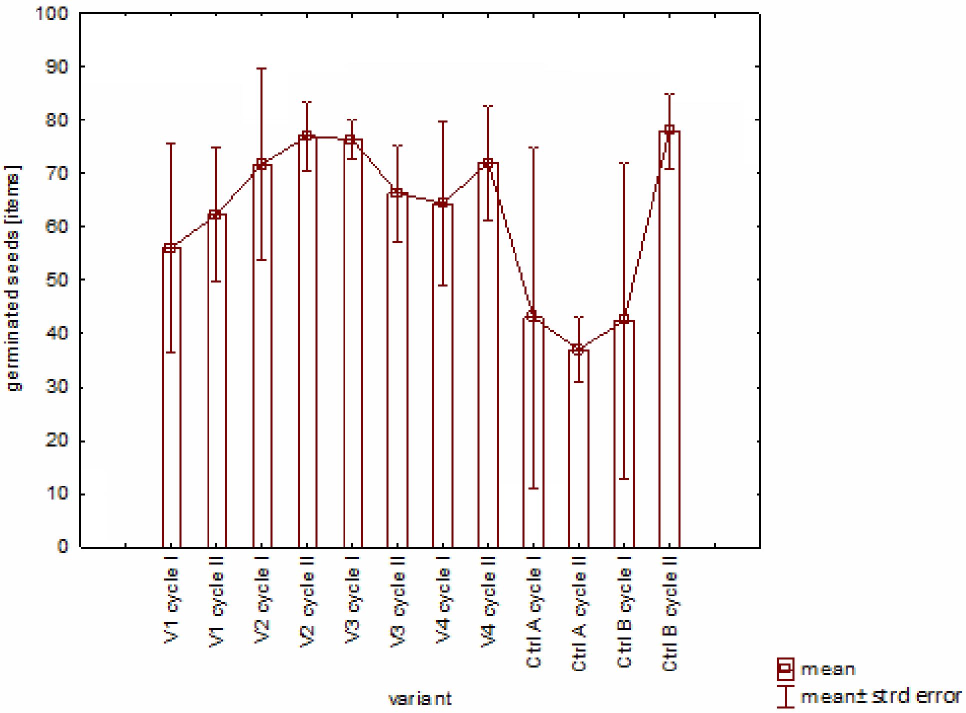

Figure 1. Variation in germination level of tall wheatgrass *Agropyron elongatum* L. 'Bamar' in all pots within a given experimental variant – two experimental cycles (I and II).

Table 4. Coefficients of variations for seed germination within each variant

Variant	I Cycle	II Cycle
Variant 1 – V1	0.604	0.353
Variant 2 – V2	0.432	0.146
Variant 3 – V3	0.087	0.236
Variant 4 – V4	0.409	0.257
Control variant A	1.052	0.229
Control variant B	0.982	0.127

These pots were characterized with minimum germination at the level of <20 seeds, which meant that germination efficiency, was below 15%. High variability in the germination level in the first cycle was also recorded for variant V1 with a low percentage of halloysite. The amount of germinated seeds in the cultivations within the variants V2, V3 and V4 was characterized with lower coefficients of variation. The average germination level for these was oscillating at the level of 40-50%. The most efficient germination was found for the cultivations from the variant V3 whose values remained at comparable levels. In the second cycle, the variation in germination level was lower than in the first cycle. For each variant, there were significant variations in the germination levels. There was no significant statistical relationship between the cultivation variant and the test plant's germination level.

The observed variability in the test plant's germination may be the result of participation of heavy metal forms bioavailable for plants, which derive from sewage sludge. High toxicity of heavy metals causes necrosis of plants at the level of seedling as reported by Navari-Izzo & Rascio (2010). According to Campana et al., (2014) as well as Levy & Taylor (2003), the higher salinity of sewage sludge may limit the process of germination as well. The conducted observations showed that the addition of halloysite to the growing substrate with sewage sludge may facilitate the test plant's germination level and growth rate –the cultivation variants with a significant addition of halloysite were characterized with the faster germination level; moreover, less variation in the germination efficiency was observed, which may have a positive impact on the cultivation stability, also under field conditions. The pot experiment indicated that already the second sowing on the growing substrate allowed for achieving good germination parameters for tall wheatgrass.

The studies conducted on the growing substrates with sewage sludge brought good crop yield effects which may be comparable to those with manure or mineral salts (Koncewicz-Baran et al., 2014). This is of great importance, especially in the case of reclamation treatments where the cover of soils with plants is one of the key elements for conducting cultivation. Sewage sludge provides plants with nutrients such as magnesium and potassium (Abdu et al., 2011); however, high heavy metal concentrations may limit the growth of plants.

The studies indicated that the addition of halloysite did not limit the test plant's germination level. The test plant's germination efficiency was at the comparable level for all variants, but those enriched with halloysite (especially V2, V3 and V4) were characterized with the lowest variation in this regard. This indicates the need for further field works in order to fully verify the impact of halloysite on crop parameters.

In each substrate variant, 10% of germinated seeds were selected, which then were marked with colorful rubber bands. The increase in height was measured according to appropriate frequency. The measurement was made using a scale ruler. The growth of biomass in the pot experiment was varied; however, in each analyzed variant, a similar trend in the test plant's growth was observed, i.e. the growth was dynamical at each stage of the cycle, but the highest repeatability in the growth dynamics was noted for the first two weeks of the experiment. The growth adopted the shape similar to a logarithmic curve (Fig. 2).

The test plant was growing throughout the whole experiment in both experimental cycles. There were no statistically significant relationships indicating direct variations in the biomass growth with regard to each cultivation variant.

The pot experiment involving tall wheatgrass 'Bamar' was to indicate whether this plant may be used in cultivations on low-quality growing substrates, mainly with high concentrations of heavy metals. The mean crop yield obtained from each variant showed that although using varied growing substrates, there were no statistically significant relationships which would limit the test plant's biomass growth (Table 5). The test plant indicated good yielding parameters in each variant, however, there were significant variations in the obtained crop yield values.

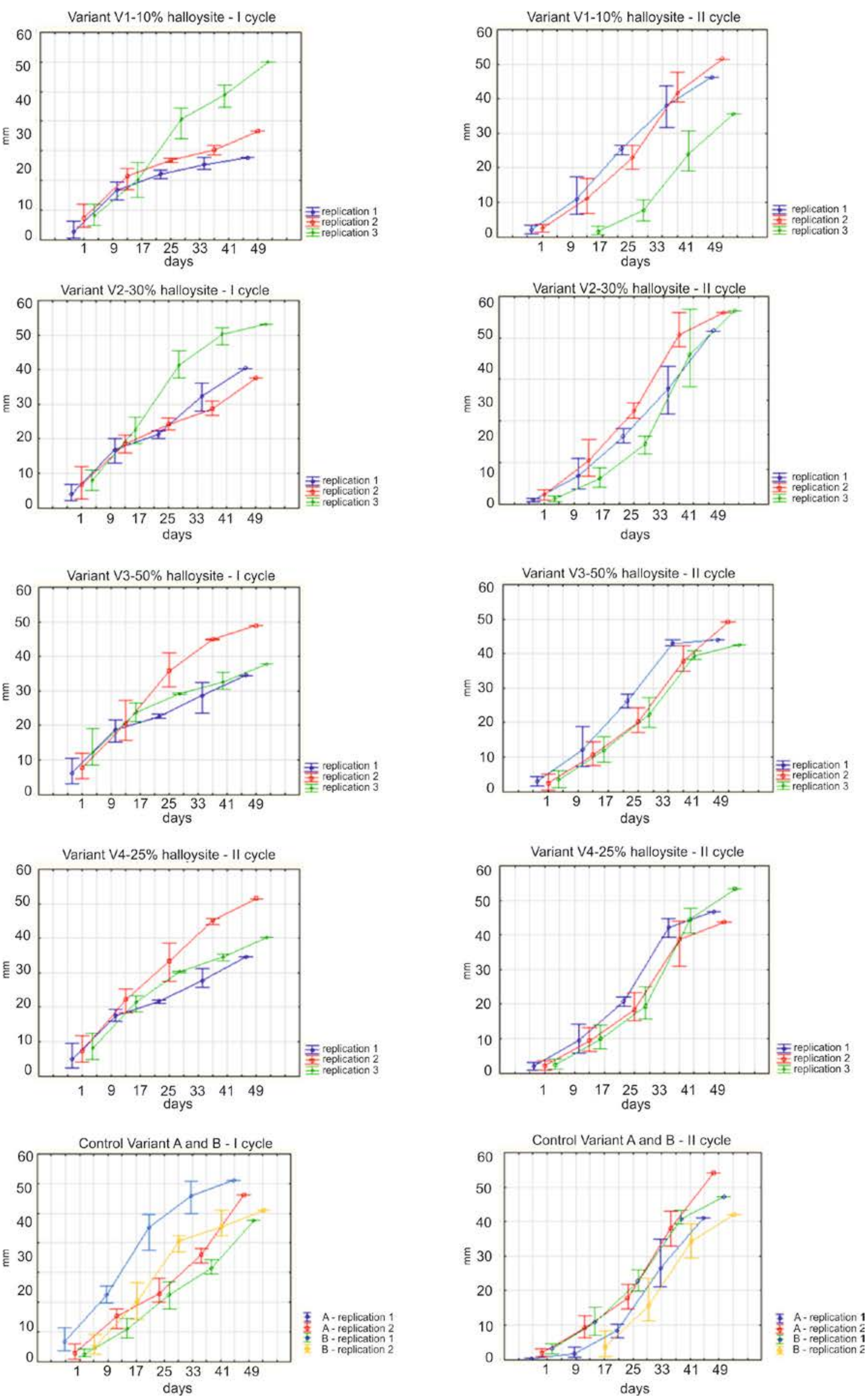

Figure 2. Course of tall wheatgrass' biomass growth [mm] in pot cultivations – I cycle and II cycle

Table 5. Biomass crop yield obtained in each experimental variant [g]

Variant	I Cycle					II Cycle				
	replication					replication				
	1	2	3	mean	Standard Deviation	1	2	3	mean	Standard Deviation
Variant 1 – V1	0.10	0.60	6.09	2.26	3.32	2.18	3.88	0.90	6.96	1.49
Variant 2 – V2	0.18	0.60	5.95	2.24	3.21	1.73	4.09	2.81	2.88	1.18
Variant 3 – V3	0.40	4.04	0.57	1.67	2.05	2.91	2.40	2.94	2.75	0.30
Variant 4 – V4	0.20	3.89	0.61	1.56	2.02	3.25	3.33	4.08	3.55	0,46
Control variant – A	7.01	2.38	-	4.70	3.27	0.84	2.30	-	1.57	1.03
Control variant – B	1.44	6.18	-	3.81	3.35	3.55	3.23	-	3.39	0.27

A strong and positive correlation (the correlation coefficient – 88%) was noted for the average crop yield and average germination level. The crop yield obtained for the cultivations enriched with halloysite was comparable to that reported for the control cultivations.

Redjala et al., (2010) and Radziemska et al., (2013) indicate that the growth of aerial parts of plants may be limited by high doses of heavy metals concentrated in growing substrates based on sewage sludge. Sewage sludge is characterized with extremely variable properties, including heavy metal concentrations, as evidenced by the control studies conducted for the sewage sludge used in the pot experiment. The variability in the level of crop yield may also be affected by the growing substrate structure. Sewage sludge, when completely dry, becomes hard and creates aggregates which weakly absorb water, and thus disturbs air-water relationships. The pot experiment indicated that the cultivations enriched with halloysite were absorbing and retaining water much better than the control ones; and after being dried, they more easily underwent mechanical processing. These relationships were observed in each cycle of the pot experiment. Also, Grobelak et al., (2013) prove the positive impact of non-organic additives to growing substrates based on sewage sludge, which improve the crop efficiency.

3.2. Growing substrate pH

From the environmental point of view, pH is one of the key factor determining the biological activity of soils, plants' growth and development, as well as availability of chemical elements for plants (Sosnowski & Jankowski, 2010). The acidic soil pH facilitates the migration of heavy metals and their bioavailability (Favas et al., 2014; Elekes, 2014; Laghlimi et al., 2015). The pH of growing substrates at the beginning of the experiment was acidic and was ranging from 5.92 to 6.08, which may facilitate heavy metals in migrating within the soils solution. Sewage sludge, due to variable parameters of sewage, may be characterized with varied pH values. The addition of halloysite to the growing substrates within the variants V1-V4 stabilized the pH values at a slightly acidic level. The pH values were oscillating at the level of 6.27–6.56 (Fig. 3).

Halloysite increased the pH in the growing substrates. Generally, the growth of pH in the soil (alkalization) determines the stabilization of heavy metals in the soil and reduces both their availability for plants and their migration in the soil-water environment (Marques et al., 2009; Kumar & Maiti, 2015).

3.3. Content of heavy metals in growing substrates and biomass

Table 6 shows the total content of heavy metals after conducting the two-cycled experiment ($p<0.05$). The values were given as the mean of the three repetitions.

The results of the experiment indicated a significant reduction in the content of heavy metals in the growing substrates in comparison to the values reported for sewage sludge (all analyzed heavy metals, except for cadmium). Pure sewage sludge due to high heavy metal concentrations (mainly zinc) could not be directly used as an addition to the growing substrates. Adding halloysite to the growing substrate allowed sewage sludge to be safely used in soil fertilization. The most preferred addition of halloysite in the above case was that of 30%. High level of reduction in heavy metal concentrations in each of the analyzed cases indicated the possibility to use growing substrates based on sewage sludge and halloysite as practice in land reclamation processes. Sewage sludge is not characterized by uniform physicochemical parameters and heavy metal concentrations, and its composition is determined by waste water treatment plant's cycles and waste water parameters. After conducting the second experimental cycle, the content of heavy metals was varied. There were no statistically significant relationships between the content of heavy metals and each cultivation variant.

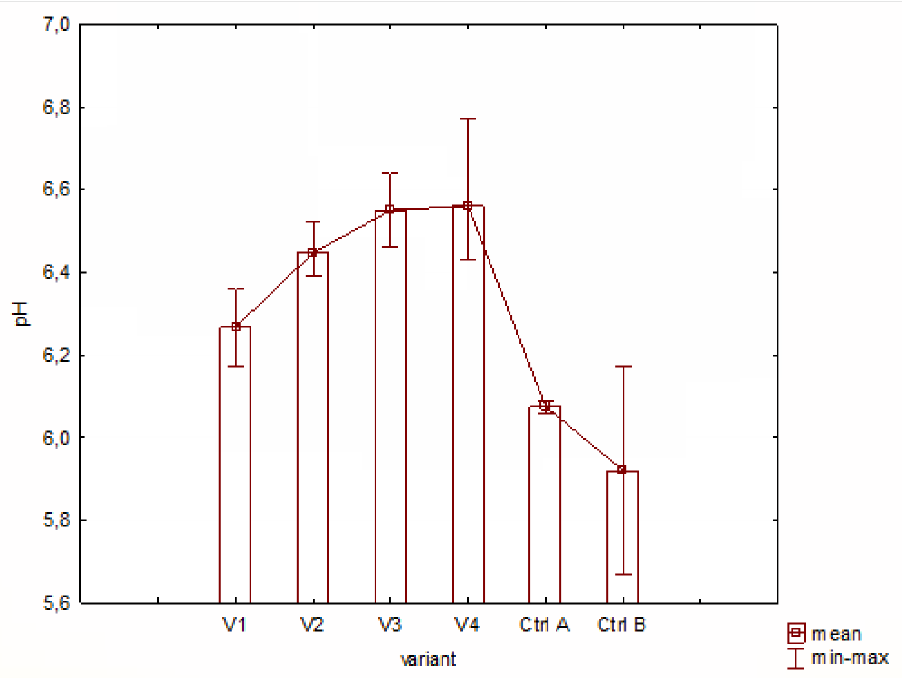

Figure 3. Average pH values of growing substrates ±

Table 6. Total content of heavy metals in biomass ($p<0.05$) after conducting pot cultivation and percentage of reduction in heavy metal concentrations in comparison with sewage sludge

Parameter		Variant					
		V1	V2	V3	V4	A	B
		Percentage of halloysite [%]					
		10	30	50	25	0	0
Cr [mg kg^{-1}/d.m.]	I Cycle	2.50±0.02	5.00±0.25	20.8±1.00	18.3±0.26	24.1±0.15	2.50±0.22
	II Cycle	2.50±0.15	24.2±0.65	2.50±0.10	21.4±0.32	2.50±0.10	12.9±0.70
	Total	5.00±0.17	29.2±0.90	23.3±1.10	39.7±0.58	26.6±0.25	15.4±0.92
	Growing substrate*	318±9.01	303±8.71	556±22.0	364±4.50	252±3.60	48.90±1.16
Zn [mg kg^{-1}/d.m.]	I Cycle	166±3.46	169±6.08	215±29.2	18.0±0.61	114±2.00	206±8.62
	II Cycle	169±5.00	251±23.3	7.10±0.15	79.4±1.29	154±8.89	260±8.54
	Total	335±8.46	420±29.4	222±29.4	97.4±1.90	268±10.1	466±17.1
	Growing substrate*	1570±18.6	1289±10.0	1062±17.8	1135±17.8	3359±52.5	1050±44.6
Cd [mg kg^{-1}/d.m.]	I Cycle	1.35±0.06	1.32±0.27	0.96±0.38	0.84±0.03	0.74±0.13	1.55±0.17
	II Cycle	2.75±0.07	0.25±0.03	2.37±0.09	0.81±0.05	2.62±0.18	0.93±0.05
	Total	4.10±0.13	1.57±0.30	3.33±0.47	1.65±0.08	3.36±0.31	2.48±0.23
	Growing substrate*	2.10±0.06	1.51±0.11	0.25±0.01	2.23±0.26	4.37±0.29	1.20±0.10
Cu [mg kg^{-1}/d.m.]	I Cycle	21.3±0.15	19.0±1.00	29.9±1.41	36.0±4.00	26.6±0.90	21.7±0.15
	II Cycle	19.6±0.41	35.2±0.82	17.7±0.47	27.1±0.47	17.1±0.21	27.6±1.00
	Total	40.9±0.56	54.2±1.82	47.6±1.88	63.1±4.47	43.7±1.01	49.6±1.15
	Growing substrate*	112±1.15	123±1.73	168±4.36	119±4.36	247±6.66	73.0±3.06
Pb [mg kg^{-1}/d.m.]	I Cycle	8.10±0.11	8.10±0.55	5.70±0.53	6.50±0.35	26.9±0.64	8.15±0.46
	II Cycle	12.3±0.41	2.50±0.32	12.5±0.31	2.50±0.15	12.8±0.32	6.58±0.13
	Total	20.4±0.52	10.6±0.87	18.2±0.84	9.00±0.50	39.7±0.96	14.7±0.59
	Growing substrate*	33.0±2.01	31.5±0.50	20.6±1.59	35.1±1.05	54.2±1.61	27.0±2.00

* Study results after conducting the second experimental cycle

Heavy metal concentrations in the test plant had no steady trend – there were no statistically significant relationships between the content of analyzed heavy metals and each cultivation variant. Depending on the variant of experiment, the concentrations of particular heavy metals in the test plant were oscillating at varied levels.

The studies conducted by Kalembasa & Malinowska (2008) indicated that fertilization of crops with sewage sludge may lead to an increase in heavy metal concentrations in biomass. The studies indicated that the addition of halloysite led to the differentiation of heavy metal concentrations in the test plant. The impact of halloysite on the content of analyzed heavy metals in the biomass was differentiated. That is why a decision on the percentage of halloysite addition to sewage sludge should depend on intended purposes of land reclamation. Significant differentiation in heavy metal concentrations in the biomass for each of the cycle indicated the need for both further experimental works involving field cultivations and studies to identify exchangeable forms of heavy metals in soils. Determined content of heavy metals did not directly point out the specific experimental variant which would be the most beneficial to a given process. Considering the crop parameters, i.e. the rate and efficiency of germination and biomass growth, the variants V2 and V3 seemed to be the most beneficial in preparing the growing substrates based on sewage sludge. They were characterized with relatively low concentrations of zinc and lead. Increased values of chromium were, in turn, reported for the control variants.

Grasses belong to those plant species which intensively absorb heavy metals, and this absorption intensity is proportional to the content of these heavy metals in the growing substrates. This concerns especially such heavy metals as Cd, Pb and Zn. Zinc, copper and cadmium are accumulated in aerial parts of plants, and lead – in their roots (Lubin & Tychinin, 2003; Ociepa et al., 2014). The pot experiment results proved that tall wheatgrass is a plant which accumulates heavy metals from sewage sludge well; however, no statistically significant relationships between the content of heavy metals and their absorption were found. In such conditions, after two experimental cycles, the highest accumulation of lead, and most optimal accumulation of cadmium, chromium and copper in the test plant were obtained. This aspect may be limited through adding halloysite to sewage sludge, which thanks to appropriate halloysite doses would reduce the mobility of heavy metals in the soil by limiting their bioaccumulation in aerial parts of plants and minimize heavy metal concentrations in growing substrates by mechanical mixing.

4. CONCLUSIONS

Halloysite added to the growing substrates based on sewage sludge improves the reduction of heavy metal concentrations and allows for unrestricted use of the substrate. Zinc concentrations in municipal sewage sludge were blocking its use in a land reclamation process. The studies conducted in two experimental cycles indicate that halloysite has differentiated the growing substrate parameters by influencing the change of the crop structure as well as its physical and mechanical properties. The addition of halloysite stabilizes the growing substrate pH at a slightly acidic level – the pH values in experimental variants were ranging from 6.27 to 6.58 and from 5.92 to 6.08 in the control cultivations. A positive impact of halloysite on the rate and efficiency of the test plant's germination was noted – the variants with halloysite were characterized with lower differentiation in germination than in the control cultivations. The germination level in the variants enriched with halloysite was oscillating at the level of 40–50%. The pots within each variant were characterized by less dispersion at the level of germination. Halloysite influences the level of heavy metals absorption as well – a varied impact of halloysite on the uptake of heavy metals by the test plant, depending on the percentage share of halloysite, was reported. Also noteworthy is that the addition of halloysite has significantly reduced the concentrations of heavy metals in the growing substrates, except for chromium whose higher values were recorded for the control cultivations.

The test plant used in the pot experiment – tall wheatgrass *Agropyron elongatum* L. 'Bamar' is a grass suitable for seeding on growing substrates based on municipal sewage sludge. It is characterized with good biomass growth and efficiency of germination. The crop parameters were comparable in each of the analyzed variant. This indicates that tall wheatgrass *Agropyron elongatum* L. 'Bamar' is tolerant to high concentrations of heavy metals. Tall wheatgrass may be used on sandy soils of low soil valuation classes as well – due to its rapid growth rate, it provides good protection against soil erosion and leaching.

The ambiguous results of the pot experiment and significant variations in the analyzed properties, which did not show one-direction trends, indicate the need for further and extended field works in this regard, which will be continued at further stages of the studies.

REFERENCES

Abdu A., Aderis N., Abdul-Hamid A., Majid N.M. & Jusop S., 2011. *Using Orthosiphon Stamineus B. for Phytoremediation of Heavy Metals in Soils Amended with Sewage Sludge.* Am. J. Applied Sci. 8, 323-331.

Adriano D.C., Wenzel W.W., Wangronsveld J. & Bolon

N.S., 2004. *Role of Assisted Natural Remediation in Environmental Cleanup*. Geoderma 122, 121-142.
Ahmed H.K., Fawy H.A. & Abdel-Hady E.S., 2010. *Study of Sewage Sludge Use in Agriculture and its Effect on Plant and Soil*. Agric. Biol. J. N. Am. 1, 1044-1049.
Bauman-Kaszubska H. & Sikorski M., 2014. *Conditions of Agricultural and Natural Use of Sewage Sludge in Rural Areas*. Eng. Prot. Environ. 17, 105-115.
Burlakovs J., Karasa J. & Klavins M., 2013. *Devonian Clay Modification for the Improvement of Heavy Metal Absorption Properties*. Environ. Clim. Techn. 13, 22-26.
Campaña D., Echevarría M., Airasca A. & Couce M., 2014. *Physicochemical and Phytotoxic Characterisation of Residual Sludge from the Malting of Barley*. J. Pollut. Eff. Cont. 2, 2, 1-6. http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1522-2624
Chibuike G.U. & Obiora S.C., 2014. *Heavy Metal Polluted Soils: Effect on Plants and Bioremediation Methods*. Appl. Environ. Soil Sci. 14, 1-12.
Cholewa M. & Kozakiewicz Ł., 2012. *The Properties of Moulding sand with Halloysite*. Arch. Foundry Eng. 12, 205-210.
Elekes C.C., 2014. *Eco-technological Solutions for the Remediation of Polluted Soil and Heavy Metal Recovery*. Environ. Risk Assess. Soil Contamin. 3, 309-335.
Favas P., Pratas J., Varun M., D'Souza R. & Paul M., 2014. *Phytoremediation of Soils Contaminated with Metals and Metalloids at Mining Areas: Potential of Native Flora*. Environ. Risk Assess. Soil Contamin. 14, 485-517.
Fytili D. & Zabaniotou A., 2008. *Utilization of Sewage Sludge in EU Application of Old and New Methods – a Review*. Renew. Sust. Energ. Rev. 12, 116-140.
Grobelak A., Kacprzak M., Grosser A. & Napora A., 2013. *Chemical Stabilisation of Soils Contaminated with Cadmium, Zinc and Lead*. Ann. Set The Environ. Prot. 15, 1982-2002.
Kalembasa D. & Malinowska E., 2008. *Influence of Fertilisation with Waste Activated Sludge and Urea on Content of Selected Elements in Biomass of Miscanthus Sacchariflorus*. Acta Agrophysica 11, 657-666 (in Polish).
Koncewicz-Baran M., Gondek K. & Korol J., 2014. *Influence of Biological and Thermal Transformed Sewage Sludge Application on Manganese Content in Plants and Soil*. J. Ecol. Eng. 37, 17-30 (in Polish).
Kumar A. & Maiti S.K., 2015. *Effect of Organic Manures on the Growth Cymbopogon Citratus and Chrysopogon Zizanioides for the Phytoremediation of Chromite-Asbestos Mine Waste: A Pot Scale Experiment*. Int. J. Phytoremediation 17, 437-447.
Laghlimi M., Baghdad B., El Hadi H. & Bouabdli A., 2015. *Phytoremediation Mechanisms of Heavy Metal Contaminated Soils: A Review*. Open J. Ecol.5, 375-388.
Lalak J., Kasprzycka A., Martyniak D. & Tys J., 2016. *Effect of Biological Pretreatment of Agropyron elongatum 'BAMAR' on Biogas Production by Anaerobic Digestion*. Bioresour. Technol. 200, 194-200.
Levy J.S. & Taylor B.R., 2003. *Effects of Pulp Mill Solids and Three Composts on Early Growth of Tomatoes*. Bioresour. Technol. 89, 297-305.
Liphadzi M.S. & Kirkham M.B., 2006. *Heavy-Metal Displacement in Chelate-Treated Soil with Sludge during Phytoremediation,* J. Plant Nutr. Soil Sci. 169, 737-744.
Lubin Y.V. & Tychinin D.N., 2003. Phytoremediation in Russia. In N. Willey, ed. *Phytoremediation: Methods and Reviews*. Humana Press, New York, 423-434.
Marques A.G.C., Rangel A.O.S.S. & Castro A.M.L., 2009. *Remediation of Heavy Metal Contaminated Soils: Phytoremediation as a Potentially Promising Clean-Up Technology*. Crit. Rev. Environ. Sci. Technol. 39, 622-654.
Martyniak D., Fabisiak E., Zielewicz W. & Martyniak J., 2011. *The Biological-Chemical Properties of Tall Wheat Grass (Agropyron elongatum Host. Beauv.) in terms of Potential Use as Biomass for Energy Production*. Bull. Plant Breed. Acclim. Instit.260/261, 375-384 (in Polish).
Navari-Izzo F. & Rascio N., 2010. Heavy Metal Pollution: Damage and Defense Strategies in Plants. In P. Mohammad, ed. *Handbook of Plant and Crop Stress*.CRC Press, Boca Raton, 635-674.
Ociepa E., Pachura P. & Ociepa-Kubicka A., 2014. *Effect of Fertilization Unconventional Migration of Heavy Metals in the Soil-Plant System*. Eng. Protect. Environ. 17, 325-338(in Polish).
Oh K., Cao T., Li T. & Cheng H., 2014. *Study on Application of Phytoremediation Technology in Management and Remediation of Contaminated Soils*. J. Clean Energy Technol. 2, 216-220.
Ostrowska A., Gawliński S. & Szczubiałka Z., 1991. *Methods of Analysis and Evaluation of Soil Properties and 455 Plants – Catalogue*. Institute of Environmental Protection, Warsaw (in Polish).
Radziemska M., Mazur Z. & Jeznach J., 2013. *Influence of Applying Halloysite and Zeolite to Soil Contaminated with Nickel on the Content of Selected Elements in Maize (Zea mays L.)*. Chem. Eng. Trans. 32, 301-306.
Raymond A., Wuanna I. & Okieimen F.E., 2011. *Heavy Metals in Contaminated Soils: A Review of Sources, Chemistry, Risks and Best Available Strategies for Remediation*. Int. Schol. Res. Net. Ecol. 17, 20-41.
Redjala T., Sterckeman T., Skiker S. & Echevarria G., 2010. *ContributionofApoplast and Symplast to Short Term Nickel Uptake by Maize and Leptoplax Emarginata Roots*. Environ. Exp. Botany 68, 99-106.
Sewalem N., Elfeky S. & El-Shintinawy F., 2014. *Phytoremediation of Lead and Cadmium Contaminated Soils using Sunflower Plant*. J. Stress

Physiol. Biochem. 10, 122-134.
Słomkiewicz P. & Świercz A., 2011. *Technological Assumptions of Initiating the Halocompost Production from Utilized Waste Plant Deposits and from Deposits Polluted by Heavy Metals*. Sci. Didact. Equip. 16, 79-90 (in Polish).
Sosnowski J. & Jankowski K., 2010. *Effect of Soil Fertilizer on the Floristic Composition and Yield of Braun's Festololium Mixtures with Red Clover and Alfalfa*. Grassland Science in Poland 13, 157-166 (in Polish).
Świercz A. & Słomkiewicz P., 2015. *Vegetative Flowerpot for Measurements of Studied Plants in the Conditions of the Diverse Condensation of the Soil in the Roots System*. Sci. Didact. Equip. 15, 12-23(in Polish).
Świercz A., Smorzewska E., Słomkiewicz P. & Suchanek G., 2016. *Possible Use of Halloysite in Phytoremediation of Soils Contaminated with Heavy Metals*. J. Elem. 21, 559-570.
Usman K., Khan S., Ghulam S., Khan M.U., Khan N., Khan M.A. & Khalil S.K., 2012. *Sewage Sludge: An Important Biological Resource for Sustainable Agriculture and its Environmental Implications*. Am. J. Plant Sci. 3, 1708-1721.
Wang X., Guo Y., Yang L., Han M, Zhao J. & Cheng X., 2012. *Nanomaterials as Sorbents to Remove Heavy Metal Ions in Waste Water Treatment.* J. Environ. Anal. Toxicol. 2, 1-7.

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