Mercury Concentration in Technosols and Alder Tissue from a Plantation on a Combustion Waste Disposal Site

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Abstract Combustion of fossil fuels including coal is one of the sources of mercury pollution. Combustion waste from fly ash disposal sites poses a problem for the environment and constitutes a potential source of Hg, thus phytostabilisation is a crucial goal in the mitigation of fossil fuel impact. The paper presents mercury (Hg) concentration in technosols from combustion waste and in individual biomass components (fine roots, bark, stem wood and leaves) of alder species (black, gray and green alder) introduced as part of a long-term experiment to develop a method of phytostabilisation and afforestation of a lignite combustion disposal site. Mercury content in the combustion waste was elevated compared to the data for natural soils from uncontaminated forest areas, however, it did not exceed the amounts considered to be toxic. Hg content in technosols was related to clay and silt fraction content and phosphorus content. Mercury in the alder biomass

accumulated mainly in the underground part, especially in the fine roots and displayed a positive correlation with acid and alkaline phosphatase and sulfur content, with no differences in the accumulation of Hg between the alder species. The obtained results indicate that the fine roots are the frontier of Hg biosorption in developed alder systems on combustion waste disposal sites.

Keywords Trace elements · Contamination · Mercury · Reclamation · Fly ash

1 Introduction

Mercury is one of the most toxic and dangerous elements for human health and the natural environment (Rice et al. 2014), therefore many measures are being implemented to reduce its emission, including through agreements and international legal acts such as The Minamata Convention on Mercury (Selin 2014). In the case of long-term exposure, mercury causes damage to the human brain which leads to disruption of metabolic processes, convulsions, psychopathological symptoms, sensory organ impairments, speech disorders and memory loss (Gibb and O’Leary 2014).

Mercury released into the natural environment may be due to natural factors, such as volcanic eruptions, evaporation from ocean surface, geothermal processes, weathering of mercury-containing minerals (Kabata-Pendias 2001; Gustin et al. 2008). However, the majority, i.e. up to 70–80% of total mercury emission to the environment used to come from anthropogenic

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emissions (Mason et al. 1994; De Simone et al. 2016). As a result of the international agreements mentioned above, the proportion of mercury deposition from anthropogenic sources is now decreasing. Estimates indicate that since 2000, anthropogenic mercury emissions on the global scale have already dropped down to the level of mercury emissions from natural sources (Pacyna et al. 2006). Currently, it is estimated that anthropogenic emissions account for approx. 30% of the global total mercury emission to the atmosphere, while 70% is due to natural factors (primary mercury emissions and re-emissions) (De Simone et al. 2016; Pacyna et al. 2016). The main global source of anthropogenic emissions to the atmosphere, i.e. 60%, is the combustion of fossil fuels (Pacyna et al. 2006). Poland, due to fact that the energy industry still hinges on coal combustion (in 2016, 81% of electricity was produced from the combustion of lignite and coal, Source: International Energy Agency (2017) is considered one of the countries with the highest mercury emission in Europe (Glodek and Pacyna 2009). Approximately half of the total mercury emissions to the environment in Poland are accounted for by fossil fuel combustion processes in the course of electricity production (Bartnicki et al. 2017). Other sources of mercury emissions to the environment include production processes in the cement, chemical and metallurgical industries (Pacyna et al. 2006, 2016). Currently, it is estimated that the total mercury emission in Poland in 2015 was about 10 Mg and that it has remained stable since 2000 (Bartnicki et al. 2017).

Despite the decline, mercury emissions are still an important environmental problem, for example around fossil fuel combustion power plants (Martín and Nanos 2016). As a result of coal combustion in power plants, mercury is released into the environment in two main ways: in gas form in exhaust fumes and in solid form, for instance in combustion waste such as ashes and slags (Hassett and Eylands 1999; Glodek and Pacyna 2009). Combustion waste may be reused to a certain extent, including in the production of building materials (Sua-iam and Makul 2014), as an amendment enhancing the properties of degraded soils in agriculture and in the reclamation of post-industrial sites (Ram and Masto 2014). However, a significant proportion of combustion waste is deposited on various types of disposal sites. Combustion waste disposal sites in various ways affect adjacent areas, mainly through dust pollution and alkaline and strongly saline leachate (Haynes 2009; Weber

et al. 2017). For these reasons, there is still a problem of effective reclamation and biological protection and termination of the impact on the natural environment of combustion waste disposal sites (Krzaklewski et al. 2012; Pietrzykowski et al. 2018a).

A problem in the reclamation of combustion waste disposal sites is presented by a lack of appropriate quality mineral soil used for topsoiling, therefore to achieve phytostabilisation, and even afforestation in the long-term, many studies have been devoted to the development of soilless methods (Krzaklewski et al. 2012). Due to nitrogen deficit and an unusual phytomelioration capacity at Lubień combustion waste disposal site (Central Poland), different alder species (black (*Alnus glutinosa* Gaertn.), gray (*Alnus incana* (L.) Moench) and green alder (*Alnus viridis* (Chaix) DC.)) were used experimentally as N-fixing species (Krzaklewski et al. 2012; Pietrzykowski et al. 2015; Pietrzykowski et al. 2018a). Mercury in the combustion waste may be absorbed by plants, accumulated in their tissues and introduced to the subsequent links of the food chain (Kabata-Pendias 2001). Mercury, just like other trace elements such as lead and cadmium, has no specific vital functions for plants. One of the negative effects of excessive mercury accumulation in plants is the disruption of cellular respiration processes. Toxic mercury content in plants may lead to chlorotic stains, browning of leaf blades and deformation of root systems. Some plant species may absorb significant amounts of mercury which may be accumulated in biologically inactive tissues, e.g. the bark and wood (Kabata-Pendias 2001).

The aim of the paper was to determine mercury content in the technosols and bioaccumulation in individual biomass components of alder species introduced at the lignite combustion waste disposal site. The novelty of the study is the determination of the mobility and bioaccumulation of mercury in the technosol-plant system on lignite combustion waste disposal site. However a lot of studies about bioaccumulation of heavy metals were published around the world only several were connected to lignite combustion wastes, especially in the context of soil development of afforested wastes. These soils are formed on completely artificial substrate and the main goal in the first phase of ecosystem developed is successful revegetation and plant- vegetation system nutrients circulation. Additionally we conducted very unique experiment with alders species introduction, as N fixing system, whereas Nitrogen is strongly

deficient nutrient. An important fact, as well, is a global problem and monitoring of mercury emission and bioaccumulation in nutrient chain in novel ecosystem, which is a key factor for environmental health in strong polluted industrial areas. The following research hypotheses have been formulated: i) mercury bioaccumulation in alder biomass is related to chemical and microbiological properties of technosols produced from combustion waste, ii) mercury content and bioaccumulation vary significantly between alder species, iii) mercury concentration in individual biomass components varies, and accumulation mainly occurs in the roots which constitute the frontier in Hg biosorption by alders.

2 Material and Methods

2.1 Study Site

Lubień disposal site of Bełchatów power plant is located in Central Poland (51.2752 N; 19.2624 E). The climate at the site is temperate with mean annual precipitation ranging from 576 mm yr⁻¹ and an average annual temperature of approximately 8.8 °C (data for 1990–2012 from meteorological stations, source: tutiempo.net). Lubień disposal site has been in operation since 1980 and currently occupies ca. 440 ha. Combustion waste containing about 85% ash and 15% slag is deposited there with the use of hydro-transport. The main components of combustion waste are thermally processed aluminosilicates. The average content of Al₂O₃ and SiO₂ compounds is 60–70%, and of CaO, about 20%. The content of trace elements (Zn, Cu, Pb, Cd, and Cr) does not exceed the values reported for natural soils (Krzaklewski et al. 2012; Pietrzykowski et al. 2018b). Freshly deposited combustion waste (CW) typically displayed a strongly alkaline reaction (pH in KCl – 8.60) and had low unburned carbon (1.95%) and nitrogen (0.025%) content (Pietrzykowski et al. 2018b).

2.2 Description of the Experiment

The experiment was started in spring 2006 in a part of a sedimentation pond flat shelf set up between 2003 and 2004. Prior to the experiment and the planting of trees, the experimental area was subject to hydro-seeding with biosolids (sewage sludge of 4 Mg dry mass ha⁻¹) mixed with seeds (200 kg ha⁻¹) of Cock's-foot grass (*Dactylis*

glomerata L.) and Italian ryegrass (*Lolium multiflorum* Lam.). Next, NPK start-up mineral fertilizing was applied at a rate of N: 60, P: 36 and K: 36 kg ha⁻¹. Later, 24 plots measuring 6 m × 13 m were laid out and planted with either black, gray or green alder. The plots were separated by a 2-m-wide buffer strip. A total of 50 seedlings of black, gray or green alder (6410 trees ha⁻¹) were planted on the plots in 40 cm × 40 cm × 40 cm planting holes containing two soil treatment variants (with four replications for each variant): lignite (CCW + L) and a control without any soil amendments (CCW) (Krzaklewski et al. 2012). So far, the following assays were carried out in the course of regular monitoring of the experiment: alder survival rate and growth parameters (Krzaklewski et al. 2012; Pietrzykowski et al. 2018a) as well as the determination of the chemical and microbiological soil properties (Pietrzykowski et al. 2018b).

2.3 Soil and Biomass Sampling

For Hg concentration monitoring and basic current soil chemical and microbial characteristics, soil samples were collected twice in the autumn of 2016 and 2017 from the 0 to 5 cm horizon (mineral horizon) at 5 locations regularly distributed along the diagonal of each plot. A total of 24 mixed samples of technosols (1.0 kg mass of fresh sample) representative of individual plots were selected.

The bark and wood samples were collected in 2017 from 5 trees regularly distributed along the diagonal of each plot. The leaves were collected in summer from 5 trees regularly distributed along the diagonal of each plot from crown tops of the SW exposition. Fine root biomass (diameter < 2.0 mm) samples were determined using the core methods (Böhm 1979). For this purpose, holes in the soil (with a diameter of 5 cm and depth and 30 cm, three replications per research plot) were obtained using a soil auger. The obtained root samples were put in plastic bags and taken to the laboratory.

2.4 Laboratory Tests

In the laboratory, the soil samples were divided into two subsamples. The first group of subsamples were dried at 65 °C and sieved through a 2.0 mm sieve. Next, the subsamples were measured for texture using laser diffraction methods (Fritsch GmbH Laser Particle Sizer ANALYSETTE 22 NanoTec). Soil organic carbon

(SOC), total nitrogen (Nt) and sulfur (St) content were measured using a LECO TruMac® CNS analyzer; prior to the SOC measurement, the samples were treated with 10% HCl to remove carbonates. The soil pH was determined potentiometrically in 1 M KCl at a 1:2.5 w/v ratio. The exchangeable acidity (Hh) was measured in 1 M Ca(OAc)₂; basic exchangeable cations (Ca²⁺, Mg²⁺, K⁺, Na⁺) in 1 M NH₄Ac by Thermo Scientific™ iCAP™ 6000 Series ICP-OES. Cation exchange capacity (CEC) was calculated as the sum of exchangeable cations and exchangeable acidity (Hh). Total phosphorus (Pt) was determined by digestion in a mixture of HNO₃ (d = 1.40) and 60% HClO₄ acid in a 4:1 ratio by Thermo Scientific™ iCAP™ 6000 Series ICP-OES (Ostrowska et al. 1991; Van Reeuwijk 1995). In order to calculate the content of SOC and Nt in technosols, the amount of carbon determined in a sample of freshly deposited ash (C = 1.95%, according to Pietrzykowski et al. 2018b) was subtracted from the carbon content in the samples collected from the alder plots (Pietrzykowski et al. 2018b). It was assumed that carbon content in the samples of freshly deposited substrates represented the carbon remaining in the ashes due to incomplete combustion of lignite at a power plant (Klotz et al. 2004; Uzarowicz et al. 2017). The total content of Hg in technosol samples was measured with a Milestone DMA-80 Direct Mercury Analyzer by drying and thermal decomposition (Gruba et al. 2019).

The second group of subsamples was sieved when still fresh through a 2 mm sieve, the roots, soil fauna were removed and stored in a refrigerator at 4 °C. The maximum storage period was 2 months. According to literature data, samples can be stored at 4 °C for up to 6 months (Nielsen and Winding 2002). Before the individual microbiological tests were carried out, the humidity content of the samples was determined and then they were incubated at 22 °C and at constant soil humidity of 50% of the maximum soil water capacity (WHC) for 7 days (Nielsen and Winding 2002). The WHC was determined gravimetrically according to Schlichting and Blume (1966). To measure basal respiration (RESP) and microbial biomass (Cmic), samples (50 g d.w.) unamended for RESP measurements and amended with 8 mg glucose monohydrate for Cmic measurements were incubated at 22 °C in gas-tight jars. The incubation time was 24 h for the determination of RESP and 4 h for Cmic. The jars contained small beakers with 5 ml 0.2 M NaOH to trap the evolved CO₂. After the jars were opened, 2 ml 0.9 M BaCl₂

was added to the NaOH; excess hydroxide was titrated with 0.1 M HCl in the presence of phenolphthalein as an indicator. Cmic was calculated from the substrate-induced respiration rate according to the equation given by Anderson and Domsch (1978):

$$\text{Cmic} [\text{mg g}^{-1}] = 40.04 y + 0.37, \text{ where } y \text{ is ml CO}_2 \text{ h}^{-1} \text{ g}^{-1}.$$

Urease activity (URE) was determined as described by Kandeler (1996). The soil samples (5 g d.w.) were mixed with 2.5 ml urea (720 mM) and 20 ml borate buffer (pH 10) and incubated at 37 °C for 2 h. The released ammonium was extracted with acidified potassium chloride solution, colored in modified Berthelot reaction and measured photometrically at 690 nm (Hach DR/4000 U Spectrophotometer). Urease activity was expressed as $\mu\text{g N g}^{-1} \text{ h}^{-1}$.

Acid (AcPHP) and alkaline (AlPHP) phosphomonoesterase activity was determined as described by Margesin (1996). After the addition of a buffered p-nitrophenylphosphate solution, soil samples (1 g d.w.) were incubated for 1 h at 37 °C. The p-nitrophenol released by phosphomonoesterase activity was extracted and colored with sodium hydroxide and determined photometrically at 400 nm (Hach DR/4000 U Spectrophotometer).

Fine root samples were stored for a maximum of 2 weeks at 4 °C and successive assays were conducted in the laboratory. The roots were rinsed from the soil samples, measured with a caliper with an accuracy of 0.1 mm, and then divided into the fine root fraction with root diameter up to 2.0 mm.

The biomass component samples (fine roots, leaves, bark, and wood) were then dried at 65 °C and ground. The content of Hg in the biomass was measured with a Milestone DMA-80 Direct Mercury Analyzer by drying and thermal decomposition (Gruba et al. 2019).

2.5 Statistical Analyses and Data Evaluation

The bioaccumulation factor (BAF) and the translocation factor (TF) (Bonanno 2013) have been calculated:

$$\text{BAF} = \text{Hg in biomass component} / \text{Hg in technosol}.$$

$$\text{TF} = \text{Hg in aboveground biomass component} / \text{Hg in fine roots}.$$

The experimental design was a completely randomized 2-way factorial (soil treatment in planting holes and alder species: Tr × Sp). Data sets were first tested for normality using the Kolmogorov–Smirnov test and for variance homogeneity by Leven's test. The effects of Tr

and Sp on the soil parameters were analyzed by 2-way ANOVA for the majority of response variables. The Tukey's honestly significant differences (HSD) test for equal sample sizes was performed if any significant differences were found ($p < 0.05$).

The correlations between Hg concentration in alder biomass and technosol properties were described using Pearson's correlation matrix and the multiple forward stepwise regression method. The significance of individual independent variables in multiple regression equations was tested using the t-test at a significance level of $p < 0.05$.

The statistical analyses were carried out using STATISTICA 13.1 software (StatSoft Inc. 1984–2018).

3 Results

3.1 Soil Parameters

The investigated technosols had a silt content of 14 to 18%, an average clay content of 1% and a slightly alkaline reaction (pH on average 7.42). CEC ranged from 26.34 to 29.09 cmol(+) kg⁻¹. Corg content ranged from 2.27 to 2.91%. The alder species significantly affected the Nt content (from 0.09% under the green alder to 0.14% under the black alder) and Mg (from 0.89 cmol(+) kg⁻¹ under the green alder to 1.28 cmol(+) kg⁻¹ under the black alder) (Table 1).

The Cmic in the investigated technosols ranged from 241.43 to 290.49 µg g⁻¹, and RESP from 2.91 to 3.25 µM CO₂ g⁻¹ 24 h⁻¹. The activity of soil enzymes was respectively: urease (URE) from 10.87 to 12.84 µg Ng⁻¹ 2 h⁻¹, acid phosphomonoesterase (AcPHP) from 216.71 to 273.95 µg NP g⁻¹ h⁻¹, alkaline phosphomonoesterase (AlPHP) from 500.72 to 602.02 µg NP g⁻¹ h⁻¹. No impact of alder species and soil treatment on the examined microbiological parameters of technosols was found (Table 1).

3.1.1 Mercury Content in Technosols and Individual Alder Biomass Components

Alder species (Sp), soil treatment (Tr) and the Sp × Tr interaction had no impact on Hg concentration in the technosol mineral horizon (0–5 cm). Mercury content in this horizon ranged from 0.519 mg kg⁻¹ under the black alder to 0.570 mg kg⁻¹ under the green alder. In the organic horizon (Oi + Oe), Hg content was significantly

higher under the green alder (0.635 mg kg⁻¹) compared to the horizons under the remaining alder species (Table 2).

The highest Hg content among the studied biomass components was displayed by fine roots (from 0.119 to 0.202 mg kg⁻¹), while the lowest was found in wood (0.001 mg kg⁻¹ for all the investigated species). No impact of Sp, Tr and the Sp × Tr interaction on Hg content was found in fine roots, bark, and wood of the alder. Only Hg content in the leaves significantly varied between CCW (0.048 mg kg⁻¹) and CCW + L (0.059 mg kg⁻¹) (Table 2).

3.1.2 Mercury Bioaccumulation in Alder Biomass Individual Components

Alder species (Sp), soil treatment (Tr) and the Sp × Tr interaction had no impact on the bioaccumulation factors (BAF) in alder biomass components. The highest BAF was found in the fine roots (from 0.30 to 0.37), followed by the leaves (from 0.10 to 0.13), and the lowest in wood (from 0.002 to 0.003) (Fig. 1).

Alder species (Sp), soil treatment (Tr) and the Sp × Tr interaction had no impact on the translocation factors (TF) in bark, wood, and leaves of alders. The highest CF was found in the leaves and the amount varies significantly between the applied soil treatments (0.33 in CCW and 0.49 in CCW + L). Like in the case of BAF, the lowest CF was found in alder wood (an average 0.01 for all the alder species) (Fig. 1).

3.1.3 Correlation between Mercury Content in Technosols and its Content in Alder Biomass

Hg content in the fine roots correlated with silt and macroelement (Nt, St, Ca²⁺, K⁺, Mg²⁺) content, RESP, AcPHP and AlPHP activity. No significant correlation was found between Hg content in the bark and leaves and the investigated soil properties. Hg content in wood only correlated significantly with AcPHP activity (Table 3).

BAF root/soil negatively correlated with Hg content in technosols, BS, clay and Pt content. BAF bark/soil, BAF wood/soil and BAF leaves/soil negatively correlated with the majority of the investigated soil parameters except for pH, St, Hh, K⁺ and Mg²⁺ (in case of BAF bark/soil and BAF wood/soil) (Table 3).

No correlation was found between the TF wood/root and the investigated soil properties. TF bark/root

Table 1 Physical, chemical and microbial properties of technosols (partial results from the first year of monitoring were published by Pietrzykowski et al. 2018b)

Effect	Silt (0.05–0.002 mm) [%]	Clay (<0.002 mm) [%]	pH	Corg [%]	Nt	St	Ca ²⁺ [cmol(+) kg ⁻¹]	K ⁺	Mg ²⁺	Na ⁺
Sp ¹	N.S. ⁴	N.S.	N.S.	N.S.	S., F=4.01 p=0.0364	N.S.	N.S.	N.S.	S.; F=11.97 p=0.0005	N.S.
Gr.a	17 ± 5 ^{a 5}	1 ± 0 ^a	7.42 ± 0.02 ^a	2.27 ± 0.49 ^a	0.09 ± 0.01 ^a	0.05 ± 0.01 ^a	25.16 ± 1.78 ^a	0.35 ± 0.03 ^a	0.89 ± 0.08 ^a	0.06 ± 0.01 ^a
B.a.	15 ± 5 ^a	1 ± 0 ^a	7.42 ± 0.02 ^a	2.91 ± 0.36 ^a	0.14 ± 0.02 ^b	0.10 ± 0.05 ^a	25.12 ± 1.51 ^a	0.40 ± 0.03 ^a	1.52 ± 0.12 ^b	0.06 ± 0.01 ^a
G.a.	17 ± 3 ^a	1 ± 0 ^a	7.42 ± 0.02 ^a	2.63 ± 0.44 ^a	0.11 ± 0.01 ^{ab}	0.05 ± 0.01 ^a	25.33 ± 1.37 ^a	0.47 ± 0.05 ^a	1.28 ± 0.11 ^b	0.05 ± 0.00 ^a
Tr ²	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CCW	18 ± 4 ^a	1 ± 0 ^a	7.42 ± 0.02 ^a	2.72 ± 0.37 ^a	0.12 ± 0.01 ^a	0.08 ± 0.03 ^a	26.52 ± 1.27 ^a	0.42 ± 0.03 ^a	1.28 ± 0.13 ^a	0.06 ± 0.01 ^a
CCW + L	14 ± 3 ^a	1 ± 0 ^a	7.41 ± 0.01 ^a	2.48 ± 0.33 ^a	0.11 ± 0.01 ^a	0.05 ± 0.00 ^a	23.89 ± 1.09 ^a	0.40 ± 0.03 ^a	1.18 ± 0.10 ^a	0.05 ± 0.00 ^a
Sp × Tr ³	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	S.; F=3.52 p=0.0492	N.S.

Effect	Hh [cmol(+) kg ⁻¹]	CEC	BS [%]	Pt [%]	RESP [μM CO ₂ g ⁻¹ 24 h ⁻¹]	Cmic [μg g ⁻¹]	URE [μg N g ⁻¹ 2 h ⁻¹]	AcPHP [μg NP g ⁻¹ h ⁻¹]	AIPHP
Sp ¹	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Gr.a	0.80 ± 0.03 ^a	27.26 ± 1.89 ^a	96.96 ± 1.89 ^a	0.024 ± 0.002 ^a	3.07 ± 0.36 ^a	241.43 ± 27.37 ^a	12.84 ± 0.86 ^a	216.71 ± 35.71 ^a	500.72 ± 60.03 ^a
B.a.	0.82 ± 0.03 ^a	27.92 ± 1.67 ^a	96.99 ± 1.67 ^a	0.023 ± 0.002 ^a	3.25 ± 0.34 ^a	290.49 ± 32.18 ^a	12.78 ± 1.55 ^a	273.95 ± 48.15 ^a	602.02 ± 75.88 ^a
G.a.	0.83 ± 0.03 ^a	27.96 ± 1.51 ^a	96.97 ± 1.51 ^a	0.023 ± 0.001 ^a	3.04 ± 0.23 ^a	251.54 ± 17.07 ^a	10.87 ± 1.21 ^a	259.01 ± 42.05 ^a	594.69 ± 89.77 ^a
Tr ²	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
CCW	0.82 ± 0.03 ^a	29.09 ± 1.38 ^a	97.13 ± 1.14 ^a	0.025 ± 0.001 ^a	3.33 ± 0.28 ^a	270.10 ± 24.70 ^a	11.92 ± 0.92 ^a	253.06 ± 37.43 ^a	555.25 ± 60.69 ^a
CCW + L	0.82 ± 0.01 ^a	26.34 ± 1.20 ^a	96.82 ± 1.20 ^a	0.021 ± 0.001 ^a	2.91 ± 0.20 ^a	252.21 ± 18.37 ^a	12.41 ± 1.12 ^a	246.73 ± 31.28 ^a	576.37 ± 63.83 ^a
Sp × Tr ³	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

1 Sp – alder species: Gr.a. – green alder, B.a. – black alder, G.a. – gray alder;

2 Tr – soil treatment: CCW – without any soil amendments and CCW + L – combustion waste with lignite culm in planting holes;

3 Sp × Tr – interaction between alder species and soil treatment;

4 Results of two-way ANOVA for the effect of species and treatment: S. – significant; N.S. – differences not significant ($p = 0.05$)

5 mean ± SE; within columns, means followed by different letters (a, b, c) are significantly different

Table 2 Mercury content in soils and particular alder biomass components

Effect	Hg [mg kg ⁻¹]					
	Technosols (0–5 cm)	Litter horizon (Oi + Oe)	Fine roots	Bark	Wood	Leaves
Sp ¹	N.S. ⁴	S.; F = 39.31 <i>p</i> = 0.0000	N.S.	N.S.	N.S.	N.S.
Gr.a.	0.570 ± 0.120 ^{a 5}	0.635 ± 0.027 ^b	0.119 ± 0.012 ^a	0.023 ± 0.002 ^a	0.001 ± 0.000 ^a	0.055 ± 0.004 ^a
B.a.	0.519 ± 0.095 ^a	0.292 ± 0.029 ^a	0.159 ± 0.028 ^a	0.028 ± 0.003 ^a	0.001 ± 0.000 ^a	0.054 ± 0.003 ^a
G.a.	0.554 ± 0.073 ^a	0.328 ± 0.028 ^a	0.202 ± 0.031 ^a	0.023 ± 0.002 ^a	0.001 ± 0.000 ^a	0.052 ± 0.005 ^a
Tr ²	N.S.	N.S.	N.S.	N.S.	N.S.	S.; F = 6.86 <i>p</i> = 0.0174
CCW	0.620 ± 0.088 ^a	0.415 ± 0.052 ^a	0.172 ± 0.021 ^a	0.024 ± 0.002 ^a	0.001 ± 0.000 ^a	0.048 ± 0.002 ^a
CCW + L	0.478 ± 0.062 ^a	0.422 ± 0.051 ^a	0.148 ± 0.024 ^a	0.026 ± 0.002 ^a	0.001 ± 0.000 ^a	0.059 ± 0.003 ^b
Sp × Tr ³	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

¹ Sp – alder species: Gr.a. – green alder, B.a. – black alder, G.a. – gray alder;

² Tr – soil treatment: CCW – without any soil amendments and CCW + L – combustion waste with lignite culm in planting holes;

³ Sp × Tr – interaction between alder species and soil treatment;

⁴ Results of two-way ANOVA for the effect of species and treatment: S. – significant; N.S. – differences not significant (*p* = 0.05)

⁵ mean ± SE; within columns, means followed by different letters (a, b, c) are significantly different

negatively correlated with AcPHP activity and TF leaf/root with Mg²⁺ content, CEC and acid and alkaline PHP activity (Table 3).

Multiple regression analysis showed that Hg content in technosols was related to the texture (silt and clay content) and Pt content. The determination coefficient

Fig. 1 Bioaccumulation and translocation factors (BAF and TF) in alder biomass components on the disposal site

explains 95% variation of Hg content in technosols (Table 4).

Hg content in technosols $[\text{mg kg}^{-1}] = 0.017 \times (\text{silt} + \text{clay}[\%]) + 16.38 \times \text{Pt}[\%]$.

Hg content in the roots was related to the content of St and the activity of acid and alkaline PHP in technosols. The determination coefficient explains 46% variation of Hg content in the roots (Table 5).

Hg content in roots $[\text{mg kg}^{-1}] = 0.42 \times \text{St}[\%] + 0.0001 \times (\text{AcPHP} + \text{AlPHP}[\mu\text{g NP g}^{-1} \text{h}^{-1}])$.

The BAF root/soil was related to the St content and a negative with BS in technosols. The determination coefficient explains 45% variation of BAF in the roots (Table 6).

$\text{BAF root/soil} = 1.06 \times \text{St}[\%] - 0.23 \times \text{BS}[\%] + 22.64 \pm 0.14$.

4 Discussion

Mercury concentration in technosols ($0.478\text{--}0.620 \text{ mg kg}^{-1}$ in 0–5 cm and $0.292\text{--}0.635 \text{ mg kg}^{-1}$ in litter horizons) and in alder biomass (from 0.001 mg kg^{-1} in wood to 0.202 mg kg^{-1} in fine roots) in the experiment on combustion waste disposal site was higher compared to the amounts found in natural forest ecosystems, however, it did not exceed the values considered toxic (Kabata-Pendias 2001). The values given for Poland in soils under stands of different tree species (Scots pine, black alder, Norway spruce, silver birch, deciduous oak, silver fir and European beech) ranged from 0.06 to 0.23 mg kg^{-1} for the O horizon and from 0.02 to 0.08 mg kg^{-1} for the 0–10 cm horizon (Gruba et al. 2019). It is estimated that the average Hg content in European soils is about 0.04 mg kg^{-1} in top organic-mineral horizons and about 0.02 mg kg^{-1} in sub-soil (FOREGS 2005). In natural soils, mercury concentration in the litter layer is usually higher than in mineral soil (Obriest et al. 2011; Gruba et al. 2019). The opposite phenomenon occurs in technosol on combustion waste disposal site. There is another regularity in combustion waste technosols due to a different type of mercury compound deposition, which originally comes from combustion waste and may be added to the biological cycle by means of technosol extraction or dust deposition on above-ground parts. Hg concentration in the leaves of gray alder growing in the riparian peatland wetland in the USA was about 0.003 mg kg^{-1} (Selvendiran et al. 2008) which was lower compared to

the results obtained in alders leaves ($0.048\text{--}0.059 \text{ mg kg}^{-1}$) at a combustion waste disposal site. Hg concentration in alder species biomass at the combustion waste disposal site was also lower compared to the amounts found in the leaves ($0.02\text{--}0.970 \text{ mg kg}^{-1}$) and the stems ($0.02\text{--}0.20 \text{ mg kg}^{-1}$) of green alder in a contaminated area of a former mercury mine in Alaska. However, the Hg content in the soil of mercury mine attained much higher amounts, even up to 1500 mg kg^{-1} . The green alder and willow (*Salix* sp.) in such habitat conditions displayed the highest (up to 20-fold) mercury concentration in the tissues (the leaves, stems and flowers) compared to the other investigated species: white spruce (*Picea glauca*), cottonwood (*Populus balsamifera*), black spruce (*Picea mariana*), blueberry (*Vaccinium uliginosum*), paper birch (*Betula papyrifera*) and dwarf birch (*Betula nana*). In natural soils of areas adjacent to the investigated mercury mine, the green alder displayed slightly higher Hg content in the leaves ($<0.02\text{--}0.19 \text{ mg kg}^{-1}$) and in the trunk ($<0.02\text{--}0.03 \text{ mg kg}^{-1}$) (Bailey and Gray 1997) compared to the amount reported in our study at the combustion waste disposal site.

Mercury by plants is absorbed directly from the water and soil through the root system and a lesser extent from the air for example by foliar uptake of Hg^0 volatilized from the soil (Patra and Sharma 2000). The mobility of mercury in the technosol-plant system in the experiment on the combustion waste disposal site was relatively low. The highest mercury concentration and BAFs were found in fine roots, while the lowest in stem wood and the TF was low. This is consistent with the literature data, indicating low mobility of this element in ecosystems and the tendency of Hg accumulation in roots and limited translocation to biomass of aboveground plant parts (Patra and Sharma 2000). Mercury has a high affinity to sulfhydryl (-SH) groups. Mercury after uptake by roots can be bound by sulfur and to a limited extent move to the aboveground part of the alders (Patra and Sharma 2000). The fine roots are therefore the frontier in Hg bioaccumulation. This is important in the phytostabilisation of contaminated soils. The presence of plants that effectively translocate heavy metals to aboveground parts may contribute to the spread of pollution in the environment (Stefanowicz et al. 2016).

Hg content in fine roots significantly correlated with the content of the silt fraction in the combustion waste. On the other hand, negative correlations between BAF in the biomass components and the silt and clay content

Table 3 Pearson correlation coefficients (*r*) between Hg content in biomass components, BAFs, TFs, and technosol properties

Properties	Hg in soil [mg kg ⁻¹]	Hg in soil [mg kg ⁻¹]	Hg in roots	Hg in bark	Hg in leaves	Hg in wood	BAF root/soil	BAF bark/soil	BAF wood/soil	BAF leaves/soil	TF bark/root	TF wood/root	TF leaves/root
Hg in soil	1.00	0.40	-0.11	-0.12	0.20	0.20	-0.46*	-0.76**	-0.67**	-0.79**	-0.38	-0.27	-0.33
Silt (0.05–0.002 mm)	0.95**	0.41*	-0.08	-0.06	0.26	0.26	-0.37	-0.63**	-0.54**	-0.65**	-0.37	-0.22	-0.31
Clay (<0.002 mm)	0.92**	0.30	-0.07	-0.02	0.21	0.21	-0.43*	-0.62**	-0.54**	-0.63**	-0.27	-0.16	-0.20
pH	0.27	-0.10	0.15	0.22	0.07	0.07	-0.30	-0.12	-0.21	-0.09	0.04	0.06	0.04
Corg	0.60**	0.40	0.35	0.06	0.20	0.20	-0.28	-0.44*	-0.52**	-0.61**	-0.12	-0.29	-0.26
Nt	0.50*	0.42*	0.28	-0.03	0.38	0.38	-0.28	-0.48*	-0.44*	-0.64**	-0.21	-0.22	-0.36
St	0.16	0.52**	-0.06	-0.22	0.16	0.29	0.29	-0.21	-0.11	-0.27	-0.34	-0.32	-0.36
Ca ²⁺	0.91**	0.48*	0.07	-0.15	0.17	-0.40	-0.40	-0.72**	-0.70**	-0.84**	-0.34	-0.36	-0.39
K ⁺	0.43*	0.45*	0.18	0.18	0.19	-0.08	-0.08	-0.38	-0.36	-0.41*	-0.28	-0.35	-0.28
Mg ²⁺	0.48*	0.58**	0.35	-0.06	0.35	-0.08	-0.08	-0.35	-0.37	-0.55**	-0.25	-0.34	-0.44*
Na ⁺	0.69**	0.32	0.23	-0.20	0.16	-0.31	-0.31	-0.49*	-0.51*	-0.66**	-0.17	-0.25	-0.35
Hh	0.09	0.28	0.16	-0.18	0.00	0.09	0.09	-0.08	-0.13	-0.22	-0.16	-0.30	-0.32
CEC	0.89**	0.51*	0.10	-0.14	0.19	-0.38	-0.38	-0.71**	-0.69**	-0.84**	-0.34	-0.37	-0.41*
BS	0.83**	0.35	0.04	0.01	0.16	-0.49*	-0.49*	-0.72**	-0.71**	-0.78**	-0.23	-0.23	-0.20
Pt	0.78**	0.24	0.01	0.08	0.07	-0.55**	-0.55**	-0.76**	-0.72**	-0.80**	-0.24	-0.24	-0.25
RESP	0.76**	0.42*	0.19	-0.08	0.20	-0.26	-0.26	-0.52**	-0.52**	-0.70**	-0.23	-0.32	-0.36
Cmic	0.67**	0.38	0.25	-0.10	0.27	-0.22	-0.22	-0.43*	-0.43*	-0.63**	-0.18	-0.27	-0.36
URE	0.68**	0.26	-0.09	-0.03	0.19	-0.30	-0.30	-0.55**	-0.42*	-0.60**	-0.23	-0.13	-0.22
AcPHP	0.81**	0.61**	0.05	-0.11	0.42*	-0.15	-0.15	-0.56**	-0.41*	-0.65**	-0.44*	-0.32	-0.47*
AlPHP	0.80**	0.61**	0.20	0.01	0.32	-0.16	-0.16	-0.49*	-0.46*	-0.61**	-0.35	-0.38	-0.42*

*-, significant at $p < 0.05$ **-, significant at $p < 0.01$

Table 4 Regression summary for Hg content in technosols (mg kg^{-1}) as the dependent variable and silt, clay content (%), Pt (%) as independent variables, $R^2_{\text{adj}} = 0.95$; $F(2,21) = 216.31$; $p < 0.00001$

Predictors	b	Standard error of b	t(21)	p value
Intercept	-0.11521	0.0677393	-1.70953	0.102091
silt + clay	0.01653	0.001312	12.59142	0.000001
Pt	16.38077	3.381839	4.84374	0.000087

indicate difficulties in Hg uptake from finer fractions of fly ash. The finer the fraction of fly ash grains, the more Hg it contains. However, the majority (up to 70%) of Hg may be permanently absorbed in fly ash and have limited migration capacity in the soil-plant system (Kobyłecki et al. 2009).

A strong correlation between microbiological properties (RESP, AcPHP, and AIPHP) of technosols and Hg content in the fine roots is interesting. Also, a multiple regression analysis showed that Hg content in the roots depends on the activity of PHP. It is frequently indicated in literature on the subject that that mercury has a limiting effect on the development of the microflora and on the activity of soil enzymes (Casucci et al. 2003). The effect of excessive Hg concentration on phosphatase activity may be smaller than e.g. in the case of amidohydrolases (Tazisong et al. 2012). Soil enzymes are produced not only by microorganisms, but they can also be products of plant root secretions or be of animal origin (Orcewska et al. 2012). For example, a slight stimulatory effect of mercury on the production of phosphatases in the roots of barley (*Hordeum vulgare* L.) has been found (Tamás et al. 2008). At relatively low concentrations found in the combustion waste disposal site, no limiting effects of Hg on the microbiological properties of technosols were found. There was even a positive correlation between Hg content in combustion waste and all the analyzed microbiological parameters (RESP, Cmic, URE, acid, and alkaline PHP). This does not, of course, indicate an absolute beneficial effect of mercury or an increase in microbiological activity due to this element because the soil system is, firstly, rather

complex, and moreover such impact may only be concluded for some given concentrations.

One of the factors impacting the increase of Hg bioavailability in soils may be the activity of soil microorganisms (de Souza et al. 1999; Kabata-Pendias 2001). Acid and alkaline PHP are enzymes that catalyze the hydrolysis of phosphorus-containing compounds to phosphates ($\text{H}_2\text{PO}_4^{4-}$ and HPO_4^{2-}) which are available to plants (Nannipieri et al. 2011). Mercury forms sparingly soluble compounds with phosphorus that are unavailable to plants (Kabata-Pendias 2001). In the hydrolysis of these compounds with the participation of phosphatases, in addition to phosphorus, mercury may be released and then absorbed by the plants. A correlation between mercury and phosphorus in combustion waste is also indicated by a multiple regression analysis which indicated a correlation between Hg content and Pt content in combustion waste. The correlation between mercury and phosphorus content was also found in peats and organic-rich soils in Florida, USA (Arfstrom et al., 2000). Microbiological activity, and in particular phosphatase, may be of key importance in increasing mercury bioavailability in combustion waste disposal sites. Soil enzymes with low concentrations of trace elements may affect the mobility of trace elements in the soil-plant system (Kabata-Pendias 2001). A strong correlation between phosphatase activity in the soil and mercury content in above-ground biomass is also reported in the case of barley (*Hordeum vulgare* L.) in a pot experiment under controlled conditions

Table 5 Regression summary for Hg content in fine roots (mg kg^{-1}) as the dependent variable and St (%), AcPHP and AIPHP ($\mu\text{g NP g}^{-1} \text{ h}^{-1}$) as independent variables, $R^2_{\text{adj}} = 0.46$; $F(2,21) = 8.882$; $p < 0.00160$

Predictors	b	Standard error of b	t(21)	p value
Intercept	0.049500	0.033074	1.496650	0.149364
AcPHP + AIPHP	0.000093	0.000035	2.661238	0.014610
St	0.419371	0.158970	2.638049	0.015376

Table 6 Regression summary for BAF root/soil as the dependent variable and St (%), BS (%) as independent variables, $R^2_{\text{adj}} = 0.45$; $F(2,21) = 8.485$; $p < 0.00199$

Predictors	b	Standard error of b	t(21)	p value
Intercept	22.63972	6.003309	3.77121	0.001121
BS	-0.23076	0.061979	-372.319	0.001257
St	1.05883	0.379260	2.79184	0.010928

(Wyszkowski and Wyszkowska 2006). A positive correlation between dehydrogenase activity and Cd content in pine needles was observed in the course of reclamation treatments of post-mining sites in Poland (Pietrzykowski et al. 2014). Multiple regression analysis also indicates that in addition to phosphatase activity, Hg content in the roots is affected by St content, which may result from the formation of compounds of sulfur and mercury (Kabata-Pendias 2001). A high correlation was also found between the calcium content and the Hg content in combustion waste and in fine roots. However, no significant relationship was found in multiple regression analysis. Calcium is used to produce of sorbents for mercury emissions control from combustion processes (Ghorishi and Gullett 1998). Studies in China have found that Hg can increase Ca uptake by winter wheat (*Triticum aestivum*) (Liu et al. 2010).

In the course of the research, no correlation was found between mercury content in the aboveground biomass components (bark, wood, and leaves), and Hg content in technosols or chemical and microbiological properties of technosols. Only Hg content in wood positively correlated with AcPHP activity. This may be due to the above-mentioned trend of mercury accumulation in the roots and hindered translocation of Hg from the roots to above-ground biomass, as well as from Hg volatilization from technosols and the subsequent uptake by stomata described in the literature (Patra and Sharma 2000; Graydon et al. 2009). Even at very low levels of Hg, foliar uptake of Hg from the air can play an important role (Patra and Sharma 2000). In addition, small particles of combustion waste are susceptible to eolic erosion (Haynes 2009). They can then be deposited on the biomass of above-ground parts of plants and from there the substances contained in them may be absorbed by plants. For these reasons, it is best to assume mercury concentration in fine roots as the indicator of mercury biosorption by alders and in the assessment of the level of technosol contamination in the experiment on the combustion waste disposal site.

5 Conclusion

Mercury content in combustion waste was higher compared to the data given for non-contaminated forest areas and was related to the texture (silt and clay content) and total phosphorus content. However, the disposal site is not a dangerous mercury emitter although it is significant for the environment. All the alder species (black, gray and green) displayed similar mercury accumulation capacity in biomass. The alders tended to accumulate mercury in the underground biomass (fine roots), and the translocation of this element to above-ground biomass was poor. Hg content in the roots was significantly impacted by sulfur content and acid and alkaline phosphomonoesterase activity. The obtained results indicate that it is best to take Hg concentration in fine roots as an indicator of Hg biosorption by alders and in the assessment of technosol contamination at combustion waste disposal sites.

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