# Phytotoxicity assessment of soils from industrial areas in varying degrees of contamination with metals

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**Abstract.** The determination of the impurities presented in the soil not always defines the full hazard and risk to human health and the environment. Bio content of pollutants in the soil environment is an important factor affecting the toxic effect on plants. The aim of the study was to evaluate the phytotoxicity of soils from industrial areas in varying degrees of contamination with metals. This objective was achieved by determination of the total and potentially available to plants content of metals in the soil along with the determination of metal content in the tested plant (*Medicago falcata L.*). Within the work there was analyzed the effect of soils with different degrees of contamination with metals and containing sorbents on tested plant, i.e. on inhibition of germination of seeds and reduction of the growth of roots and stems, based on the microbiotest toxicity Phytotoxkit$^{TM}$ and pot experiment. The studies found that greater sensitivity to metals' exposure was observed for the rate of inhibition of root growth than the parameter of seed germination and inhibition growth of stems. For the most contaminated soil there was observed toxic effect of metals influence on the tested plant. The use of sorbents lessened the phytoavailable forms of metals and thus resulted in a reduction of phytotoxicity of soils. Phytotoxicity tests together with a pot experiment and chemical studies are an integrated approach to obtain information about the potential risks to human health and the environment.

**Keywords:** metals, soil pollution, phytotoxicity, *Medicago falcata* L., sorbents, industrial areas, environmental risk

## 1. Introduction

Studies on the presence of metals such as copper, nickel, lead, zinc, cadmium or chromium in ecosystems indicate that many areas associated with the metallurgical and steel-making industries contain high levels of those metals. Areas which are particularly problematic are those where soils contaminated with metals are stored in heaps, mixed with steel dust and other wastes with high contents of metals. Most often such soils were, or are, deposited in post-mining excavations or on industrial landfills without any protection of the substrate.

This work has used various types of sorbents which, thanks to their high sorption capacity, can be a method of in situ stabilisation and, thus, a method of immobilisation of metal forms in polluted soils. The aim of the study was to assess the phytotoxicity of soils originating from industrial areas with varying degrees of metal contamination. This goal was pursued by determining the total metal content and the mental content in the soil potentially available for plants, and by determining the metal content in the plant tested (*Medicago falcata L.*). Under the study, work was carried out to analyse the effect of soils contaminated with metals to a varying degrees with added sorbents on the tested plant, i.e. inhibition of seed germination and reduction of root and stem growth, based on the PhytotoxkitTM phytotoxicity microbiotest and a pot experiment.

## 2. Materials and methods

### 2.1 Characteristics of soils

Three soils, labelled as G1, G2 and G3, were used for the experiment. G1 came from the landfill of metallurgical waste and was used to clean wagons contaminated, among others, with steel dust. The analysed soil was alkaline (pH in 1 M KCl ranged from 7.54 to 8.02) and it was a light soil with the granulometric composition of slightly loamy sand. Based on the analysis of the 12 metals listed in Annex 1 to the Regulation of 1 September 2016 *on the method of conducting groundwater pollution assessment* (hereinafter referred to as 'Regulation of the Environment Minister') it was found that the permissible content of 6 metals (zinc, copper, lead, chromium, nickel and cadmium) for Group IV of land, i.e. industrial areas, was exceeded in G1.

G2 and G3 were sampled from the heap within the area of the foundry in Ostrowiec Świętokrzyski after the construction of the facilities in 2011 and 2015. The studied soils were alkaline (the pH in 1 M KCl was in the range of 6.36-6.45) and they were medium soils with the granulometric composition of light clay. No permissible concentrations of metals were exceeded in G2 and G3, in accordance with the Regulation of the Environment Minister, both for industrial areas and Group I and II of land, i.e. arable land and meadows.

The total metal content in G1, G2 and G3 is presented in Table 1. In view of the results obtained, only six metals were analysed in further research i.e. those where the permissible levels in G1 were exceeded.

**Table 1.** Total metal concentration in soils G1 and G2

| Gleba | Total metal concentration [mg/kg s.m.] | | | | | |
|---|---|---|---|---|---|---|
| | $Zn^{2+}$ | $Cu^{2+}$ | $Pb^{2+}$ | $Cr^{2+}$ | $Ni^{2+}$ | $Cd^{2+}$ |
| Permissible value for Group IV of land according to the Regulation of the Environment Minister | 2000 | 600 | 600 | 1000 | 500 | 15 |
| G1 | 7992.5 | 2021.6 | 9766.0 | 683.7 | 1741.0 | 38.3 |
| Permissible value for Group II of land according to the Regulation of the Environment Minister | 300 | 100 | 100 | 150 | 100 | 2 |
| G2, G3 | 19.2 | 3.54 | 3.16 | 4.90 | 4.99 | 0.075 |

**Table 2.** Design of the experiment

| Combinations | G1:G2 G1:G3 (without carbons) | Dose of coals per pot | | | | | |
|---|---|---|---|---|---|---|---|
| | | lignite | | active | | charcoal | |
| | | 200 g | 400 g | 200 g | 400 g | 200 g | 400 g |
| 1:1 | - | $wb_1$ | $wb_2$ | $wa_1$ | $wa_2$ | $wd_1$ | $wd_2$ |
| 1:3 | - | $wb_1$ | $wb_2$ | $wa_1$ | $wa_2$ | $wd_1$ | $wd_2$ |
| 1:7 | - | $wb_1$ | $wb_2$ | $wa_1$ | $wa_2$ | $wd_1$ | $wd_2$ |
| 1:9 | - | $wb_1$ | $wb_2$ | $wa_1$ | $wa_2$ | $wd_1$ | $wd_2$ |

### *2.2 Design of the experiment*

In order to obtain soils with varying levels of contamination with metals, G1 and G2 were mixed in 2011 whereas G1 and G3 were mixed in 2015 in the following proportions: 1:1; 1:3; 1:7 and 1:9. Four combinations of G1 with G2 and G1 with G3 did not contain sorbents, but were contaminated with metals to a varying degree. Coals (lignite, active coal and charcoal) were used in the other combinations as sorbents to stabilise the metal forms. Coals were used in two different dosages: single and double. In total, 32 combinations of soils were obtained. The design of the experiment is provided in Table 2.

### *2.3 Experimental procedure*

The total content of six metals (copper, nickel, lead, zinc, cadmium and chromium) in the tested samples was determined using atomic spectroscopy, ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) with the Thermo Scientific - iCAP 6500 or Varian Axial Vista 720-ES spectrometer. For each series of measurements, blind samples were prepared in parallel, and their values were included in the calculation of the results for the specific samples. The correctness of the methodology was verified by the standard addition method, in accordance with PN-ISO 11466 and PN-EN ISO 11885, whereas the actual solubility of metals in the soil solution was assessed by determining the metal forms in soil extracts by using an extraction solution (0.02 M EDTA).

The modified Phytotoxkit$^{TM}$ test from Tigret [Phytotoxkit, 2004] was used to assess the phytotoxicity of soils G1, G2 and G3 and soils contaminated with metals to a varied degree (32 prepared soil combinations). The test consists in determining the inhibition of germination and the growth of the roots and shoots of a plant exposed to contamination versus the germination and growth of the same plant in the control sample. The control soil was alkaline and had the granulometric composition of slightly loamy sand.

*Medicago falcata L.* was used in the study. The Phytotoxkit$^{TM}$ test was performed in three repetitions under laboratory conditions.

In the first part of the test, the tested soils were placed on test plates and then wetted with distilled water to reach the full (100%) capacity of the aqueous sample. The plates prepared in this way were covered with a paper filter and then seeds were placed at appropriate intervals. The prepared plates with the tested plant were incubated vertically at 25° in the dark for 72 hours. Plant responses to contaminants in soil samples were determined by measuring inhibition of seed germination and the length of roots and shoots.

Toxicity was determined using the following formula:

$$I\,[\%] = \frac{100 \times (L_K - L_T)}{L_K}$$

where:

$L_K$ – average length of root/stem for the control sample [mm],

$L_T$ – average length of root/stem for the test sample [mm] [Janecka, Sobik-Szołtysek, 2009].

In order to assess the phytotoxicity of the soils studied (Table 2), an experiment with the use of Wagner type pots was also conducted. In total, in three pot experiments, 32 combinations were set up with three replicates each, which totalled 96 experimental pots. Due to the uniformity of the experimental units, the pot experiments were set up in a completely randomised manner.

In the experiment, uniform fertilisation with nitrogen, phosphorus and potassium (NPK) was applied in all pots. During the entire vegetation period constant soil humidity was maintained at 60% of field water capacity.

The seeds of the selected species (*Medicago falcata L.*) were sown on 30 April 2015, while the crop, with roots, was gathered on 14 August 2015.

## 3 Results and discussion

### 3.1 *Contamination of studied soils*

In order to determine the efficacy of stabilisation methods after the application of lignite, active coal and charcoal, the total metal concentration was determined (Table 3). According to the Regulation by the Environment Minister, the studied soils were classified as Group IV land, i.e. land covering industrial areas, mining land and transport areas.

Cadmium and nickel values in the tested substrates were, respectively, in the range of 2.35 mg/kg s.m. (1:9 $wd_2$) to 11,6 mg/kg s.m. (1:1$R_1$(G2)) and from 38.3 mg/kg s.m. (1:7 $wa_2$) to 118.0 mg/kg s.m. (1:1$R_1$(G2)). The total chromium content in the substrates ranged from 53.4 mg/kg s.m. (1:9 $wa_2$) to 298.0 mg/kg s.m. (1:1$R_1$(G2)), whereas the copper content ranged from 108.0 mg/kg s.m. (1:9 $wa_2$) to 615.0 mg/kg s.m. (1:1$R_1$(G2)). The values of lead and zinc in the tested soils ranged, respectively, from 529.0 mg/kg s.m. (1:9 $wa_1$) to 3912.0 mg/kg s.m. (1:1$R_1$(G2)) and from 738.0 mg/kg s.m. (1:9 $wa_2$) to 5415 mg/kg s.m. (1:1$R_1$(G2)).

In neither of the tested soils from industrial areas the permissible limits for cadmium, chromium, nickel and copper, as presented in the Regulation of the Environment Minister, were not exceeded. Only in the 1:1 combination without sorbents, the permissible value for copper was slightly exceeded, i.e. 600 mg/kg s.m. On the other hand, in the case of a substrate without added carbon in the 1:1 combination for zinc, the limit values provided for in the Regulation of the Environment Minister for Group IV land were exceeded more than five times, with the respective permissible values for lead being exceeded more than six times.

There are different forms of metals in the environment. The metal compounds in the soil affect the environment to varying degrees. However, water-soluble forms and interchangeable forms represent an important problem as they are mobile in the environment and readily accessible for plants and organisms, and, as such, can cause an increase in toxicity to fauna and flora [Pusz 2013].

Soil tests based on single extraction are used for practical purposes to assess the solubility, toxicity or bioavailability of metals [Pusz, 2013]. The purpose of such extraction is to determine the metal forms soluble in extraction solutions selected to reflect the biological availability of the metal concerned for plants or microorganisms and the susceptibility of metals to leaching, and this is particularly relevant to heavily contaminated soils and land [Karczewska 2002, Karczewska *et al*. 2008; Pusz 2013].

In order to assess metals potentially available to plants in soil combinations, extraction with 0.02 M EDTA complexing reagent was performed. This method cannot be used to determine one particular form of an element but, instead, a certain group of forms, while the results obtained are overestimated in relation to actual phytoavailability, since the complexing reagent extracts much larger amounts of metals than the soluble and exchangeable pool [Crosland *et al*. 1993; Lo, Yang 1999; Pusz 2013].

The actual solubility of metals in soil solutions is shown in Table 3. The cadmium and nickel values in the tested substrates fell respectively in the range from 0.78 mg/kg s.m. (1:9 $wa_2$) to 4.08 mg/kg s.m. (1:1 $R_1$(G2)) and from 1.81 mg/kg s.m. (1:9 $wa_1$) to 7.00 mg/kg s.m. (1:1 $R_1$(G2)). The actual chromium content in the substrates was in the range from 0.36 mg/kg s.m. (1:9 $wa_1$ and 1:9 $wa_2$) to 1.52 mg/kg s.m. (1:1 $R_1$(G2)), whereas the copper content ranged from 44.9 mg/kg s.m. (1:9 $wa_1$) to 334.5 mg/kg s.m. (1:1 $R_1$(G2)). The values for lead and zinc in the studied soils ranged, respectively, from 281.0 mg/kg s.m. (1:9 $wa_1$) to 2400.0 mg/kg s.m. (1:1 $R_1$(G2)) and from 184.0 mg/kg s.m. (1:9 $wa_2$) to 1435 mg/kg s.m. (1:1 $R_1$(G2)).

In all combinations of soils with added sorbents the forms of studied metals potentially available to plants were limited. However, a significant effect of the two doses of sorbents on the reduction of metal forms potentially available to plants was found for chromium, copper, nickel and zinc.

Active carbon was more efficient in sorbing phytoassimilable forms of metals in the soil than lignite and charcoal. The application of the aforementioned sorbents leads to a reduction in potentially available metal forms in the first two links of the trophic chain (soil-plant), as well as to a potential possibility of metal forms being leached into groundwater [Pusz 2013].

**Table 3.** Metal concentration in soils

| Combinations | Total metal concentrations in soils [mg/kg s.m.] | | | | | | Metal concentration in EDTA [mg/kg s.m.] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **$Cd^{2+}$** | **$Cr^{2+}$** | **$Cu^{2+}$** | **$Ni^{2+}$** | **$Pb^{2+}$** | **$Zn^{2+}$** | **$Cd^{2+}$** | **$Cr^{2+}$** | **$Cu^{2+}$** | **$Ni^{2+}$** | **$Pb^{2+}$** | **$Zn^{2+}$** |
| 1:1$R_1$ (G2) | 11.6 | 298 | 615 | 118 | 3912 | 5415 | 4.08 | 1.52 | 334.5 | 7.00 | 2400 | 1435 |
| 1:1 $wb_1$ | 11.2 | 239 | 560 | 109 | 3876 | 5402 | 3.74 | 1.33 | 329.5 | 6.85 | 2265 | 1380 |
| 1:1 $wb_2$ | 10.3 | 248 | 573 | 112 | 3566 | 5242 | 3.69 | 1.38 | 324.0 | 6.75 | 2125 | 1350 |
| 1:1 $wa_1$ | 9.94 | 247 | 602 | 109 | 3295 | 4720 | 3.73 | 1.32 | 317.0 | 6.45 | 2060 | 1280 |
| 1:1 $wa_2$ | 9.8 | 244 | 605 | 106 | 3211 | 4350 | 3.64 | 1.18 | 319.0 | 6.30 | 1880 | 1225 |
| 1:1 $R_1$ (G3) | 10.2 | 256 | 471 | 98 | 3174 | 4195 | 3.99 | 1.49 | 318.9 | 6.99 | 2397 | 1418 |
| 1:1 $wd_1$ | 10.2 | 247 | 486 | 102 | 3146 | 4152 | 3.73 | 1.40 | 324.0 | 6.75 | 2085 | 1355 |
| 1:1 $wd_2$ | 10.1 | 259 | 462 | 98.4 | 3141 | 4093 | 3.69 | 1.30 | 306.5 | 6.65 | 1860 | 1260 |
| 1:3 $R_1$ (G2) | 6.1 | 148 | 365 | 67.5 | 1772 | 2325 | 2.26 | 0.88 | 178.5 | 4.32 | 1160 | 770 |
| 1:3 $wb_1$ | 5.38 | 132 | 279 | 60.4 | 1745 | 2123 | 1.97 | 0.70 | 161.0 | 3.61 | 915 | 630 |
| 1:3 $wb_2$ | 5.4 | 135 | 286 | 58.7 | 1642 | 1993 | 1.83 | 0.68 | 160.0 | 3.56 | 900 | 555 |
| 1:3 $wa_1$ | 5.19 | 118 | 254 | 54.7 | 1631 | 2053 | 1.82 | 0.58 | 138.0 | 3.56 | 850 | 520 |
| 1:3 $wa_2$ | 5.06 | 124 | 265 | 54.3 | 1465 | 2025 | 1.80 | 0.58 | 138.5 | 3.55 | 840 | 585 |
| 1:3 $R_1$ (G3) | 5.01 | 139 | 253 | 61.7 | 1513 | 1990 | 2.22 | 0.99 | 173.2 | 4.28 | 1155 | 763 |
| 1:3 $wd_1$ | 4.93 | 130 | 251 | 62.8 | 1514 | 1952 | 2.18 | 0.93 | 152.0 | 4.07 | 945 | 665 |
| 1:3 $wd_2$ | 4.95 | 144 | 268 | 60.4 | 1523 | 2004 | 2.14 | 0.93 | 141.5 | 4.08 | 935 | 630 |
| 1:7 $R_1$ (G2) | 3.49 | 99.2 | 164 | 47 | 753 | 1130 | 1.17 | 0.50 | 122.0 | 3.09 | 670 | 448.5 |
| 1:7 $wb_1$ | 2.97 | 72.2 | 142 | 39.8 | 683 | 1082 | 1.14 | 0.47 | 78.5 | 2.55 | 515 | 337 |
| 1:7 $wb_2$ | 2.68 | 75.3 | 145 | 41.9 | 677 | 891 | 1.12 | 0.43 | 74.0 | 2.20 | 505 | 334.5 |
| 1:7 $wa_1$ | 2.81 | 68.8 | 141 | 38.9 | 680 | 806 | 1.02 | 0.37 | 47.3 | 2.12 | 328.5 | 274 |
| 1:7 $wa_2$ | 2.75 | 72.4 | 134 | 38.3 | 670 | 789 | 1.01 | 0.37 | 47.0 | 2.07 | 324 | 262 |
| 1:7 $R_1$ (G3) | 2.86 | 80.7 | 130 | 49.6 | 767 | 998 | 1.12 | 0.62 | 114.4 | 3.01 | 663 | 440.1 |
| 1:7 $wd_1$ | 3.01 | 80.4 | 132 | 42.4 | 753 | 934 | 1.05 | 0.56 | 75.0 | 2.51 | 435 | 306.5 |
| 1:7 $wd_2$ | 3.09 | 85.5 | 141 | 46.1 | 767 | 992 | 1.10 | 0.51 | 49.0 | 2.40 | 425 | 311.5 |
| 1:9 $R_1$ (G2) | 2.97 | 66.4 | 144 | 45.8 | 650 | 817 | 1.05 | 0.44 | 99.6 | 2.43 | 464 | 342.6 |
| 1:9 $wb_1$ | 2.71 | 65.8 | 136 | 41 | 576 | 807 | 0.99 | 0.41 | 70.5 | 2.13 | 399.5 | 265.5 |
| 1:9 $wb_2$ | 2.82 | 60.1 | 143 | 40.2 | 582 | 805 | 0.97 | 0.38 | 71.0 | 1.94 | 370 | 257 |
| 1:9 $wa_1$ | 2.79 | 63.1 | 123 | 40.1 | 529 | 784 | 0.85 | 0.36 | 44.9 | 1.81 | 281 | 225.5 |
| 1:9 $wa_2$ | 2.78 | 53.4 | 108 | 40.7 | 544 | 738 | 0.78 | 0.36 | 46.2 | 1.83 | 284 | 184 |
| 1:9 $R_1$ (G3) | 2.44 | 56 | 118 | 42.8 | 647 | 770 | 1.01 | 0.43 | 99.2 | 2.37 | 461 | 339.8 |
| 1:9 $wd_1$ | 2.48 | 53.9 | 119 | 43.6 | 651 | 758 | 0.90 | 0.42 | 63.0 | 2.29 | 399.5 | 238 |
| 1:9 $wd_2$ | 2.35 | 53.7 | 123 | 44.2 | 635 | 740 | 0.86 | 0.40 | 49.5 | 2.01 | 360.5 | 204.5 |

**Table 4.** Disclosure of growth of root and sprout suppression results for used planting soils G1, G2 and G3

| Sample No. | Inhibition of root growth [%] | Inhibition of shoot growth [%] | Inhibition of germination [%] | G1 | G2 |
|---|---|---|---|---|---|
| G1 | 94 | 96 | 75 | | |
| G2 | 23 | 0* | 100 | | |
| G3 | 18 | 0* | 100 | | |

*Inhibition was not calculated if the roots of the test plant were elongated relative to control sample

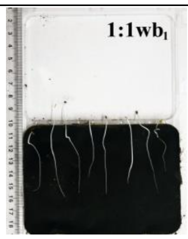

**Table 5.** Disclosure of growth of root and sprout suppression results for used planting soils without sorbents

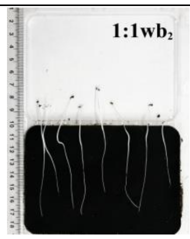
| Sample No. | Inhibition of root growth [%] | Inhibition of shoot growth [%] | Inhibition of germination [%] |
|---|---|---|---|
| 1:1R1 (G2) | 44 | 25 | 100 |
| 1:1R1 (G3) | 39 | 20 | 100 |
| 1:3R1 (G2) | 37 | 23 | 100 |
| 1:3R1 (G3) | 34 | 16 | 100 |
| 1:7R1 (G2) | 30 | 2 | 100 |
| 1:7R1 (G3) | 26 | 0* | 100 |
| 1:9R1 (G2) | 21 | 0* | 100 |
| 1:9R1 (G3) | 17 | 0* | 100 |

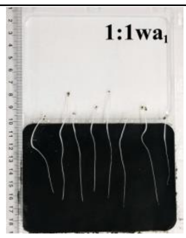
*Inhibition was not calculated if the roots of the test plant were elongated relative to control sample

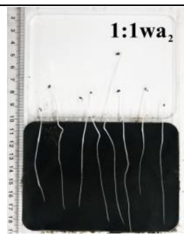
**Table 6.** Disclosure of growth of root and sprout suppression results for used planting soils with sorbents

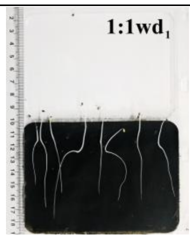
| Sample No. | Inhibition of root growth [%] | Inhibition of shoot growth [%] | Inhibition of germination [%] | 1:1 $wb_1,a_1,d_1$ | 1:1 $wb_2,a_2,d_2$ |
|---|---|---|---|---|---|
| 1:1 $wb_1$ | 31 | 0* | 100 | | |
| 1:1 $wb_2$ | 0* | 0* | 100 | | |
| 1:1 $wa_1$ | 0* | 0* | 100 | | |
| 1:1 $wa_2$ | 0* | 0* | 100 | | |
| 1:1 $wd_1$ | 18 | 0* | 100 | | |
| 1:1 $wd_2$ | 0* | 0* | 100 | | |
| 1:3 $wb_1$ | 23 | 0* | 100 | | |
| 1:3 $wb_2$ | 0* | 0* | 100 | | |
| 1:3 $wa_1$ | 0* | 0* | 100 | | |
| 1:3 $wa_2$ | 0* | 0* | 100 | | |
| 1:3 $wd_1$ | 17 | 0* | 100 | | |
| 1:3 $wd_2$ | 0* | 0* | 100 | | |
| 1:7 $wb_1$ | 0* | 0* | 100 | | |
| 1:7 $wb_2$ | 0* | 0* | 100 | | |
| 1:7 $wa_1$ | 0* | 0* | 100 | | |
| 1:7 $wa_2$ | 0* | 0* | 100 | | |
| 1:7 $wd_1$ | 0* | 0* | 100 | | |
| 1:7 $wd_2$ | 0* | 0* | 100 | | |
| 1:9 $wb_1$ | 0* | 0* | 100 | | |
| 1:9 $wb_2$ | 0* | 0* | 100 | | |
| 1:9 $wa_1$ | 0* | 0* | 100 | | |
| 1:9 $wa_2$ | 0* | 0* | 100 | | |
| 1:9 $wd_1$ | 0* | 0* | 100 | | |
| 1:9 $wd_2$ | 0* | 0* | 100 | | |











* Inhibition was not calculated if the roots of the test plant were elongated relative to control sample

### *3.2 Toxicity of the studied soils*

To assess the phytotoxicity of soils, various authors [An 2004; Baran, Jasiewicz, Klimek 2008; Oleszczuk 2008; Oleszczuk, Hollert 2011; Bakopoulou, Emmanouil, Kungolos 2011; Baran 2013; Kopeć, Gondek, Baran 2013; Baran, Tarnawski 2013, 1015; Babajide, Aribisala 2014] use seed germination inhibition rates and inhibition rates for root and stem growth. These parameters were also determined in the Phytotoxkit$^{TM}$ acute toxicity microbiotest. The test revealed significant differences between the studied soil combinations.

The inhibition of germination of lucerne seeds was found only in G1 at 75% (Table 4). In the case of G2 and G3 and other soil combinations containing sorbents at different doses, no inhibition of seed germination was observed for any of the tested plants.

Many authors [Adam, Duncan 2002; An 2004; Baran, Jasiewicz, Klimek 2008; Oleszczuk 2008; Oleszczuk, Hollert 2011; Baran 2013; Kopeć, Gondek, Baran 2013] believe that the seed germination deterioration parameter is less sensitive to environmental pollutants than the root growth inhibition parameter and, thus, does not reflect the actual effects of environmental pollutants on the flora [An 2004; Baran, Jasiewicz, Klimek 2008]. Despite the high levels of metal forms in the environment plants *do* germinate but then cease to grow due to the effects of pollutants on roots [An 2004; Baran, Jasiewicz, Klimek 2008]. This dependence can also be observed for lucerne, where despite the 100% germination of the seeds for G2 and G3 soil and all soil combinations (Table 4, 5 and 6), root growth inhibition was found for G2 and G3 in all combinations of soils without added sorbents and in combinations 1:1 and 1:3 with a single dose of lignite and charcoal.

Root is the element of a plant that first reacts to soil contamination because it has direct contact with xenobiotics [Hadam, Obidorska 2009; Kopeć, Gondek, Baran 2013]. The greatest toxic effect of metals on lucerne occurred for contaminated soils from an industrial area (G1). Metals caused a reduction in root growth at the level of 94%.

The inhibition of root growth in lucerne was also observed in G2 and G3 at respectively 23% and 18%, in all soil combinations without sorbents, ranging from 44% (1:1$R_1$(G2)) to 17% (1:9$R_1$(G3)) and in combinations 1:1 and 1:3 with a single dose of lignite and charcoal at the level from 31% (1:1w$b_1$) to 17% (1:3w$d_1$). No growth inhibition of lucerne root was found only in the combination of soils with different doses of active carbon (Table 6).

The next parameter to be analysed is the growth inhibition index for the stems of the tested plant. Contaminated soil from an industrial area (G1) was toxic to lucerne. The percentage of stem growth inhibition was high and amounted to 96% for lucerne (Table 4).

Metals caused a reduction in the growth of lucerne stems in the most contaminated soil combinations without sorbents from 25% (1:1$R_1$(G2)) to 2% (1:7$R_1$(G2)). In combinations of soils with active coal, lignite and charcoal at various doses, no growth inhibition was found in roots or stems (Table 6).

### *3.3 Metal concentration in Medicago falcata L.*

For the purposes of the analysis of metal intake efficiency by the selected plant species, the content of metals in the above-ground parts of lucerne (*Medicago falcata L.*) was determined in the case of metals that exceeded the permissible content of specific elements in the environment (i.e. zinc, copper, lead, chromium, nickel and cadmium). The analysis of metals was performed for roots and for above-ground biomass after the end of the growing season (Table 7).

The content of zinc, copper, lead, chromium, nickel and cadmium was varied, depending on the dose of active carbon, lignite and charcoal, and the analysed part of the plant. The content of individual metals in the tested plant increased with the increase in soil contamination with metals. The content of the studied metals in roots and above-ground parts of lucerne was as follows: zinc > lead > copper > chromium > nickel > cadmium (Table 7).

The addition of sorbents to the tested soils reduced bioavailability of metals for *Medicago falcata L.* (Table 3) and, thus, their migration to roots and above-ground parts. The roots contained smaller amounts of metals than the above-ground parts of lucerne in all combinations of soils. Some authors [Korzeniowska, Stanisławska-Gubiak 2003], who observed a higher accumulation of metals in plant roots, found that roots are a much better indicator of phytotoxic activity of metals than above-ground parts of plants, but this depends on the species and even on the type of plant tested.

Numerous authors [Ciećko *et al*. 2001; Maciejewska 2004; Kwiatkowska-Malina, Maciejewska 2009; 2011; Pusz 2007; 2013] obtained similar results in the case of lignite. Ciećko *et al*. [2001] as well as Kwiatkowska-Malina and Maciejewska [2009, 2011] observed that the use of lignite reduced the metal content of the tested plants. This was caused by the decreased content of their bioavailable forms in the soil, by immobilising these elements [Karczewska *et al*. 1996] by macromolecular organic colloids as well as an improvement in physical and chemical properties of soils [Kwiatkowska-Malina, Maciejewska 2009, 2011].

In her research conducted, among others, with lignite, Pusz [2007, 2013], observed a limitation of phytoavailability forms of metals and a reduced percentage of the share of the above-mentioned forms relative to the total metal concentration in the soil.

**Table 7.** Metal concentration in root and sprout suppression of *Medicago falcata L.*

| Combinations | Metal concentration in roots [mg/kg s.m.] | | | | | | Metal concentration in above-ground parts [mg/kg s.m.] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Cd^{2+}$ | $Cr^{2+}$ | $Cu^{2+}$ | $Ni^{2+}$ | $Pb^{2+}$ | $Zn^{2+}$ | $Cd^{2+}$ | $Cr^{2+}$ | $Cu^{2+}$ | $Ni^{2+}$ | $Pb^{2+}$ | $Zn^{2+}$ |
| 1:1 $R_1$ (G2) | 0.998 | 14.57 | 25.9 | 7.98 | 41.7 | 107.2 | 2.01 | 21.5 | 31.8 | 11.0 | 104 | 224 |
| 1:1 $wb_1$ | 0.787 | 12.17 | 22.1 | 5.69 | 36.6 | 80.1 | 1.54 | 19.4 | 28.3 | 8.55 | 83.5 | 196 |
| 1:1 $wb_2$ | 0.772 | 11.46 | 21.2 | 4.9 | 28.7 | 66.4 | 1.49 | 16.4 | 26.6 | 7.93 | 76.8 | 191 |
| 1:1 $wa_1$ | 0.722 | 10.9 | 18.4 | 5.29 | 29.6 | 71.1 | 1.55 | 15.2 | 22.6 | 8.1 | 71.8 | 165 |
| 1:1 $wa_2$ | 0.708 | 10.7 | 17.8 | 4.3 | 24.7 | 61.8 | 1.53 | 12.3 | 20.5 | 6.33 | 57.4 | 150 |
| 1:1 $R_1$ (G3) | 0.699 | 10.0 | 21.9 | 7.56 | 32.7 | 80.3 | 1.42 | 13.6 | 29.3 | 9.3 | 73 | 198 |
| 1:1 $wd_1$ | 0.636 | 9.77 | 18.2 | 5.07 | 22.7 | 51.4 | 1.04 | 7.77 | 19.6 | 6.26 | 52 | 150 |
| 1:1 $wd_2$ | 0.523 | 9.6 | 16.4 | 4.34 | 21.0 | 54.6 | 1.05 | 9.1 | 19.4 | 5.3 | 53.6 | 130 |
| 1:3 $R_1$ (G2) | 0.829 | 10.2 | 19.8 | 6.16 | 31.3 | 89.5 | 1.36 | 16.5 | 22.8 | 8.97 | 65.8 | 151 |
| 1:3 $wb_1$ | 0.705 | 8.26 | 18.7 | 5.04 | 25.6 | 60.3 | 1.07 | 14.1 | 20.4 | 7.2 | 49.7 | 140 |
| 1:3 $wb_2$ | 0.671 | 7.83 | 17.8 | 4.88 | 23.5 | 58.7 | 1.06 | 13.3 | 19.3 | 7.09 | 43.8 | 135 |
| 1:3 $wa_1$ | 0.665 | 6.38 | 14.9 | 4.63 | 25.2 | 56.8 | 1.02 | 12.2 | 19 | 6.19 | 40.3 | 122 |
| 1:3 $wa_2$ | 0.649 | 5.74 | 14.5 | 4.58 | 20.1 | 54.6 | 1.05 | 9.8 | 17.5 | 5.24 | 41.5 | 118 |
| 1:3 $R_1$ (G3) | 0.492 | 7.86 | 16.7 | 5.43 | 21.4 | 67.2 | 1.094 | 13 | 19.2 | 7.13 | 48.4 | 135.3 |
| 1:3 $wd_1$ | 0.401 | 7.41 | 15.3 | 4.75 | 16.6 | 48.6 | 0.7 | 11.5 | 16.9 | 5.37 | 39.4 | 110 |
| 1:3 $wd_2$ | 0.369 | 5.95 | 14.5 | 3.98 | 14.4 | 43.5 | 0.739 | 10.3 | 15.9 | 5.09 | 40.2 | 99.1 |
| 1:7 $R_1$ (G2) | 0.59 | 9.1 | 17.1 | 4.05 | 22.3 | 53.3 | 0.873 | 11 | 20.8 | 5.41 | 35.6 | 131 |
| 1:7 $wb_1$ | 0.491 | 7.66 | 15.2 | 3.03 | 15.2 | 48.4 | 0.715 | 8.8 | 17.8 | 3.77 | 28.8 | 117 |
| 1:7 $wb_2$ | 0.494 | 6.72 | 13.8 | 2.98 | 13.7 | 48.1 | 0.722 | 6.87 | 16.1 | 3.18 | 18.8 | 111 |
| 1:7 $wa_1$ | 0.482 | 5.88 | 13.8 | 3.01 | 15.2 | 47.9 | 0.657 | 5.48 | 15 | 3.55 | 23.3 | 99.5 |
| 1:7 $wa_2$ | 0.471 | 5.61 | 12.7 | 2.86 | 12.4 | 42.9 | 0.507 | 4.94 | 14.4 | 2.81 | 24.1 | 97.5 |
| 1:7 $R_1$ (G3) | 0.417 | 8.18 | 15.6 | 3.77 | 17.4 | 47.2 | 0.674 | 10.5 | 18 | 5.43 | 28.9 | 104 |
| 1:7 $wd_1$ | 0.31 | 6.11 | 13.8 | 3.02 | 12.8 | 40.2 | 0.412 | 6 | 15.2 | 3.09 | 21 | 87.1 |
| 1:7 $wd_2$ | 0.292 | 5.93 | 13.1 | 2.78 | 11.2 | 39.8 | 0.487 | 5.8 | 14.8 | 3.33 | 18.8 | 79 |
| 1:9 $R_1$ (G2) | 0.665 | 8.205 | 16.9 | 3.85 | 20.3 | 51.1 | 0.937 | 11.2 | 20.6 | 6.22 | 24.4 | 118 |
| 1:9 $wb_1$ | 0.588 | 6.65 | 15.2 | 3.27 | 18.2 | 42.6 | 0.684 | 8.3 | 16.2 | 4.79 | 13.5 | 102 |
| 1:9 $wb_2$ | 0.486 | 5.05 | 12.9 | 2.8 | 17.3 | 42.3 | 0.574 | 6.86 | 16 | 3.07 | 12.9 | 98.4 |
| 1:9 $wa_1$ | 0.479 | 4.33 | 11.8 | 2.72 | 16.4 | 39.6 | 0.495 | 5.9 | 15.9 | 3.28 | 10.4 | 98.1 |
| 1:9 $wa_2$ | 0.451 | 4.05 | 11.3 | 2.52 | 15.3 | 30.4 | 0.458 | 5.2 | 14.8 | 3.02 | 11.3 | 91.2 |
| 1:9 $R_1$ (G3) | 0.366 | 5.45 | 14.4 | 3.11 | 20.1 | 48.9 | 0.693 | 9.3 | 18.6 | 5.47 | 23.5 | 114 |
| 1:9 $wd_1$ | 0.295 | 4.18 | 12.5 | 2.65 | 15.7 | 38.5 | 0.485 | 6.3 | 16.3 | 3.48 | 13.4 | 89.2 |
| 1:9 $wd_2$ | 0.256 | 3.91 | 11.8 | 2.54 | 13.4 | 34.2 | 0.469 | 5.96 | 13.6 | 3.51 | 13 | 90.2 |

Babajide and Aribisala [2014] also observed that organic matter can reduce the toxic effects of metals in soil on plants. Similar relationships were found in this study. Negative effects of metal forms on *Medicago falcata L.* were observed in the combination of soils without sorbent and in combinations 1:1 and 1:3 with a single dose of lignite. For the same soil samples with lignite in combinations 1:1 and 1:3 combinations with double doses of sorbent and in combinations 1:7 and 1:9 in two doses of sorbent, no toxic effects of contamination on the tested plant were observed and even a positive effect on its growth was noted.

Subsequent sorbents under study included active coal and charcoal in two different doses. The use of charcoal reduced the amount of potentially available metal forms in all soil combinations, and the content of metals in the roots and above-ground parts was lower than in soil combinations without sorbent.

The greatest reduction in pollutant accumulation in the roots and above-ground parts of the tested plant was observed in the case of substrates with active carbon added. Darvishi *et al*. [2012] demonstrated that the adsorption of pollutants on active carbon significantly limits the availability of metals in soil to plants, thus positively contributing to the reduction of soil toxicity. Consequently, the accumulation of lead and cadmium in roots and shoots of spinach was significantly lower than in soils without sorbent. Pusz [2013] observed that the use of active carbon as a soil fertiliser reduced the content of phytoassimilable metal forms, i.e. forms of metals currently and potentially available to plants, and reduced the percentage of the above-mentioned forms relative to the total metal content in the soil. This is due to the improvements in the physical and chemical properties of the soil, including its reaction and sorption capacity. Bezak-Mazur [2006] showed that the use of active carbon increased the sorption capacity of the soil and, consequently, the adsorption proper of metals (chromium, lead and nickel) increased, with their migration to the soil being limited. These properties were also observed by Semenyuk *et al*. [2014] in their research, where bio- and phytotoxicity of soil was significantly reduced, both for lucerne and for microorganisms, thus reducing the risk of migration of petroleum-related compounds into the soil and possible contamination of groundwater. The addition of active carbon also influenced the yield of lucerne. It was higher in the case of soils with carbon than in the soil without the addition of the product, and the process of bioremediation was faster and more efficient.

Similar dependencies were also observed in the present research for active carbon. The use of sorbent reduced the amount of potentially available metal forms in all soil combinations and, hence, the toxic effect on lucerne was minimised. The root length and stem length parameters for *Medicago falcata L.* were higher in comparison with the control sample, whereas the content of metals in the roots and above-ground parts was lower than in the soil combinations without added carbon.

## 4 Conclusions

1. The use of active carbon, lignite and charcoal on all substrates has reduced the potentially available forms of metals in the first two links of the trophic chain (soil-plant), as well as the potential possibility of metal forms being leached into groundwater. Active carbon more efficiently sorbed phytoavailability forms of metals in the soil than lignite and charcoal.
2. In the case of the most heavily polluted soil (G1), a toxic effects of metals on *Medicago falcata* L. was observed. Metals reduced the growth of lucerne roots at the level of 94%, while the growth of above-ground parts was reduced at 96%
3. A higher sensitivity to metals was found in root growth inhibition versus the seed germination and stem growth inhibition.
4. The use of sorbents reduced the amount of potentially available metal forms in all soil combinations and thus reduced the phytotoxicity of the studied soils.
5. The content of the individual metals in the test plant increased with increasing soil contamination with metals. The roots contained higher amounts of metals than above-ground parts of lucerne in all combinations of soils.
6. The content of tested metals in roots and above-the-ground parts was lower than in combinations of soils with no added carbon. Their amount in the roots and above-ground parts of lucerne was as follows: zinc > lead > copper > chromium > nickel > cadmium.

**Acknowledgements** This work was supported by a grant from the Warsaw University of Technology.

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Rozporządzenie Ministra Środowiska z dnia 1 września 2016 r. w sprawie sposobu prowadzenia oceny zanieczyszczenia powierzchni ziemi (Dz. U. z 2016 r. poz. 1395).

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