Leachate and contact test with *Lepidium sativum* L. to assess the phytotoxicity of waste

M. Bożym · A. Król · K. Mizerna

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Abstract

The study evaluated the use of *Lepidium sativum* L. to assess the phytotoxicity of three types of waste such as hazardous waste (slags from zinc to copper smelters) and nonhazardous waste (mineral–organic composite). Previous studies evaluated heavy metal leaching and the environmental impact of the tested waste. The purpose of phytotoxicity studies was to increase knowledge about this waste. Two types of tests were used to assess the phytotoxicity of wastes: germination index and accumulation test. Both tests were carried out for leachate (leachate test) and waste (contact test). The results of both tests were compared to assess the phytotoxic effects of tested waste. Phytotoxicity tests have shown that the mineral–organic composite leachate was stimulated plant growth, copper smelter slag leachate was characterized by no phytotoxicity and zinc slag leachate inhibited the growth of plants. In contrast, contact test showed inhibitory effects from all tested waste. Wherefore, the contact test was indicated as more sensitive in the assessment of phytotoxicity. However, this is not a clear assessment, because the germination index values for both tests were a result of differences of root length for both control samples (deionized water and sand). The study confirmed the usefulness of *L. sativum* for the assessment of phytotoxicity of various types of waste. Additionally, it was found that *L. sativum* was resistant to high concentrations of heavy metals in the leachate, without causing any negative physiological effects.

Keywords *Lepidium sativum* · Lump copper slag · Mineral–organic composite · Phytotoxicity · Sewage sludge · Inc slag

Introduction

An important aspect of environmental protection is the assessment of the toxicity of waste and its impact on biota. Waste may contain toxic inorganic compounds, which include heavy metal and organic compounds. Landfilling of waste containing toxic substances can cause their leaching into groundwater. Therefore, in the assessment of waste toxicity, the content of toxic substances and their leaching should be determined. According to Polish law, leaching tests are used to classify waste into hazardous, inert and non-hazardous waste classes (Journal of Law 2015, item 1277). The classification method consists of a comparison of the pollution leaching result (liquid to solid ratio as L/S, 1/10) from waste with limits for one of the classes. The evaluation of leaching pollutants from landfilled waste may be carried out using several methods (e.g., TCLP, SPLP and ASTM) (Mizerna and Kuterasińska 2016; Bożym 2017; Mizerna et al. 2017; Mizerna and Król 2018; Bożym 2019). These methods are also used to assess the leaching of heavy metals from solidified hazardous waste in concrete (Król and Jagoda 2012; Halim et al. 2018). In addition, in the assessment of the mobility and bioavailability of heavy metals, one–step or sequential extraction methods dedicated to soils, sewage sludge or sediments are useful (Bożym 2017; Król et al. 2019). All these methods are based on leaching metals using various reagents such as salts, inorganic and organic acids, and complex compounds. However, leaching with water reflects the natural conditions in the environment.

Although the physicochemical analyses of waste deliver much information about their toxicity, this information is not enough to evaluate the potential impacts on biota. Complementary phytotoxicity tests are usually used to assess the toxicity of waste (Sjöberg et al. 2014; Machado et al. 2016; Gu et al. 2018; Sharma et al. 2018). Phytotoxicity is defined
as an intoxication and cumulation of toxic substances in plants growing on contaminated medium (substrate) (Chang et al. 1992). Phytotoxicity tests may concern various stages of plant development. Most often the phytotoxicity tests are based on the assessment of the germination of seeds or root elongation (length). It was found that seed germination test may be less sensitive than the length of the roots test for the evaluation of phytotoxicity (Fuentes et al. 2004; Mitelut and Popa 2011). Seed cover may isolate germplasm from the negative external factors. It was found that the root is in direct contact with the substrate (medium), and is exposed to toxic substances. While during germination, the germplasm is isolated by the seed cover from negative factors (Gyuricza et al. 2010). On the other hand, the roots assimilate nutrients from aqueous solutions, therefore the root length test may be used to assess the effect of the medium on stimulating plant growth. Both tests, the germination and root length test are used to assess phytotoxicity. The results of both tests are used to calculate the germination index (GI) (Fuentes et al. 2004; Mitelut and Popa 2011). In addition to the GI, the plant growth, size and color of leaves, chlorophyll content or biomass and accumulation of pollutants are also examined (Gyuricza et al. 2010; Awasthi et al. 2017). Many factors should be considered in the assessment of phytotoxicity. Water extracts indicate the toxicity of water–soluble substances present in the waste. Leachate tests may be supplemented with contact tests, where the plant grows directly on the waste substrate or soil (Gyuricza et al. 2010). The problem in the contact test may be used to assess root elongation. The solution to this problem is covering the waste with a paper filter, wetting it with water, whereby the next step is sowing seeds (Czop et al. 2016). Phytotoxic tests may be useful in assessing hazardous waste with high heavy metal pollution, e.g., metallurgical slags or other waste, like sewage sludge, fly ashes and their mixtures (Su and Wong 2002; Fuentes et al. 2006; Ramirez et al. 2008; Phoungthong et al. 2016; Awasthi et al. 2017; Sharma et al. 2018). Some heavy metals are micronutrient, such as Zn, Cu, Cr, Ni, Mo and Co. Hence, at certain concentrations they are essential for plant metabolism and physiology, becoming toxic above specific threshold values. However, some heavy metals (Pb, Cd) and metalloids (As, Sb) are phytotoxic also at low concentrations (Kabata-Pendias and Pendias 2001; Kranner and Colville 2011). Heavy metals may inhibit seed germination and root elongation at high concentration in the medium (Seneviratne et al. 2017; Nedjimi 2020). However, at lower concentrations in the substrate, heavy metals may stimulate the plant growth. The interaction of metals, form of metal (organic, inorganic), plant species, environmental conditions, e.g., pH may also affect the GI (Sreekanth et al. 2013).

According to Manas and De las Heras (2018) it is difficult to select plant species for phytotoxicity tests. It is suggested to reach agreement in order to standardize this type of analysis. Among the plant species, cress (Lepidium sativum L.) is often used in research for evaluation of phytotoxicity. The advantage of cress is high sensitivity to contamination, including heavy metals, high tolerance of humidity, rapid growth and prevalence. According to Janecka and Fijalkowski (2008), L. sativum is characterized a high sensitivity to heavy metals, petrochemicals and polycyclic aromatic hydrocarbons, therefore it allows the use for the assessment of soil or waste contamination. Moreover, cress is recommended to use in many biotoxicity tests (ISO 11269, XP U 44–167, ÖNORM 2021, VDLUFA Methodenbuch) (Baumgarten and Spiegel 2004). Cress was used to assess the phytotoxicity of composts (Aslam et al. 2008), sewage sludge (Manas and De las Heras 2018), soil saturated with wastewater (Mekki and Sayadi 2017) or soil mixed with bottom sediments (Bianchi et al. 2008), contaminated soil (Serrano et al. 2009), cattle dung (Hoekstra et al. 2002) etc. L. sativum was also used to assess the phytotoxicity of aqueous solutions of heavy metals (Visioli et al. 2014; Smolinska and Leszcynska 2017) or other hazardous substances, e.g., benzalkonium chlorides (Khan et al. 2018) or low-weight carboxylic acids (Himanen et al. 2012) in laboratory tests. In the assessment of phytotoxicity, the GI on leachate from waste or soil is most often used. The development and condition of plants or the accumulation of heavy metals can also be assessed by sowing L. sativum directly on the substrate (Bianchi et al. 2008).

Materials and methods

Sampling

Three types of waste—two slags from a zinc and copper smelter and a mineral–organic composite based on sewage sludge and bottom ash were used for phytotoxicity tests using L. sativum. Sampling was performed in accordance with Polish standards (PN–EN 932–1; PN–EN 932–2; PN–EN 14899). Before the analyzes, the waste samples were ground to the particle size < 1 mm.

Lump copper slag (LCS)

The first waste sample was slag from the Polish copper smelter. The slag was formed in the shaft kiln after the copper concentrate smelting process. Due to the low percentage of copper, the slag is the final type of waste of the smelting process. The liquid slag at the temperature of 1200 °C is transported and landfilled on a waste heap, where is slowly cooling and solidifies. The form of the slag was lumps of irregular shapes.
Zinc slag (ZS)

The second type of waste was collected from the one of Polish zinc smelter. It was slag from the rotary kiln after the lead refining process. Due to the high percentage of heavy metals, the slag is deposited in a hazardous waste landfill located in the area of the smelter. The slag was characterized by the grain size 0.1–10 mm and contained metallic and ceramic inclusions.

Mineral–organic composite (MOC)

The third type of waste was a mineral-organic composite based on a mixture of two types of waste, i.e., sewage sludge and bottom ash from households. The sludge was stabilized by aeration and dehydrated on the filter press and on settling plots (> 20% DM). Fresh sewage sludge was mixed with ash in a 1:1 (wt.) ratio. The sewage sludge came from the municipal sewage treatment plant, while the bottom ash came from the selective collection of municipal waste. Both wastes came from the area of one of Polish commune (Opole Voivodeship). The mineral-organic composite is used for reclamation and strengthening of the slopes of the landfill located in the commune area.

Phytotoxicity tests

Two types of tests were used to assess the phytotoxicity of wastes: the germination index (GI) and accumulation test using a leachate of waste (variant 1) and directly on waste as contact test (variant 2). Usually, the results of contact tests are more reliable and reflect the natural conditions (Gyuricza et al. 2010). Cress seeds (L. sativum) were used in phytotoxicity tests. The seeds were untreated and derived from commercially available certified material (No. PL–EKO–01). A leachate from waste was prepared according to Polish standard PN–EN 12457–2 (1:10 wt. after 24 h of shaking in room temperature) using a rotary shaker WL 2000 (JW Electronic).

Germination index (GI)

The germination tests were carried out according to the methodology of the testing of Zucconi et al. (1985) with modification (Visioli et al. 2014). Each test was conducted in triplicate for each waste. As a control sample, deionized water (leachate test) and sand (contact test) were used. The sand was pretreated and mixed for 12 h at room temperature with acid (10% HNO₃), then washed with deionized water to clean from the acid and other contaminants. The sand and the tested waste were sieved < 1 mm. The tests were carried out in Petri dishes with a diameter of 90 mm.

- Variant 1: Whatman #1 paper filters were used. Leachates from waste were prepared in accordance with classification of landfilled waste in Poland (Journal of Law 2015, item 1277). The filters were moisturized with waste leachate (5 ml).
- Variant 2: into Petri dishes 10 g of dry waste was added and moisturized with deionized water. After about 24 h, the wet waste was covered with a Whatman #1 paper filter.

Into each Petri dish was placed of 20 undamaged seeds. After placing the seeds, the Petri dishes were covered and incubated in the dark in room temperature using incubator ST2BD Smart (Pol-EKO-APARATURA SP). After 72 h, the number of germinating seeds was counted and the root length was measured. In order to assess the germination index (GI), two components were assessed: Relative Seed Germination (RSG,%)) and Relative Root Growth (RRG,%)) as the ratio of germinated seeds to their total number or root length of seeds from the tested Petri dishes to control, respectively. The root length was measured from the tip to the radicle of root. The accuracy of measuring the root length was 1 mm. The positive germination effect (RSG) was considered to be the appearance of a sprout of at least 1 mm in length. The GI was calculated as quotient of RSG and RRG related to 100%.

Accumulation test

The test was extended with the analysis of the accumulation of heavy metals, metalloids and nutrients. The test was carried out in two variants: 1) using leachates from waste and 2) directly on waste.

- Variant 1): each Petri dish was filled with cotton wool and watered with 10 ml of leachate. The cotton wool was pretreated by washing in deionized water for 24 h and drying in temp. 105 °C. To each Petri dish 1.0 ± 0.1 g of cress seeds were added.
- Variant 2): the Petri dishes were filled with tested waste and moisturized with deionized water (10 ml) within 24 h. Next 1.0 ± 0.1 g of L. sativum seeds were added. Seeds were placed uniformly on the surface of a waste at the bottom of a Petri dish. As a control sample, Petri dishes were prepared with sand pretreated with acid (HNO₃), Ø 1 mm.

The seeds were placed evenly over the entire surface of the dish. Both variants were conducted for seven days. The dishes were kept in the white light (16 h) and in the dark...
(8 h) at room temperature using incubator ST2BD Smart (Pol-EKO-APARATURA SP). From day 1 to day 7 of test, the growth and condition (cotyledon color) of the plants were assessed. The stem length was additionally measured after 7 days of test. After 7 days of experiment, the plants were separated from the substrate. The plant material was washed in deionized water, dried at 105 °C and ground in a mortar. All seedlings from each waste were collected and mixed, creating a composite plant sample of each waste.

**Physico–chemical analysis**

Mineralization of waste and plant samples was carried out using a microwave oven (Start D, Milestone) in Teflon vessels with *aqua regia* (waste) or HNO₃ and H₂O₂ mixture (plants). All reagents were *ultra pure* (Merck). During the analysis, deionized water with a EC < 0.055 µS/cm was used (HLP 5, Hydrolab). The analysis of heavy metals, metalloids and nutrients was carried out by the FAAS method (Solaar 6, Thermo). Heavy metals, metalloids and nutrients in the leachate and mineralizate were determined by the FAAS method using a spectrometer Solaar 6 (Thermo).

The chemical compositions of waste were determined by the XRF method (Axios Cement, Panalytical). Loss of ignition (LOI) was determined at 550 °C in laboratory oven (B 180, Nobertherm). The leachate determined the pH and conductivity using pH–conductometer CPC–501 (Elmetron), concentration of heavy metals, metalloids and nutrients using spectrometer Solaar 6 (Thermo), as well as chlorides, sulfates, fluorides, ammonia, cyanides, phenol, formaldehyde by spectrophotometric methods using a UV–1601pc spectrometer (Shimadzu).

**Quality control**

All analyzes were carried out in triplicate accordance with Polish standards (PN–EN 12457–1–3; PN–EN 15169:2011; PN–79/C–04566/10; PN–75/C–04588; PN–ISO 7150–1; PN–82/C–04576/08; PN–EN 27888; PN–71/C–04593; PN–EN 15169; PN–ISO 11262; PN–ISO 6439; PN–EN 1541; PN–74/C–04540). For quality control, three certified reference materials were tested, i.e., as plant control ‘Lichen (trace elements)’ (IRMM, No. BCR–482), as waste control ‘Metals in soil’ (Merc. No. SQC001), as a leachates control ‘CA WET Metals in soil—QC’ (Sigma Aldrich, No. SQC006) and ‘Anions in soil’ (Sigma Aldrich, No. SQC013). For the analyzes of metals and metalloids, the recovery was in the order of 85–115%, for other analyzes 90–110%. The control of the analytical range for the determination of heavy metals and metalloids was based on the analysis of samples of the certified standard (‘ICP–multielement standard XI’ (Merck, Lot: HC394644) and performed for each measurement series. The acceptable range of recovery was 90–110%. If no recovery value was obtained in this range, the analysis was repeated. The means and standard deviation (SD) were calculated for triplicate data using Statistica ver. 13.3, considering a significance level of $p < 0.05$. Limit of quantification (LOQ) of metals and metalloids analysis by the FAAS method (Solaar 6 M, Thermo): Cd 0.005; Pb 0.05; Cu 0.02; Zn 0.03; Ni 0.02; Cr 0.05; Mo 0.05; Co 0.01; Fe 0.1; Mn 0.01; Ba 0.25; K 0.02; Na 0.02; Mg 0.01; Ca 0.05; As 0.003; Se0.001; Sb 0.01 mg L⁻¹.

**Results and discussion**

**Characteristics of waste and leachates**

Table 1 presents loss of ignition (LOI) and oxide composition of the tested waste. The MOC sample characterized high LOI value (19% wt.), which indicates a high percentage of organic matter in this waste. The LOI value was below the limit of quantification in LCS, which indicates the mineral character of this waste. The ZS sample was characterized by a low LOI value (3% wt.), because similarly to LCS, this waste is of mineral character. The main component of LCS and MOC was silica (SiO₂). In the MOC sample, apart from silica, a high percentage of Fe₂O₃ and MgO was found. The percentage of oxides in ZS depends mainly on the composition of fly ash added to sewage sludge, which is usually characterized by a high percentage of silica, iron and aluminum oxides (Phuongthong et al. 2018). In the current study, Fe₂O₃ was the dominant component of ZS. The high percentage of Fe₂O₃ in slag from the copper and zinc smelters is confirmed by other authors (Murari et al. 2015; Dash et al. 2016; Prince et al. 2016; Nadirov et al. 2019). In both slag samples (ZS and LCS), a high percentage of Al₂O₃ was also found. The percentage of Na₂O and K₂O in the slag samples (LCS, ZS) was varied. The content of other oxides

| Parameter (% wt.) | LCS | ZS | MOC |
|------------------|-----|----|-----|
| SiO₂             | 43 (± 4) | 10 (± 1) | 38 (± 4) |
| SO₄²⁻            | 0.1 (± 0.0) | 7.6 (± 0.8) | 1.9 (± 0.2) |
| Al₂O₃            | 16 (± 2) | 15 (± 1) | 2 (± 0) |
| Fe₂O₃            | 17 (± 2) | 42 (± 4) | 6 (± 1) |
| CaO              | 12 (± 1) | 2 (± 0) | 1 (± 0) |
| MgO              | 7 (± 1) | 1 (± 0) | 5 (± 1) |
| Na₂O             | 1.1 (± 0.1) | 3.4 (± 0.3) | 1.1 (± 0.1) |
| K₂O              | 3.4 (± 0.3) | 0.3 (± 0.0) | 1.0 (± 0.1) |
| LOI              | <0.1 | 3.0 (± 0.0) | 19.0 (± 2.0) |
was low. The oxide composition of the waste influences their physical properties and its potential for reuse e.g., as additive to construction materials (Gorai and Jana 2003; Chung et al. 2006; Lin et al. 2006; Murari et al. 2015).

Table 2 presents the total content of heavy metals, metalloids and nutrients in the waste. In the slag samples (ZS, LCS), very high content of some heavy metals (Cd, Pb, Cu, Zn, Ni) was found. The metallurgical slag samples are characterized by a high content of heavy metals, depending on the type of ore smelted and the stage of the technological process (Jiang et al. 2012; Murari et al. 2015; Nadirov 2019). Due to the high content of heavy metals in slag from the copper and zinc smelters, some metals may be recovered by physical and chemical methods (Gorai and Jana 2003, Jiang et al. 2012; Murari et al. 2015; Prince et al. 2016). In current study, the content of other metals in both slags (ZS, LCS) was at a lower level. The content of nutrients (Na, K, Ca, Mg) was also high in LCS, and slightly lower in ZS. The high percentage of those elements in metallurgical slags is the result of adding of alkali materials during smelting of the metal (Rusen et al. 2016). The content of heavy metals in MOC was low in comparison to metallurgical slags samples. The source of heavy metals in these composites may be sewage sludge or bottom ash. The tested composite (MOC) meets the requirements of heavy metals content in sewage sludge used in agriculture according to Polish law, which proves low pollution (Journal of Laws 2015, item. 257).

The phytotoxicity of heavy metals and metalloids depends on the type and its concentration in the substrate and plant species. The metals form is important in their phytotoxicity (Arambašić et al. 1995; Masarovičová and Kráľová 2017).

The main problem concerns waste landfilling or its used for agrotechnical purposes, e.g., soil additive. It has been proved that the phytotoxicity of heavy metal in soils depends on the type of metal and plant species, e.g., phytotoxic content of Pb was 0.005 mg kg\(^{-1}\) DM for *Lactuca sativa* L. and *Daucus carota* L.; Ni was 10–20 g m\(^{-2}\) for *Brassica oleracea* L.; Fe was 100 mg kg\(^{-1}\) DM for many plants; Mo was 135 mg kg\(^{-1}\) DM for barley and 200 mg kg\(^{-1}\) DM for oats; Zn was 450 mg kg\(^{-1}\) DM for soybeans; Cr was 80 mg kg\(^{-1}\) DM for *Seccalium officinarum* L., 125 mg kg\(^{-1}\) DM for *Triticum aestivum* L. and 1000 mg kg\(^{-1}\) DM for *Hipsicus esculentus* L.; Co was 5000 mg kg\(^{-1}\) DM for *T. aestivum* (Gautam et al. 2016). Arambašić et al. (1995) found heavy metal toxicity in aqueous solutions for *L. sativum* in the order of Cu > Pb > Zn (CuSO\(_4\) 2.43 mmol L\(^{-1}\), Pb(NO\(_3\))\(_2\) 3.37 mmol L\(^{-1}\), ZnSO\(_4\) 8.42 mmol L\(^{-1}\), respectively). Whereas, Pokorska-Niewiada et al. (2018) determined the metal phytotoxicity for *L. sativum* at 50 mg L\(^{-1}\) for Cu, Zn, Pb, Hg and 400 mg L\(^{-1}\) for Cr. According to Visioli et al. (2014) the phytotoxic effect of Ni on *L. sativum* is 30 mg L\(^{-1}\).

Table 3 presents the content of contaminants in the leachate from waste, the waste classification and the content of other contaminants that are not included in the classification. The directions of further use of waste are governed by separate legal regulations based on the assessment of contaminants leachability.

According to Polish classification, LCS has been classified as hazardous due to slightly exceeded limit value of Pb in the leachate. The content of other pollutants in the leachate from this waste did not exceed the limits set for inert waste (As, Ba, Cd, Co, Ni, Zn, Cl\(^{-}\), F\(^{-}\), SO\(_4\)\(^{2-}\)) or

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**Table 2.** The heavy metals, metalloids and nutrients total content (mg kg\(^{-1}\) DM) in tested waste and sand used as control in contact test, n = 3

| Parameter | LCS | ZS | MOC | Sand (as control) |
|-----------|-----|----|-----|-------------------|
| Cd        | 0.5 (± 0.1) | 223 (± 20) | 0.2 (± 0.1) | < 0.2 |
| Pb        | 2580 (± 150) | 87,213 (± 450) | 45 (± 4) | < 1.0 |
| Cu        | 4027 (± 200) | 95,216 (± 250) | 283 (± 20) | < 0.5 |
| Zn        | 7076 (± 50) | 71,757 (± 350) | 642 (± 32) | < 0.5 |
| Ni        | 291 (± 25) | 1890 (± 150) | 62 (± 5) | < 0.5 |
| Cr        | 690 (± 25) | 274 (± 11) | 47 (± 2) | < 0.50 |
| Mo        | 58 (± 4) | 186 (± 11) | 6 (± 1) | < 2 |
| Co        | 1049 (± 56) | 531 (± 22) | 28 (± 2) | < 0.5 |
| Ba        | 54 (± 4) | 487 (± 35) | 587 (± 32) | < 10 |
| As        | 3.4 (± 0.5) | 2.8 (± 0.2) | 0.3 (± 0.0) | < 0.1 |
| Se        | 0.4 (± 0.0) | 0.4 (± 0.0) | 0.1 (± 0.0) | < 0.1 |
| Sb        | 162 (± 11) | 75 (± 5) | 9 (± 1) | < 0.5 |
| Fe        | 3200 (± 200) | 9600 (± 900) | 39,900 (± 1500) | 1500 (± 200) |
| Mn        | 1800 (± 200) | 600 (± 100) | 700 (± 100) | 300 (± 30) |
| Mg        | 6200 (± 200) | 2000 (± 100) | 3100 (± 200) | < 1 |
| Ca        | 11,600 (± 500) | 1900 (± 100) | 11,000 (± 500) | < 1 |
| K         | 20,200 (± 500) | 4400 (± 100) | 4700 (± 100) | < 1 |
| Na        | 8300 (± 800) | 31,500 (± 900) | 6000 (± 500) | < 1 |
nonhazardous waste (Cr, Cu, Sb, Se). ZS waste has been classified as hazardous due to the very high leachability of Cd, Pb, Cu and Zn. The values obtained exceed the requirement for hazardous waste intended for landfilling. Concentrations of other pollutants in leachate from ZS did not exceed the limit values for inert waste (As, Ba, Sb, Cl\(^-\), F\(^-\)) or nonhazardous waste (Cr, Mo, Ni, Se, SO\(_4^{2-}\)). MOC was characterized by the low leachability of heavy metals, metalloids and other impurities. Most of leached pollutants did not exceed the limit values for inert waste. However, several pollutants, including chlorides and sulfates, are classified as nonhazardous waste. It is known that the addition of liming materials to sewage sludge may increase the salinity of the leachate (Su and Wong 2002). Sulfates and chlorides affect the salinity of natural waters. Therefore, the leaching of these anions from waste should be monitored.

Many substances in waste leachate may be phytotoxic (Sjöberg et al. 2014; Machado et al. 2016; Gu et al. 2018; Sharma et al. 2018). Their toxicity depends on the concentration in the leachate. For this reason, more parameters than required by Polish law in the classification of waste were analyzed (Table 3). The additionally analyzed parameters included Co, Fe, Mn, nutrients, nitrogen compounds, cyanides, formaldehyde, pH and EC. For example, ionic strength as EC, Fe content and pH changes should be taken into account as an important factor in phytotoxicity studies (Bartakova et al. 2001). Likewise cyanides and other nitrogen compounds can have a negative effect on

- **Table 3** The leachate characteristic and waste classification based on the guidelines for its leachates before landfilling, n = 3

| Parameter (mg kg\(^{-1}\) DM) | LCS | ZS | MOC | Classification of i/nih/h waste |
|-------------------------------|-----|----|-----|--------------------------------|
| As <0.03 (i)                  | <0.03 (i) | <0.03 (i) | <0.03 (i) | 0.5/2/25 |
| Ba <2.5 (i)                   | <2.5 (i) | 9.5 (± 0.9) (i) | 20/100/300 |
| Cd <0.05 (i)                  | 144 (± 40) (h\(*) | <0.05 (i) | 0.04/1/5 |
| Cr 0.6 (± 0.1) (nh)           | 0.8 (± 0.1) (nh) | <0.5 (i) | 0.5/10/70 |
| Cu 3.9 (± 0.4) (nh)           | 360 (± 36) (h\(*) | 8.7 (± 0.9) (nh) | 2/50/100 |
| Mo <0.5 (i)                   | 0.6 (± 0.1) (nh) | <0.5 (i) | 0.5/10/30 |
| Ni <0.2 (i)                   | 0.9 (± 0.1) (nh) | <0.2 (i) | 0.4/10/40 |
| Pb 13.5 (± 1.5) (h)           | 221 (± 20) (h\(*) | 9.0 (± 1.0) (nh) | 0.5/10/50 |
| Sb 0.7 (± 0.1) (nh)           | <0.1 (i) | <0.1 (i) | 0.06/0.7/5 |
| Se 0.15 (± 0.02) (nh)         | 0.35 (± 0.04) (nh) | <0.01 (i) | 0.1/0.5/7 |
| Zn 3.1 (± 0.3) (i)            | 18175 (± 200) (h\(*) | 11.8 (± 2.0) (nh) | 4/50/200 |
| Cl\(^-\) 370 (± 30) (i)       | 384 (± 30) (i) | 1040 (± 40) (nh) | 800/1500/25,000 |
| SO\(_4^{2-}\) 336 (± 30) (i) | 19,656 (± 220) (nh) | 19,205 (± 2) (nh) | 1000/20,000/50,000 |
| F\(^-\) 0.6 (± 0.1) (i)       | 1.8 (± 0.1) (i) | 1.5 (± 0.1) (i) | 10/150/500 |
| phenol 2.2 (± 0.2) (nc)       | 1.9 (± 0.2) (nc) | <0.5 (i) | 1/n.r/n.r. |
| Co 1.2 (± 0.1)                | 0.2 (± 0.0) | <0.2 | n.r. |
| Fe 0.5 (± 0.0)                | 3.0 (± 0.3) | 5.0 (± 0.5) | n.r. |
| Mn 4.2 (± 0.4)                | 1.1 (± 0.1) | 0.5 (± 0.0) | n.r. |
| Mg 22 (± 2)                   | 11 (± 1) | 20 (± 2) | n.r. |
| Ca 11 (± 1)                   | 2 (± 0) | 19 (± 2) | n.r. |
| K 18 (± 2)                    | 790 (± 25) | 82 (± 8) | n.r. |
| Na 7740 (± 200)               | 7020 (± 150) | 50,600 (± 550) | n.r. |
| N–NH\(_4^{+}\) 10 (± 1)      | 9 (± 1) | 9 (± 1) | n.r. |
| N–NO\(_3^{-}\) 11 (± 1)      | 7 (± 0) | 675 (± 50) | n.r. |
| CN\(^-\) 0.41 (± 0.04)       | 0.35 (± 0.04) | 0.37 (± 0.04) | n.r. |
| Formaldehyde 8.0 (± 0.5)      | <0.2 | <0.2 | n.r. |
| pH 8.0 (± 0.1)                | 7.5 (± 0.1) | 7.5 (± 0.1) | n.r. |
| EC (uS/cm) 105 (± 5)          | 4100 (± 50) | 1980 (± 25) | n.r. |
the germination of plants (Yu and Gu 2007; Palmieri et al. 2014). Ammonia may have a phytotoxic effect (Hoekstra et al. 2002; Ramírez et al. 2008). In contrast, nutrients are necessary for plant growth, but their excess may affect the salinity of solutions and decrease germination (Arambašić et al. 1995). The content of heavy metals in the leachate with LCS and MOC should not decrease the germination and growth of cress. Only higher Pb content in LCS may negatively affect test plants. However, the very high content of Cd, Pb, Cu and Zn in the leachate from ZS may be phytotoxic to cress. For example, phytotoxic concentrations of metals in aqueous solutions for selected plants are: Ni 200 μM (wheat); Cu 10 μM (Withania somnifera L., Zea mays L.) and > 50 μM (Solanum nigrum L.); Pb > 400 μM (Brassica juncea L.), 1500 μM (Sinapis arvensis L.) and 3000 μM (T. aestivum); Cd 100–200 μM (mustard and soybean) (Gautam et al. 2016) and 50–100 μM (rice) (Afzal et al. 2019). Visioli et al. (2014) found a negative impact on GI of L. sativum at Ni concentrations: 7 mg L−1 (EC10), 17 mg L−1 (EC30) and 30 mg L−1 (EC50).

The concentration of metalloids in the leachate of tested waste was lower than heavy metals, except Sb in the LCS and ZS leachates. Among the metalloids, arsenic is the most toxic, especially the As(III) (Kabata-Pendias and Pendias 2001). Arsenic may negatively affect the roots and shoots to toxic, especially the As(III) (Kabata-Pendias and Pendias 2001). Arsenic may negatively affect the roots and shoots of plants (Seneviratne et al. 2017). For example, the phyto-

toxic As concentration for varieties of T. aestivum was determined between 0 and 16 mg L−1 (Gautam et al. 2016). The toxic effect of antimony depends on the plant species (Liang et al. 2018). In contrast to arsenic and antimony, selenium is one of the micronutrients that stimulates the germination and growth of plants (Praveen et al. 2017). In the current study, the concentration of metalloids in the leachate from the tested waste was low and should not be phytotoxic for L. sativum.

Nutrients are essential elements for plant growth, but their excess in the medium may be phytotoxic (Tripathi et al. 2014; Reich 2017; Antonkiewicz et al. 2020). Sodium plays an important role for turgor pressure inside plant cells. Its excessive concentrations produce toxic effects on plants, causing water stress. Increased sodium uptake affects the potassium deficiency in the plant. Sodium is responsible for the salinity of the soil (Arambašić et al. 1995; Tutteja 2007). Plant resistance to salinity depends on the species, e.g., for Solanum melongena L., a toxic concentration of Na is 150 mmol L−1, for Capsicum annuum > 50 mmol L−1, for Chrysanthemum sp. L. > 9 g L−1 (Gautam et al. 2016). Potassium is a basic macronutrient required for plant physiological and metabolic processes. However, excessive K uptake may reduce P uptake and water balance in plant. Calcium is a macronutrient that is necessary for the stability of plant cell walls, influences the transport of ions and controls enzymatic activity. But excessive calcium uptake may be phytotoxic to plants, causing such symptoms as necrosis, chlorosis, leaf curling and puckering (Gautam et al. 2016). Magnesium may become toxic when available in excess, although Mg is necessary for photosynthesis, respiration and energy metabolism. Excess Mg causes chloroplasts damage and reduce of potassium transport. For example, the toxic content of Mg in soil for tea is 5000 mg kg−1 DM (Gautam et al. 2016). In current study, the leachate from ZS was characterized by the highest potassium concentration, although the total potassium content in this waste was not high (Table 2). While, the highest total K content was determined in LCS, but its leaching of this waste was low. This may suggest that potassium may be associated with the structure of this waste. Whereas, the highest Na concentration was found for the leachate from MOC, although the low total content of this nutrient was determined in this waste. The highest total content of Na was determined in the ZS, but low concentration of this nutrient was determined in leachate. High Na content in the leachate or substrate (waste) may negatively affect germination and root growth of L. sativum. Compared to Na and K, the Ca and Mg concentrations were lower in the leachate from all wastes.

The concentration of chlorides in all tested leachates was low. However, the concentration of sulfates in the ZS and MOC samples was high. A high EC value was also found for these samples. Therefore, it can be concluded that the salinity of tested leachates may be affected by the concentration of sulfates, and for the ZS sample also by other ions. LCS leachates were characterized by the lowest concentrations of sulfates (336 ± 30 mg kg−1 DM) and chlorides (370 ± 30 mg kg−1 DM). In contrast, Murari et al. (2015) determined the content of sulfates in copper slag at 1000 mg kg−1 DM (0.1%) and chlorides 10 mg kg−1 DM (0.001%). According to Polish regulations, the salinity parameters and the sum of the concentration of chlorides and sulfates are used interchangeably. Electrical conductivity (EC) is a measurable indicator of leachate salinity. High salinity may have a negative effect on seed germination and plant growth. Hoekstra et al. (2002) report that the negative EC effect of the leachate on seed germination is only reached when the value of 2 mS cm−1 is exceeded. The ZS leachate
was characterized by a very high EC value (4.1 mS cm$^{-1}$), which may result in the inhibition of plant germination and growth in phytotoxicity tests. The leachate from MOC was also characterized by a high EC. However, this value did not exceed the phytotoxicity limit, which should not adversely affect the plants. The lowest EC values were determined for LCS leachate.

The concentration of fluorides in the leachate of the tested waste was low. However, the toxicity of fluorides to the biota is much higher than chlorides and sulfates. Even at low concentrations of F$^-$ in the leachate, it can affect the germination and development of plants (Palmieri et al. 2014). The toxicity of fluoride to plants depends on the species (Stevens et al. 2000). The pH of the leachate and the presence of heavy metals may neutralize the negative effects of fluoride (Palmieri et al. 2014). Gupta et al. (2009) found a negative impact of fluorides on rice germination above 20 mg L$^{-1}$ and root elongation above 30 mg L$^{-1}$. In current research, the fluoride concentration in the leachate from the tested waste should not be phytotoxic.

The presence of nitrogen compounds in the leachate may stimulate the germination and growth of plants. When the optimum concentration is exceeded, especially of ammonia, it may be toxic and inhibit germination (Hoekstra et al. 2002; Ramirez et al. 2008). Bennet and Adams (1970) found the phytotoxic effect of ammonia in water at 13 mM (i.e., 220 mg L$^{-1}$). The concentration of nitrogen compounds (N–NH$_4$, N–NO$_3$) was low in slags leachate (LCS and ZS). The leachate from MOC was characterized by a high concentration of nitrates. Unlike ammonia, nitrates are not toxic to plants and stimulate their growth, even at high concentrations. The content of nitrogen compounds in the leachate from the tested waste should not negatively affect germination and growth of L. sativum. Nitrogen compounds also include cyanides, which may be present in leachate from industrial waste (Bożymin 2020, Dungan et al. 2006). A high concentration of cyanides in the leachate may inhibit the germination of plants (Hoekstra et al. 2002), but plants have the ability to detoxify cyanides and convert to asparagine amino acid (Yu and Gu 2007). The content of cyanides in the tested leachates was very low and should not be phytotoxic.

Phenol and formaldehyde may be present in industrial waste, but mainly in foundry waste (Bożymin 2020, Dungan et al. 2006; Siddique et al. 2010). These compounds are toxic to biota. The content of both pollutants in the tested leachates was very low and should not have been phytotoxic.

The tested wastes were characterized by a neutral or slightly alkaline pH. The pH of the leachate affects the germination of plants, as it affects the leaching of metals and their toxicity. Phoungthong et al. (2016) claimed that the pH can negatively affect the germination of plants only in very acidic (pH < 2) or alkaline (pH > 13) solution only with presence of heavy metals. In the case of tested waste, pH should not adversely affect the germination and root growth of plants in phytotoxicity tests.

Based on the result of leachates analysis it can be stated that the germination and growth of plants in phytotoxicity tests may be influenced by the high content of sulfates and heavy metals, and also EC value. The assessment of

![Fig. 1](image-url) The germination index (GI) of L. sativum from waste leachate (1) and from waste (contact test) (2), mean(± SD), n = 3
inhibition or stimulation (promotion) of germination and plant growth was assessed in the next part of the paper.

**Phytotoxicity assessment**

To assess the phytotoxicity of the waste, the GI and the accumulation test was used, which uses *L. sativum* growing on leachate and directly on the substrate (contact test).

**Germination index**

Figure 1 shows the GI values of waste calculated for leachate (GI1) and substrate (GI2). Figure 1 also presents the phytotoxicity classification based on the GI value according to Zucconi et al. (1985): for GI < 50% mean high phytotoxicity, GI ranged between 50 and 80% mean moderate phytotoxicity, and for GI > 80% indicate that the material present is not phytotoxic. A GI value higher than 100% indicates a plant–stimulating effect. For comparison, Fig. 2 shows the RRG and RSG values from which the GI value was calculated. The research shows that the GI value was affected by root length (RRG), not by germination value (RSG). Therefore, it can be stated that the root length value is better for phytotoxicity assessment, not the number of germinating seeds.

The average root length of *L. sativum* control sample was 19 ± 4 mm. For slags leachate the average root length was LCS 16 ± 4 mm and ZS 11 ± 5 mm, respectively. The longest roots characterized *L. sativum* growing on MOC leachate (mean 34 ± 5 mm). The GI values for *L. sativum* from the leachate were: LCS 88%, ZS 60% and MOC 182%, respectively. This means that the leachate from LCS was not phytotoxic to *L. sativum*. In contrast, the leachate with MOC stimulated the growth of *L. sativum* seeds. High levels of heavy metals could influence the moderate phytotoxicity of ZS leachate. In contrast, the stimulating effect of MOC leachate could have been the result of a low content of heavy metals and the presence of nitrogen compounds (ammonia content was at a non–toxic level). However, some authors have stated that leachate from processed sewage sludge may be phytotoxic, due to high fatty acids, ammonia and heavy metals concentration (Fuentes et al. 2004, 2006; Yang et al. 2017; Manas and De las Heras 2018). Fatty acids in sewage sludge from MOC may have been neutralized by bottom ash, therefore no phytotoxic effect of this waste was found.

In current studies, the GI values for *L. sativum* growing directly on the substrate (contact test) were lower than on leachate. *L. sativum* seeds did not germinate on ZS, probably due to the high salinity of this waste. After 72 h, salt crystals on the edge of the Petri dish were observed. This effect was not found for ZS leachate and this was probably related to dilution of the salt in the leachate. The leachate was prepared as an aqueous extract in the ratio L/S = 10. Probably for a leachate prepared in a different L/S ratio, e.g., 2, the salt concentration would be higher and probably more phytotoxic than L/S = 10. The average root length of *L. sativum* from the control (sand) was 56 ± 5 mm. The average root length for LCS was 38 ± 6 mm, and MOC 19 ± 5 mm. It was found that the root length of *L. sativum* growing on the substrate in the contact test was higher than on the leachate of waste. Due to the higher root length of *L. sativum* for sand (56 mm) compared to deionized water (19 mm) as control samples, the GI value for the contact test was lower than for the leachate. The root length of *L. sativum* growing on the substrate in the contact test was higher than
on the leachate of waste. Due to the higher root length of *L. sativum* for sand compared to deionized water (control samples), the GI value for the contact test was lower than for leachate. The GI value for the contact test was LCS 66%, ZS 0% and MOC 31%, respectively. It can be stated that LCS was moderately phytotoxic for *L. sativum*. An unexpected effect was the high phytotoxicity of MOC substrates for *L. sativum* seeds, although similar values for germination/root length of *L. sativum* for both tests of MOC were obtained. The reason for this effect could be considerable differences between the root length of the controls (deionized water and sand), which affected the GI value.

**Accumulation test**

The accumulation test evaluated the content of heavy metals, metalloids and nutrients in *L. sativum* and the condition of plants. In the visual assessment of plant condition, cotyledon coloring and stem height are important to indicate the condition of the plant. Improper coloring of the cotyledons may indicate nutrient shortages in the plant or pollution with heavy metals. In plant tissues, heavy metals may be destructive for photosynthesis as they take part in the destabilization of enzymes (Seneviratne et al. 2017). High concentration of certain metals (Cr, Cu and Pb) in the leachate may be cytotoxic, causes stress and damage to plants, which causes leading to retardation of plant growth and chlorosis of leaf (Phoungthong et al. 2016). In current study, the condition of *L. sativum* growing on waste leachates was good, the cotyledon was properly colored, with no signs of chlorosis. This means that pollutants in the leachates did not adversely affect the photosynthesis process. The height of the stem, measured from the root tip to the cotyledon base, varied between the leachates and the control sample (Fig. 3).

However, higher variability of *L. sativum* stem height was found for the contact test. The average length of stems of *L. sativum* from control was about 90 mm. The longest stems (150 mm) were determined for *L. sativum* growing on MOC. The leaves of plants from the control and MOC were well colored, with no signs of chlorosis. The plants growing on LCS were less colored and the stem length was lower than control and MOC (40–50 mm) (Fig. 3). The lower root length could have been affected by the high content of heavy metals in this waste. According to Phoungthong et al. (2016) the inhibition of plant growth, but also cotyledon coloration may be affected by the excess of some heavy metals, such as Cr, Cu and Pb in the substrate. For this waste, high Pb content (Table 2), which was also found to be high in the leachate (Table 3), could have negatively affected on plant growth. The seed germination did not occur on ZS substrate. The seeds did not absorb water, did not expand and germinate. This means that the composition of the waste made it impossible their germination. Such an effect was most likely caused by high salinity of the substrates and a high content of heavy metals. Heavy metals, especially Cd, reduce plant tolerance to water stress. Since water absorption is the main requirement for seed germination, the negative impact of heavy metals on the water content of seeds is considerable (Różyło et al. 2015; Seneviratne et al. 2017).
L. sativum roots from LCS and ZS leachate were shorter by 14% and 41%, respectively, than the control. On the other hand, the roots of L. sativum from the MOC leachate were 67% longer than the control. In the case of the contact test, the L. sativum root length was shorter than the control, i.e., by 34% for LCS and 67% for MOC. It follows that due to the differences in the results obtained for both tests, it may be necessary to conduct both tests simultaneously to assess the phytotoxicity of the waste.

Figures 4, 5, 6 show the content of heavy metals, metalloids and nutrients in L. sativum growing on control and waste (substrates). The content of heavy metals in L. sativum growing on the leachate from LCS (LCS1) was similar to the control (Fig. 4). In contrast, a high increase in the content of heavy metals, especially Pb, Cu, Zn, Fe, Mn was found in L. sativum from the contact test, growing directly on the substrate. The reason could be heavy metals in the waste in forms available to plants, except for water-soluble forms. The content of Zn in L. sativum from the ZS leachate
(ZS1) was high, related to the high content of this metal in the water extract. *L. sativum* did not germinate directly on the substrate. Probably as a result of high sodium and heavy metals content (Table 2). *L. sativum* growing on the MOC leachate (MOC1), the heavy metal content was slightly higher than the control (Fig. 4). In the contact test (MOC2), *L. sativum* from this waste accumulated higher heavy metal content (especially Fe and Zn) than those from the leachate test. In this case, heavy metals assimilated by plants may have been present in the waste in other forms than just water-soluble.

Figure 5 shows the content of metalloids in *L. sativum* from leachate (1) and waste (2). The content of metalloids in *L. sativum* from all samples was low, not exceeding 1 mg kg\(^{-1}\) DM for each metalloid. For controls and leachate, the Sb content of the plants was below the limit of quantification. The content of metalloids in *L. sativum* from LCS leachate (LCS1) was slightly higher than the control. The content of As and Se in *L. sativum* from the LCS leachate in both tests was similar, except Sb, which content was higher for the contact test (LCS2). A similar effect was found for MOC. The content of As and Se was at a similar level for the leachate and the contact test, in contrast to Sb. Thus, it can be concluded that the content of metalloids was low, especially in plants from the leachate of the tested waste. This could be due to the low concentration of these elements in the leachate. Only Sb was accumulated by *L. sativum* directly from the waste in contact test.

Among nutrients, the highest Ca content was found in *L. sativum* from the leachate and the contact test. *L. sativum* from LCS leachate was characterized by a low content of nutrients, slightly higher than in the control (Fig. 6). In contrast, *L. sativum* from the contact test (LCS2) was characterized by a higher Ca content than from the leachate test (LCS1). The nutrient content, specially Ca and Na, in *L. sativum* from the ZS leachate was higher than the control. The leachate from this waste was characterized by a higher Na concentration (Table 3). Therefore, it can be assumed that the accumulation of sodium by *L. sativum* was due to its higher concentration in leachate. The highest nutrient content was found in *L. sativum* growing on MOC in the leachate and the contact test. The plants characterized a highest Ca content and a high Na content from the contact test. It was found that higher accumulations of Ca and Na in *L. sativum* from the contact tests compared to the leachate tests.

Based on the results of the accumulation and GI tests, it can be concluded that the contact test was more sensitive than the leachate test as confirmed by Gyuricza et al. (2010). In the case of GI, it was a result of high differences between the root length for both controls (deionized water and sand). For the accumulation test, a significantly higher content of heavy metals and other components in *L. sativum* from contact test was found, which may indicate the possibility of accumulation of these elements from waste as other forms than soluble in water (from leachate).
Conclusion

The tested wastes were characterized by wide range of contents of heavy metals, metalloids and alkali metals. The most contaminated by heavy metals were zinc slag (ZS) samples. Very high concentrations of Cd, Pb, Cu and Zn were also determined in the leachate from this waste, which resulted in its classification as hazardous waste. Lump copper slag (LCS) were less contaminated with heavy metals than ZS. However, total Pb content in this waste was high, which affected high concentrations of this metal in its leachate. Due to the slightly exceeded limit value of Pb in the leachate, LCS was classified as hazardous waste. The third tested waste sample, mineral–organic composite (MOC), was characterized by a low content of heavy metals. The leachability of heavy metals and other pollutants from this waste was low and classified it as nonhazardous waste.

It was stated that through the increased concentrations of sulfates in the leachate from ZS to MOC, as a consequence, the increased EC value of these leachates was determined. The high salinity of these leachates had a negative effect on the germination and development of L. sativum plants in phytotoxicity tests. The high content of heavy metals could also affect the phytotoxicity of slags and their leachate.

The results of phytotoxicity tests carried out from the leachate test and contact test were varied. The MOC leachate was stimulating plant growth. However, contact studies showed inhibitory effects from this waste. The highest phytotoxicity was observed for ZS, on which L. sativum seeds did not germinate in the contact test. Also, the leachate from this waste inhibited the growth of plants. Ambiguous results were obtained for LCS. The leachate from this waste did not show any phytotoxic effects, whereas the waste in the contact test was characterized by moderate phytotoxicity. The contact test proved to be more sensitive in assessing the phytotoxicity of waste than the leachate test. However, the GI value for both tests was affected by large differences in the root length of L. sativum from the control samples, i.e., deionized water and sand. It can be stated that both tests needed to be carried out to fully assess the phytotoxicity of the waste. This was confirmed in the accumulation tests; in the contact test L. sativum accumulated more heavy metals, metalloids and nutrients than in the leachate test.

The study confirmed the usefulness of cress (L. sativum) for the assessment of phytotoxicity of various types of waste. Additionally, it was found that L. sativum is resistant to high concentrations of heavy metals in the leachate, without causing any negative physiological effects, e.g., cotyledon chlorosis. However, this species may be sensitive to the salinity of the leachate.

Based on the obtained results, it can be concluded that phytotoxicity tests should be carried out both on the leachate and directly on the substrate. This is important for landfilled waste or used for various purposes. This solution is useful in biologically remediated landfills. Biological remediation requires an assessment of the phytotoxicity of the waste before planting. Another direction of use these tests is the assessment of waste used for agrotechnical purposes, e.g., mineral-organic composite as strengthen material of slopes in landfills. The biological remediation with various plant species is also used on waste landfills, after mechanical strengthening of slopes. Therefore, it is important to choose the right plant species for each application. In further stages of our research, it is planned to conduct all tests with other plant species, most often used for biological remediation in Poland.

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Compliance with ethical standards

Conflict of interest The authors declare that has no any conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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