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Preliminary acute toxicity studies of calcium and magnesium sorbent extracts on selected test organisms

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ABSTRACT

The growing population generates a lot of waste, including industrial waste. These wastes can be used as sorbents, for example in the form of aggregates, which have a porous structure and the ability to absorb pollutants. The use of natural sorbents, which are characterised by low toxicity and biodegradability, is in line with the idea of sustainable development. In ecotoxicological studies, the effects of calcium and magnesium sorbent extracts on two plant species *A. sativa L.* and *L. sativum*, and earthworms *E. andrei*, were evaluated in acute toxicity tests. The conclusions of the studies indicate that calcium and magnesium sorbents do not pose a threat to the organisms tested with a short exposure time, which allows their use in industry and environmental protection. Further research on natural sorbents is recommended, as it is necessary to understand the long-term effects of these sorbents. Their effects may also depend on the specific environment conditions in which they are used, suggesting the need for further research on the effects of sorbents on organisms under different conditions, such as in soil, and the use of other test organisms.

Keywords: *Avena sativa L.*, *Lepidum sativum*, *Eisenia andrei,* waste management, ecotoxicology.

INTRODUCTION

Human population growth, as well as the development of industry and new technologies, contribute to the rapid growth of production and consumption, which is directly linked to the overconsumption of natural resources and the generation of increasing amounts of waste (Mir et al., 2022). This is one of the reasons why ensuring sustainable production and consumption patterns is one of the key sustainable development goals set out in the UN 2030 Agenda (Ararat, 2020; Henriksen et al., 2021; Walsh et al., 2022). This is important in the context of reducing environmental degradation and natural resource depletion (Fonseca et al., 2020).

Waste is any material, substance or other object that a person has deemed unnecessary and has subsequently discarded or intends to discard (Journal of Laws, 2013). Each person in the world produces an average of 0.74 kg of waste per day, although this figure can range from 0.11 kg to 4.54 kg depending on the level of urbanisation and income (Kaza et al., 2018; Eurostat). Including all types of waste, the global total was estimated at around 20 billion tons in 2017 and is expected to increase to 46 billion tons by 2050 (Maalouf and Mavropoulos, 2023). According to the report "Environmental Protection 2024" published by the Central Statistical Office (CSO, 2024), in 2023 Poland produced 122.8 million tons of waste, of which 13.4 million tons, or about 11%, was municipal waste. Unfortunately, waste management is the most neglected environmental sector in Poland, as in 2010, for example, more than 97.5% of municipal waste was landfilled (Turlej and Banaś, 2017; Ciuła et al., 2023). However, the situation is improving over the years as, according to the

Central Statistical Office, this figure fell to 38.7% of waste per year in 2023 (CSO, 2024).

The Act on Waste in Poland of 14 December 2012 (Journal of Laws, 2013) does not directly define the term ‘industrial waste’ as a separate category. On the other hand, it classifies waste according to its source and type into categories such as: waste from production processes, waste from raw material processing, hazardous waste, which may also originate from industrial activities such as manufacturing, mining and milling. These include: oils, slag and ash, mineral wastes, packaging and metal wastes (Samuelson, 2009; Kareem et al., 2023). Wastes from mining and industrial activities can be used as sorbents. The use of natural sorbents in industrial applications is a recommended approach as part of the green transition (Smol and Włóka, 2022).

Sorbents are materials characterised by a porous structure and are widely used to absorb and retain liquids such as oils, acids, bases, petroleum products, water or other liquids. They absorb hazardous liquids through the processes of adsorption, i.e. retaining liquid molecules on the surface, and absorption, i.e. absorbing these molecules into the interior of the material with which they come into contact (Willis, 2009). Currently, with growing environmental awareness and stricter environmental regulations, sorbents are being used, for example, in environmental remediation (Singh and Ambika, 2018; Islam et al., 2018; Beljin et al., 2024; Osman et al., 2024) and in various industries (Sabdo, 2006). Sorbents are divided into three groups based on their composition and origin: organic (of plant origin, e.g. straw or wood sawdust), inorganic (of mineral origin, e.g. sand, aggregates, peat, organic wool or glass wool) and chemical (produced under laboratory conditions) (Crini, 2006; Lonin et al., 2021).Their effectiveness depends on their physical structure, pore size, surface chemistry and the nature of functional groups (Beaugeard et al., 2020).

Sorbents, i.e. materials used to absorb and neutralise pollutants, play an extremely important role in waste management, especially for industrial waste (Lartey-Young and Ma, 2020; Lima et al., 2022). After use, sorbents that have absorbed hazardous substances become waste that can pose a threat to the environment and human life. Used sorbents are classified as hazardous waste under the Minister of the Environment’s Regulation of 2 January 2020, which means that they cannot be disposed of in standard waste containers or left in the environment (Journal of Laws, 2020).

Sorbents, if properly selected and used under the right conditions, should be safe for the environment when used, for example, in environmental remediation and pollution control, but due to their different compositions they may have different effects on organisms. Some, at high concentrations, can inhibit the growth of certain soil microorganisms and affect biofilm formation (Ogbudu et al., 2024). Natural sorbents, characterised by low toxicity, high biodegradability and availability from renewable sources, provide a sustainable and effective solution. Their use helps to minimise environmental impact compared to synthetic sorbents (Godage and Gionfriddo, 2020). Sorbents such as silica gel and various clays can mitigate the inhibitory effects of cationic surfactants on microorganisms, thereby enhancing biodegradation processes. This suggests that sorbents can play a role in reducing the toxicity of certain chemicals, thereby supporting microbial activity (Timmer et al., 2020). The overall environmental impact of sorbents varies depending on their composition and the specific contaminants with which they interact. Some sorbents may have minimal toxicity, while others may significantly alter the bioavailability and toxicity of contaminants (Jonker and Mourik, 2014).

Many different mineral sorbents with different chemical compositions are used in industry and their effect on the functioning of organisms is not fully understood. In this context, the aim of this work is to evaluate the effect of Ca and Mg sorbents on the germination and growth of *Avena sativa L.* and *Lepidium sativum* as model organisms in ecotoxicological studies. The response of *Eisenia andrei* earthworms in contact with these sorbents was also investigated.

MATERIAL AND METHODS

Water extracts

Ecotoxicological tests were carried out on waste material in the form of a mineral raw material that could be used, for example, as a sorbent in the treatment of industrial waste water. Two types of raw material were chosen: ‘calcium sorbent’ and ‘magnesium sorbent’, classified according to the dominant content of each element. The 'raw' calcium sorbent is mainly characterised by its content of Ca – 24.30 m/m%, Si – 15.18 m/m%, Fe – 2.61 m/m%. After thermal treatment, the calcium sorbent contains, among others, Ca – 21.67 m/m%, Si – 16.48 m/m% and Fe – 3.64 m/m%. In contrast, the 'raw' magnesium sorbent contains, among others, Mg – 23.65 m/m%, Si – 4.37 m/m%, Ca – 4.36 m/m% and Fe – 3.96 m/m%. The variants tested differed in the activation method (CaAO – activated at 900 °C for 1.5 h and stored for 2 years, CaNO – non–activated sorbent stored for 2 years, CaAF – sorbent immediately after activation at 900 °C for 1.5 h) or in the degree of fragmentation of the mineral raw material (Mg > 5 mm, Mg > 1.5 mm, Mg > 1 mm, Mg < 1 mm). The experiment used samples from a company involved in the extraction and distribution of this type of raw material and the production of sorbents for wastewater treatment based on these materials. C4In is located in the Podkarpackie province. The calcium and magnesium sorbent produced by C4IN is used as a component in the synthesis of the final sorbent for the removal of biogenic substances.

Samples of mineral raw materials were filled with distilled water in a ratio of 1:10 and shaken for 24 h at a speed of 150 rpm. The sedimented suspensions were filtered by gravity through cellulose filters. The extracts thus prepared were subjected to tests.

Acute toxicity – plants

The experiment, which consisted in studying the germination strength and the early growth phase of roots and stems, was carried out using two test plants. In the first experiment, 20 *A. sativa L.* seeds were placed in Petri dishes lined with filter paper (*Watmann No. 1,* diameter 5 cm) and moistened with successive extract samples (15 ml). In the second experimental variant, 20 *L. sativum* seeds were placed in plastic boxes (allowing vertical plant growth) lined with cellulose lignin and moistened in the same way as before. For each experimental variant, the control group received distilled water. The seeds thus prepared were protected from excessive evaporation and placed in an incubator (25±1 °C, 72 h, 24D) (ISO 18763:2016). The samples were then transferred to a freezer (-18 °C, 24 h) to stop the process of further germination and growth. The experiment was carried out in five replicates. The germinated plants were then counted and, after scanning, measured using the *ImageJ* program with an accuracy of 1 mm (Figure 1).

Acute toxicity – animals

In the acute toxicity studies, earthworms of the species *E. andrei* were used according to the filter paper contact test (OECD, 1984). This method is commonly used to assess the toxicity of various chemicals to earthworms (e.g. Wang et al., 2012; Yang et al., 2015).

Figure 1. Determination of the length of roots and stems of *L. sativum* in the ImageJ programme

For the study, adult earthworms (*clitellum*) with similar body mass and average body condition were selected. Prior to exposure to the following concentrations of the tested extracts, the digestive tracts of the earthworms were cleaned by placing them on moist filter paper for 24 h. Individuals were placed on petri dishes containing moist filter paper discs (Watmann No. *1,* diameter 5 cm). Toxicity tests were carried out for 24 h under constant laboratory conditions (18 ± 2 °C, 24 D). Mortality of earthworms was checked after the specified time.

Statistical analysis

Statistica V.13.1 (StatSoft) was used for statistical calculations. Results were interpreted at a significance level of $\alpha = 0.05$. The non-parametric Kruskal-Wallis test was used for the corresponding analyses, followed by an appropriate post hoc test, as the data did not meet the assumptions for performing parametric tests (Shapiro-Wilk W test, Brown-Forsythe test).

RESULTS AND DISCUSSION

Due to the wide variety of materials that act as sorbents, many users find it very difficult to select a sorbent that will properly fulfil its role and have a very low, or at least acceptable, negative impact on the environment. Therefore, many studies are carried out to illustrate the effects of different types of sorbents on representatives of different species (Zetterberg et al., 2005). For example, bacteria, fungi, algae, but also invertebrates or higher plants are used in such studies (Godlewska-Żyłkiewicz, 2006; Fiset et al., 2008). The results of our preliminary studies on the effect of the tested extracts of calcium and magnesium sorbents on plants were presented as germination strength expressed as a percentage of two plant species commonly used in ecotoxicological research. Results were also obtained showing the effect of individual extract variants on root and stem growth in relation to the test plants analysed. The studies performed took into account the degree of activation for Ca sorbents and the different degree of fragmentation of the raw material for Mg sorbents. Results from other authors suggest that the fragmentation of the feedstock may be important. For example, in the study by Smol and Wólka (2022), Opoka rock was analysed to contain approximately 70% calcium carbonate ($CaCO_3$) and 26 to 27% silica (SiO_2) and was classified as heavy Opoka rock. The results showed that the P removal efficiency was 96.6% for the fine-grained sorbent (grain size < 2 mm) and 90.8% for the coarse-grained sorbent (2–4 mm) in the batch tests, while lower efficiencies were observed in the column tests, reaching 67.8% and 54% respectively. The N ($NH4+$) removal efficiency was 84% for both types of sorbents in the batch tests and 47.7% in the column tests. The use of materials of natural origin in industrial applications is a recommended direction, part of the green transition. The analysed samples of opoka rock came from deposits, and opoka rock has not been analysed for its possible use in water and wastewater treatment technology. Therefore, further research is recommended for this low-cost sorbent, which could be a competitive material for commercial products (Smol and Wólka, 2022).

Sorbents used for presented testes were selected for their unique physical and chemical properties, which are crucial for the synthesis of the final adsorbent for the adsorption of biogenic substances or heavy metals. Different fractions were used because of the desire to obtain a final material with a specific fraction, and the powdery fraction is a waste fraction after material processing. In order to utilise the entire material, the process is carried out on different fractions and the resulting adsorbents are also used in powder and granular form. The use of different temperatures and fractions allowed us to obtain starting materials with appropriate crystal structures and surface properties. These properties are essential for optimising the synthesis processes and increasing the efficiency of the final material. It was shown that the applied variants of water extracts from the tested mineral raw materials had little effect on the percentage of germinated seeds of the two plant species. Despite the fact that most *A. sativa* plants germinated as a result of contact with the CaAO extract, which was prepared using a calcium sorbent activated 2 years previously, while the least germinated seeds were observed after contact with the Mg<1mm extract, the differences were too small to be considered significant (Figure 2). When analysing the percentage of *L. sativum* germination, the highest number of germinated plants was observed in the case of CaNO and CaAF, i.e. unactivated and activated before the experiment, respectively. The least germinated seeds were observed for the Mg > 5 mm extract (Figure 2).

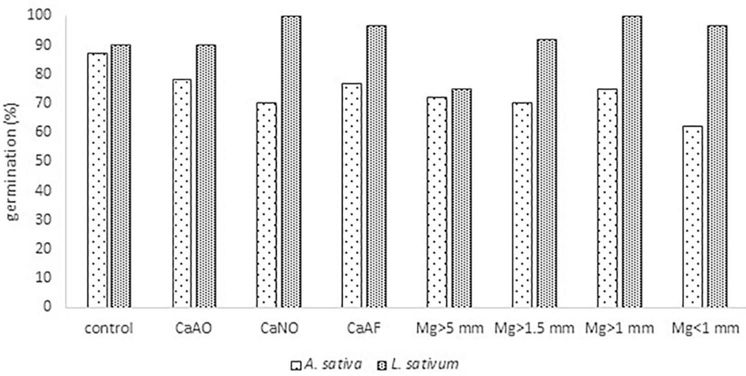

Figure 2. Germination strength of *A. sativa* and *L. sativum* seeds as a result of contact with different variants of extracts of Ca and Mg sorbents in comparison with the control group (%)

The proper mineral nutrition of plants depends on the correct ratio of nutrients extracted from the soil, classified among others as macroelements, i.e. elements present in quantities greater than 0.1% of the dry mass. In addition to calcium and magnesium, the most important elements for plants include nitrogen, potassium, phosphorus and sulphur (Chauhan et al., 2014; Saleem et al., 2022; Tariq et al., 2023). Plants take up macro elements in large quantities as it is necessary for their proper development and functioning, but both deficiency and excess of these elements can adversely affect germination, growth and development of plants as well as formation of root system (Gulzar et al., 2020; Saleem et al., 2022).

Abiotic stresses include a variety of environmental factors that prevent proper plant growth and limit crop productivity. Extensive research has been conducted to elucidate the mechanisms by which plants respond to these stresses. Calcium plays a crucial role in numerous plant pathways and has been shown to effectively mitigate stress by enhancing both resistance and tolerance (Feng et al., 2023). While magnesium is one of the most important nutrients involved in plant growth and development, both deficiency and excess of this element can negatively affect plants. Magnesium is the main component of chlorophyll and is involved in the activation of enzymes (Saleem et al., 2022). Stress responses include changes in the secretion and distribution of phytohormones such as abscisic acid (ABA) or auxins (Guo et al., 2015). The results obtained in this experiment confirm that excess magnesium in the solution has a negative effect on seed germination rate. However, this was not confirmed by our research results (Figure 2).

The influence of extracts of selected mineral raw materials was studied to determine the length of roots and stems of seedlings (Figure 3). No significant differences in root length were found for *A. sativa*. When the data for *L. sativum* were analysed, it was found that most of the extract variants used significantly reduced root length compared to the control group. Interestingly, studies on fenugreek (*Trigonella foenum-graecum*) have shown that an appropriate calcium/magnesium ratio in the nutrient solution has a positive effect on root length and other growth parameters (Begum et al., 2024). This suggests that maintaining a balance between these two elements is critical for optimal root system development (Salehi-Eskandari et al., 2018). In addition, the results of other ecotoxicological studies of sorbents confirm that plants are relatively more resistant to their effects than, for example, *Vibrio fisheri*, algae or zebrafish (Zetterberg et al., 2005).

Earthworms play a key role in the functioning of the soil ecosystem. By digging underground corridors, they contribute to soil aeration and facilitate water flow, which benefits plants (Clause et al., 2014; Schon et al., 2017). They also feed on dead organic matter in the soil, forming stable organic-mineral compounds that improve soil structure and provide nutrients for plants (Guhra et al., 2020). Earthworms prefer soils with higher pH, which are richer in calcium, which they then excrete in the form of calcium carbonate (Hodson et al., 2015). This is highly beneficial for many plants, as calcium carbonate improves the availability of macronutrients such as nitrogen, phosphorus and potassium, while reducing the availability of toxic metals (e.g. aluminium, manganese) (Dinkecha and Tsegaye, 2017). Research on the

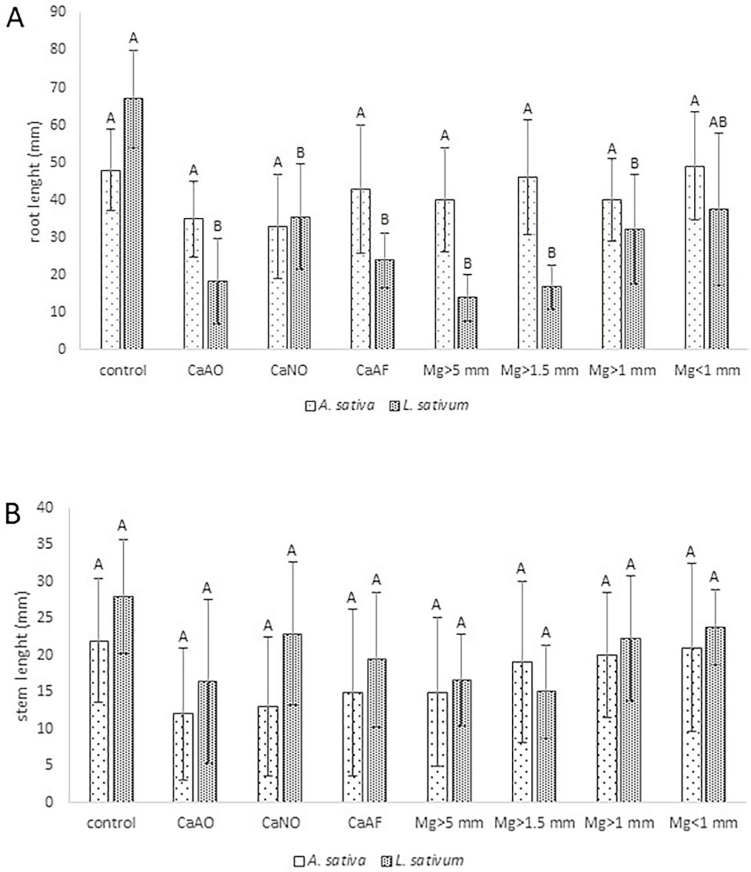

Figure 3. Root (A) and stem (B) length (mm) of *A. sativa* and *L. sativum* as a result of contact with the following variants of extracts of Ca and Mg sorbents compared to the control group (different letters indicate statistically significant differences compared to the control)

effects of sorbents on earthworms is extremely important, as under natural conditions there is an interaction between earthworms and sorbents. The simultaneous use of earthworms and sorbents can improve soil remediation processes. Due to their high activity and movement in the soil, earthworms can improve the distribution and effectiveness of sorbents, helping to reduce various types of pollution faster and more effectively (Zhao et al., 2016). The mucus they secrete, in combination with the microorganisms present, can interact with sorbents to form

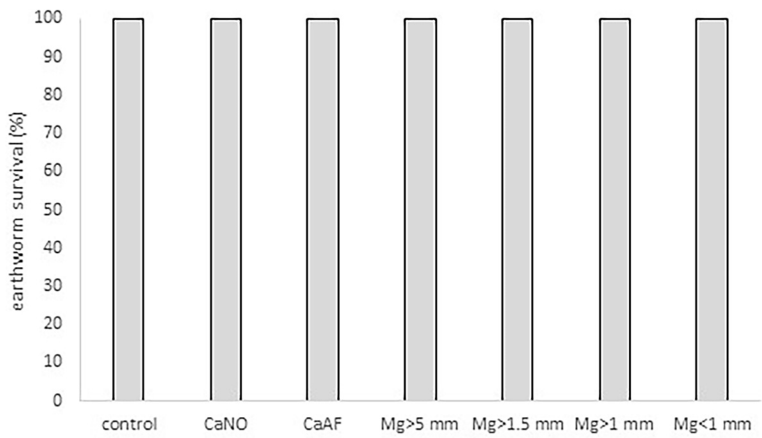

Figure 4. Survival of *E. andrei* earthworms (%) as a result of 24 h contact with the following Ca and Mg sorbent extract variants compared to control

stable complexes that bind pollutants more effectively, reducing their availability in the soil (Clause et al., 2014; Guhra et al., 2020).

The results of our tests indicate that the water extracts of Ca and Mg sorbents used did not have a negative effect on earthworms in the acute toxicity test using filter paper tests. Analysis of the survival rate of earthworms after contact with the tested variants of the extracts confirmed that all individuals survived the 24-hour experiment (Figure 4). The results confirm that the sorbent variants tested did not cause any adverse effects in the 24-hour acute toxicity test, even for the selected species. The studies certainly need to be extended to investigate the effects of the sorbents in combination with soil on earthworms over a longer period and under changing conditions.

CONCLUSIONS

Technological progress and economic development of the 20th century, apart from obvious advantages, led to environmental pollution, which we have to deal with. However, modern technologies using mineral sorbents represent an opportunity for dynamic economic development of Poland, increasing the competitiveness of domestic industry in the international arena and improving the condition of the natural environment. Based on the research conducted on the effect of calcium and magnesium sorbent extracts on selected test organisms, it was shown that the extracts used did not have a significant effect on the germination rate of *A. sativa* and *L. savitum* seeds. Although calcium and magnesium are important nutrients for plants, an excess of calcium and magnesium may have a negative effect on the germination rate of seeds, but this was not confirmed in the study. The results of the study on the effect of sorbents on earthworms suggest that the aqueous extracts did not have a negative effect on earthworms in the acute toxicity test, indicating that the sorbents did not cause harmful effects in the short term. Due to the short contact time of the tested extracts with the test organisms, it is necessary to extend the research to include chronic toxicity tests to assess the long-term effects of the elements contained in the sorbents on the test organisms. Their effects may also depend on the specific environment in which they are used, suggesting the need for further research on the effects of sorbents on organisms under different conditions, e.g. in soil, and the use of other test organisms. In addition, further research on naturally occurring sorbents is warranted in the context of their use in industrial activities, including wastewater treatment technology. The research confirmed that natural sorbents (Mg and Ca) are not toxic to test organisms, making them an excellent product for a wide range of applications.

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