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**Investigation of phytoremediation of soil contaminated with
toxic elements using model experiments**

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1. Introduction and objectives

Contamination by potentially toxic metallic species in soil is considered one of the most serious environmental problems that has been increasingly recognized in recent decades. Toxic metals are non-biodegradable like organic pollutants and remain in the environment for a long time. Soil is a fundamental resource, which provides direct support to agriculture, ecosystem, and the health of living organisms. However, when soil is contaminated, they not only act as a reservoir of toxic substances but also become a source of pollutants. When human activities such as mining, smelting, industrial production, inappropriate waste disposal, or overuse of fertilizers and agrochemicals can significantly increase the level of toxic substances when they contaminate the soils (Kumar et al. 2022). For example, mining activities usually release large amounts of cadmium and lead, while smelting operations are major sources of zinc and copper.

Cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) are the most common heavy metals that are especially dangerous. When these accumulate in soils, they destroy the fertility of the soil, damage agricultural productivity, and can eventually enter the food chain. Once in the food chain, they may cause serious risks to human health. As an example, cadmium and lead are known to cause neurological diseases and kidney damage, whereas copper and zinc are important micronutrients, but when consumed in large amounts, they are toxic. All these health effects are well-documented and cover both chronic exposure risks, bioaccumulation in a human body, and a long-term imbalance in the ecology. Therefore, not only is toxic metal contamination a dangerous threat to soil fertility, but it also poses a risk to food security and human health globally.

Over the years, different methods have been developed to address the problem of metal-contaminated soils, such as excavation, soil washing, chemical stabilization, and a range of physical or chemical treatments. Although these techniques can be effective in some cases, they are generally expensive and require significant energy input, and they are commonly linked to environmental side effects that risk causing secondary pollution (Xu et al. 2024). For example, excavation results in the permanent loss of cultivated land, while washing soil can lead to the loss of nutrients needed by the soil and lower its fertility. The toxic elements can be immobilized through chemical stabilization that can cause changes in the soil chemistry that reduce plant growth. Moreover, these technologies cannot be effectively applied over large areas due to high cost and logistical challenges. Therefore, it is necessary to find more sustainable, cost-effective, and eco friendly management methods.

Phytoremediation is one of the most common techniques that uses plants to absorb, accumulate, transform, or reduce heavy metals and toxins in soil to less harmful forms (Priya et al. 2023). Some of these plants have the special ability to accumulate high levels of heavy metals without significantly affecting their growth. These plants, often referred to as hyperaccumulators, have been studied in contaminated soil remediation research. The main mechanisms of phytoremediation include phytoextraction, where the metals are absorbed and accumulated in the plant tissues; the phytostabilization whereby the metals are immobilized in the rhizosphere and no longer spread; and phytovolatilization where some of the elements are converted into volatile substances which are released into the leaves of the plant. For example, plant extraction has been found to be effective in removing cadmium in contaminated soils by collecting the aboveground biomass that accumulates the metal. The benefits of using plants in remediation consist of the low cost, broad coverage, limited soil destruction, and landscape and environmental value improvement.

Brassica juncea, or Indian mustard, is one of the most suitable plants to use in remediation of polluted soils among the species analyzed. This species is a fast-germinating plant that grows successfully in a wide range of conditions, has an extensive root system and can accumulate heavy metals that include copper, cadmium, zinc, and lead, among others, at rather high concentrations (Awa and Hadibarata 2020; Małecka et al. 2021). Moreover, *Brassica juncea* is a simple to cultivate and maintain plant, which has become a useful model plant for both laboratory-scale studies and applied phytoremediation trials.

To evaluate the effectiveness of this plant, the study of phytoremediation usually includes the use of bioassays that measure the impact of contaminants with the help of living organisms. In this method, involved is the observation of the germination, growth, and reaction of plants to heavy metal contaminated soil (Kaur et al. 2019). A model experiment, also known as pot experiment, was constructed in this study where several factors such as soil type, contamination level, and species of plant were clearly controlled. Using bioassays in this experiment does not only indicate the true impact on living organisms, but also allows for the analysis of *Brassica juncea*'s ability to accumulate and absorb heavy metals under different conditions.

This study is therefore of both academic and practical significance. In addition to understanding the germination, growth, and heavy metal accumulation behavior of *Brassica juncea* under bioassays, the data obtained can be used to determine the plant's potential for sustainable and environmentally friendly soil remediation. The results of this study may provide guidelines for

environmental management in areas affected by mining or other industrial activities and may also serve as a base for further research in the future.

The following research tasks were done to accomplish the main objective:

- The effects of toxic element contamination on the germination and growth of *Brassica juncea* were studied using a bioassay in a model experiment.
- Heavy metals uptake and accumulation (Cd, Cu, Pb, Zn) in *Brassica juncea* was determined in the shoot tissues of plant.
- To evaluate the potential of *Brassica juncea* as a model plant to be used in phytoremediation of heavy metal – contaminated soil.

2. Literature review

2.1 Heavy metal contamination in soil

Soil is an important natural resource that is important for agriculture, ecosystems and food security. However, during the past decades, heavy metal pollution of soils has become a major environmental problem worldwide. These metals are non-biodegradable and remain in the environment for long periods (Alloway 2013; Angon et al. 2024). Contaminated soils affect both ecosystems and human health because they reduce fertility and productivity, act as reservoirs and resources of pollutants, and allow toxic substances to enter food chains (Alengebawy et al. 2021; Jaishankar et al. 2014).

2.1.1 Sources of heavy metals

Heavy metals enter the environment from two different sources, including natural (geological) and anthropogenic sources (Angon et al. 2024).

Natural sources of heavy metals include volcanic eruptions and weathering of rocks, which cause elements like cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) to spread in the environment. Although the contribution from natural resources is relatively low, their accumulation over time can affect soil quality (Angon et al. 2024; Wuana and Okieimen 2011).

Anthropogenic sources human activities are considered the main cause of heavy metal contamination in soils include mining and smelting activities, industrial emissions, fossil fuel burning, and the use of phosphate fertilizers and metallic pesticides in agriculture (Angon et al. 2024; Briffa et al. 2020).

These (potentially toxic) metals are emitted during mining and extraction of various elements from their ores. Heavy metals that are emitted into the atmosphere during mining, smelting, and other industrial activities return to the land surface through dry and wet deposition. The release of wastewaters including industrial effluents and household sewage add heavy metals to the environment (Wuana and Okieimen 2011). The use of chemical fertilizers and burning of fossil fuels also contribute to the anthropogenic input of heavy metals in the environment. Phosphate fertilizers are particularly important because they often contain higher quantities of heavy metals (Wuana and Okieimen 2011). Wastes are the main source of soil contamination originating from mining, chemical, metal processing industries. These wastes contain various substances like heavy metals, phenols and other organics, non-metals, etc. (Asati et al. 2016).

2.1.2 Characteristics of heavy metals in soil

Heavy metals in soil have several properties that make them highly persistent, difficult to remediate, and harmful to the environment. The first and perhaps most important characteristic of heavy metals is their persistence. Heavy metals are not biodegradable either by biological, chemical, or physical processes to produce harmless products. When these elements have entered the soils, they may remain for decades or even centuries, accumulating in soil particles or sediment layers and causing long-term environmental and human health impacts (Alloway 2013; Tchounwou et al. 2012). Another important property is mobility, which depends on the physical and chemical conditions of the soils. Factors including pH, redox potential, soil texture, and cation exchange capacity determine whether the metals will remain bound to soil particles or be released into soil solution, where plants can absorb them. As an example, cadmium (Cd) and lead (Pb) are more soluble in acid soils because the solubility of these elements increases under low pH conditions. In comparison, neutral or alkaline types of soil tend to fix metals and lead to mineral precipitation or their complexation (Alloway 2013; De Matos et al. 2001).

Interactions with soil organic matter are another important property. The organic matter in the soil, like humus, can fix heavy metals and reduce their short-term toxicity. However, they may release the metals again during their decomposition in a more soluble and toxic form (Asati et al. 2016; Lasota et al. 2020). Previous studies have also found that heavy metal accumulation is related to the amount of organic matter and organic carbon in the soil, which determines the soil's ion sorption and exchange capacity (Jaworska and Lemanowicz 2019).

The bioaccumulation and biomagnification potential of heavy metals is another characteristic that causes serious concern. After being absorbed by plants, the metals may be accumulated in edible tissues and can be transferred to animals and humans through food consumption. Long-term accumulation results in increased metal concentrations in organisms higher up the food chain, such as herbivores and humans, which can cause health impacts even at low concentrations (Ali et al. 2019; Nnaji et al. 2023). Small amounts of contamination may cause changes in the balance of soil microorganisms, as well as inactivate enzymes in the cycle of nutrients, including nitrogen and phosphorus, which would result in the loss of soil fertility and the capacity to facilitate plant growth (Tchounwou et al. 2012). These changes reduce the quality of soil and affect large-scale agricultural ecosystems.

2.1.3 Environmental and health effect

Heavy metal contamination in soil has severe impacts on the environment and health of human beings. These impacts are caused by the basic properties of heavy metals, which are persistence, toxicity, and bioaccumulation capacity. After being released into the environment, they are more likely to stay in the environment over decades and have cumulative impacts, which include the endangerment of soil quality, agricultural systems, aquatic life and health in the population (Alloway 2013; Jaishankar et al. 2014).

Impacts on soil and ecosystems

Reduction in the fertility of the soil is one of the major environmental impacts of heavy metal pollution. Heavy metals like cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) disrupt the natural cycling of essential nutrients by attaching to soil particles and replacing the useful cations, like calcium and magnesium, which inhibit the growth of plants (Ali et al. 2019). Moreover, the existence of high levels of heavy metals concentrations reduces soil microorganisms and reduces the activity of enzymes in the cycling nutrients including nitrogen and phosphorus, which result in soil degradation and low agricultural production (Jaworska and Lemanowicz 2019; Tchounwou et al. 2012).

Apart from directly degrading soil fertility, heavy metals can also migrate through leaching and runoff can also help the spread of heavy metals to water bodies outside agricultural lands. The washed metals in polluted soils might end up in rivers, lakes, and sediments, where they survive and accumulate in the bodies of water. This contamination reduces biodiversity, damages fish populations, and disruption in the operations of ecosystems, including primary production (Jaishankar et al. 2014).

Impacts on plants and agriculture

From an agricultural perspective, heavy metal contamination results in low crop quality and productivity. Metals like cadmium (Cd) and lead (Pb) may prevent seed germination, root growth and chlorophyll synthesis, thereby affecting photosynthesis and overall growth (Asati et al. 2016; Benavides et al. 2005). When concentrations of heavy metal increase, plants experience oxidative stress, cell membrane damage, and metabolic failure.

Although some plants can survive, heavy metals often accumulate in edible parts, including leaves and seeds that can present a direct threat to the consumer (Mawari et al., 2022; Nagajyoti et al., 2010). In Asian region, such as in China and Japan, rice is a high-cadmium accumulator and a key pathway of entry of the heavy metals into the human food chain. This contamination

is not only affecting food security but also the economic aspects related to it, as food produced in contaminated areas is often rejected in the world market (He et al. 2013; Uraguchi and Fujiwara 2012).

Human health impacts

Heavy metals cause serious impacts on human health, even at low concentrations. For example, cadmium (Cd), which is linked to chronic kidney disease, bone resorption, and, as a notable case study, Itai-itai disease in Japan caused by cadmium accumulation over a long period (Nordberg et al. 2015; WHO 2019). Another extremely dangerous metal, especially among children, is lead (Pb), which may lead to neurological disorders, learning disabilities, and behavioral issues, even in tiny amounts. Accumulated lead exposure among adults increases cardiovascular disease and high blood pressure (WHO 2021).

Copper (Cu) and zinc (Zn) are crucial micronutrients that are necessary in many enzyme processes in humans. They are, however, toxic when consumed in large quantities. Both excessive zinc and excess copper levels can cause gastrointestinal distress, impaired immune response, and copper metabolism interference (NIH ODS 2022a; NIH ODS 2022b), whereas too much copper can result in liver damage and neurotoxicity (ATSDR 2024). Copper (Cu) and zinc (Zn) are difficult to regulate in soil and food due to two distinct roles; they are vital nutrients and simultaneously toxicants.

Bioaccumulation and food chain risks

Another important aspect is the bioaccumulation and biomagnification of heavy metals within food chains. After entering the agricultural systems, they can be transferred to herbivores, carnivores, and eventually, humans. For example, mercury and cadmium can accumulate in fish and aquatic animals, which may affect communities that eat fish as their main protein source (Noman et al. 2022; Ray and Vashishth 2024). This is at the level of the food chain, which is why comparatively low environmental concentrations of the same may lead to severe damage to human beings (Ray and Vashishth 2024).

Public health and socioeconomic consequences

Beyond direct health impacts, heavy metal contamination also has broader impacts on public health and socioeconomic factors. Some of the communities living close to the mining regions, smelting stations, or a highly industrialized area are usually at increased risks of exposure by way of polluted water, food, and soils. Prolonged exposure not only leads to medical burdens but also low labor productivity and high healthcare expenses. According to the World Health

Organization, its exposure to cadmium and lead is among the highest global health priorities because of their widespread occurrence and severe effects (WHO 2019; WHO 2021). In addition, polluted farms become economically worthless, which may lead to the loss of livelihoods in rural areas and impact the national food chains.

2.2 Conventional method for soil remediation

The process of remediation of heavy metal contaminated soils has long been based on engineering-related strategies, which are aimed at either physically eliminating the contaminated soils, stabilizing the pollutants chemically, or isolating the contaminated zones of the soils from further interaction with the environment. The practices are commonly known as traditional remediation practices. Although they have been successfully implemented in most polluted sites across the world, their sustainability and applicability are low because they are very expensive and energy-consuming, and there is also a risk of secondary environmental effects.

2.2.1 Physical methods

Physical methods are the most direct remediation methods, which aim at removing or isolating the contaminated soils to eliminate immediate risks. These techniques are widely considered as engineered solutions due to the fact that they are based on heavy machinery, transport logistics, and engineered disposal systems. Excavation, landfilling, and soil replacement are the most common methods of remediation and are the main components of traditional remediation projects (Liu et al. 2018; Song et al. 2022).

Excavation is the most common technique, which is also known as the “dig-and-haul” method. In this method, the contaminated soil is mechanically removed from the site and transported to an off-site treatment or disposal facility. Excavation is particularly popular in emergency situations or localized and shallow contamination. However, excavation can be prohibitively costly. A recent review pointed out that the costs increase significantly with depth of contamination, the volume of soil, and the distances of transport and often cost more than several hundred dollars per ton (Ahmed et al. 2022a). Moreover, excavation causes secondary impacts such as dust, noise, and fuel emissions during soil handling and transport, which causes both environmental and health problems (Sánchez-Castro et al. 2023).

Once contaminated soils are excavated, they are often treated under landfill conditions, where they are disposed of in engineered cells that are lined, have leachate gathering systems, and have gas control technologies. Landfilling separates the contaminants from the original location, mitigating the dangers of immediate exposure. However, this method only moves contaminants around instead of getting rid of them, which creates long-term problems with monitoring landfills and possible leachate migration (Ali et al. 2019; Liu et al. 2018). Sustainability is another problem that is associated with reliance on landfill space, because in most areas, there is not a lot of disposal space for hazardous soils. In addition, regulatory requirements for engineered containment significantly increase the cost of this option.

A related technique is soil replacement (backfilling), which is often combined with excavation. Once some contaminated material has been removed, the space in between is filled up with clean or treated soil to regain the physical structure and usability of the site. This practice makes it possible to use land for agricultural, construction, or recreational use. However, the ecological effects of disturbance of natural soil horizons and microbial communities can have long-lasting effects, and the quality of the clean soil that is used to backfill must be of high quality to avoid recontamination (Song et al. 2022). Also, the procurement and distribution of the mass of the replacement soil also contribute to another cost aspect of the remediation process.

Physically, excavation, landfilling, and soil replacement are simple and effective and ensure immediate removal of contaminated material. However, they are most appropriate in small-scale or hotspot remediation projects as they are very expensive, logistically complex, and disruptive to the ecology (Sánchez-Castro et al. 2023; Xu et al. 2024). Physical methods are usually considered unworkable for achieving high-scale contamination, so researchers suggest combining them with other technologies, including chemical or biological treatment methods, to enhance cost-effectiveness and sustainability.

2.2.2 Chemical methods

Chemical processes used in soil remediation are dependent on the use of reagents to transform, immobilize, or extract contaminants. Unlike physical techniques, where the contaminated soils are simply removed, chemical techniques aim to decrease the mobility, toxicity, or concentration of the contaminants in the soil matrix. Soil washing, chemical stabilization, and immobilization are the most widely used chemical techniques (Liu et al. 2018; Xu et al. 2024). These strategies are particularly relevant for heavy metals, which cannot be biodegraded and have to be immobilized or removed.

One of the most developed ex situ chemical treatments is soil washing. Excavated contaminated soils are treated with aqueous solutions, which are frequently water containing additives such as surfactants, acids, or chelating agents. These solutions desorb or dissolve heavy metals from soil particles. The pollutants are then moved to the liquid phase, which is separable and treated more (Zhang et al. 2022). Soil washing has demonstrated that the metals cadmium (Cd), lead (Pb), zinc (Zn), and copper (Cu) are greatly removed during soil washing, particularly in sandy or loamy soils where metals are weakly sorbed (Xu et al. 2024). However, its effectiveness declines in soils having high cation exchange capacity, and the production of polluted wastewater is another problem (Zhang et al. 2022).

Another effective method is chemical stabilization whereby soil is fixed with chemicals like lime, phosphates, biochar or cementitious binders. These additives combine with the heavy metals to precipitate insoluble, or increase sorption to the stable mineral surfaces, causing the reaction to lower leachability and bioavailability (Priya et al. 2023). Stabilization can be conducted in situ, which causes minimal soil disturbance and low operating expenses relative to excavation. It has been widely used at mine-affected sites with high cadmium or lead levels. Stabilization, however, does not eliminate contaminants but, rather, is based on the maintenance of good redox and pH levels; changes in the soil chemistry over time may potentially remobilize the metals (Xu et al. 2024).

Immobilization is more closely related and involves applying binding material like fly ash, clay minerals, or cement to entrap contaminants in a lower mobility material. This method is especially useful in the prevention of leaching into ground water and has been applied to rehabilitate the old industrial and smelting premises (Santos et al. 2025). Geotechnical stability is also enhanced by immobilization and is useful for construction purposes. However, similar to stabilization, immobilization does not remove the contaminants and may also change the soil structure, restricting its use as an agricultural soil.

2.2.3 Limitations of conventional methods

Economic cost is one of the most significant disadvantages of the traditional remediation. Physical techniques like excavation and landfilling involve heavy machinery, labor, transport logistics and engineered landfill facilities. The cost of excavation can be range from USD 150 to 450 per ton of soil and varies based on the depth, the level of contamination, and the distance to the disposal locations (Sharma et al. 2018). Soil washing, while effective, also requires large

amounts of additives such as chelating agents, surfactants, or acids, which increase operating costs and require further wastewater treatment. These expenses make conventional remediation only possible for small, isolated hotspots but impossible for large-scale areas that are polluted, like mining areas or industrial areas (Dermont et al. 2008).

The other significant limitation is the risk of secondary pollution. The result of soil washing is wastewater streams that are dissolved in metals (e.g., cadmium, lead and zinc) and in surfactants which are left behind as well as chelating agents such as EDTA, which further persist in the environment and are difficult to treat (Tandy et al. 2004). If not managed properly, this wastewater may lead to the pollution of nearby water sources, transferring the issue of the soil to the waterways. Similarly, chemical stabilization does not eliminate pollutants, only minimizes bioavailability; when the pH or redox potential varies with time, other metals like lead or cadmium may be mobilized, which will reduce the efficiency of the treatment over the long term (Sharma et al. 2018).

Conventional methods are also characterized by high energy consumption and material requirements thereby compromising their sustainability. Excavation involves using a lot of fuel to operate the machinery and transport it. The stabilization of chemicals and immobilization depend on lime, cement, and fly ash. As an example, massive stabilization works can use thousands of tons of cementitious binders, which are also a source of greenhouse gas emissions and additional environmental pollution (Ahmed et al. 2022b). Besides, manufacturing and consumption of these additives are linked to drainage of resources and energy resources intensive activities which augment the total environmental footprint of a remediation project.

2.3 Phytoremediation as a green economy

2.3.1 Definition and principles of phytoremediation

Phytoremediation refers to the utilization of green plants and their interactions with the rhizosphere to reduce contamination of the environment in soils, sediments, and water. This method has become an ecologically friendly alternative to conventional practices, as it involves biology to control pollution instead of the use of mechanical or chemical treatment. The main principle is to use the natural capacity of plants to absorb, stabilize or detoxify chemical substances like heavy metals, pesticides and hydrocarbons to decrease their environmental and human health impacts (Ali et al. 2013; Awa and Hadibarata 2020).

The strategy integrates both the physiology of plants and soil chemistry with the physiological aspects of the roots as absorptive and reactive surfaces. Plants absorb soluble contaminants and nutrients, bring them to the upper biomass, and store or transform them into less toxic levels. Meanwhile, the rhizosphere, which is the thin layer of soil affected by root exudates, is extremely essential as it changes pH, releases organic acids, and supports the formation of microbial communities, promoting the transformation of contaminants (Yan et al. 2020). Therefore, phytoremediation is not only a plant-based ecological process but rather a plant-microbe association that enhances partnership that improves efficiency and resilience.

An important concept in phytoremediation is the presence of hyperaccumulator plants which are species that are naturally tolerant and accumulate abnormally high concentrations of heavy metals without being inhibited to grow. For example, *Brassica juncea* (Indian mustard) has been shown in experimental research studies to absorb lead, cadmium, copper, and zinc at levels that are many times higher than most other plant species (Kamal et al. 2023; Roychowdhury et al. 2017). Similarly, *Thlaspi caerulescens* (now *Noccaea caerulescens*) is a hyperaccumulator of zinc and cadmium, and *Pteris vittata* is a hyperaccumulator of arsenic. These plants demonstrate the concept of phytoextraction and can be used as examples for remediation studies (Ali et al. 2013).

The dual purpose of phytoremediation in remediation and ecosystem restoration is another principle of the phytoremediation. Planting on polluted soil can help prevent soil erosion, stabilization of sediments as well as assist in carbon sequestration. The phytoremediation process can also increase organic matter and biodiversity; as well as eliminate contaminants, unlike excavation and landfilling, which usually impairs the quality of the soil (Ashraf et al. 2019). This makes it a green technology that is in line with the sustainable development goals. Critically, phytoremediation is also inexpensive, and utilizes solar energy and natural plant mechanisms as its primary effective tools, so it can be applied in developing countries or contaminated areas of significant size and high volume where expensive engineering solutions are impractical (Awa and Hadibarata 2020).

However, the ability of phytoremediation is site-specific. It is determined by the type and level of contaminant, the pH of soil, texture, the redox potential and climate. As an example, acidic soils frequently enhance the solubility of cadmium and lead and, therefore, make them more bioavailable to plants, while alkaline soils tend to immobilize metals through precipitation. For example, some cases metal solubility is enhanced by adding an amendment like EDTA or biochar to allow plants to take up the metal (Beesley et al. 2011; Kamal et al. 2023). These

principles highlight the importance of the application of phytoremediation in combination with the soil management practices to maximize the results.

2.3.2 Mechanisms of phytoremediation

Phytoremediation depends on various processes in which plants react with contaminants found in the soil, sediments, or water. These are the processes that define how pollutants are stored, stabilized, changed or eliminated in the environment. The four primary processes are phytoextraction, phytostabilization, phytovolatilization and rhizofiltration, each of them has specific applications and limitations depending on the pollutant and site conditions.

Phytoextraction refers to the process by which plants take in the contaminants in soil or water through their root system and carry them to the aboveground biomass. This process works well when it comes to heavy metals like cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) that are able to build up in the stems and leaves (Kamal et al. 2023). The contaminated biomass can then be harvested and safely disposed or used to obtain valuable metals when accumulated. Hyperaccumulator plants such as *Brassica juncea* and *Noccaea caerulea* are widely studied on due to their high efficiency in phytoextraction. However, the process can be slow, which demands repeated growth-harvesting, and can be restricted to high contaminant levels on account of plant tolerance (Yan et al. 2020).

Phytostabilization refers to immobilization of pollutants in the rhizosphere, which does not allow the contaminants to migrate by leaching or erosion. Plants carry out this by releasing organic chemicals that adjust soil pH and redox potential, which makes contaminants less soluble and bioavailable. Root systems provide the soil with physical stabilization, and chemical modifications of the rhizosphere reduce the chances of metals being enter into groundwater or food chains. For example, some studies have established that plants like *Vetiveria zizanioides* and *Brassica juncea* would stabilize lead and cadmium in mining-contaminated soils and thereby significantly reduce the levels of leachate (Ashraf et al. 2019; Beesley et al. 2011). This method is particularly useful in locations where elimination of contaminants is not practical, but where reduction of the risks is urgent.

Phytovolatilization is one of the processes when plants absorb contaminants, transform them into volatile forms, and release them to the atmosphere through the process of transpiration. For example, selenium (Se) can be volatilised to dimethylselenide and mercury (Hg) could be reduced to less toxic gaseous forms. This is a process that reduces concentrations in soils but is

controversial by the fact that it introduces pollutants into the atmosphere. However, phytovolatilization can be used as a substitute method of reduction in contaminants in specific scenarios where excavation or stabilization is not practical (Awa and Hadibarata 2020).

Rhizofiltration is aimed at eliminating pollutants in water bodies by absorbing them by roots, adsorption, or precipitation. Plants with extensive root systems, such as sunflowers (*Helianthus annuus*), can be grown in hydroponic system or directly into the contaminated water bodies to collect metals such as lead, cadmium, and uranium (Ali et al. 2013). Saturated roots can be harvested and replaced to provide a low-cost wastewater treatment and mine drainage remediation alternative. But rhizofiltration is limited by the fact that harvested biomass should be managed properly to prevent secondary pollution.

2.3.3 Advantages and limitations of phytoremediation

Phytoremediation is often considered one of the most promising alternatives to conventional soil remediation techniques because of its low cost, environmental sustainability, and ecological benefits. Phytoremediation utilizes natural plant reactions with the power of the sun, unlike engineering-based methods, which require excavation, soil washing, or use chemicals to stabilize it, and doesn't need as many external inputs or equipment (Shen et al. 2022). For this reason, it is often referred to as an environmentally friendly solution that is particularly well suited for large, low-to-moderately contaminated sites where physical or chemical treatment would be too expensive or disruptive. The other significant benefit is the rehabilitative impact on the soil ecosystems. Excavation or landfilling generally degrade soil structure and reduce fertility, but phytoremediation can enhance soil quality by increasing organic matter, promoting microbial activity, and mitigating erosion (Fernández-Braña et al. 2023). It also has long-term benefits because it restores vegetation cover, which helps stabilize the environment and increase biodiversity.

Despite these benefits, phytoremediation has several limitations. Among the most serious disadvantages is its low rate of contaminant extraction. Since it requires plant development cycles, the process can take years or even decades to reach target cleanup levels, which is not the best option on the sites that urgently require intervention (Yan et al. 2020). Moreover, root depth and bioavailability of contaminants are limiting factors of phytoremediation. Most plant roots are shallow and cannot access pollutants located deeper in the soil profile. The other significant limitation is related to the treatment of contaminated biomass. Plants that were found to have high concentrations of heavy metals should be harvested and disposed in safe so that does not lead to secondary contamination. Uncontrolled burning or composting can lead to the

reintroduce of pollutants into the environment (Shen et al. 2022). Also, phytoremediation is very site-specific and relies on climate, soil chemistry, contaminant type, and plant tolerance. Overall, phytoremediation is a cost-effective and eco-friendly alternative to standard soil remediation techniques, supplemented by ecological advantages, including soil restoration and the possibility of resource recovery. However, its relative slowness, difficulties in biomass disposal, and location-specific performance indicate that it cannot entirely supplant engineering-based solutions. Rather, phytoremediation works best as a component of a combined remediation plan in which the ecological and economic advantages of phytoremediation are integrated with quicker traditional methods to achieve sustainability over the long term.

2.4 Brassica juncea as a Model Plant

2.4.1 Biological characteristics of Brassica juncea

Brassica juncea (Indian mustard) is a rapidly growing yearly plant belonging to the family Brassicaceae and has drawn interest in phytoremediation studies because of its versatility and heavy biomass generation. It germinates rapidly, often within three to five days, and establishes seedlings capable of tolerating a range of environmental stresses. This rapid establishment allows it to outcompete weeds and makes it suitable for remediation programs where multiple planting–harvest cycles are required within a season. Moreover, its short life cycle and ability to thrive under diverse climatic conditions make it one of the most practical candidates for field-scale remediation projects (Zeremski et al. 2021). The species is highly adaptable to different soil types and conditions, including soils contaminated with heavy metals. *B. juncea* can grow in soils with varying textures, ranging from sandy to loamy soils, and tolerates a broad pH range. Studies show that the plant can sustain growth even under acidic soils that increase the solubility of metals, as well as in slightly alkaline conditions where metals tend to precipitate. This tolerance is partly due to its physiological plasticity plants are able to adjust nutrient uptake pathways and root exudate production in response to external stressors. With this flexibility, *B. juncea* is able to colonize marginal or degraded soils where other conventional crops would not survive (Bortoloti and Baron 2022).

The root system of *B. juncea* is another key trait that supports its use in phytoremediation. Its roots are fibrous, deep, and highly branched, which provides large surface area contact with the soil. This structure promotes the absorption of nutrients and contaminants thus the accumulation of metals like cadmium (Cd), lead (Pb), and zinc (Zn). Importantly, the roots also release exudates including organic acids and enzymes that modify soil chemistry in the rhizosphere,

increasing the availability of certain metals for uptake. As an example, stress-induced citrate and malate exudation has been associated with enhanced soil particle-metal binding solubility (Małecka et al. 2019). At the same time, *B. juncea* roots are relatively tolerant to oxidative stress caused by metal exposure because of the activation of antioxidant enzyme systems such as peroxidase, superoxide dismutase, and catalase. These biochemical defenses assist in sustaining the condition of roots and supporting the growth of plants in severely polluted conditions.

Another important biological characteristic is its tolerance to metal stress and ability to sustain biomass production under contaminated conditions. *B. juncea* has continuously demonstrated improved germination and biomass yields than other leafy vegetables and oil seed crops in contaminated soils in comparative studies. (Roychowdhury et al. 2017) stated that *B. juncea* grown in soils with high levels of cadmium and lead germinated successfully and concentrated metals in shoots and roots many times more than did other species. (Kamal et al. 2023) have said that further demonstrated that even in chelate-assisted conditions, where plants are subjected to higher solubility of toxic metals, *B. juncea* maintained growth and continued to sequester large quantities of cadmium and copper in aboveground tissues. This stress resistance explains why the plant is believed to be a model species in experimental phytoremediation experiments.

In addition to physiological tolerance, *B. juncea* also benefits from symbiotic interactions with soil microorganisms. Rhizospheric microbes, particularly plant growth-promoting bacteria (PGPB), enhance its ability to establish in poor soils. For example, (Mendoza-Hernández et al. 2019) showed that inoculation with PGPB increased root length, chlorophyll content, and overall biomass of *B. juncea* grown in mine tailings, which improved both plant survival and metal uptake. These interactions also stimulate soil enzyme activity, which contributes to nutrient cycling and remediation outcomes. The integration of microbial symbionts with *B. juncea* cultivation has therefore become a common approach to improve remediation efficiency.

Finally, *B. juncea* holds significance because of its dual role as a phytoremediation plant and an agronomically valuable crop. It is already widely cultivated for its seeds, which are used as a source of mustard oil, condiments, and leafy vegetables. This dual use means that large-scale cultivation methods, breeding programs, and management practices are already available and can be adapted for remediation purposes. The familiarity of local farmers with this species reduces barriers to its application in contaminated sites compared to wild hyperaccumulators that lack agricultural relevance. Moreover, its widespread availability ensures that research

findings can be more easily scaled up for field application (Bortoloti and Baron 2022; Zeremski et al. 2021).

2.4.2 Metal uptake ability

The ability of *Brassica juncea* (Indian mustard) to absorb, translocate, and tolerate heavy metals has been one of the main reasons why it is extensively studied as a model plant for phytoremediation. *B. juncea* has shown a remarkable capacity to endure high content of toxic metals when compared to most conventional crops, but still able to produce biomass, which is essential to phytoextraction efficiency. This capacity is associated with three key processes: (i) very efficient uptake and transport pathways in roots, (ii) internal detoxification mechanisms, including chelation and vacuolar sequestration, and (iii) adaptive physiological processes, such as antioxidant enzyme activation. These factors make *B. juncea* one of the most adaptable hyperaccumulator species for soils that are polluted (Ashraf et al. 2019; Kamal et al. 2023).

Cadmium is considered one of the most hazardous metals because of its high soil mobility and strong toxicity to living organisms. *B. juncea* exhibits an efficient Cd uptake system that allows significant accumulation in both roots and shoots. Uptake often occurs through pathways that normally transport essential divalent cations like calcium and zinc, due to the ionic similarity of Cd^{2+} (Roychowdhury et al. 2017). Once absorbed, Cd is bound by phytochelatins and glutathione, which form stable Cd–thiol complexes and transport them into vacuoles. This compartmentalization prevents free Cd ions from disrupting enzyme activity and damaging cellular membranes. In addition to sequestration, *B. juncea* enhances its tolerance by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), which protect cells from oxidative stress. A number of greenhouse experiments have shown that *B. juncea* is capable of accumulating Cd contents of up to 100-150 mg/kg in shoots without affecting photosynthetic activity and growth (Ashraf et al. 2019). Soil supplements like EDTA and citric acid make Cd even more soluble and go into shoots, but too much Cd can be harmful and lower biomass (Kamal et al. 2023). The fact that *B. juncea* can both tolerate and take up Cd makes it extremely helpful for clearing up Cd in agricultural and industrial soils.

Lead contamination is widespread in soils near mining areas, smelting industries, and roadside environments. Pb is generally immobile in soils because of its strong binding to organic matter and clay particles. Despite this, *B. juncea* demonstrates a notable ability to mobilize Pb and accumulate it in both roots and shoots under certain conditions. Most of the Pb remains in the

roots, where it attaches to cell walls, pectins, and root exudates. This prevents it from moving around and protects the aerial tissues (Małecka et al. 2019). Studies have shown that the use of chelating chemicals, like EDTA or citric acid, significantly improves Pb solubility, leading to better transfer to shoots (Kamal et al. 2023). In hydroponic systems, stems, and leaves have been found to contain up to 30-40% of absorbed Pb, emphasizing the possibilities of using the plant to extract phytoaccessively under the ideal circumstances. The physiological effect of Pb stress is the accumulation of proline, which stabilizes the protein and membrane and an increase in antioxidant enzyme activity which counteracts the effect of Pb on oxidative stress. Roots also release organic acids like citrate and malate, which help dissolve Pb and make it easier for plants to take up (Ashraf et al. 2019). This combination of biochemical and physiological strategies underlines the adaptability of *B. juncea* in Pb-contaminated soils.

Zinc is an essential micronutrient involved in plant growth, unlike Cd and Pb, acting as a cofactor in numerous enzymes and transcription factors. However, too much Zn can be harmful to plants, causing chlorosis, stunted growth, and oxidative damage. *B. juncea* has been shown to be exceptionally tolerant to Zn toxicity due to various processes including vacuolar sequestration, organic ligand chelation (histidine, citrate), and xylem loading to shoots. Zn uptake is facilitated by ZIP (ZRT/IRT-like protein) family transporters, which control absorption and translocation according to the external availability of Zn (Roychowdhury et al. 2017). According to the experimental studies, the accumulation of Zn was more than 500 mg/kg in aerial samples, especially in the leaves without severe growth inhibition (Ashraf et al. 2019). Interestingly, because Zn and Cd compete for the same uptake channels, soils contaminated with both metals present a challenge for selective absorption. Nonetheless, *B. juncea* demonstrates flexible gene expression of metal transporters, allowing simultaneous uptake and detoxification. This adaptability makes it especially effective in areas with multi-metal contamination. Moreover, it has high Zn accumulation potential, which could be used in phytomining where the biomass harvest undergoes processing to extract metals of economic value.

Copper is another essential element required for electron transport, photosynthesis, and enzyme activity, but at high levels it can cause severe oxidative stress and inhibit root growth. *B. juncea* has shown effective Cu uptake and tolerance by sequestering Cu ions into vacuoles and binding them with phytochelatin and metallothioneins, which prevent the accumulation of free Cu^{2+} ions in the cytoplasm. The uptake of Cu is possible mainly via high-affinity copper transporters present in root membranes (Małecka et al. 2019). *B. juncea* increases the activity of enzymes like peroxidase (POD) and glutathione reductase (GR) under Cu stress and these

enzymes have a protective effect against reactive oxygen species produced by high amounts of Cu. According to the field and pot experiments, the accumulation of Cu in the roots and shoots was between 200-300 mg/kg without significant biomass losses (Ashraf et al. 2019). Like Pb and Cd, Cu uptake and translocation are significantly enhanced by chelating agents such as EDTA, which increase Cu solubility. However, similar to Cd, it is important to indicate the dose optimization because over-application of amendments can result in reduced growth (Kamal et al. 2023).

2.4.3 Previous studies on *B. juncea*

Numerous studies have been conducted on *Brassica juncea* to evaluate its potential as a model plant for phytoremediation of heavy metal-contaminated soils. Such investigations include laboratory pot experiments and field-scale tests, and continue to show that it can withstand, absorb and concentrate various metals including cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu). The earliest major study of *B. juncea* demonstrated that the species was able to accumulate large amounts of Cd and Pb in shoots without an apparent decline in biomass, which cemented its status as a potential hyperaccumulator. Roychowdhury et al. (2017) reported that the metals accumulated in leaves and stems of *B. juncea* were several times greater than the metals in most other non-accumulator plants produced in the same conditions, which shows that it is a suitable indicator of polluted soils.

Later experiments have focused on how soil amendments enhance the metal uptake efficiency. Kamal et al. (2023) studied the effect of ethylenediaminetetraacetic acid (EDTA) on *B. juncea* and found that while moderate application enhanced the uptake of Pb and Cd, excessive concentrations inhibited growth due to chelate-induced stress. This research has shown that the mobilization of metals to be taken up and the health of the plants is a delicate balance towards the success of phytoextraction of the plant.

Other researchers have evaluated *B. juncea* under multi-metal contamination. Ashraf et al. (2019) showed that the plant could take up Zn and Cu at the same time as Pb and Cd, which showed that it could handle oxidative stress caused by metals. They found that the defense mechanisms of the plant, including the activity of antioxidant enzymes, facilitated its survival in the presence of high levels of contamination. These results are significant important because many contaminated sites are polluted with mixtures of metals rather than a single contaminant, and plants used for phytoremediation must cope with this complexity.

More recent studies have focused on the mechanisms of uptake and tolerance in *Brassica juncea*. Shehzad et al. (2023) demonstrated that the species employs strategies such as vacuolar sequestration, chelation with organic acids, and activation of antioxidant enzymes to cope with heavy metal stress. These biochemical and physiological adaptations allow the plant not only to survive, but also to retain considerable biomass in the contaminated environment, which makes it particularly useful as a remediation agent.

Field-scale experiments have also supported laboratory results. Awa and Hadibarata (2020) observed that *B. juncea* grew quickly in the soils strongly polluted with the mining activities, lowered metal concentration in the upper soils after several growth cycles, and offered other environmental advantages such as soil stabilization and soil erosion control. These practical observations prove that the species is not only practical under controlled conditions, but also adaptable to a variety of environmental factors.

2.5 Bioassays for soil toxicity assessment

2.5.1 Concept of bioassays

Bioassays are biological tests which use living organisms to determine the toxicity, bioavailability and ecological hazards of soil, sediment and water contaminants. Unlike conventional chemical analyses, which only measure total concentrations, bioassays capture the biological responses of organisms to pollutants, providing more ecologically relevant information (Andreu-Sánchez et al. 2022). This contributes to their importance in the analysis of environmental risks, especially in polluted soils where pollutants are frequently found in complicated combinations.

The principle of bioassays is simple: samples of the environment are added to test organisms including microorganisms and plants as well as invertebrates are exposed to environmental samples under controlled conditions, and their biological responses are measured. Common endpoints include germination rates, root elongation, reproduction success, enzymatic activity, or mortality. These reactions are delicate ecosystem warning signals. For example, microbial enzyme assays can reveal functional disturbances in soil communities, while seed germination and root length tests are widely used to detect the phytotoxic effects of heavy metals (Kim et al. 2021). One of the major advantages of bioassays is their ability to account for bioavailability and mixture toxicity. Contaminated soils usually contain several pollutants, such as heavy metals, hydrocarbons, and pesticides, that interact in ways chemical analysis alone cannot predict. Bioassays reflect the combined toxicity of these mixtures, as organisms naturally

respond to the total stress present in the environment. This makes them more realistic indicators of ecological risk compared to analytical chemistry data (Mao et al. 2022).

Bioassays are also common with the monitoring of soil remediation projects. They detect the toxic hotspots before remediation. They are used during treatment and after remediation to determine whether the soils are back to their ecological functionality. Some bioassays have already been standardized by regulatory bodies, including the OECD, such as the seedling emergence and growth test, with recognition of their usefulness in environmental safety tests (OECD 2006). Finally, bioassays operate across different ecological levels. Microbial tests measure soil enzymatic activity and respiration, plant assays evaluate seed germination and growth, and invertebrate bioassays with species such as earthworms assess survival and reproduction. These tests have been combined to provide a complete view of soil health and this way remediation not only reduces contaminant levels but restores an ecological balance.

2.5.2 Seed germination and seedling growth test

Seed germination and early seedling growth bioassays are among the most widely used tools for assessing soil phytotoxicity because they are rapid, inexpensive, and sensitive to a broad range of contaminants. Seeds are subjected to actual field soils or water/soil extracts, at controlled conditions in these assays, and endpoints of germination percentage, time to germination, root (radicle) length, shoot (hypocotyl) length, and in some cases fresh/dry biomass are measured. These endpoints are sensitive to stress caused by heavy metals (e.g., Cd, Pb, Zn, Cu), agrochemicals, and mixed industrial pollutants, early and biologically significant indicating soil quality that complements chemical analyses (Andreu-Sánchez et al. 2022). One of the benefits of these plant-based bioassays is that they reflect bioavailability and not simply total metal content. The extent of the availability of a metal to seeds and roots is dependent on soil properties (pH, organic matter, texture) and any co-occurring pollutants. Root elongation may be the most sensitive endpoint because during the germination and early growth, roots are in close contact with the soil solution and has a way of accumulating sub-lethal toxicity when overall germination percentages appear normal. During germination and early growth, roots are in close contact with the soil solution causing root elongation is generally the most sensitive endpoint, showing sub-lethal poisoning even when total germination percentages look normal. Reviews of soil and freshwater bioassays regularly highlight seed germination and root development tests as primary instruments in ecological risk assessment and monitoring remediation success (Andreu-Sánchez et al. 2022; Fernández-Marcos 2024).

Species commonly used include *Lactuca sativa* (lettuce), *Brassica juncea* (Indian mustard), and cereals such as *Triticum aestivum* (wheat), because they germinate quickly, uniformly, and have a well-characterized sensitivity. Lettuce has been used as a benchmark: several researchers have reported effective, dose-dependent inhibition of germination and radicle growth by exposure to Cd, Pb, Zn, Cu and other metals in contaminated soils or soil extracts and has been a proven indicator of phytotoxicity. Recent open access literature once again supports the idea that germination and initial seedling development are the most metal sensitive phase due to the lack of a well-developed defense system, and, therefore, even low levels of bioavailable metals may inhibit root development and slow germination (Moğ et al. 2019; Parera et al. 2023). For contaminated-site management, seed germination bioassays are valuable before, during, and after remediation. Prior to intervention, they help locate “hot spots” of phytotoxicity and prioritize treatment. During remediation (e.g., stabilization, soil washing, or amendments), repeated assays quantify whether phytotoxicity is decreasing in step with reductions in bioavailable metals. After treatment, recovery of normal germination and root growth indicates that soils have regained basic ecological function and are more suitable for revegetation or agricultural use. This monitoring purpose of plant bioassays is underscored in recent syntheses of soil ecotoxicology, which integrate biological endpoints with chemical speciation information within integrated assessment frameworks (Andreu-Sánchez et al., 2022; Fernández-Marcos 2024).

The methodological control is temperature, moisture, light regime and exposure time (usually 3-7 days) and necessitate adequate controls (control soils without contamination) and replication to ensure statistical confidence. Effect levels are typically obtained by analyzing endpoints as percentages of inhibition compared to controls or concentration-response modeling (e.g., EC10/EC50 of root length). Since seed assays are susceptible to soil physical parameters (e.g., salinity or poor aeration), recent reviews suggest that seed bioassays should be coupled with other levels (microbial or invertebrate tests) in order to differentiate between chemical toxicity and physical confounders and to have a more comprehensive view of soil health (Andreu-Sánchez et al. 2022).

2.5.3 Applications of bioassays

Bioassays have become a cornerstone of environmental research because they provide direct evidence of biological effects of contaminants, complementing chemical analysis that only measures concentrations. Bioassays provide a more realistic estimate of ecological and health risks compared to purely analytical methods, which do not provide any measure of whether

pollutants are biologically active (Andreu-Sánchez et al. 2022). This is why they are so important in both academic research and practical soil risk assessment systems. One of the most common applications is in monitoring contaminated industrial and mining sites. Plant bioassays, including seed germination and root elongation tests, have been widely used to evaluate soil from smelters, abandoned mines, and e-waste recycling areas. Such findings highlight the ability of bioassays to detect residual toxicity overlooked by chemical assays.

Bioassays are also valuable in tracking the effectiveness of remediation technologies. Seed germination assays are used as a fast test in cases where soils have been chemically stabilized or washed, or the biochar amendments have been made to ensure that phytotoxicity has decreased in parallel with changes in extractable metal fractions. Recent reviews in ecotoxicology suggest incorporating seed-based bioassays into monitoring procedures due to their sensitivity, reproducibility, and capability to identify early enhancements in soil quality prior to observable vegetation recovery (Fernández-Marcos 2024). In addition to heavy metals, bioassays are now used to analyse complex contaminant mixtures (pesticides, petroleum hydrocarbons and microplastics). Under such conditions, chemical analysis frequently fails to reflect the synergies or antagonisms of mixtures of pollutants. Plant-based assays, and microbial bioassays, have been shown to indicate combined stressors on soil performance. As an example, recent studies with High Andean native species shown that seedling growth bioassays exhibited significant sensitivity to hydrocarbon residues, emphasizing the feasibility of utilizing indigenous or endemic plants as region-specific indicators (Parera et al. 2023).

One more use is in ecological risk assessment and policy formulation. Bioassays, as used in supplement to chemical monitoring of soil health, are becoming the recommendation of the international guidelines, including the European Union and the OECD. Bioassays are considered a crucial tool for validating whether remediation has restored soils to a condition that can sustain vegetation, agriculture, or ecological function (Andreu-Sánchez et al. 2022). They are indispensable in the context of science and regulation of the environment as they bring together soil chemistry, bioavailability, and biological response.

Overall, bioassays are not limited to theoretical use, but offer effective environmental management practices. They are applied to contaminated-site diagnosis, remediation monitoring, ecological risk assessment, and even policy frameworks. Connecting soil contamination to biological consequences that can be observed in the field, bioassays can be used to ensure that environmental studies are not concerned with abstract chemical values.

3. Material and method

3.1 Study site and soil collection

The soils used in the experiment were collected at a contaminated location in the city of Mátraszentimre in Hungary, which has a history of mining. Due to the long-term impact of mining, the soils in this area are characterized by a high level of metals, especially, cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn). These metals are usually present in polluted areas and they may influence the quality of soil or plant growth. This location was selected because the contaminated soil provides a good basis for studying the potential of *Brassica juncea* for phytoremediation.

Soil samples were collected from different points within the site in 2020. Soil B and Soil D were selected for this studies, which represent moderately and heavily contaminated soil (Table 1), respectively, based on the work of Opeña (2024). In each sampling point, surface soils were collected using soil auger from 0-25 cm depth. Soil samples were mixed thoroughly in each sampling point to make composite samples. Around 1 kg composite sample from each sampling point was taken and brought for laboratory analysis in the Hungarian University of Agriculture and Life Sciences, Institute of Environmental Sciences, Gödöllő, Hungary. The samples were air dried, ground and sieved in 2 mm pore opening sieve. Around 50 g soil sample was used for chemical analysis.

Table 1. Pseudototal element content of Soil B and D determined after H_2O_2/HNO_3 microwave digestion (Opeña 2024)

B	Cadmium (Cd)	below the limit of detection		
	Copper (Cu)	40.10	± 0.03	mg/kg
	Lead (Pb)	797	± 4	mg/kg
	Zinc (Zn)	162.6	± 0.4	mg/kg
D	Cadmium (Cd)	7.89	± 0.06	mg/kg
	Copper (Cu)	62.23	± 0.17	mg/kg
	Lead (Pb)	4349	± 10	mg/kg
	Zinc (Zn)	1239	± 1	mg/kg

The soils were stored in plastic containers with lids in a shaded place to protect them from moisture and sunlight. This helped preserve their characteristics until they were used for the experiment. By applying soil of an actual polluted area, one can get real conditions about the plant behavior under heavy metal stress and the effectiveness of the phytoremediation in such conditions.

3.2 Soil preparation and experimental setup

The contaminated soils were prepared and diluted on 1 July 2025, a couple of days before the start of the experiment. The aim of this step was to create growing media with different contamination levels so that the response of *Brassica juncea* could be tested under various soil conditions. For the dilution, quartz sand and mould (uncontaminated potting soil) were used. Quartz sand was selected because it is a chemically inert substance, and it contains no heavy metal, which makes it an appropriate diluting material and a control substance. Sand was applied to enhance the drainage and the structure of the soil and mould was used to retain moisture and facilitate germination of seeds. These clean materials were added to the contaminated soils in a weight ratio of 1:8, which mean one part of the contaminated soils was added to eight parts of the clean material. This dilution reduced the level of contamination and provided a better environment of germination of seeds and growth of plants.

The mixing was done manually in plastic containers and buckets. Each portion of contaminated soil was measured and combined with the correct amount of sand or mould. The soil and clean materials were mixed thoroughly by hand using plastic spoons. Soil B and Soil D were prepared in various tools and containers to avoid cross-contamination. After mixing, the soils were labeled according to the treatment type.

Figure 1. Preparation of soil and sand mixtures



Figure 2. Experimental pot setup with labeled



Two cultivars of *Brassica juncea*, Southern Giant Curled and Amsoi Swatow Thick Stem were used in this experiment to compare their germination and growth performances in the contamination conditions and under control conditions with the varying levels of contamination. The seeds were purchased from Valeyrac Exotic Kft. (Fót, Hungary). In the first experiment, a total of 36 plastic pots were used which were divided into six treatments: B-sand,

B-mould, D-sand, D-mould, control-sand and control-mould. Each treatment had 3 replicates and the pots were filled with about 10 g of the prepared soil mixture.

In order to study the uptake of elements by plants, a second experiment was set up, simpler experiment with the aim of producing sufficient plant material for analytical measurement. Based on the preliminary results, mould was not used in this experiment due to its heterogeneity and inconsistent performance. In this round, only quartz sand was used as the diluting medium and control substrate. Six pots were used with 20 g of soil (soil B or D mixed with sand, and sand control) in each pot. Twenty seeds of *Brassica juncea*, either the Southern Giant Curled or Amsoi Swatow were sown in each pot.

The pots were stored inside the house directly under indirect natural light and the room temperature maintained at approximately 22–25°C. Distilled water was added every morning, and excess water was allowed to drain freely beneath the pots to avoid mixing of leachates between treatments. The pots were grouped according to the treatment and placed together on a tray to keep the layout organized. Ambient temperature and relative humidity were also checked every day using a digital thermo-hygrometer to record microclimatic conditions for further analysis.

Figure 3. Experimental pots placed under indirect natural light inside the house with labeled treatments and daily watering with distilled water



3.3 Germination and harvesting

Germination was monitored on a daily basis after sowing to determine when the seeds began germination as well as the seedlings progress in the first stage. After a few days after sowing, the first seedlings appeared and most of the seeds germinated within the first week. This early period was important to see how the plants reacted to different soil treatments.

No additional nutrients or fertilizer were used. This allowed the plants to grow only with the nutrients already in the prepared soils. All seedlings that germinated in each pot were left to grow together, without removing any plants, to show the real germination rate in each treatment. The experiment was done in two rounds. The first experiment was started on 8. July and harvested on 24. July 2025 and the second experiment was started on 25. August and harvested on 23. September 2025. During the harvest, plants from each pot were carefully removed from the soil to avoid breaking or damaging the roots. The soil that stuck to the roots was removed gently by shaking it off or washing it away with small amount of distilled water.

The shoot and root lengths were measured with a simple ruler. In the first experiment, both the roots and shoots were measured. In the second experiment, we only measured and collected the shoots. The shoot length was measured between the tip and the base of the stem and the root length was measured between the base of the stem to the end of the main root. The number of germinated seeds in each pot was counted to calculate the germination rate. The general appearance of the plants, such as strength, color, and height, was also observed.

Figure 4. Brassica juncea seedlings during growth



The shoots and roots were separated and dried at 65°C for six hours, until the weight was stable. This was done to prepare dried plant samples for later heavy metal analysis.

3.4 Heavy metal analysis

The dried plant samples from the experiment were used to measure the heavy metal concentrations. The digestion of plant samples was performed using a microwave-assisted digestion system by Dr. Márk Horváth (Department of Environmental Analysis and Technology, Institute of Environmental Sciences, MATE Gödöllő). The samples were placed into special containers and digested in a microwave digestion system. This process helps break down the plant material completely so that the metals inside the plants can be measured.

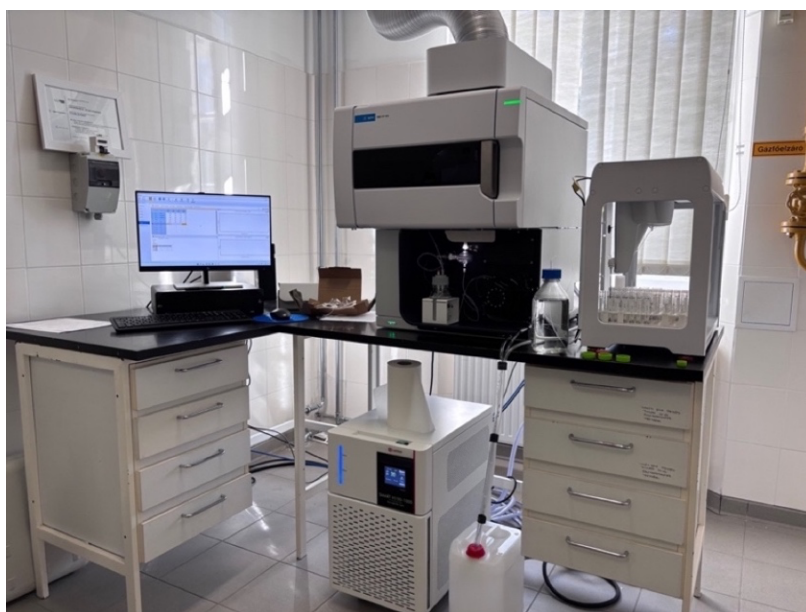
Instrument: CEM MARS5 microwave digestion system

Reagents: 5 mL 65 m/m% HNO₃ and 3 mL 30 m/m% H₂O₂

This protocol is used usually to digest 0.3000-0.4500 g air-dry sample, however, the plant samples only weighed 0.0084-0.0947 g in the second experiment.

After digestion, the solutions were filtered and filled up to 25 mL final volume with deionized water. Measurement of the concentration of cadmium (Cd), lead (Pb), copper (Cu), and zinc (Zn) was performed by Dr. Márk Horváth using ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) according to the standard method MSZ 21470-50:2006 (Figure 5).

Figure 5. ICP-OES (Agilent 5800) used in this study. Photo by Dr. Márk Horváth



The measurements were performed with an Agilent 5800 ICP-OES with CCD camera polychromatic imaging. The plasma power was 1300 W, the solution samples were introduced with a OneNeb nebulizer with cyclone cloud chamber. Flow rate for plasma gas was approximately 16 l/min, sheath gas 0.3 L/min, sample gas pressure was 2.7 bar.

This analysis helped to understand how much heavy metal was taken up by the plants grown in different soil treatments.

The capacity to remove potential toxic elements by plants can be characterized by the bioconcentration factor (BCF) which is a measure of ratio between the concentration of elements accumulated in the whole plant or plant parts over the concentration in the contaminated soil (Zayed et al. 1998). It is calculated using the following formula:

$$BCF = \frac{\text{Concentration of potential toxic elements in plant tissues}}{\text{Concentration of potential toxic elements in soil}}$$

Where the unit of metal concentration in the plant is mg/kg dry weight and the unit of metal concentration in the soil is mg/kg. A BCF greater than 1 indicates that the plant accumulates the metal in higher concentration than what is found in the soil, indicating that the plant is a good accumulator. If the BCF is less than 1, it means the plant accumulates relatively less of that metal.

3.5 Data analysis

The data collected from the germination test, shoot and root length measurements, biomass, and metal concentration were processed and summarized using Microsoft Excel. All raw data were organized in spreadsheets to make the results easier to manage and compare between treatments. The standard deviations and mean values were calculated of each of these treatments to show the variation and consistency of the data.

The data were analyzed to compare differences between the contaminated soils, control soils, and the two dilution materials (sand and mould). Basic statistical calculations were used to describe how the plants responded to the different soil treatments. Graphs and tables were created to present the results in a clear and simple way, which made it easier to interpret the patterns and trends of plant growth and metal uptake. Moreover, the ambient temperature and relative humidity data were also recorded and included into the analysis to determine possible correlations with the rate of germination, plant growth, and biomass development.

4. Results and evaluation

4.1 Germination test

- Experiment 1:

The germination test results for Brassica juncea seeds under various soil treatments in experiment 1 are summarized in Table 2. The treatment codes can be explained as below:

- B, D, C = Soil source (B and D are contaminated soils from different areas and C is control i.e. only the quartz sand or mould without the contaminated soil).
- S, M = Dilution material (S = Sand, M = Mould).
- A, B = Cultivar types (A = Southern Giant Curled, B = Amsoi Swatow Thick Stem).

For example, BSA refers to Soil B mixed with sand, using ratio A. CMA (Control + Mould + Southern Giant Curled) was used as the reference treatment to calculate inhibition because it had the highest germination among control soils.

The highest germination rate was observed in BSA (100%), which even exceeded the clean control CMA (88.9%). This indicates that the quartz sand was enhancing the soil condition and reduced the immediate toxicity of the contamination, allowing good seed germination despite the presence of pollutants. On the other hand, some treatments showed very poor germination. No seeds germinated in both BMA and DMA treatment (0 %) indicating that the contamination level or the mixture of soil and dilution substance in both treatments provided a very unfavorable environment of seed germination. Other treatments including BMB and CSA exhibited relatively good germination, while BSB, DSB and DMB having moderate germination.

Among the control treatments, CMA was the most germinated, followed by CMB and CSA and CSB was the lowest. This indicates that germination is affected even in clean conditions by the type of diluting material. The inhibition values supported these observations. For example, BSA had a negative inhibition of – 12.50%, meaning seeds germinated slightly better than in CMA. In contrast, BMA and DMA had 100% inhibition reflecting complete failure to germinate compared to the control. Some other treatments such as BSB (62.50%) and DMB (50.00%) had moderate inhibition of germination.

Overall, the results indicate that germination is highly sensitive to both soil contamination and the type of diluting material. The quartz sand also had better germination than the mould which probably enhanced the stress in the contaminated condition.

Table 2. Germination performance of *Brassica juncea* under different treatments

<i>Treatment</i>	<i>Seed sown</i>	<i>Total germinated</i>	<i>Germination (%)</i>	<i>Inhibition (%)</i>
<i>BSA</i>	9	9	100	-12.5
<i>BSB</i>	9	3	33.33	62.5
<i>DSA</i>	9	6	66.67	25.0
<i>DSB</i>	9	4	44.44	50.0
<i>BMA</i>	9	0	0.00	100.0
<i>BMB</i>	9	7	77.78	12.5
<i>DMA</i>	9	0	0.00	100.0
<i>DMB</i>	9	4	44.44	50.0
<i>CSA</i>	9	6	66.67	25.0
<i>CSB</i>	9	4	44.44	50.0
<i>CMA</i>	9	8	88.89	0.0
<i>CMB</i>	9	6	66.67	25.0

- Experiment 2:

The germination results are shown in Table 3. The treatment codes follow the same as the previous experiment. The highest level of germination was observed in BSB treatment (Soil B + sand + Cultivar B) where 13 out of 20 seeds germinated follow by DSB, which was even higher than the sand-only control for the same cultivar (CSB) which may partly be due to the fact that quartz sand alone is not an ideal medium for cultivation, which could have contributed to the lower germination rates in the control treatment.

However, Cultivar A was significantly less germinated in all treatments. BSA and DSA were both significantly lower than their control (CSA) showing a strong negative impact of the contaminated soils and indicating that this cultivar was more sensitive to the contamination.

Table 3. Germination performance of *Brassica juncea* under different treatments

<i>Treatment</i>	<i>Seed sown</i>	<i>Total germinated</i>	<i>Germination (%)</i>	<i>Inhibition (%)</i>
<i>CSA</i>	20	5	25	44.44
<i>CSB</i>	20	9	45	0.00
<i>BSA</i>	20	4	20	55.56
<i>BSB</i>	20	13	65	-44.44
<i>DSA</i>	20	3	15	66.67
<i>DSB</i>	20	11	55	-22.22

Among the controls, CSB was chosen as the reference to calculate the germination inhibition percentage because it represents an uncontaminated soil with the highest germination performance. These results indicate that cultivar B is more tolerant to contamination than cultivar A, particularly in Soil B, where its germination even exceeded the control. The comparison with Experiment 1 also shows that using quartz sand instead of mould improved germination, likely because it reduced the toxic effect of the contaminated soils. Overall, cultivar B showed better germination performance and appears more suitable for further phytoremediation studies.

4.2 Shoot and root growth

In the first experiment root and shoot lengths of *Brassica juncea* seedlings were measured for each treatment. Table 4 below summarizes the average shoot and root lengths (mean \pm standard deviation) of the two cultivars (“Southern Giant Curled” and “Amsoi Swatow Thick Stem”) under each soil treatment.

Amsoi Swatow Thick Stem was better than Southern Giant Curled in highly contaminated treatments, particularly with the organic matter. Most effective growth was in the uncontaminated mould control (CMA) where Southern Giant grew to an average shoot length of about 9.5 cm approximately twice the height grown in any contaminated soil. In the sand-mixed contaminated soils (BS and DS) both cultivars also grew but their shoot lengths were small (3–4 cm) while mould in contaminated soils (BMA and DMA) resulted in poor or no growth for Southern Giant. Cultivar root growths were also different: Southern Giant developed longer root in B+Sand, and Amsoi grew better in D+Sand. These results indicate that Amsoi is more tolerant to contaminated conditions than Southern Giant especially in mould media.

Table 4. Average shoot and root lengths of *Brassica juncea* cultivars in Experiment 1 using various soil treatments s (1:8 soil dilution). Values are in centimeters – means that no seedlings germinated in that treatment

<i>Treatment</i>	<i>Southern Giant</i> – Shoot (cm)	<i>Southern</i> <i>Giant</i> – Root (cm)	<i>Amsoi Swatow</i> – Shoot (cm)	<i>Amsoi Swatow</i> – Root (cm)
<i>B + Sand</i>	3.2 \pm 0.87	6.5 \pm 2.3	4.3 \pm 2.8	4.3 \pm 2.8
<i>D + Sand</i>	3.4 \pm 1.0	2.8 \pm 1.4	3.75 \pm 0.9	6.0 \pm 3.4
<i>B + Mould</i>	–	–	6.2 \pm 2.6	1.7 \pm 0.6
<i>D + Mould</i>	–	–	7.75 \pm 2.3	2.1 \pm 0.5
<i>Control (Sand)</i>	3.0 \pm 0.8	3.1 \pm 0.5	3.8 \pm 1.0	1.4 \pm 0.5
<i>Control (Mould)</i>	9.5 \pm 1.1	1.8 \pm 0.6	8.0 \pm 2.1	2.6 \pm 0.5

In the second experiment, only shoot length was measured. Table 5 shows the average shoot lengths of both cultivars. Amsoi Swatow outperformed Southern Giant in both seed germination and shoot length their average shoot length stayed around 4–5 cm in all treatments, particularly in soil B. Meanwhile, the shoot length of Southern Giant in the B soil treatment fell sharply only to an average of about 2.3 cm (shortest of all treatments), while in the D soil treatment it was about 5.6 cm on average nearly twice the height observed in soil B. Interestingly, even though soil D in Southern Giant was highly contaminated, its mean shoot in D+Sand (5.6 cm) was slightly higher than even the clean sand control (5.3 cm). Amsoi, at the same time, had germinated much higher in the B soil (65%), and maintained a decent shoot size (~4.3 cm), at least the Southern Giant did not do so in a similar B soil (only 20% germination and very stunted shoots). These findings support the conclusion that Amsoi is not so sensitive to the soil contaminants, not only it germinated better in soil B, but its shoots were almost two times higher than those of Southern Giant in the same treatment.

Table 5. Average shoot length (cm) of *Brassica juncea* seedlings in Experiment 2 under different soil treatments

<i>Treatment</i>	<i>Southern Giant – Shoot (cm)</i>	<i>Amsoi Swatow – Shoot (cm)</i>
<i>Control (Sand)</i>	5.3 ± 2.0 (25% germ)	4.9 ± 1.5 (45% germ)
<i>Soil B + Sand</i>	2.3 ± 0.6 (20% germ)	4.3 ± 1.9 (65% germ)
<i>Soil D + Sand</i>	5.6 ± 1.1 (15% germ)	4.4 ± 0.9 (55% germ)

4.3 Biomass production

Fresh and dry biomass of the two mustard cultivars (Amsoi Swatow and Southern Giant Curled) were measured at the end of Experiment 2. Whole plants were weighed at harvest as a fresh biomass and then dried in the oven until constant weight (to find out the dry biomass). Overall, plants grown in clean sand produced the greatest biomass, while soils that were combined with contaminated samples (Soil B or Soil D) gave lower biomass. Table 6 below presents the average weight of the fresh and dry weight of each cultivar across the three treatments. In the two cultivars, the plants in Soil D (the more contaminated soil) had the lowest biomass, while plants in the clean sand were found to have the highest biomass. It is important to note that Southern Giant Curled was more likely to generate a bit more biomass compared to Amsoi Swatow in polluted soils.

Table 6. Fresh and Dry Biomass of *Brassica juncea* cultivars under different soil treatments from Experiment 2, – mean not measured

<i>Cultivar</i>	<i>Treatment</i>	<i>Fresh weight (g/plant)</i>	<i>Dry weight (g/plant)</i>
<i>Amsoi Swatow</i>	Control Sand	0.78	0.06
<i>Amsoi Swatow</i>	Soil B + Sand	0.79	0.07
<i>Amsoi Swatow</i>	Soil D + Sand	0.56	0
<i>Southern Giant Curled</i>	Control Sand	0.26	0.03
<i>Southern Giant Curled</i>	Soil B + Sand	–	–
<i>Southern Giant Curled</i>	Soil D + Sand	0.14	0.01

These results indicate that *Amsoi Swatow* was more tolerant to heavy metal stress with its biomass being stable even with contaminated conditions as compared to *Southern Giant Curled* which was more delicate especially in contaminated soils.

However, these biomass results should be interpreted carefully. Because only a small amount of plant material was produced in this experiment, the data are not very strong or representative. So, the biomass values are considered supporting information not the main evidence for phytoremediation. A more reliable evaluation of the plant's performance can be obtained from the elemental content and BCF values.

4.4 Heavy metal accumulation

The accumulation of heavy metal (Cd, Cu, Pb, and Zn) in the shoots of *Brassica juncea* was examined to determine how the plant can absorb metals in the contaminated soils. Table 7 contained the initial soil concentrations that were used as a reference value to determine treatment/control ratios and bioconcentration factors (BCF). The results showed that Soil D contained 0.88 mg/kg Cd, 6.91 mg/kg Cu, 483 mg/kg Pb, and 138 mg/kg Zn, while Soil B had lower levels, with Cd below the detection limit, 4.46 mg/kg Cu, 88.6 mg/kg Pb, and 18.1 mg/kg Zn. Control soil was considered uncontaminated. Control plants (CSA and CSB) were also tested to determine the metal concentrations.

Clean quartz sand was chosen as the control soil because it is made of pure silica (SiO₂) and it does not contain any toxic elements. That's why it was also used to mix with contaminated soil to reduce the metal levels. Potting soil was only used in the first experiment, but we stopped using it later because it was not consistent and gave unreliable results.

Table 7. Initial heavy metal concentrations in contaminated soils (D and B, 1:8 dilution, bdl: below the limit of detection)

		mg/kg
D	Cadmium (Cd)	0.88
	Copper (Cu)	6.91
	Lead (Pb)	483
	Zinc (Zn)	138
B	Cadmium (Cd)	bdl
	Copper (Cu)	4.46
	Lead (Pb)	88.6
	Zinc (Zn)	18.1

The concentrations of metals in plant shoots after the experiment are shown in Table 8. The highest accumulation of Pb and Zn was observed in DSA, reaching 821 mg/kg and 829 mg/kg, respectively. High levels were also detected in DSB for Zn (412 mg/kg) and Pb (354 mg/kg). Control plants contained no detectable Cd and Pb, confirming that the metals accumulated in the shoots originated from the contaminated soils. The low concentration of zinc in the control treatment can be explained by the use of quartz sand, which is almost pure silicon dioxide and contains no nutrients or heavy metals. Therefore, plants grown in sand had limited zinc uptake compared to those planted in contaminated soil mixtures. However, copper concentration was highest in the control plants and decreased in the contaminated treatments. This indicates that plants accumulated more zinc from contaminated soils, copper uptake was decreased which may be because of competition between the two elements during uptake.

Table 8. Element concentration in the shoots, mg/kg dry weight (bdl: below the limit of detection)

	mg/kg		mg/kg		mg/kg			
CSA	Cd	bdl	BSA	Cd	bdl	DSA	Cd	3.76
	Cu	188		Cu	62.5		Cu	316
	Pb	bdl		Pb	89.3		Pb	821
	Zn	77.0		Zn	277		Zn	829
CSB	Cd	bdl	BSB	Cd	1.24	DSB	Cd	2.75
	Cu	49.1		Cu	16.1		Cu	23.7
	Pb	bdl		Pb	57.7		Pb	354
	Zn	38.3		Zn	174		Zn	412

To further assess metal uptake, treatment/control ratios and BCF values were calculated. The treatment/control ratios highlighted the strong uptake of Zn by both cultivars. For soil D, Zn accumulation reached 10.8 times higher than in control plants for both Amsoi Swatow and Southern Giant. Southern Giant also accumulated Cu at 1.7 times the control concentration. For soil B, Zn accumulation was 4.5 times higher for Amsoi Swatow and 3.6 times higher for Southern Giant. These results confirm that both cultivars have high Zn accumulation capacity, with variety-specific differences in Cu uptake.

Bioconcentration Factor (BCF) Analysis

The uptake efficiency can be measured by the use of the bioconcentration factor (BCF), which is defined as the ratio of metal concentration in the plant tissue (mg/kg DW) divided by the soil concentration (Table 9). In this measure, the active accumulation was observed in both cultivars. In our results, both cultivars showed BCF >1 for Zn and Cu, indicating active accumulation. For example, Southern Giant’s BCF for Zn was approximately 6.0 in Soil D (vs. 3.0 for Amsoi) and 15.3 in Soil B (vs. 9.6 for Amsoi). Similarly, Southern Giant’s Cu BCF was 45.7 in Soil D (vs. 3.4 for Amsoi). These high BCF values (especially for Zn and Cu) indicate that Southern Giant is the most effective at translocating metals to shoots. In contrast, Pb had BCF <1 reflecting Pb’s strong retention in roots rather than shoots.

Table 9. Bioconcentration factors (BCF) of Cd, Cu, Pb, and Zn in *B. juncea* cultivars

	Soil D Amsoi Swatow	Soil D Southern Giant	Soil B Amsoi Swatow	Soil B Southern Giant
<i>Cd</i>	3.1	4.3	–	–
<i>Cu</i>	3.4	45.7	3.6	14.0
<i>Pb</i>	0.7	1.7	0.7	1.0
<i>Zn</i>	3.0	6.0	9.6	15.3

These values can be compared with the results of Opeña (2024), that studied plant material collected at the sampling sites. Compared to the herbaceous species *Holcus lanatus* and *Poa nemoralis*, *B. juncea* may accumulate zinc in a similar or higher extent, especially the cultivar Southern Giant. The following tables from Opeña’s thesis summarize the BCF values for different species (Tables 10 and 11).

Table 10. Bioconcentration Factor of different tree species for the uptake of As, Cd, Cu, Pb, and Zn (Opeña 2024)

Potential Toxic Elements	Tree Species				p-value	Remarks
	<i>Betula pendula</i>	<i>Carpinus betulus</i>	<i>Fagus sylvatica</i>	<i>Salix caprea</i>		
As in Roots	0.067±0.051 a	0.049±0.029 a	0.066±0.105 a	0.055±0.047 a	0.985	Ns
As in Stem	0.017±0.006 a	0.019±0.002 a	0.014±0.010 a	0.013±0.010 a	0.624	ns
As in leaves	0.066±0.092 a	0.033±0.022 a	0.017±0.006 a	0.015±0.007 a	0.780	ns
Cd in roots	0.183±0.173 a	1.131±1.009 a	0.158±0.242 a	1.577±0.346 a	0.067	ns
Cd in Stem	0.359±0.590 b	0.332±0.140 b	bdl	4.299±2.516 a	0.008	**
Cd in Leaves	0.475±0.792 b	0.050±0.055 b	bdl	4.387±1.134 a	< 0.001	***
Cu in Roots	0.169±0.034 a	0.457±0.142 a	0.077±0.035 a	0.420±0.352 a	0.058	ns
Cu in Stem	0.254±0.370 a	0.273±0.158 a	0.203±0.219 a	0.188±0.158 a	0.975	ns
Cu in leaves	0.301±0.328 a	0.370±0.376 a	0.117±0.025 a	0.222±0.120 a	0.707	ns
Pb in roots	0.425±0.285 a	0.698±0.595 a	0.393±0.431 a	0.173±0.161 a	0.612	ns
Pb in Stem	0.216±0.227 a	0.148±0.035 a	0.003±0.001 a	0.067±0.005 a	0.257	ns
Pb in Leaves	0.202±0.335 a	0.069±0.059 a	0.037±0.063 a	0.013±0.006 a	0.651	ns
Zn in roots	1.368±1.259 a	1.208±0.510 a	1.172±0.558 a	1.111±0.056 a	0.983	ns
Zn in Stem	4.396±5.459 a	2.056±1.775 a	0.835±0.112 a	1.447±0.244 a	0.556	ns
Zn in Leaves	3.030±1.284 a	0.568±0.574 b	0.648±0.274 b	2.071±0.205 a	0.016	ns

bdl- below detection limits

* Mean ± Standard Deviation (SD). Mean values in the same row with different letters as statistically different (P<0.05) using Tukey's test ns-not significant

*significant at 0.05

**significant at 0.01

***highly significant at 0.01

Table 11. Bioconcentration Factor of different herbaceous species for the uptake of As, Cd, Cu, Pb, and Zn (Opeña 2024)

Potential Toxic Elements	Herbaceous Species					
	<i>Holcus lanatus</i>	<i>Luzula albida</i>	<i>Poa augustifolia</i>	<i>Dactylis glomerata</i>	<i>Arrhenatherum elatius</i>	<i>Poa nemoralis</i>
As in Roots	0.05	0.04	0.07	0.02	0.09	0.02
As in Stem and Leaves	0.02	bdl	bdl	bdl	bdl	0.01
Cd in Roots	bdl	bdl	bdl	bdl	0.07	0.19
Cd in Stem and Leaves	bdl	bdl	bdl	bdl	bdl	bdl
Cu in Roots	0.35	0.19	0.38	0.17	0.31	0.88
Cu in Stem and Leaves	0.08	0.06	0.05	0.03	0.03	0.20
Pb in Roots	0.38	0.13	0.08	0.04	0.06	3.35
Pb in Stem and Leaves	0.02	0.01	bdl	0.01	0.01	0.35
Zn in Roots	4.06	1.61	0.43	1.52	0.25	3.30
Zn in Stem and Leaves	1.45	0.61	0.17	0.39	0.05	1.10

bdl- below detection limits

In comparison, the high BCF values of *B. juncea*, particularly the Southern Giant cultivar (Zn BCF up to 15.3 and Cu BCF up to 45.7), which is much higher than most of the herbaceous species listed in Opeña's work indicate that this species can be as effective or more effective at metal uptake than native plants.

In case of Southern Giant, the BCF values are higher for the studied elements in from both contaminated soils, this proves that *Brassica juncea* can be used in phytoremediation, and the cultivar of the Southern Giant Curled is the most effective in terms of metal accumulation ability especially for zinc and copper removal from contaminated soils.

5. Conclusion and Proposals

This experiment was done to determine the tolerance and accumulation ability of *Brassica juncea* cultivars (Amsoi Swatow and Southern Giant Curled) and to evaluate their phytoremediation potential. Quartz sand was added to the soils to lower the level of contamination and provide growth environment that could be compared among the treatments.

The results clearly demonstrated that soil contamination had a negative effect on plant growth. Plants grown in clean sand produced the highest biomass, while in those contaminated soils had lower growth performance especially Soil D. Amsoi Swatow was more tolerant to heavy metal stress and had a relatively constant biomass under the contaminated conditions compared to Southern Giant Curled which was more sensitive to the level of pollution. However, as very little plant material was obtained during the experiment these data should be treated carefully and considered as supporting information rather than as strong evidence for phytoremediation efficiency. Among the analyzed elements, the highest concentrations in the shoots were zinc. Lead and cadmium were detected at lower levels in all treatments and only the control plants had concentrations below the detection limits. Copper accumulation was moderate but still significant. These results proved that *Brassica juncea* is able to accumulate and translocate some metals in the soil to the shoots. Based on the calculated bioconcentration factors (BCF), Southern Giant Curled cultivar showed the highest BCF values for copper (45.7) and zinc (15.3) in soil B and lead and cadmium showed values below 2. These data show that *Brassica juncea* is selective in its uptake ability and more effective in zinc and copper phytoextraction.

For future studies, long term experiments and field studies are recommended. It would be valuable to determine the metal concentration in both roots and shoots to better understand the uptake and translocation processes. Remediation could be enhanced by experiments using larger plants, or with repeated cycles of crop planting, or adding soil amendments, which increase the availability of metals. Experiments in testing other high accumulator species or cultivar combinations can also be useful in increasing the performance of metal removal. Increase in the production of biomass and better uptake and mobility of less accessible elements like lead and cadmium could be further investigated by using of organic matter, fertilizers, or chelating agents to enhance biomass production.

In conclusion, *B. juncea* had significant potential in phytoextraction, especially on zinc and copper. Despite the fact that phytoremediation techniques had a limited potential to produce biomass under the specified experimental conditions, the research provided useful baseline data for the further development of phytoremediation methods.

6. Summary

This thesis examined the possibility of *Brassica juncea* (Indian mustard) for phytoremediation of heavy metal contaminated soils that have been collected in Mátraszentimre, Hungary. The aim of the study was to evaluate the tolerance, growth and accumulation capacity of two cultivars Amsoi Swatow and Southern Giant Curled, under different contamination levels. The cadmium (Cd), copper (Cu), and lead (Pb) and zinc (Zn) were combined with clean quartz sand in a 1:8 proportion in order to minimize contamination and provide uniform growing environment. Germination, growth, and biomass were measured and the metal concentrations in plant shoots were analyzed by ICP-OES after microwave digestion.

The results showed that soil contamination reduced plant growth and biomass production. Amsoi Swatow was more tolerance to metal stress and Southern Giant Curled was more sensitive. The highest accumulation of zinc and copper was observed in the shoots, cadmium was very low and detected only in plants grown in contaminated soils and remained below detection limits in the controls. The calculated bioconcentration factors proved that *Brassica juncea* is capable of absorbing and translocating Zn and Cu showing good potential for phytoextraction.

In conclusion, this study has shown that *Brassica juncea* has significant potential for phytoremediation especially in removing zinc and copper from contaminated environments. Although, the levels of biomass production were low in the conditions of the experiment the results provide valuable baseline information for future studies and for the development of phytoremediation techniques under field conditions.

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9. Student's Declaration

DECLARATION

the public access and authenticity of the thesis

Student's name: Souphaphone Keomany
Student's Neptun code: P045E0
Title of thesis: Investigation of phytoremediation of soil contaminated
with toxic elements using model experiments
Year of publication: 2025
Name of the consultant's institute: Institute of Environmental Sciences
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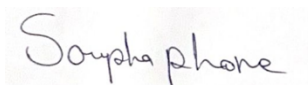
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1. general information:

Name of the student:	Souphaphone Keomany
Neptun ID:	P045E0
Level of program (mark with X):	<input checked="" type="checkbox"/> BSc/BA <input type="checkbox"/> MSc/MA <input type="checkbox"/> Doctoral School (PhD) <input type="checkbox"/> Other:
Name and code of the subject*:	Environmental Engineering - B-GOD-N-EN-KORNY
Title of the work:	Investigation of phytoremediation of soil contaminated with toxic elements using model experiments

* Not required to be completed in the case of a doctoral dissertation.

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Purpose of Use	Name and Version of the AI Tool Used	Affected Section (if not applicable to the entire text)
Paraphrasing sentence structure	StealthWriter (web platform)	Chapter 2 - 4
Assistance in searching for relevant references, background materials	Google Search, ChatGPT (OpenAI, GPT-5 version), Connected Papers (web service)	Chapter 2
Language polishing	QuitBot (web platform)	Chapter 2 - 4

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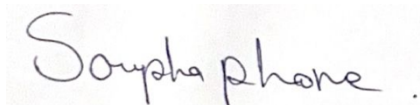
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10. Consultancy Statement

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I, Gábor Endre Halász as a consultant of the student Souphaphone Keomany (Neptun code: P045E0) declare that I have reviewed the final thesis and that I have informed the student of the requirements, legal and ethical rules for the correct handling of literature sources.

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Date: 2025 November 1st



Gábor Endre Halász
supervisor