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Evaluation of tree species, *Aésculus hippocastanum* as a phytoremediator in conditions of artificial soil contamination

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ABSTRACT
The article explores the potential use of *Aesculus hippocastanum* (horse chestnut) as a phytoremediator under conditions of artificial soil contamination by heavy metals in an urban environment. The research was conducted in Almaty City, where a significant excess of the maximum permissible concentrations of heavy metals, particularly lead has been detected. The study involved growing horse chestnut seeds in soils with varying contamination levels and treating them with solutions of toxic metals (Pb, Cd, Hg, etc.). Morphological and anatomical changes in the plants were analyzed, and levels of physiological stress were assessed. The results confirmed the high ability of *A. hippocastanum* to accumulate heavy metals, making it a promising bioindicator and a practical tool for ecological restoration of urban areas.

Keywords: Phytoremediation, Heavy metals, Horse chestnut, Urbanization, Soil pollution, Environmental safety, Lead, Cadmium, Mercury.
Article type: Research Article.

INTRODUCTION
By looking at the rapid urbanization of Almaty over the past decade, it was impossible not to notice the growing environmental problems. Soil contamination with heavy metals is of particular concern a problem that is still not given due attention, not only in Almaty, but also around the world. Urban air pollution is a worldwide problem. In addition, the main sources of pollution are intensive industrial production, transport infrastructure, energy, construction industry and household waste, which leads to an inevitable increase in environmental impact, especially if there are no or insufficient measures to protect against anthropogenic impact. One of the most acute environmental problems is the pollution of the city environment with heavy metals (HM). Under urban conditions, the air space of densely populated locations is cut off from natural air flows, as happened in Almaty, where decades of active construction work have trapped residents, depriving the area of natural ventilation, which causes a constant stagnation of polluted air, and can be seen by the thick smog that gathers in the evening every day due to the active air pollution and transport traffic. These toxic elements accumulate wherever possible: in soil and water, in the air and on porous surfaces, and living objects such as vegetation and biological tissues of animals, including humans, are no exception. Presenting a long-term threat to ecosystems and public health, through a cumulative effect, creating conditions for passive poisoning on a permanent basis, slowly accumulating in tissues, getting inside microdoses that are imperceptible without proper control. Heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), nickel (Ni), copper (Cu) and zinc (Zn) can cause a variety of effects: mutagenic and carcinogenic effects, as well as damage to the respiratory tract, central nervous system (CNS) and cardiovascular system. In addition, HMs

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can significantly reduce soil fertility, undermining the entire balance of elements in it, while at the same time disrupting natural biochemical processes in the tissues of all living organisms (Chernykh *et al.* 2003; Alibaeva *et al.* 2013).

This is due to the following factors:

Population growth, which increases the consumption of resources, and emissions into the environment;

Elevation in transport activity, leading to an upraise in heavy metal emissions into the atmosphere;

Increasing urbanization, accompanied by an elevation in the density of buildings and a reduction in green areas that serve as natural filters (Bolshakov *et al.* 1993).

Social and economic factors, including changes in consumption levels, rising cost of living, and the development of new industrial clusters. In this regard, studying the processes of accumulation of heavy metals is an important stage in assessing the current environmental situation in cities, becoming increasingly relevant due to the growth of industrial and transport loads. All this requires a regular assessment of the level of pollution and the search for effective methods to reduce it and solutions to improve the situation (Oxengendler *et al.*1991).

The accumulation of heavy metals in plants leads to the following consequences:

Disruption of photosynthesis and metabolic processes, which reduces the growth of plants and their resistance to adverse conditions;

Changes in physiological processes, including violation of the water balance and deformation of cellular structures, which further leads to diseases and death of plant masses;

Transmission of toxic substances through the food chain, which poses a threat to animals and humans, leads to the fact that plants accumulate heavy metals in their stems, roots and leaves, which are part of the permanent diet of not only animals, but also humans (Parvaiz *et al.* 2015).

In addition, heavy metal contamination of the soil also has a larger long-term impact. From a global point of view, this can affect not only the sphere of environmental problems, but also the level of economics, politics, and social aspects: economic consequences deterioration of soil quality reduces its suitability for agriculture and forestry, increasing the cost of its restoration; food hazard accumulation of toxic substances in agricultural crops threatens the environment. health impacts. Heavy metals cause chronic diseases affecting the human nervous, endocrine, and immune systems; and depletion of natural resources loss of fertile land and destruction of ecosystems lead to a reduction in biodiversity and deterioration of the ecological balance. The need to develop methods for reducing pollution (Davydova *et al.* 2002; Sadyrova *et al.* 2025). To prevent the negative effects of toxic metals on the environment, it is necessary to develop and implement methods of soil reclamation, such as:

Phytoremediation: using plants for the natural absorption and storage of heavy metals.

ENG phytoremediation operates through several core mechanisms: it may involve the breakdown of contaminants, their uptake and accumulation by plants, inhibition of their effects, or a combination of these strategies. Furthermore, these processes can be categorized based on how pollutants are either eliminated or neutralized. Among the main pathways are the isolation of contaminants from soil or water, accumulation of harmful substances in plant tissues, degradation via biological or chemical means, release of volatile pollutants into the atmosphere through transpiration, and stabilization of toxins within the root zone (Issayeva *et al.* 2022; Bukharbayeva *et al.* 2024). Chemical cleaning methods – using sorbents, bioactive substances and composting to bind toxic elements;

Agrochemical methods: using fertilizers that increase the resistance of plants to pollution;

Monitoring: conducting systematic studies of the level of soil pollution and its impact on the environment;

Using plant growth accelerators developed at the Kazakh National Women's Teacher Training University (Baitasheva *et al.* 2024).

Therefore, studying the mechanisms of heavy metal accumulation in soil and plants is a key area for assessing the ecological state of the urban environment and developing effective remediation strategies.

For this purpose, it is necessary to study the level of soil contamination with heavy metals in various districts of Almaty, identify the impact of pollution on the state of woody plants, using the example of horse chestnut (Latin *Aesculus*), and conduct a detailed study of their adsorption from the environment in urban conditions (Alekseev *et al.* 1989). This work is complex in nature, as it also covers the study of many overlapping aspects related to the environmental consequences of toxic substances released into the atmosphere (Mussina *et al.* 2018; Nurmahanova 2024). This study focuses on the study of transport exhaust gases as the leading source of anthropogenic air pollution in large cities. Analyzing the impact of emissions on the biosphere and human health, we rely on modern scientific research, and also consider possible ways to minimize environmental damage (Levina *et al.* 1972). The effect of the leaves of woody plants is considered as a kind of biofilter that can adsorb pollutants and thereby reduce their concentration in the air (Baitasheva *et al.* 2024). Studying plants, as natural adsorbents of heavy metals, makes it possible not only to objectively assess the ecological state of the urban environment, but also to offer solutions to reduce the risk of negative effects on public health. Studying the impact of emissions on vegetation, we address the problem of improving the quality of life of people, since the health of plant communities is directly related to the ability of the environment to self-clean and maintain ecological balance (Ovcharenko *et al.* 1995; Mussina *et al.* 2018). Despite the abundance of research, effective solutions to reduce the negative impact of motor vehicle emissions have not yet been found, which is with a whole range of social, economic and technological factors.

MATERIALS AND METHODS

Analysis of the research object

The object of research in this paper is a woody plant-horse chestnut, collected in advance and planted in compliance with the norms and relevant requirements for botany.

Description of the progress of the work

During the preparatory phase of the study (October 2024), approximately 90 fruits of *Aesculus hippocastanum* were collected from trees growing in different districts of Almaty. The selection was based on the following criteria:
Uneven distribution by city's administrative districts;
The main sources of pollution (industrial zones, highways);
Distance of trees (only visually healthy specimens were selected).
According to preliminary examination, about 20% of the collected fruits had visible morphological anomalies, which probably already indicates the impact of negative environmental factors.
This work is multi-stage and the following materials and equipment were used for its implementation:

First stage

Preparation of biological material - fruits of *A. hippocastanum*, collected during the period of their full ripening and falling;
Soil preparation: natural soils from different city districts were used for planting:
Northern district: Alatau Shopping Center, Ryskulov Avenue;
Southern district: HidePark, Al-Farabi Avenue;
Western district: Tole bi/Sain intersection;
Eastern district: Sayahat Business Center;
Preparation of 3-liter plastic planting pots.

Second stage

Preparation of toxic metal solutions using "chemically pure" grade reagents;
Solutions concentration: 0.01 M, volume: 5 liters;
Irrigation following a strict treatment schedule.

Third stage

Preparation of fixing solutions: a mixture of ethanol, distilled water and glycerol for preserving horse chestnut leaf samples for subsequent anatomical studies.

Fourth stage

Planting seeds of horse chestnut collected from different areas of the city and preparing them for treatment with heavy metal solutions;
Before planting, the seeds were soaked in water at room temperature for 10 days. The total number of seeds exceeded 90, but after soaking some were discarded due to defects;
Seeds were planted in prepared soil at a depth of 3-4 cm. Each pot contained 4-5 seeds (optimal amount for 3 kg of soil). In total, more than 65 seeds were planted;
Germination began after several days, and by November 20 most seeds had developed small shoots. This process is shown in Fig. 1. Plants were watered on a fixed schedule, twice a week.

Fifth stage

Preparation of heavy metal salt solutions

To simulate technogenic soil pollution, aqueous solutions of heavy metal salts with a concentration of 0.01 M were used. Data on the solutions are presented in Table 1.

Fig. 1. The process of germination of *Aesculus hippocastanum* seeds.

Table 1. Content of heavy metals in irrigation solutions.

	Chemical compound	Salt weight (g)
1	Lead Lead (II) nitrate	16.36
2	Cadmium nitrate	14.59
3	Carbonate hydroxide of copper (II)	11.054
4	Nickel sulfate	13.151
5	Zinc sulfate	14.156
6	Mercury (I) nitrate	28.06

The preparation of solutions was carried out at room temperature.

Sixth stage

Watering and treatment of seedlings with heavy metal solutions

Treatment of plants with heavy metal solutions began on February 17, 2025, three months after planting, when the seedlings had become sufficiently established. According to the irrigation schedule, watering was performed twice a week. For each toxic element, two pots with chestnut seedlings were allocated. Four pots with seedlings grown without exposure to heavy metals (in artificially enriched soil collected from environmentally clean areas of the city) were used as control samples. Each plant received an equal volume of solution, which was adjusted during watering due to changes in temperature and plant growth. The control group received an equivalent volume of irrigation with plain water, with the same consideration of external factors (Baitasheva *et al.* 2024).

RESULTS AND DISCUSSION

The study of the degree of soil contamination in Almaty was carried out on the example of lead content. Before planting and working directly with horse chestnuts, it was decided to analyze the soil for the content of heavy metals in it. As a test heavy metal, lead was chosen as one of the most common pollutants in the urban soil profile. The analysis of soil samples was carried out using an atomic adsorption spectrometer, which were then analyzed also by other analytical methods, in particular, by inversion voltammetry to detect the lead content (Orlov *et al.* 1991; Musina *et al.* 2018). Table 1 shows the results of the analysis. The work was carried out in accordance with GOST 31870-2012.

Table 1. Lead content in soil samples from different districts of Almaty.

Soil samples from different parts of Almaty	Pb concentration, mcg L^{-1}	Excess of the maximum permissible concentration of lead in a solution of 10 mcg L^{-1}	Comments
on Eco-friendly	17.656	1.76 times	Moderate excess
Eastern	31.361	3.1 times	Significantly-a dangerous excess of pollution
South	22.445	2.2	Times Higher than 2 times higher, requiring attention
Western	67.728	6.7 times	Very high pollution
Northern	44.756	4.5 times	Dangerous lead content

Analysis progress

Samples weighing 1.00–2.00 g were taken from the prepared and sifted soil and placed in a chemically resistant glass or test tube. Then 10-15 mL of concentrated HNO_3 was added to the samples. The resulting mixture was left at room temperature for a day to pre-loosen the soil and oxidize the organic components of the substance. Then the solution was carefully heated on an electric stove at a temperature of 80-90 °C, without bringing it to a boil for 1-2 hours, until the release of gases stopped. After the reaction was completed, the solution was cooled and diluted with distilled water to a volume of ~100 mL, then filtered into a measuring flask, bringing the distilled water to the exact mark. Then the methods of atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and other analytical methods were used. Based on the data obtained, it can be concluded that the lead content in the soil is really serious, especially in the samples taken in the Western and Northern points, since there is increased traffic in these parts of the city, as for air pollution caused by heavy metals in Almaty .In July 2023, the concentrations of heavy metals were determined (Table 2).

Table 2. Characteristics of air pollution with heavy metals in Almaty.

Impurity	Average concentration (mg m^{-3})	Multiplicity of exceeding the MPC
Cadmium	0.001	0.00
Lead	0.010	0.03
Arsenic	0.001	0.00
Chromium	0.005	0.00
Copper	0.008	0.00
Nickel	0.000	0.00
Zinc	0.023	0.00

Analysis of the obtained data shows that the most significant excess of the maximum permissible concentration (MPC) was recorded for lead. This metal is one of the most dangerous pollutants, as it easily accumulates in the body and has a neurotoxic effect, especially on children and pregnant women. The problem of heavy metal pollution requires a comprehensive approach that includes monitoring pollutants, developing environmental programs, and using natural filters such as green spaces. The research results obtained confirm the need to introduce additional measures to reduce the concentration of heavy metals in the urban environment in order to maintain ecosystem balance. Plants perform a natural filtration function, absorbing toxic substances from the atmosphere and soil, helping to reduce the concentration of harmful substances.

The main functions of urban vegetation include:

Absorption of carbon dioxide (CO_2);

Cleaning the air from solid particles and toxic compounds;

Increased oxygen levels in the urban environment;

Maintaining a comfortable microclimate and reducing the temperature regime.

For a deeper understanding of the properties of plants used as natural adsorbents, studies were conducted on their ecological and biological properties, as well as studying the adaptation of this species to low temperatures. The authors note that the most optimal conditions for growing chestnuts are observed in temperate regions of the European part of Russia. However, in regions with severe winters, such as the Urals and Western Siberia, trees are exposed to significant stress, which negatively affects their growth and fertility (Baidina *et al.* 1999). Research conducted in Omsk has shown that by the age of 34, a horse chestnut tree can reach a height of 8.3 meters. At the same time, there is a steady growth rate of shoots and individual variability of winter hardiness. The authors conclude that chestnut is a promising option for new territories and deserves further study. It is important to note that the climatic conditions of Almaty are not stressful for this species, so adaptation factors do not play a decisive role in our study. In this regard, it is important not only to maintain existing green areas, but also to actively plant new trees and shrubs that are resistant to pollution. These studies provide detailed information on the mechanisms of adsorption in plants and soil, as well as on the effect of heavy metals on their structure and vital activity. Studies show that pollutants disrupt the natural metabolism, slow down growth processes, and alter the physiological properties of plants. Special attention was paid to the study of juniper, as a perennial plant with high resistance to pollution and having adsorption abilities in relation to heavy metals. A number of toxicities have been identified.

Its physiological features are largely similar to chestnut, which makes it possible to use these studies in our work (Mussina *et al.* 2018). *Aesculus hippocastanum* (horse chestnut) is a deciduous tree of the Sapindaceae family, widely distributed in temperate climates. In urban environments, it is used for landscaping parks, alleys and streets due to its decorative qualities, resistance to air pollution and ability to filter harmful substances.
The horse chestnut performs several important ecological functions in urban environments:
Air filtration: leaves absorb dust, carbon dioxide and toxic compounds, reducing atmospheric pollution levels;
Noise reduction: the dense crown effectively reduces noise levels along roadways;
Soil protection: the powerful root system prevents soil erosion;
Wildlife habitat: chestnuts serve as a food source for squirrels and certain bird species;
Another reason for selecting this particular plant species is its comparative advantages over other types of chestnuts. Table 3 presents comparative characteristics of some chestnut representatives.

Table 3. Comparative morphology and biological characteristics of chestnuts.

Characteristics of chestnuts	**Horse chestnut (*Aesculus hippocastanum*)**	**Edible chestnut (Castanea sativa)**
Properties of the root system	Strong, branched, surface	Deep core root system
Resistance to high temperature	Medium, requires moderate humidity	High, tolerates dry conditions
Frost	Resistance High, can withstand up to -30 °C	Medium, can withstand up to -18 °C
Adaptability to urban environments	Good resistance to atmospheric and lithospheric gas	pollution Less resistant to air and soil pollution
Life span	200-300 years	To 500 years

The horse chestnut is characterized by rapid germination and high germination rates (up to 85-90%), especially when properly stratified (2-3 months at +3...+5 °C). Horse chestnuts germinate faster (typically within 2-3 weeks after planting). It is worth noting the phytoncidal properties of horse chestnut and its ecological impact. The leaves and flowers of horse chestnut emit phytoncides that purify the air of harmful impurities and suppress the development of pathogenic microorganisms (Mussina *et al.* 2018).

The ability to adsorb heavy metals

Studies show that horse chestnut is able to efficiently accumulate heavy metals (HM) from the air and soil, including: Lead (Pb) – one of the main pollutants in the vicinity of highways; cadmium (Cd) - a toxic element accumulating in soil and plants; copper (Cu) and zinc (Zn) are metals found in exhaust gases and industrial emissions (Mussina *et al.* 2018).

Adsorption capacity

Horse chestnut leaves have a rough surface, which increases their ability to trap dust particles with heavy metals. The root system absorbs metals from the soil, after which they are partially deposited in the plant tissues.

Due to its ability to accumulate heavy metals, horse chestnut can be used for

As a bioindicator of air and soil pollution.
For cleaning the urban environment (phytoremediation): it is planted near roads and industrial areas.
However, these works are only descriptive in nature, requiring experimental confirmation. For growing in an urban environment and cleaning the air from toxic impurities, for a combination of these reasons, it is horse chestnut that is preferred as an object of research.

Effects of toxic metals on woody plants

During the research, such metals as lead, cadmium, nickel, copper, mercury and zinc were selected, most often acting as components of pollutants in urban environments. In this study, special attention is paid to the three most toxic heavy metals: lead, cadmium, and mercury, exhibiting the most pronounced symptoms of depression: lead caused severe physiological stress, manifested in yellowing, leaf fall, growth retardation and significant anatomical damage to the stem; cadmium, being a highly toxic element, led to curvature of the stem, leaf necrosis, decreased photosynthetic activity and serious cellular disorders; mercury had a pronounced toxic effect, causing metabolic depression, impaired cellular differentiation, and damage to key structural and functional components of the stem, which led to a decrease in life-supporting functions and a weakening of the overall plant resistance to external influences (Alekseev *et al.* 1989).

Lead

In the early stages of the experiment, the test plants exposed to lead showed active growth. Strong stems were formed, the root system developed normally, and leaves grew with a rich green color. This indicated that at this stage, the lead concentration in the soil probably had not yet reached the toxicity threshold and the plants were temporarily resistant to contamination. Healthy seedlings are shown in Fig. 2. However, over time, noticeable changes began to occur in the vegetative organs, which indicates a gradual accumulation of lead in the tissues and its negative impact on physiological processes. A month later, on January 25, the first signs of poisoning became noticeable: the leaves began to turn yellow at the edges. The plant still managed to adapt to the increased toxicity, which shows a relatively healthy appearance in Fig. 3.

As shown in Fig. 4, by March 11, the plant showed clear symptoms of lead poisoning.

Discoloration of leaves. Green pigment weakened, signs of chlorosis appeared, which indicates a violation of chlorophyll synthesis.

Leaf fall. The decrease in leaf mass can be caused by damage to cell membranes and increased activation of programmed cell death (apoptosis) mechanisms.

Fig. 2. Healthy seedlings treated with a lead solution during the first week.

Fig. 3. Condition of the plant after a month of etching with a lead solution.

Fig. 4. Visual damage to the plant after two months of treatment.

Turgor loss. Cells have lost their ability to retain water, indicating an unstable water metabolism. This process was probably associated with damage to root cells, disruption of membrane ion transport proteins, and inhibition of aquaporins. There was a delay in plant growth: A decrease in biosynthetic activity and inhibition of cell division may be the result of a violation of the enzyme systems involved in nitrogen and carbohydrate metabolism. The toxic effect of lead can be caused by the following processes:

Inhibition of enzymatic activity. Lead binds to the active sites of enzymes, which leads to their inactivation. This disrupts the antioxidant systems, increases oxidative stress, and promotes the accumulation of reactive oxygen species (ROS). The interaction of lead with the lipid components of cell membranes causes their destruction, which leads to ion leakage, changes in the osmotic balance, and weakening of the cell wall. It suppresses the synthesis of chlorophyll and damages photosynthetic proteins, which leads to a decrease in the light absorption coefficient and a drop in ATP production. Ketones with important macro-and microelements, such as: Ca^{2+}, Mg^{2+} and Fe^{2+}, thereby preventing their assimilation and transport through the plant. This leads to a violation of the balance and proper distribution. nutrients and, as a result, slowing down the growth of the plant itself.

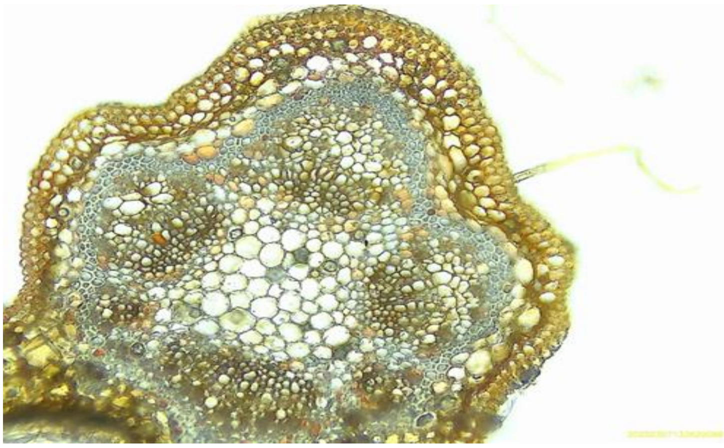

Fig. 5. Anatomy of a horse chestnut leaf exposed to lead etching.

The presented microscopic image of a cross-section of the stem of a horse chestnut, *A. hippocastanum* shows clearly pronounced anatomical changes caused by toxic exposure to lead ions (Pb^{2+}). On the preparation, the outer cover of the stem (epidermis) looks partially destroyed. The deformation of individual cells is visible: they are flattened, the walls are thinned in places, and their contours are uneven. Such changes indicate plasmolysis, weakening of turgor and destruction of cell membranes as a result of lead exposure. Such injuries deprive the epidermis of its protective function and indicate the beginning of necrotic processes. In the cortex, which includes parenchyma and collenchyma, pronounced structural disorders are noticeable. Cortical cells are uneven in shape and size, located randomly, with clearly expanded intercellular spaces. Some of them look swollen, others-deformed. This indicates vacuolation, rupture of cell walls and disruption of intercellular connections. Lead accumulates in the cell membranes, disrupts the deposition of calcium and magnesium, which leads to loss of mechanical strength and tissue death.

Strong changes related to the conducting system. The conducting bundles look structurally disturbed: the xylem vessels are deformed, their lumen is uneven: some of the vessels are excessively expanded, some are flattened. The phloem is almost indistinguishable, and cambium is not found in the form of a clear division zone between the phloem and xylem. This indicates that under the influence of lead, cambium activity is inhibited, differentiation of conducting tissues is stopped, and their functionality is destroyed. Such disturbances lead to a sharp decrease in water and photosynthetic transport in the stem. In the central part, the core, there are also serious changes. The cells here appear loose and light, sometimes with blurred borders. Voids are observed, indicating the destruction of individual cells and lysis of the contents. Given that the parenchymal cells of the core often serve as a place of deposition of toxic substances, such pronounced changes can be interpreted as a consequence of the accumulation of lead and the associated destruction of mitochondria, vacuoles, and the nucleus. Thus, the anatomical picture clearly demonstrates typical Pb^{2+} effects: degradation of the epidermis, loosening and vacuolization of the cortex, damage to the vascular system and destruction of the core parenchyma. These changes are associated with inhibition of metabolic processes, disruption of the structure of cell walls, toxic accumulation of heavy metal ions, and activation of programmed cell death. All this indicates a severe degree of physiological stress and destruction of vital functions of the plant stem (Belimov *et al.* 2011).

Cadmium

In the case of cadmium, it is considered the most common and toxic heavy metal in the environment. Pollution caused by cadmium has increased many times in recent years. A significant part of cadmium enters the soil and water with precipitation from areas with high levels of pollution. This element by its toxicity belongs to the class I of danger to living organisms. Cadmium can accumulate in the body of humans and animals, causing the development of various diseases, especially dangerous to health-the circulatory system and the heart. Detection of 10 mg of cadmium in the human body indicates symptoms of long-term poisoning. The need for cadmium for plant life, in comparison with other heavy metals, has not yet been fully proven. The normal level of cadmium is considered to be 0.1-1 μg kg^{-1} per dry weight of the plant, while some researchers consider the normal level of 0.05–0.2 mg kg^{-1} in plant tissue and believe that 3 mg kg^{-1} is a high level. The main signs of the effects of cadmium poisoning on plants are: slowing plant growth, reduced biomass accumulation, chlorosis, reduced yield and other physiological processes (Kaznina *et al.* 2003). As shown in Fig. 6, at the initial stage of the experiment, the plant was in a healthy state, having a healthy erect stem without visible deformations. Rich green leaves, elastic, without signs of necrosis or chlorosis, and also a normal growth rate is visible, without anomalies and deviations in the development of the seedling.

Fig. 6. Initial state of the plant.

After three months, as a result of exposure to the cadmium solution, noticeable changes were observed, such as bending of the stem, which may indicate a loss of turgor caused by a violation of the water balance in the chestnut. Mechanical damage to cells is possible, which leads to a decrease in the resistance of tissues to external factors such as temperature and humidity.

Discoloration of leaves. Brown ends of leaves, a sign of tissue necrosis caused by factors such as oxidative stress due to the accumulation of reactive oxygen species (ROS), violation of the water balance due to toxic effects of cadmium.

Fig. 7. Condition after three months of etching.

Fig. 7 also shows the bending of the stem, which can be associated with mechanical damage to cells and loss of water balance in them, and leaf necrosis due to oxidative stress and impaired water metabolism. There is a decrease in photosynthetic activity, which in the future leads to a slowdown in growth. Cadmium toxicity has a serious impact on plants at the molecular level, disrupting important physiological and biochemical processes. Fig. 8

shows the change of the leaf at the cellular level. Metal penetrates the plant through the roots and quickly accumulates in the tissues, replacing important trace elements (zinc, calcium, and iron), disrupting the work of enzymes involved in photosynthesis, respiration, and protein synthesis. One of the first consequences of poisoning is the suppression of photosynthesis: cadmium damages green particles and reduces the content of chlorophyll, weakening the supply of nutrients to the plant. In addition, cadmium causes oxidative stress, which promotes the formation of reactive oxygen species that damage cell membranes, proteins, and DNA. In response, the plant activates its defense mechanisms: it produces antioxidant enzymes and cadmium-binding proteins (metallothioneins, phytochelatins). However, at high doses, these mechanisms fail. As a result, growth slows down, nutrient absorption, yield and resistance to other stressors decreases. Thus, cadmium causes serious disturbances that reduce the viability of the plant. This information is in good agreement with juniper studies (Mussina *et al.* 2018).

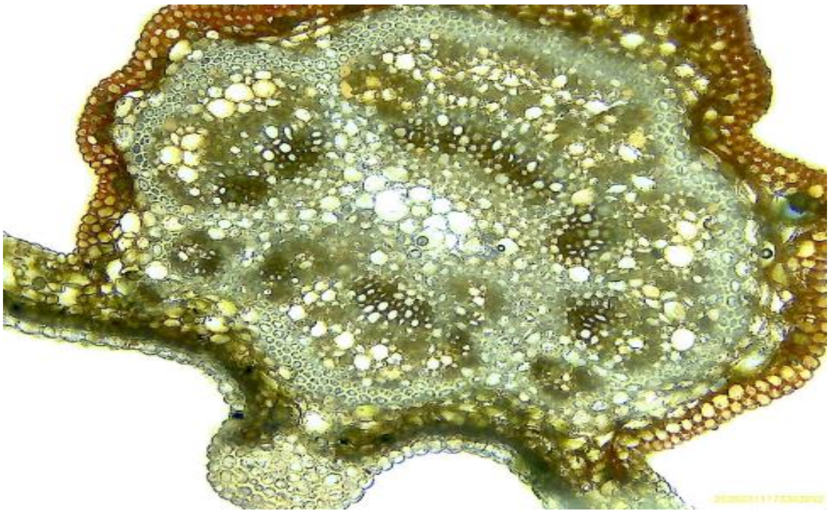

Fig. 8. Anatomy of a horse chestnut leaf after treatment with a cadmium solution.

A microscopic image of a cross-section of the stem of a horse chestnut, *A. hippocastanum* exposed to cadmium ions (Cd^{2+}) shows characteristic morphological changes that reflect the toxic stress of the plant. First of all, changes affect the integumentary tissue. The epidermal cells are partially destroyed, their deformation is noticeable: the contours of the cells are uneven, the walls are thinned in some places, and in some places. On the contrary, they are thickened. This indicates a violation of osmotic pressure and membrane integrity caused by a violation of water metabolism and the accumulation of toxic compounds in the apoplastic space. Deeper tissues, the cortex, which includes the collenchyma and the main parenchyma, also show pronounced signs of destruction. Cells are unevenly spaced, with enlarged intercellular spaces visible between them. Vacuolation is observed, and in some areas there are signs of plasmolysis and rupture of the membranes. Cadmium, known for its ability to replace calcium in cell walls, weakens the strength of tissues and disrupts structural stability. This leads to a chaotic arrangement of cells and loss of mechanical stability of the tissue. Conductive elements are particularly vulnerable to the action of cadmium. The structure of xylem vessels is disturbed: some of the vessels are deformed, the lumen is not uniform, and the walls are thinned. The phloem is poorly visualized, and the meristematic zone (cambium) is not distinguished, which indicates the suppression of mitotic activity. Such damage negatively affects the plant's ability to transport water and nutrients and disrupts the growth and development of the stem. The central part of the cut, the core looks loosened, with multiple voids. Parenchymal cells are partially destroyed, and their boundaries are indistinct, which is typical for cell lysis processes. These processes contribute to the activation of programmed cell death and loss of core tissue functions. In general, the anatomical structure of the stem shows a typical plant response to cadmium stress: destruction of integumentary cells, loosening of the bark, deformation of vascular tissues and lysis of the core.

Mercury

Toxicity study on woody plants. Initial observations showed healthy plants with deep green, turgid leaves free of visible damage or spots. Stems exhibited robust growth, and root systems appeared stable with no signs of deformation or weakening. After three months of mercury exposure, significant morphological and physiological alterations were documented.

Leaf discoloration. Pronounced yellowing indicated chlorosis due to impaired chlorophyll synthesis.
Necrotic lesions. Dark spots formed as a consequence of membrane damage and cell death.
Structural deformities. Leaf wilting and edge curling suggested disrupted water balance and cytoskeletal toxicity.
Loss of turgor. Visible wilting reflected ion transport imbalance and membrane structural damage.
Stem modifications included reduced rigidity, likely from inhibited lignin biosynthesis and calcium (Ca^{2+}) metabolism disruption. Notably, the emergence of new shoots demonstrated partial regenerative capacity despite mercury stress (Vaulina *et al.* 1978).

Fig. 9. Plant type after intoxication in three months.

Fig. 9 illustrates the visual manifestations of mercury toxicity in horse chestnut, *A. hippocastanum*, highlighting its interaction with specific protein groups and blockage of enzymatic active sites. Notably, enzymes involved in antioxidant defense are severely affected, triggering oxidative stress. Elevated levels of reactive oxygen species (ROS) exacerbate structural damage. Mercury-induced DNA damage may lead to mutations and cell death, while protein degradation disrupts core metabolic processes.
Mercury disrupts ion channel function, inducing micronutrient imbalances:
Reduced Ca^{2+} levels progressively weaken cell wall integrity and tissue mechanical strength;
Impaired K^+ and Mg^{2+} transport diminishes photosynthetic activity and energy metabolism;
Cellular water imbalance results in loss of turgor pressure and leaf wilting.
Root system toxicity further compounds these effects:
Inhibition of root apical meristem cell division slows overall root system development;
Impaired root hair formation reduces water and mineral uptake efficiency;
Elevated Hg^{2+} concentrations induce root cell apoptosis, severely restricting root growth.
This cascade of physiological disruptions ultimately compromises plant viability under mercury stress.
Microscopic analysis of a cross-section of the stem of *A. hippocastanum* exposed to mercury salts shows significant abnormalities in the anatomical organization of tissues characteristic of severe toxic stress. One of the most noticeable areas of damage is the epidermis: the cells of the integumentary tissue are partially deformed, the contours are uneven, there are areas with thickening or, conversely, destruction of the walls. This reflects a violation of turgor regulation and possible deposition of Hg^{2+} in cell membranes. The loss of structural integrity of the outer layer deprives the tissue of protective properties and increases the penetration of toxicants into the deep-lying zones (Vlasyuk *et al.* 1969; Kulagin *et al.* 2005). In the bark tissue, randomly arranged cells with enlarged intercellular spaces and pronounced vacuolization are observed. Such changes can be interpreted as a response to osmotic imbalance and damage to cytoplasmic membranes. Mercury, being highly chemically reactive, disrupts enzymatic systems and alters the state of the cell wall, leading to a reduction in the mechanical strength of the tissue. Some cells appear swollen and loosely packed, which may indicate the onset of necrosis or apoptosis. The vascular system also exhibits characteristic disruptions. Xylem vessels appear irregular: some are narrowed, while others are excessively dilated. Their walls vary in thickness, likely due to impaired lignification processes under the influence of Hg^{2+}. The phloem is indistinct, and the cambium (the zone of cell division) is visually undetectable. This suggests suppressed mitotic activity and impaired renewal of vascular elements,

directly affecting the transport of water and nutrients through the stem (Carlson *et al.* 1975; Sigel *et al.* 1998; Nurmahanova *et al.* 2024). At the pith level, extensive tissue loosening is observed. Parenchyma cells appear blurred, with clear signs of content degradation. The presence of multiple voids indicates cell lysis. It is known that mercury disrupts mitochondrial function and induces the accumulation of reactive oxygen species, accelerating organelle degradation and triggering oxidative stress in central tissues.

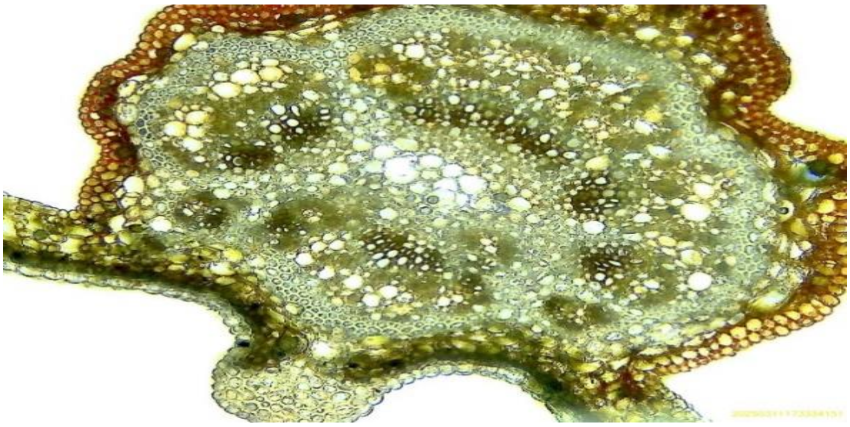

Fig. 10. Anatomy of a horse chestnut leaf after treatment with mercury solution.

The combination of identified morphological changes (destruction of the dermal tissue, vacuolization and degradation of cortical cells, vessel deformation, and pith disintegration) confirms the pronounced toxic effect of mercury. These alterations are associated with metabolic suppression, impaired cell differentiation, and damage to key structural and functional components of the stem. The result is a decline in vital physiological functions and a reduction in the plant's overall resilience to external stressors.

CONCLUSIONS

An assessment was conducted of the tree species *Aesculus hippocastanum* (horse chestnut) as a potential phytoremediator under conditions of artificial soil contamination with heavy metals. The study highlights the severity of environmental pollution amid rapid urbanization, with particular attention given to lead as a primary contaminant. The methodology involved cultivating horse chestnut in soils contaminated with various heavy metals (lead, cadmium, copper, nickel, zinc, and mercury) and analyzing their effects. A significant exceedance of maximum permissible lead concentrations was detected in the soils of Almaty, especially in areas with high traffic activity. The study revealed that exposure to lead and cadmium causes severe physiological and anatomical damage to horse chestnut, including stunted growth, chlorosis, leaf necrosis, and tissue degradation. The combination of these changes indicates a significant suppression of metabolic and cell division processes, reduced adaptive capacity, and impaired normal plant function under heavy metal contamination. Despite these adverse effects, horse chestnut demonstrated an ability to accumulate heavy metals, confirming its potential for use in phytoremediation projects and as a bioindicator of environmental pollution. The findings underscore the urgent need to develop and implement effective measures to reduce heavy metal pollution in the biosphere. Further research into the mechanisms of metal adsorption and plant resistance to heavy metals is necessary for the broader and more efficient use of green spaces in urban environmental rehabilitation programs.

CONFLICT OF INTEREST

There is no conflict of interest declared by the authors.

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