Chapter 13
The Possibility of Use of Oil Seed Plants and Grasses for Phytoremediation

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13.1 Introduction

The ecological aspect of Kazakhstan's sustainable development presupposes the preservation of the environment and the rational use of natural resources, the conservation of biological diversity, and the solution of the problem of man-made waste. The most important factor affecting the health of the population of the country is the state's steps to prevent diseases by reducing the objects polluting the environment [1]. The term "phytoremediation" means a large number of methods and technologies, in particular phytoextraction, phytoimmobilization, phytostabilization, and phytovolatilization [2, 3]. After application of phytotechnology, soils do not lose their natural properties; therefore, these technologies are soil-preserving, environmentally safe, and economically profitable.

Heavy metals that enter the soil through various ways due to human economic activity are classified as dangerous environmental pollutants. The amount of heavy metals accumulated in this way can exceed many times their natural content in the soil. Dissemination of technogenic pollution of heavy metals in the atmosphere has acquired a global character. The main sources of copper, lead, cadmium, and zinc in the environment are the mining, metallurgical and chemical industries, heat-power engineering, vehicles and chemical pesticides, and household waste.

Pollution of the atmosphere, soil, plants, and water with heavy metals in the vicinity of large industrial centers has become one of the most pressing environmental problems. In soils near industrial enterprises, the content of heavy metals exceeds the

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background content in similar soils by a factor of tens and hundreds of times [4]. The high concentration of a number of heavy metals in the soil adequately reflects the yield and quality of plant products grown within the boundaries of industrial centers in the horticultural areas. In a significant part of plant samples, the content of heavy metals exceeds the allowable concentration by 2–3.5 times [1, 4]. Excessive concentration of heavy metals in plants disrupts the natural course of the physiological and biochemical processes, suppresses the growth and development of the plant organism, and reduces the quality of the products obtained. Thus, the increasing technogenic contamination of the medium of heavy metals, migrating through the trophic bonds, leads to various unfavorable consequences in living organisms.

It is known from the literature that heavy metals adversely affect the physiological and biochemical processes of plants: change the properties of membranes [5, 6], the activity of enzymes, cause oxidative stress [7, 8, 9, 10]. The consequences of this effect are an inhibition of growth processes, delay in the onset of phenological phases, decrease of yield. In response to the negative effect of heavy metals on plants, a number of protective mechanisms are activated, such as an increase in the synthesis of metallothioneins (phytochelatins), organic acids, polyamines and antioxidant enzyme activity [11, 12, 13], aimed at reducing the toxic effect of heavy metals and maintaining homeostasis. But in different types of plants, protective mechanisms are developed to varying degrees. Different species, and even populations within a single species, can differ in their sensitivity to heavy metals and in the degree of accumulation in their organs, which may be the basis for the formation of a metallophyte flora [14]. The use of such qualities of plants as resistance to heavy metals and high metal-accumulating activity formed the basis for the technology of phytoremediation of contaminated soils, which is defined as the technology of cleaning the environment from chemical pollutants with the help of plants [15, 16].

One of the necessary steps to prevent the toxic effect of heavy metals on animals and humans is soil purification. The most effective way is at present phytoremediation of soils, i.e., cleaning of soils with the help of plant hyperaccumulators of heavy metals. Compared with physical and chemical methods, this method is less expensive, effective, and safe [3, 13]. According to some estimates, depending on the soil conditions and metal concentration, the cost of cleaning with plants (using only solar energy) can be only 5% of the costs required for other methods of restoring ecosystems contaminated with metals [17].

13.2 Heavy Metal Toxicity and Ways to Prevent It by Using Phytoremediation Technology

The term “heavy metals,” which characterizes a wide group of pollutants, has recently gained considerable popularity. As the membership criteria, numerous characteristics are used: atomic mass, density, toxicity, prevalence in the natural environment, and degree of involvement in natural and man-made cycles. Heavy metals belong to the elements of the periodic system Mendeleev with an atomic mass of more than 50 atomic units and a density of >7 g/cm^3 [18].

An important role in the definition of the term "heavy metals" is played by the following conditions: their high toxicity for living organisms in relatively low concentrations, as well as the ability to bioaccumulate [19]. Heavy metals belong to pollutants having anthropogenic origin, which are characterized by high toxic, mutagenic and carcinogenic effects. An important feature of heavy metals is that they belong to the class of nonspecific substances that are present in the "biosphere" in contrast to specific pollutants, like pesticides. Another difference between heavy metals and other pollutants is that in principle, the concept of selfpurification is not applicable to heavy metals. As a result of all the processes of migration and scattering, an irreversible increase in concentration in water, soil, air, and food takes place. There is a pollution of natural environments and biota. First of all, those metals that pollute the atmosphere to the greatest extent because of their use in significant volumes in production activity and as a result of accumulation in the external environment are of great danger from the point of view of their biological activity, and toxic properties are of interest. The most common metals that pollute the territory around metallurgical plants are zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd).

Almost all the metals falling under this definition (with the exception of lead, mercury, cadmium, and bismuth, a biological role, which are not currently detected) are actively involved in biological processes and are part of many enzymes [20].

Since many trace elements are heavy metals, soil contamination by them is essentially the accumulation of a large number of essential trace elements (Zn, Mn, Cu, Ni) or metals that can act as their counterparts (Cd, Pb, Hg). The biophilicity and toxicity of chemical elements are two sides of one phenomenon: the more amount of element is required for a living substance, the less toxic it is. It follows that trace elements are strong toxicants [14]. Hence, it follows that trace elements are strong toxicants.

Heavy metals, like Cu and Zn, are essential elements for growth of the body, as they are part of many enzymes and other proteins. Cu is the key component that provides the functioning of a number of enzymes, such as cytochrome oxidase, ascorbate oxidase, and a number of nonenzymatic proteins. It is included in the formation of the plastocyanin—a component of the electron transport chain of photosynthesis. It plays an important role in the life of organisms: it strengthens oxidative processes and promotes the formation of chlorophyll [20–22].

Zinc (Zn) is essential for the growth and normal development of most organisms. It is an important component of protoplasm, because it is associated with enzymes, regulators of cellular metabolism. Zinc participates in the synthesis of chlorophyll, prevents it from decay, affects nitrogen assimilation by plants, activates enzymes of carbohydrate and energy metabolism, and participates in the construction of a number of enzymes (some phosphatases) [20].

At high concentrations of these microelements, they have a toxic effect on plants. Excess amounts of them lead to symptoms of toxicity and suppression of plant growth, as a result of their binding to sulfhydryl groups of proteins, which leads to inhibition of activity and destruction of their structure [23–27].

Cadmium (Cd) and lead (Pb) are among the most common environmental pollutants. Cadmium is a heavy metal, usually present in soil in trace amounts. Nevertheless, human industrial activities and agricultural practices increase the level of cadmium in the soil. Everywhere used fertilizers and pesticides can contain large amounts of this metal, which for a long time enters the soil along with fertilizers [28]. Most of the Cd, contained in soil, is available for plants, since the soluble fraction reaches up to 35% of the total amount of urea [29]. Cadmium is characterized by high toxicity, possessing high mobility. There is also greater availability of Cd compared to other heavy metals, such as Zn, Cu, and Pb, which have a higher biological absorption coefficient [30]. Cadmium remains in the human body for many years, so eating food with the contents of this metal can induce chronic toxicity [31, 32]. Cadmium is a calcium antagonist. Even in soils that are considered to be uncontaminated or poorly polluted as a result of cadmium contamination coming from fertilizers or the atmosphere, some crops such as hard wheat, flax, sunflower, and potatoes can accumulate Cd in amounts exceeding the existing maximum level for consumption [28].

Lead is one of the most dangerous pollutants. The main way to enter the environment is anthropogenic pollution. Its widespread use as a liquid fuel antidetonator is one of the main reasons for increasing the content in terrestrial and aquatic ecosystems. If there are detergents in urban sewage waters, lead compounds are dissolved by these substances (polyphosphates, aminopolycarboxylic acids) [33]. Lead compounds containing a toxic anion, for example, orthoarsenates, chromates, and azide, are particularly toxic [34]. One of the necessary steps to prevent toxic effects of heavy metals on animals and humans is soil purification. To reduce global environmental pollution by technogenic pollutants, phytoremediation technology has been successfully applied worldwide. Phytoremediation is defined as the technology of using plants to clean contaminated soils, being economically advantageous and safe in comparison with other physicochemical methods of purification. In this regard, the study of metal-accumulating activity of natural species of Kazakhstan is particularly relevant and timely, and the use of the most suitable species for phytoremediation of contaminated soils is the most promising direction.

Compared with physical and chemical methods, this method is less expensive, effective, and safe. The term “over-accumulator” refers to plant species that accumulate 10–100 times more metals than conventional plants. These plants can be used to extract toxicants from the soil and thus can contribute to the restoration of the fertility of contaminated land. The accumulation of metals by plants in nontoxic form is one of the strategies used by plants to survive in conditions of severe environmental contamination [15]. One of the necessary steps to prevent toxic effects of heavy metals on animals and humans is soil purification. The most effective way at present is phytoremediation of soils, i.e., cleaning of soils with the help of plant hyperaccumulators of heavy metals. Compared to physical and chemical methods, this method is less expensive, effective, and safe [2, 15].

According to the literature data, the cost of conservative methods (chemical and physical methods) of soil purification is from $30 to $350 per hectare, and the cost of treating soils with plants is about $160 per hectare [35]. According to other estimates, depending on the soil conditions and metal concentration, the cost of cleaning with plants (using only solar energy) can be only 5% of the costs required for other methods of restoring ecosystems contaminated with metals [36]. The technology of phytoremediation has various directions. Phytoremediation technology includes phytoextraction (use of plants to extract metals from the soil), phytovolatilization (use of plants for volatilization of chemical elements), rhizofiltration (use of plant roots to extract metals from running water), and phytostabilization (use of plants to transfer metals to less toxic forms, but not extracting them from the soil) [36, 37]. For the phytoextraction of heavy metals from the soil, the use of plant hyperaccumulators of heavy metals is most beneficial. The term "over-accumulator" refers to plant species that accumulate 10–100 times more metals than conventional plants. Hyperaccumulators are of considerable interest from the point of view of phytoremediation [38], phytoextraction [39], and biofortification (improvement) in agricultural crops [40, 41].

These plants can be used to extract toxicants from the soil and thus can contribute to the restoration of the fertility of contaminated soil. Plant hyperaccumulators are endemic for those soils that are contaminated with heavy metals and do not compete with other species on unpolluted soils. Accumulation of metals by plants in nontoxic form is one of the strategies used by plants to survive in conditions of severe environmental contamination. The most well-known plant hyperaccumulators of heavy metals are *Ambrosia artemisiifolia* L. (ragwort ragweed), *Thlaspi rotundifolium* L., and *Thlaspi caerulescens* L., absorbing a significant amount of Zn, Cd, and Pb. Hyperaccumulators of Ni include *Alyssum* L. and *Arabidopsis* L. Currently, the definition of R. Brooks [42] is generally accepted, according to which those plants that accumulate zinc (Zn) >10,000, lead (Pb) >1000, and cadmium (Cd) >100 μg/g. are considered as hyperaccumulators of heavy metals. Plants-non-accumulators of heavy metals should accumulate on unpolluted soil - Zn < 100 μg/g, Pb < 10 μg/g, and Cd < 1 μg/g, respectively, and on contaminated soil - Zn < 1000 μg/g, Pb < 100 μg/g, and Cd < 10 μg/g.

The authors draw the attention of researchers to some important points in the study of plant hyperaccumulators. McGrath [43] considers that when comparing the hyperaccumulative ability of plants of different species, it is necessary to take into account not only the concentration of metal in plants (the content of metal per unit of plant weight) but also the amount of metal extracted from a given area. So, if one species strongly suppresses the accumulation of biomass of the aerial organs and the other to a lesser degree, the concentration of metal in the aerial organs of the latter may be lower than in the first due to the dilution effect. The absolute value of the metal content in plants in terms of a certain area will give a more correct picture for assessing the hyperaccumulation activity of plants in a comparative analysis [43]. Another important point is the ratio of the content of metals in the aerial plant organs to the content in the soil. As a rule, this value (up to 40 or more) is great for plant hyperaccumulators [44]. The most accurate determination of the status of hyperaccumulators can be established, the authors believe, only on a hydroponic medium, where the ability of plants to tolerate large concentrations of metals is manifested [45]. Phytoextraction is a fairly long duration of phytoremediation technology. Therefore, for the productive use of contaminated areas, it is necessary to use an economically viable and socially acceptable method for cleaning contaminated land. Technical crops, “energy crops,” possessing phytoextraction potential may be candidates for biofuel production [46].

The main disadvantage of the plants hyperaccumulators of heavy metals is due to the low growth and low biomass of these plants. If we try to imagine what an ideal plant should be, from an ecological point of view, then the plant would obviously look like this: having a long, well-developed root system and a strong transpiration current, such a plant must intensively form biomass, and this plant biomass should be characterized by tolerance to organic and inorganic toxic compounds. In addition, such a plant must necessarily quickly form conjugates and have the appropriate potential (capacity) for storing them in cellular structures and the apoplast [34]. The use of “energy” crops as phytoremediants will reduce the level of pollution from one side and on the other hand increase the productive value of contaminated soils.

Since it is not always possible to use plant hyperaccumulators, some wild grasses, as well as oil plants like sunflower, can be successfully used to clean up areas around metallurgical plants. The best candidates for use in phytoremediation are plants such as sunflower plants (Helianthus annuus L.), castor oil plants (Ricinus communis L.), and white mustard (Sinapis alba L.) [46].

As a phytoextractant, technical crops such as sunflower can be used. Sunflower is a plant that accumulates huge biomass and has the ability to store heavy metals in large amounts.

It is known from the literature that sunflower plants can accumulate large amounts of Pb, Zn, and Cd in their organs. The low bioavailability of some heavy metals in experiments was eliminated by the addition of synthetic metal chelating agents such as EDTA (0.1, 1, 3, 5, 7, and 10 mM/kg soil) and citric acid (0.01, 0.05, 0.25, 0.442, and 0.5 M/kg soil). After the use of metal chelating agents, the concentration in plant tissues increased, and, consequently, the removal of heavy metals by plant biomass increased [47]. Plants of castor oil (*Ricinus communis* L.), from which castor oil is extracted, also have a high potential for phytoextraction of metals from the soil.

When grown on a hydroponic medium that contained lead in amounts of 0, 100, 200, and 400 μmol/L, their lead hyperaccumulation potential was established. I. Raskin et al. [48] have established that plants accumulating 1.0 g/kg of dry weight in tissues can be considered plant hyperaccumulators of Pb [48]. Castor plants were accumulated on a hydroponic medium—from 10.54 to 24.61 g Pb/kg [49]. The use of metal chelating agents, such as EDTA, can increase the translocation of lead to the aerial organs.

In castor oil plants Ricinus communis L., which were grown on soil contaminated with lubricating oils (1–6% oil/soil), content of heavy metals, like Mn, Ni, and Pb were greatest in leaves and cadmium - in plant roots [50]. Application of 5 mM/kg of EDTA increased in the proportion of phytoavailable Pb, Zn, and Cd. The absorption of heavy metals increased in mustard white (*Sinapis alba*), radish (*Raphanus sativus oleiformis*), and amaranth (*Amaranthus* spp.). In mustard concentration of Pb was 479.71 mg/kg; Zn - 524.68 mg/kg; and Cd - 7.93 mg/kg, and phytoextraction potentials were 1.32 kg/ha, 1.44 kg/ha, and 0.022 kg/ha for Pb, Zn and Cd, respectively [51].

In a comparative experiment with *Helianthus annuus, Nicotiana tabacum*, and *Vetiveria zizanioides* grown in a hydroponic medium containing $Pb(NO_3)_2$ at concentrations of 0.25 and 2.5 mmol/L with or without chelating agents (EDTA or DTPA), it was found that the presence of metal chelating agents increased phytoextraction of lead. Most of the lead was accumulated in the leaves of plants. It was found that sunflower plants accumulate more lead than other species. Lead at a concentration of 2.5 mmol/L led to a strong increase in its concentration in plant tissues compared to the concentration in the growing medium. The bioconcentration factor was higher in sunflower plants than in the other two species of *N. tabacum* and *V. zizanioides*. In sunflower plants, the bioconcentration factor was 2.4 and 1.9 times more than in *Nicotiana tabacum* and *Vetiveria zizanioides*, respectively. The largest amount of lead was found in the roots, stems, and leaves of *H. annuus* grown at 2.5 mmol/L EDTA [52].

Sunflower showed good results in soil contaminated with arsenic. Pentavalent arsenate ($AsO4^-$) is very resistant in soils and is present in well-aerated soils. As a result, arsenic contamination of agricultural soils is a big problem. It is known that arsenates and phosphates ($PO4^-$) are chemically similar and therefore compete for joint sites in the soil. Therefore, in order to reduce the binding of arsenic to soil particles and to improve the phytoextraction of it from the soil, it is advisable to add ammonia. Addition of phosphate increases the content of arsenate in the soil solution by replacing arsenate at specific anion exchange sites of the soil, which will increase the bioavailability of arsenic for plant roots. Phosphate fertilizers increase As accumulation in plants by stimulating the phosphate-absorbing mechanism. It was found that sunflower (*Helianthus annuus* L.) can be a candidate for phytoextraction of arsenic when phosphorus is added as a mobilizing agent [53].

Sunflower plants in hydroponic conditions accumulated great amounts of nickel (Ni) and lead (Pb) in the shoots and roots. Accumulation of Ni and Pb (55.82 and 72.28 mg/kg) was increased in the presence of EDTA. It was shown that in sunflower concentration and total accumulation of Pb was more than Ni [54]. It was revealed that sunflower *Helianthus annuus* accumulated most amount of the lead compared to other plant species, like *Brassica juncea* (L.), *Brassica nigra* (L.), *Raphanus sativus* L., and *Ipomea triloba* L. [55]. The study of phytoextraction potential of plants such as *Helianthus annuus*, *Echinochloa crus-galli*, *Abutilon avicennae*, and *Aeschynomene indica* grown on soils polluted with cadmium (Cd), lead (Pb), and 2,4,6-trinitrotoluene (TNT) has shown that the concentration of lead was the highest in *A. avicennae* and *H. annuus*. The removal of cadmium was also high in these plants. The highest values for cadmium were found in plants *E. crus-galli* (50.1%) and *H. annuus* (41.3%) [56]. In experiment with using other chelating agent, like DTPA (3 mmol) for rapeseed (*Brassica napus*) and sunflower (*Helianthus annuus*) in soil contaminated by lead and zinc (234.6 mg/kg and 1364.4 mg/kg, respectively), concentration of these metals in sunflower plants was higher as compared to rapeseed [57].

The results of experiments with two sunflower species *Tithonia diversifolia* and *Helianthus annuus* showed that these plants have accumulated great amounts of lead and zinc in the leaves, stems, and roots. The concentrations of Pb in the leaves were 87.3 mg/kg, 71.3 mg/kg, and 71.5 mg/kg and in the stems 79.3, 77.8, and 60.7 mg/kg at 4 weeks, 6 weeks, and 8 weeks after planting, respectively. In roots, it was 99.4 mg/

kg, 97.4 mg/g, and 77.7 mg/kg at 4 weeks, 6 weeks, and 8 weeks AP, respectively. The same pattern was observed in *Helianthus annuus*. The studied plants have accumulated Zn in great amount in aboveground parts compared to roots. The translocation coefficient factor and bioconcentration factor of Pb and Zn with these plant species were greater than 1. But translocation factor of Zn was more than Pb. The authors concluded that the two species of sunflower *Tithonia diversifolia* and *Helianthus annuus* can be used in phytoremediation successfully [58]. Other researchers also state that the study of accumulation of heavy metals in *Helianthus annuus* showed that sunflower plants have accumulated great amounts of heavy metals [59, 60].

In other experiments with sunflower plants, heavy metals are accumulated mainly in the roots with little translocation of heavy metals from the roots to shoots [61]. Vermicompost amendments have increased uptake of Pb, Zn, and Cd by sunflower plants [62]. Patel et al. have determined that phytoextraction of copper by sunflower plants was higher than lead. Application of metal chelating agents like EDTA, a decrease in pH, and the addition of ammonium sulfate in the growth medium increased the uptake of metals by plants. Application of EDTA increased the heavy metal uptake by plants to a greater extent than the using of ammonium sulfate and the decreasing in pH [63]. The main reason of the application of *Helianthus annuus* L. in phytoremediation is that sunflower plants grow fast, accumulate great biomass, and are able to uptake heavy metals in large amounts [64–68].

13.2.1 Metal Accumulation Ability of Sunflower and the Mixture of Lawn Grasses

The main reason to develop phytoremediation technology using energy-rich crops is that energy valuable cultures accumulate a large biomass of aboveground organs and are able to accumulate large amounts of heavy metals in their parts. Metal chelating agents will enhance the phytoextraction of metals that have low bioavailability and will also increase the translocation of metals to the aerial organs [69, 70]. Another possibility for phytoremediation is the use of wild grass species for phytoremediation. It was investigated the metal accumulation capacity of grass species of *Poa pratensis*, *Lolium perenne*, and *Festuca rubra* [71]. The studied grass species had translocation factor <1 and bioconcentration factor for roots >1. *P. pratensis* had lower phytostabilization potential than the other grasses but had a higher translocation factor and lower tolerance to cadmium. *L. perenne* has shown more tolerance to Cd and accumulation of Cd in largest amount. The authors concluded that *L. perenne* would be useful for phytostabilization of soils characterized by a relatively small pollution by cadmium. Other researchers found that the grasses which accumulate great biomass of well-developed root system are tolerant to heavy metals. Wild grass species have shown a high ability to accumulate heavy metals in shoots and roots. These peculiarities of grass species justify their use in phytoremediation [72–74].

In our previous studies, it was found that wild grass species *Thlaspi arvense*, *Agropyron repens*, *Setaria viridis*, *Dactylis glomerata*, and *Phleum pratense* have accumulated heavy metals in large quantities in the roots. In general, all the studied species were relatively resistant to the action of heavy metals and accumulated them to varying degrees mainly in the roots of plants, with the exception of *T. arvense*. From this it can be concluded that these species can be used for phytoremediation of soils contaminated with metals such as Zn, Pb, Cu, and Cd, in particular for phytostabilization. For phytoremediation of soils contaminated by Zn, almost all these species can be used - *T. arvense*, *A. repens*, *S. viridis*, and *D. glomerata*, and for Pb-contaminated soils - *A. repens*, *S. viridis*, *T. arvense*, and *Ph. pratense*. For the cleaning of soils from Cu and Cd, the use of *T. arvense* and *A. repens* was recommended [27]. Thus, sunflower plants and wild grass species have high metal accumulation capacity. The aim of our research was to study heavy metal accumulation capacity of sunflower plants (*Helianthus annuus* L.) and wild grass species and their phytoextraction potential.

13.2.2 Material and Methods

Seeds of the mixture of lawn grasses *Poa pratensis*, *Festuca rubra*, and *Arrhenatherum elatius* (1:1:1) and sunflower (*Helianthus annuus* L.) were sown on the pots (1 m^2) on the territory of the metallurgic factory "KazZinc" in Ust-Kamenogorsk City in East Kazakhstan. On separate plots with an area of 1 m^2, 10 seeds of sunflower plants and 100 seeds of the mixture of lawn grasses were sown. After 3 months the plants were removed for analysis. Before planting and after removing of plants, the soil samples were taken for analysis. The content of heavy metals in plant parts (in aboveground organs and roots) was determined. The content of trace metals in shoots and roots was determined as described next. Plant samples (0.5 g) were digested in a mixture of 5 mL of 50% HNO_3 and 0.5 mL HCl at 95 ± 5 °C according to standards for operation procedures [75]. Samples were transferred to digestion block (section) at temperature 90 ± 5 °C, closed by glass, and heated without bringing to a boil for 10–15 min. Then they were cooled and added 5 mL of concentrated HNO_3, moved in digestion block with 90 ± 5 °C, closed by glass, and heated without bringing to a boil for 30 min before the disappearance of brown fumes. Then the samples were cooled and added 2 mL of water and 3 mL of H_2O_2, continued heating up until the volume has been reduced to about 5 mL, removed from digestion blocks, allowed to cool, filtered, and added with deionized water up to a final volume to 50 mL. Samples were analyzed using the appropriate SOP [76].

The concentrations of metals in plants and soils were measured by atomic absorption spectrophotometry using an installed Winlab A Analyst 300 (Perkin Elmer, Germany) [76] with an installed and aligned HCL/EDL lamp. HCL lamps were stabilized/aligned for 25 min and EDL lamps - for 45 min; an operating pressure for acetylene was ~0.7 kgf/cm^2 and for compressed air - 2.8–3.0 kgf/cm^2. Following calibration, samples were analyzed.

After removing of plants, the plant part biomass was measured. Plants were dismembered on the aboveground part and roots. To determine the dry biomass, the plants were placed in a thermostat and dried at temperature 105 °C to constant weight, cooled to room temperature, and weighed.

Assessment criteria for the accumulation capacity of plant bioconcentration factor (BCF) and shoot/root ratio were used. BCA was determined according to the following formula:

$$BCF = Cplant/Csoil,$$

where Cplant and Csoil are concentrations of heavy metals in plant parts and soil, accordingly [77].

Shoot/root ratio was calculated according to the following formula:

Shoot/root ratio = concentration of metal in the aboveground organs/concentration of metal in the roots.

Determination of the content of metal in plant parts in percent (%) was carried out by the following way: % (g/100 g) = metal concentration (g/kg) × 100 g/1000 g. As assessment criteria of the level of cleaning of soil, it was used the value of the removal of heavy metals by plant parts and the residual amount of metals in the soil after the experiment (mg/kg). The residual amount of metals in the soil after experiment was calculated by the following formula:

Content of heavy metals in the soil after removing of plants (% to metal concentration before planting) = (concentration of metal after removal of plants (mg/kg)/concentration of metal in the soil before planting) × 100%

The removal of metals by plant parts from the area 1 m^2 was calculated by the following formula:

The removal of heavy metals by plants (g/m^2) = concentration of heavy metals (g/kg) × yield (g/m^2)/1000 g. The concentration value in mg/kg was previously converted into g/kg.

13.2.3 Results and Discussion

13.2.3.1 The Study of the Metal Accumulation Ability of Sunflower Plants (*Helianthus annuus* L.) and a Mixture of Lawn Grasses

Sunflower plants (*Helianthus annuus* L.) and a mixture of lawn grasses—*Poa pratensis*, *Festuca rubra*, and *Arrhenatherum elatius*—were planted in the territory of the lead-zinc metallurgical factory in Ust-Kamenogorsk City. Sunflower plants and a mixture of lawn grasses were planted separately in areas of 1 m^2 in three replicates (plots of 1 m^2 under sunflower and lawn grasses). On the plots 1 m^2, 10 sunflower plants were planted, and on a site with lawn grasses, 100 plants were sown. Determination of the concentration of heavy metals, such as cadmium (Cd), copper (Cu), lead (Pb), and

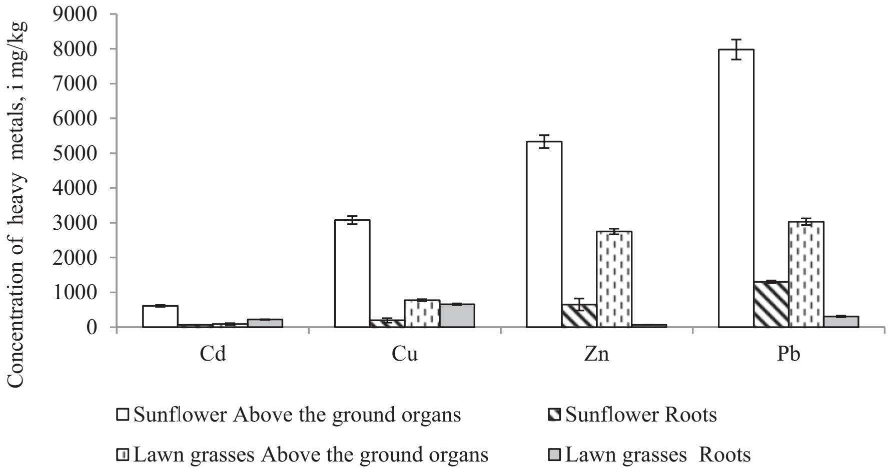

Fig. 13.1 Content of heavy metals in plants parts of sunflower and lawn grasses

zinc (Zn), showed that the content of these metals in the aerial organs of sunflower changed in the following order (mg/kg), Pb (7978.0) > Zn (5333.0) > Cu (3076.0) > Cd (612.0), and in roots Pb (1304.0) > Zn (652.0) > Cu (195.0) > Cd (65.0). The study of metal accumulating capacity of sunflower plants and a mixture of lawn grasses showed that zinc in the aboveground organs and roots of both sunflowers and in a mixture of lawn grasses is accumulated in greatest amount (Fig. 13.1). Cadmium is found in the lowest concentration in the organs of these plants.

According to the content in the aboveground organs of lawn grasses, heavy metals were arranged in the following order, Pb (3031.36) > Zn (2748.70) > Cu (775.87) > Cd (87.10), and in the roots Pb (661.0) > Zn (309.0) > Cu (220.0) > Cd (66.0). The concentration of heavy metals such as lead, zinc, copper, and cadmium in the aerial organs of sunflower was higher than in the aboveground organs of lawn grasses by 2.63, 1.94, 3.96, and 7.03 times, respectively. The highest excess in the aboveground organs is observed in cadmium and the lowest in zinc. In the roots of sunflower, the concentration of lead and zinc was also higher compared to lawn grasses. Concentration of lead in the roots of sunflower was 1.97 times higher and zinc 2.11 times more compared to the roots of lawn grasses. Concentration of copper, on the contrary, was slightly higher in the roots of lawn grasses than in sunflower (by 1.12 times), and the concentration of cadmium in the roots of sunflower and lawn grasses was almost the same (65.0 mg/kg and 66.0 mg/kg, respectively). In the roots, the highest excess was observed for zinc.

The content of heavy metals in the aerial organs of sunflower and lawn grasses was much higher, compared with the roots, probably due to strong atmospheric pollution.

The ratio of the content of metals in the aerial organs to their content in the roots is of great importance for cleaning the soil with the help of plants. The higher this value, the higher the potential ability of plants to clean the soil from metals. This index varies in sunflower in the following order: Cu (15.8.0) > Cd (9.4) > Zn

Table 13.1 Heavy metals content in plant samples in percent

Plant parts	Cu g/100 g	Pb g/100 g	Cd g/100 g	Zn g/100 g
Sunflower plants				
Aboveground organs	0.31	0.8	0.061	0.53
Roots	0.02	0.13	0.007	0.07
Lawn grasses				
Aboveground organs	0.08	0.3	0.009	0.27
Roots	0.022	0.07	0.007	0.03

(8.2) > Pb (6.1). For lawn grasses, the shoot/root ratio decreased in the following order: Zn (8.9) > Pb (4.6) > Cu (3.5) > Cd (1.3). For lawn grasses, unlike sunflower, the shoot/root ratio was higher for zinc and lead compared to copper and cadmium. A study of the content of heavy metals in plant organs showed that zinc ions were accumulated most in the aerial organs and roots of sunflower and a mixture of lawn grasses (Fig. 13.1). Cadmium is found in the lowest concentration in plant organs.

The percentage of heavy metals in plant organs is an integral indicator in the selection of plants for phytoremediation. In all studied plants, this indicator was below than 1 (Table 13.1).

The percentage of lead was highest in the aerial organs and roots of sunflower (0.8% and 0.13%, respectively) compared with other metals, and the lowest percentage of cadmium was 0.06% and 0.01% in the aerial organs and roots, respectively. Lawn grasses had the same pattern. The percentage of lead in the aerial organs was 0.3% and in the roots 0.07%; cadmium was 0.01% and 0.007% in the aerial organs and roots, respectively. The percentage of heavy metals in sunflower organs varied in the following order (%): in the aerial organs, Pb (0.8) > Zn (0.53) > Cu (0.31) > Cd (0.061), and in the roots Pb (0.13) > Zn (0.07) > Cu (0.02) > Cd (0.007).

In lawn grasses, the percentage of heavy metals decreased in the following order: aboveground organs (%), Pb (0.3) > Zn (0.27) > Cu (0.08) > Cd (0.009), and in the roots Pb (0.07) > Zn (0.03) > Cu (0.022) > Cd (0.007).

When comparing the content of metals in the aerial organs and in roots of the studied plants, it was found that the percentage of Pb content in roots of sunflower plants was 6.2 times less than in the aboveground parts. Concerning zinc, its content in the aboveground organs of sunflower exceeded that in roots by 7.57 times. The content of Cd in the aerial organs of sunflower exceeded its content in the roots by 8.7 times. The highest excess was observed for copper—its content in the aerial organs in percent was more than the percentage in the roots by 15.5 times. Lawn grasses had the same pattern. The percentage of metals in the aboveground organs exceeded its percentage in the roots—Pb, 4.29 times; Zn, 9 times; Cu, 3.63 times; and Cd, 1.29 times. If we compare the percentage of metals in the organs of sunflower and lawn grasses among themselves, we can see that in the aboveground organs of sunflower, the percentage of lead was 2.67 times more; zinc percentage content, 1.96 times more; copper, 3.88 times more; and cadmium, 6.78 times more than in lawn grasses.

In sunflower roots, the percentage of lead was 1.86 times more than that of lawn grass, and zinc content in 2.33 times greater, while the percentage of copper and cadmium in the roots of both plant species was the same—0.007% and 0.007%, respectively. Thus, the concentration of metals in the aerial plant organs was higher than in the roots. The coefficient of translocation of metals from the roots to the aboveground organs exceeded than 1. Obviously, this indicator is not a natural phenomenon and is the result of strong atmospheric pollution. Characteristically, the shoot/root ratio of cadmium and copper in sunflower was higher than that of zinc and lead, and in contrast to cadmium and copper, the shoot/root ratio of lead and zinc was greatest. The percentage of lead was greatest in the aerial organs and roots of the studied plants, and the smallest percentage of cadmium was also found.

13.2.3.2 Determination of Bioaccumulation Coefficient of Lawn Species and Sunflower Plants

Another important point is the ratio of the content of metals in the aerial plant organs to the content in the soil. As a rule, this value is great in plant hyperaccumulators [44]. To estimate the degree of bioaccumulation of heavy metals by the organs of the studied plants, the bioconcentration factor (bioaccumulation coefficient) of metals for the aerial organs and roots of sunflower and lawn grass plants was calculated (Table 13.2). Plants at the end of the experiment were collected from contaminated sites to determine the content of heavy metals in their organs. Soil samples from these sites were also taken for analysis. Using these values, the bioconcentration factor of the metals studied for sunflower and lawn grasses was determined.

In sunflower, the bioconcentration factor of Pb for aerial organs and roots was greatest and BCF of Zn the smallest. For the aboveground organs, the bioconcentration factor of all the metals studied was >1. Probably, the reason for this is strong atmospheric pollution. The bioconcentration factor in sunflower roots was >1 only for Pb (4.2).

The bioconcentration factor of heavy metals in the aerial organs compared to the roots of sunflower was higher for cadmium, copper, and zinc, while for lead, it was

Table 13.2 BCF heavy metals for sunflower and lawn grasses

Metals	Cu, mg/kg	Pb, mg/kg	Cd, mg/kg	Zn, mg/kg
Sunflower				
Soils under sunflower	870.0 ± 29.3	1900.0 ± 62.2	210.0 ± 7.6	2510.0 ± 97.8
Aboveground organs	3076.0 ± 102.3	7978.0 ± 25.16	612.0 ± 22.3	5333.0 ± 18.8
Roots	195.0 ± 6.1	1304.0 ± 4.156	65.0 ± 2.1	652.0 ± 18.3
BCF for aboveground organs	3.54 ± 0.093	4.19 ± 0.14	2.9 ± 0.09	2.12 ± 0.07
BCF for roots	0.22 ± 0.07	4.2 ± 0.09	0.31 ± 0.01	0.26 ± 0.008
Lawn grasses				
Soils under sunflower	980.0 ± 31.10	2210.0 ± 26.6	260 ± 5	2470 ± 27
Aboveground organs	775.87 ± 22.3	3031.36 ± 1.2	87.10 ± 2.5	2748.70 ± 9.3
Roots	220.0 ± 9.20	661.0 ± 21.3	66.0 ± 2.6	309.0 ± 9.2
BCF for aboveground organs	0.79 ± 0.025	1.37 ± 0.05	0.34 ± 0.015	1.11 ± 0.05
BCF for roots	0.22 ± 0.008	0.3 ± 0.013	0.25 ± 0.01	0.13 ± 0.004

approximately the same. The bioconcentration factor of sunflower plants decreases in the following order: for aerial organs, Pb (4.19) > Cu (3.54) > Cd (2.9) > Zn (2.12), and for the roots Pb (4.2) > Cd (0.31) > Zn (0.26) > Cu (0.22). In lawn grasses, as in sunflower, the lead bioconcentration factor for the aboveground organs and roots was the largest (1.37 and 0.3, respectively). In the aerial organs, the lowest value of the bioconcentration factor was observed for cadmium (0.34) and in the roots for zinc (0.13). In lawn grasses, the bioconcentration factor of lead and zinc in the aerial organs was above 1, and in the roots, this index was <1 for all metals studied. The bioconcentration factor of lawn grasses decreased in the following order: for aboveground organs, Pb (1.37) > Zn (1.11) > Cu (0.79) > Cd (0.34), and for the roots Pb (0.3) > Cd (0.25) > Cu (0.22) > Zn (0.13). In lawn grasses, the bioconcentration factor of all the heavy metals studied in the aerial organs was higher in comparison with the roots. Probably, this is a consequence of strong atmospheric pollution on the territory of the plant.

13.2.3.3 Determination of the Removal of Heavy Metals from the Soil by the Organs of Sunflower and Lawn Grass Plants

The question of whether the degree of metal accumulation is more important for the phytoremediation process or the accumulation of a significant aboveground mass is controversial for many researchers. Chaney et al. believe that the ability to hyperaccumulate metals and exhibit hyper-resistance to high metal concentrations is the most important plant properties for phytoremediation than the ability to accumulate large biomass [2]. But when comparing the hyperaccumulation ability of plants, it is also considered expedient to take into account not only the concentration of metal in plants (metal content per unit of plant weight) but also the amount of metal extracted from a given area. So, if one species strongly suppresses the accumulation of biomass of the aerial organs and the other to a lesser degree, the concentration of metal in the aerial organs of the latter may be lower than in the first due to the dilution effect. The absolute value of the metal content in plants in terms of a certain area will give a more correct picture for the estimation of the hyperaccumulation activity of plants in a comparative analysis [43]. Taking into account the concentration of heavy metals in plant organs and the yield of dry biomass from 1 m^2, the removal of heavy metals by plant organs was determined, and the degree of soil purification by the investigated plants was estimated.

For the use of plants for the purification of soils from heavy metals, the necessary index, which should be taken into account in phytoremediation, is the absolute value of the biomass of the aerial organs and plant roots from a certain area. It was determined the accumulation of biomass of the aboveground organs and roots of sunflower plants and mixture of lawn grasses grown on the area 1 m^2 around the lead-zinc metallurgical plant in Ust-Kamenogorsk City. It was calculated the value of biomass (aboveground organs and roots) per one plant for sunflower and mixture of lawn grasses and biomass of all plants, collected from the plots with an area of 1 m^2. Sunflower plants accumulated significant biomass in comparison with lawn

Table 13.3 Accumulation of biomass by aerial organs and roots of sunflower and a mixture of lawn grasses

Sunflower		
Plant parts	Dry weight per one plant, mg	mg per 1 m^2 10 plants
Aboveground organs	829.0 ± 31.9	8290.0 ± 262.6
Roots	862.0 ± 25.2	8620.0 ± 301.21
Mixture of lawn grasses		
Plant parts	Dry weight per one plant, mg	mg per 1 m^2 100 plants
Aboveground organs	297.0 ± 7.1	29,700.0 ± 934.1
Roots	21.0 ± 8.3	2100.0 ± 71.5

grasses (Table 13.3). The dry biomass of the aboveground organs of sunflower per plant exceeded the biomass of grass lawns by 2.79 times and biomass of sunflower roots by 41 times.

Biomass, collected from an area of 1 m^2, was significant. The biomass of aerial organs from 100 plants of lawn grasses per 1 m^2 exceeded than that of sunflower by 3.58 times. But the root biomass of 100 lawn grass plants from an area of 1 m^2 was less by 4.1 times than root biomass of 10 sunflower plants from the plot with the same area. Before planting plants and at the end of the experiment, the content of heavy metals in the soil was determined. According to the initial content in the soil under sunflower plants, the heavy metals were arranged in the following order (mg/kg): Pb (11,035.0) > Zn (5181.0) > Cu (3258.0) > Cd (303.0) (Table 13.4).

According to the initial content in the soil under lawn grasses, heavy metals are arranged in the following order (mg/kg): Pb (9410.0) > Zn (4871.0) > Cu (3420.0) > Cd (280.0).

Taking into account the biomass of the aboveground organs and roots of sunflower and lawn grasses from the area of 1 m^2, the removal of heavy metals by sunflower and lawn grass organs was calculated. According to the removal of heavy metals from 1 m^2 by the aboveground organs of sunflower (ten plants), metals are arranged in the following order (mg), Pb (66.138) > Zn (44.211) > Cu (25.5) > Cd (5.074), and by roots Pb (11.24) > Zn (5.62) > Cu (1.68) > Cd (0.56) (Fig. 13.2). According to the removal of heavy metals by the aerial organs of lawn grasses (100 plants), the metals are arranged in the following order (mg), Pb (90.03) > Zn (81.636) > Cu (23.043) > Cd (2.59), and by roots Pb (1.388) > Zn (0.65) > Cu (0.462) > Cd (0.139).

The removal of copper by the aerial organs of sunflower little more than that of lawn grasses (25.5 mg Cu and 23.043 mg Cu in the aerial parts of sunflower and grasses, respectively). The removal of cadmium by the aboveground organs of sunflower was higher than that of lawn plants by 1.96 times although total biomass of the aboveground organs of sunflower from the area of 1 m^2 was lower as compared to lawn grasses. It was a consequence of a large accumulation of these metals by aerial organs of sunflower, and the concentration of Cu in the aerial organs of sunflower was about four times greater than that of lawn grasses

Table 13.4 The content of heavy metals in soil sown with sunflower and lawn grasses

Soil	Cu, mg/kg	%	Pb, mg/kg	%	Cd, mg/kg	%	Zn, mg/kg	%
The content of heavy metals in the soil under sunflower before the experiment	3258.0 ± 170.0	100	11,035.0 ± 419.0	100	303.0 ± 9.6	100	5181.0 ± 218.2	100
The content of heavy metals in the soil under sunflower after the experiment	870.0 ± 25.2	27	1900.0 ± 72.4	17	210.0 ± 7.60	69	2510.0 ± 8.9	48
The content of heavy metals in the soil under lawn grasses before to the experiment	3420.0 ± 12.0	100	9410.0 ± 361.3	100	280.0 ± 8.5	100	4871.0 ± 181.4	100
The content of heavy metals in the soil under lawn grass after the experiment	980.0 ± 42.0	29	2210.0 ± 69.1	24	260.0 ± 8.2	93	247.0 ± 8.170	51

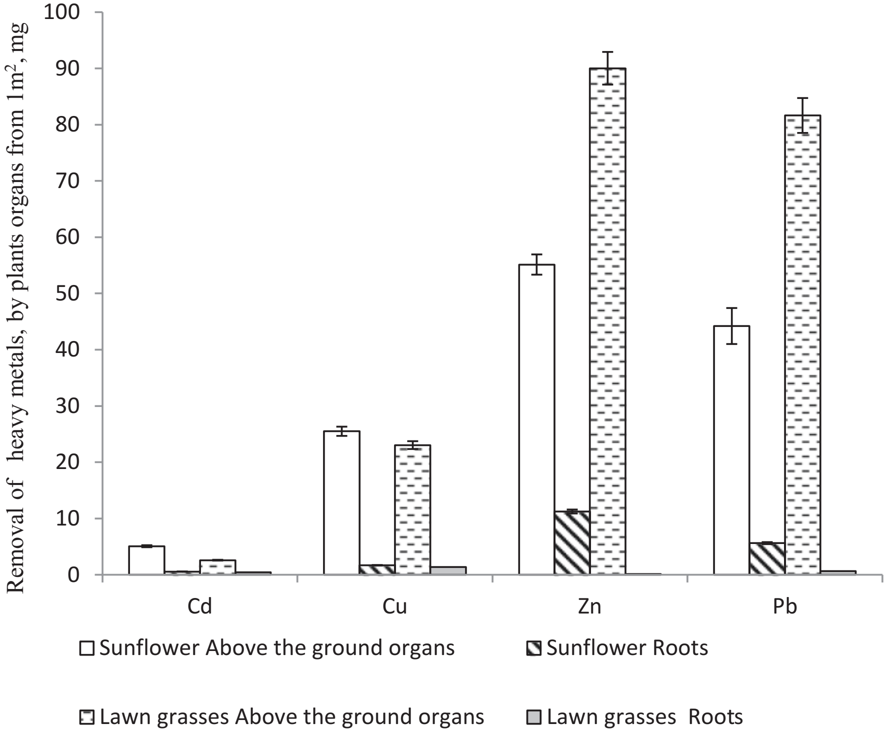

Fig. 13.2 The removal of heavy metals by plant organs

and concentration of Cd also more in sunflower aboveground organs than that in lawn grasses (1.9 times).

The removal of lead and zinc by the aboveground organs of sunflower was lower than that of the aboveground organs of lawn grasses: Pb, 1.63 times, and Zn, 1.85 times. The concentration of lead in the aerial organs of sunflower was 2.63 times and that of zinc was 1.94 times higher than in lawn grasses.

The removal by roots of all heavy metals studied was higher in sunflower plants. The removal of lead, zinc, copper, and cadmium by the roots of sunflower was higher than in lawn grasses by 8, 8.6, 3.65, and 4 times, respectively. The degree of soil purification was determined from the difference in the concentration of heavy metals in the soil before planting and at the end of the experiment. It was established that the greatest degree of soil purification from the studied metals was found in sunflower plants.

For the residual content of heavy metals in the soil under sunflower plants, the metals were arranged in the following order (%), Cd (69) > Zn (48) > Cu (27) > Pb (17), and, in the soil under a mixture of lawn grasses (%), Cd (93) > Zn (51) > Cu (29) > Pb (24) (Table 13.4). According to this indicator, it is possible to judge the degree of soil purification by the investigated plants. The higher the percentage of the metal content (residual amount) in the soil, the lower the degree of purification. From the obtained data, it follows that the degree of soil purification by plants from lead was the greatest and cadmium the lowest. The degree of soil purification by sunflower plants was higher for all metals tested than for lawn grasses.

Thus, in all the investigated plants, the bioconcentration factor of lead for the aerial organs and roots was the highest. For the aboveground organs, the bioconcentration factor of all the metals studied was above unity. The bioconcentration factor of all investigated heavy metals in the aerial organs of plants was higher in comparison with the roots. Probably, this is a consequence of strong atmospheric pollution on the territory of the plant. The removal of lead and zinc by the aboveground organs of sunflower was lower than that of the aboveground organs of lawn grasses. The removal of copper by the aerial organs of both plant species was approximately the same, and the removal of cadmium by the aboveground organs of sunflower was higher than that of lawn plants. Biomass of aerial organs from 1 m^2 in lawn grasses is 3.58 times more than in sunflower. And the biomass of roots, on the contrary, exceeds in sunflower, in comparison with lawn grasses—by 4.1 times. The degree of soil purification by plants from lead was the highest and from cadmium the lowest. The degree of soil purification by sunflower plants was higher for all metals tested than by lawn grasses.

Thus, it can be concluded that the plants of the sunflower can be successfully used for phytoremediation of soils contaminated with heavy metals.

13.3 Conclusion

Thus, bioremediation of contaminated soils is the most promising and less expensive way of cleaning the environment from contamination. To date, phytoremediation is recognized throughout the world as the most cost-effective and environmentally friendly technology. For Kazakhstan, the use of species widespread on the territory of the Republic for phytoremediation is appropriate, since the introduction of European species of plant hyperaccumulators will require additional costs. Therefore, the search for plant hyperaccumulators in the territory of Kazakhstan is the paramount task of investigators working in this direction. Oli seed plants which accumulate high biomass and are able to accumulate large amounts of heavy metals and wild grass species can be successfully used for phytoremediation.

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References

1. Suleev DK, Sagitov SI, Sagitov PI, Zhumagulov KK (2004) Ecology and nature management. The concept of a transition to sustainable development. Nauka, Almaty 391 p
2. Chaney RL, Malik M, Li YM et al (1997) Phytoremediation of soil metals. Curr Opin Biotechnol 8:279–284
3. Chaney RL, Li YM, Scott JA (1998) Improving metal hyperaccumulator wild plants to develop commercial phytoextraction systems: approaches and progress. CRC Press, Boca Raton, FL 37 p
4. The concept of ecological safety of the Republic of Kazakhstan (1996) Noosphere 1:135–146
5. Fry SC, Miller JG, Panwill JC (2002) A proposed role for copper ions in cell wall loosening. Plant and Soil 247:57–67
6. Dixit V, Pandey V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L., cv Azad). J Exp Bot 52(358):1101–1109
7. Zornoza P, Vazquez S, Esteban E, Fernandez-Pascual M (2002) Cadmiumstressinnodulated-whitelupin: strategiesto avoid toxicity. Plant Physiol Biochem 40:1003–1009
8. Reichman SM (2002) The responses of plants to metal toxicity: a review focusing on copper, manganese and zinc. The Australian Minerals & Energy Environment Foundation, Melbourne, VIC, p 54
9. Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K, Prasad MNV (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35(4):985–999
10. Kumar A, Prasad MNV, Achary VMM, Panda BB (2013) Elucidation of lead-induced oxidative stress in Talinum triangulare roots by analysis of antioxidant responses and DNA damage at cellular level. Environ Sci Pollut Res 20(7):4551–4561
11. Ebbs S, Lau J, Ahner B (2002) Phytochelatin synthesis is not responsible for Cd tolerance in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Planta 214:635–640
12. Krotz RM, Evangelou BP, Wagner GJ (1989) Relationships between cadmium, zinc, Cd-peptide and organic acid in tobacco suspension cells. Plant Physiol 91:780–787
13. Rauser WE (1995) Phytochelatins and related peptides. Plant Physiol 109:1141–1149
14. Shkolnik NY, Alekseeva-Popova IV (1983) Rastenia v extremalnih usloviah mineralnogo pitania. Nauka, Leningrad 176 p, In Russian
15. Chaney R, Brown S, Li Y-M, Angle S, Horner F, Green C (1995) Potential use of hyperaccumulator plant species to decontaminate metal polluted soils. Min Environ Manag 3(3):9–11
16. Raskin I, Smith RD, Salt DE (1997) Phytoremediation of metals: using plants to remove pollutants from the environment. Curr Opin Biotechnol 8:221–226
17. Prasad MNV (2003) Prakticheskoe ispolzovanie rastenii dlya vosstanovklenia ecosystem, zagryaznennih metallami. Fiziol Rastenii 50(3):764–780
18. Duffus JH (2002) "Heavy metals" – a meaningless term? (IUPAC Technical Report). Pure Appl Chem 74(5):793–807
19. Bandman AL, Volkova NV, Grehova TD (1998) Vrednie himicheskie veshesnva. Himia, Leningrad 592 p, In Russian
20. Chernavskaya NM (1989) Physiologia rastitelnih organizmova I rol metallov. Nauka, Moskva 156 p, In Russian
21. Harvey LJ, McArdle HJ (2008) Biomarkers of copper status: a brief update. Br J Nutr 99(S3):10–13 PubMed: 18598583
22. Stern BR (2010) Essentiality and toxicity in copper health risk assessment: overview, update and regulatory considerations. Toxicol Environ Health A 73(2):114–127
23. Tchounwou PB, Ishaque A, Schneider J (2001) Cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2) exposed to cadmium chloride. Mol Cell Biochem 222:21–28 PubMed: 11678604

24. Tchounwou PB, Yedjou CG, Foxx D, Ishaque A, Shen E (2004) Lead-induced cytotoxicity and transcriptional activation of stress genes in human liver carcinoma cells (HepG2). Mol Cell Biochem 255:161–170 PubMed: 14971657
25. Beyersmann D, Hartwig A (2008) Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82(8):493–512 PubMed: 18496671
26. Patlolla A, Barnes C, Yedjou C, Velma V, Tchounwou PB (2009) Oxidative stress, DNA damage and antioxidant enzyme activity induced by hexavalent chromium in Sprague Dawley rats. Environ Toxicol 24(1):66–73 PubMed: 18508361
27. Atabayeva S (2016) Heavy metals accumulation ability of wild grass species from industrial area of Kazakhstan. In: Ansari AA et al (eds) Phytoremediation: management of environmental contaminants. Section 4. Phytoremediation applications for metal contaminated soils using terrestrial plants. Springer, New York, NY, pp 157–200
28. Gimeno-Garcia E, Andreu V, Boluda R (1996) Heavy metals incidence in the application of inorganic fertilizers and pesticides to rice farming soils. Environ Pollut 92:19–22
29. Andreu V, Boluda R (1995) Application of contamination indexes on different farming soils. Bull Environ Pollut 104:271–282
30. Lee JS, Chon HT, Kim KW (1988) Migration and dispersion of trace elements in the rock-soil-soil plant system in areas underlain by black shales and states of the Okchon Zone, Korea. J Geochem Explor 65:61–78
31. Jackson AP, Alloway BJ (1992) The transfer of cadmium from agricultural soils to the human food chain. In: Adriano DC (ed) Biochemistry of trace metals. Lewis Publishers, Boca Raton, FL, pp 109–158
32. FAO/WHO. Joint Committee on Food Additives and Contaminants. Position paper on cadmium (prepared by France). 27th Session. The Hague, The Netherlands. 20–24 March, 1995. 32 p
33. Tasekeev M (2004) Bioremediatsia toksichnih promishlennih othodov. Promishlennost Kazakhstana 5(26):59–63
34. Kvesitadze GI, Hatisashvili GA, Sadunishvili TA, Evstigneeva ZG (2005) Metabolism antropogennih toksikantov v visshih rasteniah. Nauka, Moskva 197 p, In Russian
35. Brown SL, Chaney RL, Angle JS, Baker AJ (1995) Zinc and cadmium uptake of *Thlaspi caerulescens* grown in nutrient solution. Soil Sci Soc Am J 59:125–133
36. Prasad MN (2003) Prakticheskoe ispolzovanie rasenii dlia vosstanovlenia ekosyste, zagryaznennih tiazhelimi metallami. Fiziol Rastenii 50(5):764–780 In Russian
37. Hooda V (2007) Phytoremediation of toxic metals from soil and waste water. J Environ Biol 28(2):367–376
38. Huang JW, Cunningham SD (1996) Lead phytoextraction: species variation in lead uptake and translocation. New Phytol 134:335–342
39. Li YM, Chaney R, Brewer E, Roserberg R, Angle JS, Baker A, Reeves R, Nelkin J (2003) Development of a technology for commercial phytoextraction of nickel: economic and technical considerations. Plant and Soil 249:107–115
40. Broadly MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702
41. Palmergen MG, Clemens S, Williams LE, Kramer U, Borg S, Schjorring JK, Sanders D (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13:464–473
42. Brooks RR (1998) Plants that hyperaccumulate heavy metals, vol 53. CAB International, Wallingford
43. McGrath SP (1998) Phytoextraction for soil remediation. In: Brooks RR (ed) Plants that hyperaccumulate heavy metals. AB International, Wallingford, pp 261–287
44. Lasat MM, Fuhrman M, Ebbs SD (2003) Phytoextraction of radiocesium-contaminated soil: evaluation of cesium-137 bioaccumulation in the shoots of three plant species. J Environ Qual 27:165–169
45. Baker AJ, McGrath SP, Reeves RD (2000) Metal hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource for phytoremediation of metal polluted soils. In: Contaminated soil and water. Lewis Publishers, Boca-Raton, FL, pp 85–107

46. Kos B, Grčman H, Leštan D (2003) Phytoextraction of lead, zinc and cadmium from soil by selected plants. Plant Soil Environ 49:548–553
47. Lesage E, Meers E, Vervaeke P, Lamsal S, Hopgood M, Tack FM (2005) Enhanced phytoextraction: II. Effect of EDTA and citric acid on heavy metal uptake by Helianthus annuus from a calcareous soil. Int J Phytoremediation 7(2):143–152
48. Raskin I, PBA K, Dushenkov S, Salt DE (1994) Bioconcentration of heavy metals by plants. Curr Opin Biotechnol 5:285–290
49. Romeiro S, Lagôa AMM, Furlani PR, De Abreu CA, De Abreu MF, Erismann NM (2006) Lead uptake and tolerance of *Ricinus communis* L. Braz J Plant Physiol 18(4):18–25
50. Vwioko DE, Anoliefo G, Fashemi SD (2006) Castor oil grown in soil contaminated with spent lubricating oil. J Appl Sci Environ Manag 10(3):127–134
51. Marchiol L, Assoaris S, Sacco P, Zerbi G (2004) Phytoextraction of heavy metals by canola (*Brassica napus*) and radish (*Raphanus sativus*) grown on multicontaminated soil. Environ Pollut 132(1):21–27
52. Boonyapookana B, Parkpian P, Techapinyawat S, De Laune RD, Jugsujinda A (2005) Phytoaccumulation of lead by sunflower (*Helianthus annuus*), tobacco (*Nicotiana tabacum*), and vetiver (*Vetiveria zizanioides*). J Environ Sci Health A Toxic Hazard Subst Environ Eng 40(1):117–137
53. Gulz PA, Gupta SK, Schulin R (2003) P-enhanced phytoextraction of arsenic from contaminated soil using sunflower. Proceedings of the 7th International Conference on the Biogeochemistry of Trace Elements (7th ICOBTE), 15–19 June, 2003, Uppsala, Sweden. Book of Abstracts. – Vol I-II. P. 148–149
54. Mukhtar S, Bhatti HN, Khalid M, Haq MA, Shahzad SM (2010) Potential of sunflower (helianthus annuus L.) for phytoremediation of nickel (Ni) and lead (Pb) contaminated water. Pak J Bot 42(6):4017–4026
55. Begonia GB (1997) Comparative lead uptake and responses of some plants grown on lead contaminated soils. Department of Biology, Jackson State University, Jackson, MS 39217. http://www.msstate.edu/org/MAS/ejour3.html
56. Lee I, Baek K, Kim H, Kim S, Kim J, Kwon Y, Chang Y, Bae B (2007) Phytoremediation of soil co-contaminated with heavy metals and TNT using four plant species. J Environ Sci Health A Tox Hazard Subst Environ Eng 42(13):2039–2045
57. Solhi M, Hajabbasi MA, Shareatmadari H (2005) Heavy metals extraction potential of sunflower (*Helianthus annuus*) and canola (*Brassica napus*), Caspian. J Env Sci 31:35–42
58. Adesodun JK, Atayese MO, Agbaje TA, Osadiaye BA, Mafe OF, Soretire AA, Adesodun JK (2010) Phytoremediation potentials of sunflowers (*Tithonia diversifolia* and *Helianthus annuus*) for metals in soils contaminated with zinc and lead nitrates. Water Air Soil Pollut 207(1–4):195–201
59. Madejon P, Murillo JM, Maranon T, Cabrera F, Soriano MA (2003) Trace element and nutrient accumulation in sunflower plants two years after the Aznacolla mine spill. Sci Total Environ 37:239–257
60. Marchiol L, Fellet G, Perosa D, Zerbi G (2007) Removal of trace, metals by *Sorghum bicolor* and *Helianthus annuus* in a site polluted with zinc by industrial wastes: a field experience. Plant Physiol Biochem 45:379–387
61. Lin J, Jiang W, Lin D (2003) Accumulation of copper by roots, hypocotyls, cotyledons and leaves of sunflower (*Helianthus annuus* L.). Bioresour Technol 86:151–155
62. Angelova V, Perifanova-Nemska MN, Uzunova GP, Ivanov KI, Lee HQ (2016) Potential of sunflower (Helianthus annuus L.) for phytoremediation for soils contaminated with heavy metals. Int J Environ Ecol Eng 10(9):576–583
63. Patel SJ, Bhattacharya P, Banu S, Bai L, Namratha R (2015) Phytoremediation of copper and lead by using sunflower. Indian mustard and water hyacinth plants. Int J Sci Res 4(5):113–115 ISSN (Online): 2319-7064
64. Zavoda T, Cutright J, Szpak I, Fallon E (2001) Uptake, selectivity and inhibition of hydroponic treatment of contaminants. J Environ Eng 127:502–508

65. Prasad MNV (2007) Sunflower (*Helianthus annuus* L.) – a potential crop for environmental industry. Helia 30:167–174
66. Zadeh BM, Savaghebi-Firozabadi GR, Alikhani HA, Hosseini HM (2008) Effect of sunflower and amaranthus culture and application of inoculants on phytoremediation of the soils contaminated with cadmium. Am Eur J Agric Environ Sci 4(1):93–103
67. Herrero EM, Lopez-Gonzalvez A, Ruiz MA, Lucas-Garcia JA, Barbas C (2003) Uptake and distribution of zinc, cadmium, lead and copper in *Brassica napus* var *oleifera* and *Helianthus annuus* grown in contaminated soils. Int J Phytoremediation 5:153
68. Rivelli AR, De Maria S, Puschenreiter M, Gherbin P (2012) Accumulation of cadmium, zinc and copper by *Helianthus annuus* L. Impact on plant growth and uptake of nutritional elements. Int J Phytoremediation 14:320–334
69. Chen H, Cutright TJ (2002) The interactive effects of chelator, fertilizer and rhizobacteria for enhancing phytoremediation of heavy metal contaminated soil. J Soil Sediment 2:203–210
70. Martins CDC, Liduino VS, Oliveira FJS, Sérvulo EFC (2014) Phytoremediation of soil multi-contaminated with hydrocarbons and heavy metals using sunflowers. Int J Eng Technol 14(5):144305–147171
71. Gołda S, Korzeniowska J (2016) Comparison of phytoremediation potential of three grass species in soil contaminated with cadmium. Environ Protect Nat Resour 27(1 (67)):8–14. https://doi.org/10.1515/oszn-2016-0003
72. Aibubu N, Liu Y, Zeng G, Wang X, Chen B, Song H, Xu L (2010) Cadmium accumulation in *Vetiveria zizanioides* and its effects on growth, physiological and biochemical characters. Bioresour Technol 101:6297–6303
73. Xu P, Wang Z (2013) Physiological mechanism of hypertolerance of cadmium in Kentucky bluegrass and tall fescue: chemical forms and tissue distribution. Environ Exp Bot 96:35–34
74. Abaga NOZ, Dousset S, Mbengue S, Munier-Lamy C (2014) Is vetiver grass of interest for the remediation of Cu and Cd to protect marketing gardens in Burkina Faso. Chemosphere 113:2–47
75. LMN/SOP-06 (2006) Digestion of hard samples for heavy metal determination in the flame, 10 p
76. LMN/SOP-08 (FLAA) (2001) Work process of Analyst 300 Perkin Elmer (in the flame), 14 p
77. Agoramoorthy G, Chen F-A, Hsu MJ (2008) Threat of heavy metal pollution in halophytic and mangrove plants of Tami Nadu, India. Environ Pollut 155:320–326