# Chapter 6
# Heavy Metals Accumulation Ability of Wild Grass Species from Industrial Areas of Kazakhstan

**Saule Atabayeva**

## 6.1 Introduction

Heavy metals are prevalent man-made pollutants that cause a set of human diseases, entering a body through the food chain as a consequence of contamination of soils and vegetation. The search for effective methods of remediation of technogenic contaminated soils is an important environmental task in Kazakhstan. The urgency of the problem is associated with soil heavy metals contamination, in particular, in the area around the steel mills and tailings. Heavy metals, which come into the soil in a variety of ways as a result of human activity, are dangerous environmental pollutants. The amount of heavy metals accumulated in this way can be many times greater than its natural content in soil. Dispersion of man-made heavy metals pollution into the atmosphere has become global. The main sources of contamination of the environment by copper, lead, zinc, and cadmium are mining, metallurgical and chemical industries, heat power engineering, transport and chemical pesticides, as well as household waste. Pollution of air, soil, plants, and water by heavy metals in the vicinity of large industrial centers has become one of the most pressing environmental problems. In soils near industrial areas, heavy metal content is tens or hundreds of times higher than background levels in similar soils [1].

High concentrations of some heavy metals in the soil are adequately reflected in the yield and quality of vegetable products grown within the boundaries of the industrial centers in the garden plots. In vegetation, heavy metals content exceeds the allowable concentrations in 2–3.5 times [2, 3]. The excessive concentrations of heavy metals in plants violate the physiological and biochemical processes, inhibit the growth and development of plant organisms, and reduce the quality of the agricultural products obtained. Thus, the increasing of anthropogenic pollution by

S. Atabayeva (✉)
Department of Biology and Biotechnology, Al-Farabi Kazakh National University, Research Institute of Ecology, Almaty, Kazakhstan
e-mail: sauleat@yandex.ru



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heavy metals, the migrating of heavy metals along the trophic relations, lead to various adverse effects on living organisms. It is well-known that heavy metals negatively affect the biochemical and physiological processes of plants, change membrane properties [4–6], the activity of the enzymes, cause oxidative stress [7–9], resulting in inhibited growth processes, in changing the time required for the phenological phases, in morphological changes occurring in plant organs, in reduced yields. In response to heavy metals contamination in plants, a number of protective mechanisms are activated, such as the increased synthesis of metallothioneins (phytochelatins), organic acids, polyamines, and antioxidant enzymes [10–13] to reduce the toxic effects of heavy metals and to maintain homeostasis.

But in various kinds of plants, defense mechanisms against stress develop in varying degrees. Different species and even populations within a species may differ in sensitivity to heavy metals and the degree of their accumulation in their organs, which can be the basis of the formation of metallophytic flora [14]. The use of characteristics of plants like a resistance to heavy metals and a high metal-accumulating ability are the basis of the technology of phytoremediation of contaminated soil, which is defined as a cleaning technology of the environment from chemical contaminants using plants [15–17]. One of the necessary steps towards preventing the toxic effect of heavy metals on animal and human being is to clean soil. Currently, the most effective method is soil phytoremediation, i.e., cleaning soil using plants-hyperaccumulators of heavy metals. Compared to physical and chemical methods of soil cleaning, this method is less expensive, more efficient and safe [15, 18]. According to literature, the cost of conservative methods (chemical and physical methods) of cleaning the soil ranges from $30–$350 per hectare, and the cost of cleaning the soil using plants is about $160 per hectare [15]. According to other estimates, depending on the soil conditions and concentration of heavy metals, the cost of treatment with the help of plants (using only the sun energy) may be only 5 % of the costs required for other methods of ecosystem restoration, contaminated by metals [19]. It is necessary to use various plant species which are adapted to native environment, tolerant to heavy metals, and able to accumulate them in their organs and to implement these plants for different types of phytoremediation to reduce the risk of further spread of environment pollution.

### *6.1.1 Phytoremediation Technology*

There are different types of phytoremediation. The technology includes phytoextraction, phytovolatilization, phytostabilization, and rhizofiltration [19, 20]. Phytoextraction — the use of plants that accumulate metals mainly in the aerial organs and the further using of the aerial part for the incineration and recovery of metals from plant's ash (Figs. 6.1 and 6.2). This type of phytoremediation is used to extract metals out of the soils. The advantage of this method is the ability to extract a large amount of metals from the soil by aerial organs of plants-hyperaccumulators.

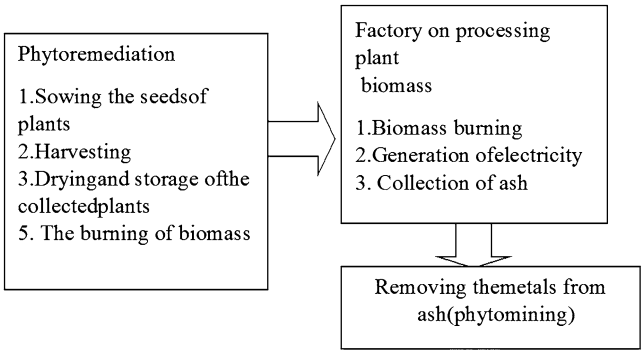


**Fig. 6.1** The scheme of phytoremediation of soils contaminated by heavy metals

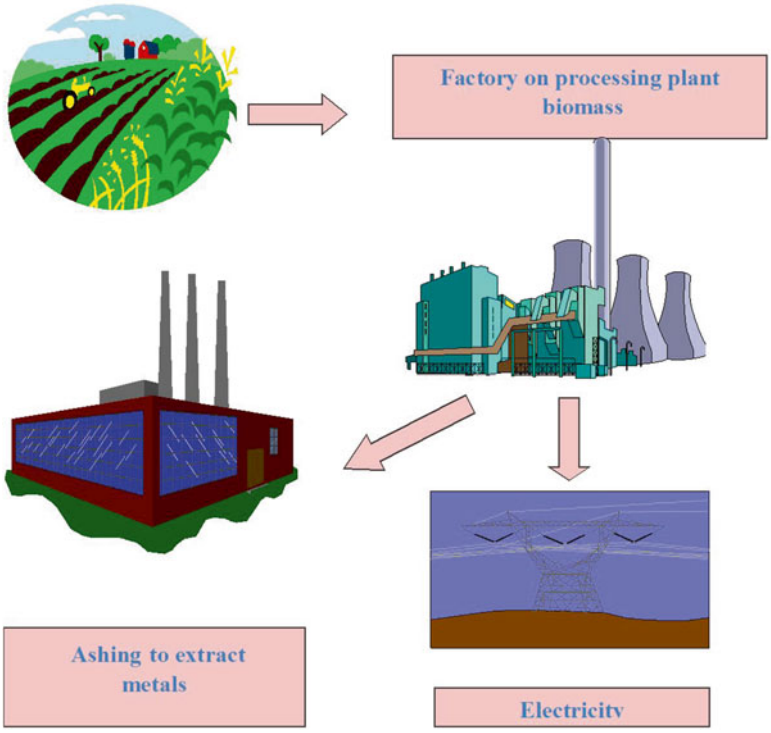


**Fig. 6.2** Scheme of phytomining

The disadvantage of the method is that the plant-hyperaccumulators suitable for this type of phytoremediation usually have a small size, low biomass that could affect the efficiency of the method. So now researchers are working on the application of conventional breeding methods and bioengineering of plants to create new forms of plants that have high capacity to accumulate metals and large biomass [20].

#### 6.1.1.1 Phytovolatilization

This includes using plants to volatilize the chemical elements. Some plant species are able to volatilize such heavy metals as Hg, Se, As [21]. The modified gene Hg-reductase (mercury-reductase) was transferred from bacteria to plants *Arabidopsis thaliana* L. to obtain species which can be used for phytovolatilization. Research is being conducted towards the production of higher plants, in which bacterial genes responsible for the hydrolysis of methyl and dimethyl-Hg-Hg are expressed. Organic mercury compounds are the main source of danger as lipophilic components are accumulated in the body of predatory birds and animals [22, 23]. The disadvantage of this method is a possible pollution of the atmosphere with toxic volatile compounds. Therefore, this method can be applied to areas that are far from crops and settlements.

#### 6.1.1.2 Phytostabilization (Phytoimmobilization)

This is the use of plants to convert metals into less toxic forms but without removing metals from the soil [24]. This kind of phytoremediation technology is possible to use towards lead (Pb) and chromium (Cr). Plants with long and strong root system are very effective for this type of phytoremediation. The roots of *Agrostis capillaris* L. (bentgrass hairlike) grown in heavily Pb/Zn-polluted soil form pyromorphites of Pb and phosphorus (P). The deposits in the roots have pyromorphites ($Pb_5(PO_4)_3C1$)-type structure and some of Cl-atoms may be substituted by OH, but the mechanism of their formation is still unknown [25]. The formation of heavy metals P-precipitates is a tolerance mechanism to heavy metals, which passively sequester Pb in a metabolically inactive form. Although it is believed that *Thlaspi rotund folium* L. is a hyperaccumulator of Pb, Zea mays (corn) can accumulate a large amount of lead (Pb) at low pH and low phosphorus concentration [26]. The addition of chelating agents (NEDTA, EDTA) increases the solubility of lead (Pb) and its mobility within the plant. Lead (Pb) content in the above ground organs can reach 1 %; this allows to extract a sufficient amount of Pb.

"Immobilization" method can be used on Pb-contaminated soils [27]. Plants that accumulate Pb in the roots could keep it from leaching down the soil profile. Therefore, inactivation of Pb-contaminated soil with soil additives (hydroxide Fe, oxides of Mn, phosphates, limestone) and the use of plants to prevent erosion is one of the ways of phytoremediation of Pb-contaminated soils [28]. The disadvantage of this method is that the metal is not completely recovered from the soil surface and it remains bound in the roots of plants. This method is not suitable for the soil, which in the future will be used for growing crops. This method can be applied to chromium (Cr) too. Soils containing 10,000 mgCr/kg of $Cr^{3+}$ are not a potential hazard, while the soil containing chromium in the form of $Cr^{6+}$ is toxic to plants and other organisms. The roots of the plants could play an important role in restoring $Cr^{6+}$ to $Cr^{3+}$ in the soil, allowing the toxic form of chromium to immobilize to an inert form, which does not represent a potential risk [29].

The disadvantage of this method is the need in periodic cleaning of the contaminated plant parts and recycling them.

#### 6.1.1.3 Rhizofiltration

This is using plant roots to extract the metal from flowing water. This technology uses plants that absorb heavy metals or radioactive elements in the aqueous medium through its root system. The plants are grown in hydroponic system and the contaminants are "filtered" through root system (rhizofiltration), which absorbs and concentrates the pollutants [30, 31]. The use of plants with a long root system or a large absorption surface with greater storage capacity (water hyperaccumulators) and tolerant to contaminants brings the best results. For effective development of phytoremediation, each element should be considered separately. We need an agronomic approach based on genetic properties of plants. Some elements can be absorbed by plant roots and turn into a volatile form as dimethylselenid [20, 21] and mercury [20]. While many plants are able to volatilize dimethylselenid, the concomitant pollution by sulfates and salinity of Se-contaminated soils inhibits this process. Therefore, it is necessary to improve soil conditions by using additives to achieve the best effect of phytoremediation.

### *6.1.2 Plants-Hyperaccumulators of Heavy Metals*

For phytoextraction of heavy metals from the soil, the most beneficial is the use of plants hyperaccumulators of heavy metals. The term "hyperaccumulators" refers to species of plants, which accumulate 10–100 times more metals than conventional plants. Plants-hyperaccumulators cause considerable interest from the point of view of phytoremediation [26], phytomining [32], and biofortification of crops [33, 34]. These plants can be used to extract toxins from the soil and thus may help restore a fertility of contaminated soils. Plants-hyperaccumulators are endemic to the soil, which is contaminated with heavy metals and they do not compete with other species in uncontaminated soils. An accumulation of heavy metals in nontoxic form is one of the strategies used by plants to survive in conditions of strong pollution. The best known plants hyperaccumulators are *Ambrosia artemisiifolia* L. (ragweed), *Thlaspi rotundufolium* L., and *Thlaspi caerulescens* L., absorbing a significant amount of Zn, Cd, and Pb. Hyperaccumulators of Ni are *Alyssum* L. and *Arabidopsis* L. The latter is considered as a convenient object for research because it has a short life cycle and the small number of chromosomes. Typically, plant hyperaccumulators of heavy metals are mostly scrubby weeds with low yields. At present, the improved by genetic bioengineering plant forms of *Alpine penycress* L. with a high yield can absorb about 500 kg/ha of zinc and 6–8 kg of cadmium per year [35]. *T.caerulescens* can accumulate 2.5 and 0.2 % of dry weight cadmium (Cd) and zinc (Zn), respectively, from contaminated soils. With these plants, there can be removed

125 kg and 10 kg Zn and Cd per hectare [36]. According to the earlier estimation, the cost of these metals extracted from 1 ha of plants will be $200 at market price of zinc — $1.33, cadmium — $4.6 per kilogram [36]. Researchers have identified some of the most specific signs of hyperaccumulators:

1. The plants must be resistant to high concentrations of metals in roots and aerial parts. Hyperaccumulation ability is the key feature that makes possible the hyperaccumulation. Hypertolerance is the result of chelation and vacuolar compartmentation of metals [35]. This was demonstrated in vacuoles of isolated protoplasts of tobacco cells, which accumulated high levels of Cd and Zn. Electron microscopic analysis of leaves of *Thlaspi caerulescens* [37] also indicates the vacuolar compartmentation of Zn.
2. The plants must be able to translocate elements from roots to aerial organs. Normally, the content of Zn, Cd, or Ni in roots is ten or more times higher than that in aerial organs. The ratio of metal content in the aerial parts to its content in the roots should be greater than one, indicating that hyperaccumulators can redistribute heavy metal ions in the aerial organs [37].

   Krameret al. [38] found that the ions of Ni, detected in leaf extracts of hyperaccumulator plant *Alyssum bertolonii* L., were chelated with citrate and malate, and in xylem exudate histidine chelates 40 % of nickel (Ni). The addition of histidine to the nutrient medium increased resistance to Ni and its translocation to the aerial organs in non-accumulator *A. montanum* L. [38].
3. Plants must absorb metals in large quantities. Plants *T. caerulescens* in natural conditions contain up to 1–4 % Zn, while the other species — less than 0.05 % of Zn. It was shown that Zn-hypertolerant genotypes of *T. caerulescens* require much more Zn in the nutrient solution ($10^4$ times more) for normal growth than non-accumulators. A high effective compartmentation of heavy metals to reduce the toxicity of Cd and Zn as it requires the plants to accumulate a large amount of metals [39]. To understand the technology of phytoremediation in detail, it is necessary to turn to classical works in this area. Currently, the definition of R. Brooks is generally accepted [40], and according to which hyperaccumulators of heavy metals are those plants that accumulate zinc (Zn) >10,000, lead (Pb) >1000, cadmium (Cd) >100 μg/g in the aerial parts (Table 6.1). Plants -non-accumulators of heavy metals should accumulate Zn <100, Pb <10, and Cd <1 on the uncontaminated soils (μg/g); on the contaminated soil — Zn <1000, Pb <100, Cd <10 μg/g.

**Table 6.1** Thresholds of metal concentrations in organs of plants hyperaccumulators

| Metals | % DW | Concentration, ppm |
|---|---|---|
| Cd | 0.01 | 100.0 |
| Co | 0.1 | 1000.0 |
| Cu | 0.1 | 1000.0 |
| Pb | 0.1 | 1000.0 |
| Ni | 0.1 | 1000.0 |
| Mn | 1.0 | 10,000.0 |
| Zn | 1.0 | 10,000.0 |

Different populations of the same species may differ in the degree of metal-accumulating ability. V. Bert et al. [41] studied several tens of populations of *Arabidopsis halleri* L. growing in polluted and unpolluted soils. Plants were tested for the ability to accumulate Zn, Cd, and Pb. It was found that all populations of *A. halleri* exhibit properties to accumulate metals regardless of the place of growth. Populations of plants from uncontaminated areas contain these metals below the threshold concentration for hyperaccumulators and higher than for non-accumulators of heavy metals. When these plants were transferred to hydroponic conditions with a high concentration of heavy metals, their hyperaccumulation status confirmed for Zn and Cd. These data indicate that in cases where the plants have relatively high metal accumulation capacity, but the value of the metal concentration in the tissue is below the commonly accepted thresholds for hyperaccumulators, additional studies with the use of hydroponic conditions to identify the real plants hyperaccumulators of heavy metals are required [37].

It was found that there is a threshold of saturation of plants hyperaccumulators by metals above which the concentration of this metal in the plant does not rise [41]. The curve has a plateau. The similar fact has also been found for Cd [42]. The authors explain this fact by blocking the flow of metals from the roots to the aerial organs. In this case, the protection mechanism is triggered, which limits the toxicity of the metal to the plants at a high concentration of metal in the medium [42]. The authors are paying attention of researchers to some important points in the study of plant-hyperaccumulators. McGrath [43] considers that while comparing hyperaccumulation ability of various kinds of plants, it is necessary to consider not only the metal concentration in plants (metal content per unit weight of the plant), but the amount of metal recovered by this plant species from a certain area. Thus, if one species is strongly suppressed in the biomass accumulation of above-ground organs and in other species biomass accumulation is reduced to a lesser extent, the concentration of the metal in the above-ground organs of the latter may be lower than the first due to the dilution effect. The absolute value of the metal content in plants, based on a certain area, will give a more accurate picture to assess hyperaccumulation activity of plants in a comparative analysis [43].

Another important point is the ratio of the metals in above-ground plant organs to its content in the soil. Typically, for plants-hyperaccumulators, this value is high (up to 40 or more) [44]. According to the authors, the most accurate definition of the hyperaccumulator status can be found only in hydroponic environment where the ability of plants to tolerate high concentrations of metals is evident [45]. The question "What is more important for the development of phytoremediation: an accumulation of metals in a large amount or a significant accumulation of biomass of above-ground organs?" is under the discussion. If high-yielding species as *Zea mays* L. and *Brassica juncea* L. grow on Zn-contaminated soils with low pH, the yield is reduced by 50 %. Under normal conditions, the yield of these plants is equal to 20 tonnes of dry biomass per hectare.

When soils are polluted by Zn and Cd (100 mg Zn : 1 mg Cd), plants suffer greatly and reduce crop yields; when the content of Zn in aerial parts is up to 500 mg/kg. The Zn toxicity of the soil is a determining factor which controls productivity

of plants. At reducing the yield by 50 % (10 t per hectare), the biomass will comprise 500 mg/kg of Zn (Zn 500 g per tonne). This will result in extraction of only 5 kg Zn per ha per year [39]. *T. caerulescens* initially has lower yield in comparison with the above-mentioned species, but can accumulate up to 25,000 mg of Zn per kg (25 kg/t) without reducing yield. Even at low yield of 5 t per hectare, zinc extraction will be 125 kg/ha [39]. Therefore, Chaney et al. [39] believes that the ability to hyperaccumulate heavy metals and a hypertolerance to high concentrations of metals are more important properties of plants for phytoremediation than the ability to accumulate a large biomass.

### *6.1.3 Phytosiderophores*

The low availability of iron (Fe) for plants due to the high pH of the soil is one of the most common abiotic stresses in the world for agricultural plants. Most crop plants get not enough iron from the soil, which leads to chlorosis, low yields, and reduces the quality of agricultural products. It is known that 30 % of the arable land are the land are the alkaline soils and they are not optimal for crop production [46]. An extremely limited bioavailability of iron from the soil plants led to the development of two established extraction strategies. Strategy I used by dicotyledonous and non-graminaceous monocotyledonous species, releasing protons by roots to decrease soil pH, induce the expression of Fe(III)-reductase to reduce Fe(III), and take up Fe(II) through Fe(II) transporters [47, 48]. Although there is a large amount of iron in the soil, Fe-deficiency in plants growing at high pH in calcareous soils is developed. Under these adverse conditions, grass species have an ability to secrete low-molecular weight secondary imino acids (mugineic acids) known as *phytosiderophores* that form soluble iron chelates.

The strategy of chelation involves the secretion of mugineic acid family phytosiderophores (MAs) [49] to uptake soluble Fe in the form Fe(III)-MAs. This way of Fe uptake was termed by Römheld and Marschner [50] as a strategy II of Fe acquisition. There are numerous data that the phytosiderophores synthes is induced by Fe-deficiency [51]. A secretion of phytosiderophores family mugineic acid increases in response to Fe-deficiency and shows an accurate circadian rhythm of intake of iron in the plant organism [48]. Among the *Poaceae* family, barley (*Hordeum vulgare*) is a most tolerant plant to iron deficiency and secretes a greatest amount of mugineic acids [52, 53]. Since phytosiderophores have the ability to form thermodynamically stable complexes with other metal cations present in the growth medium, they are also involved in the transport and bioavailability of these metals in the environment [51]. It has previously been shown that phytosiderophores promote the absorption by gramineous plants, not only iron, but also zinc [54]. It was found that phytosiderophores are capable of forming complexes and accelerate the absorption by plants not only iron, but heavy metals that can compete with the iron. Phytosiderophores, iron chelators, are allocated in cereal plants under conditions of iron restriction, but they also form complexes with other metals, including cadmium [55, 56].

It is assumed that phytosiderophores play an universal role in the consumption of other trace metals such as Zn, Mn, and Cu, which have a low solubility in alkaline soils [57]. In support of this hypothesis, it was shown that phytosiderophores form stable chelates with Zn, Mn, and Cu [58] and are effective in the extraction of these elements from the calcareous soils [59].

Phytosiderophores are strictly specific and have a high affinity for iron ligands [60]. This particular system is governed solely by the plant absorption of iron from nutrient medium, so the term *phytosiderophores* more suited to these compounds than *phytochelatins*.

Phytosiderophores is a family of linear hydroxy- and amino-substituted imino-hydroxylic acids, several members of which include 4-membered azetidine ring. It was also established that phytosiderophores can be secreted in response to a shortage of zinc in the environment [61]. Phytosiderophores are allocated in species *Aegilops tauschii* and *Triticum* species under Zn- and Fe-deficiency conditions [62]. Cadmium is not essential trace metal and frequent soil contaminant. A constant anthropogenic release of cadmium into the environment leads to permanent accumulation of Cd in the soil. Extraction and Cd accumulation in plant tissues and seeds can cause them to transfer the food chain to man. The use of synthetic chelates is proposed to increase the mobilization of metals and facilitate phytoextraction as a means for cleaning metal-contaminated soils [63].

However, most of the chelate-extractable complexes can be destroyed rather than mobilized by plant roots. Unlike synthetic chelates added to the soil, the plants produce phytosiderophores that are released in the rhizosphere [49, 64–66].

Cadmium increases the release of 2′-dioximugineic acid (DMA) under Fe-sufficient and Fe-deficient conditions [67]. It was found that the presence of cadmium in the soil causes the symptoms of Fe-deficiency [65, 67]. Symptoms of Fe-deficiency lead to more production of DMA, which bound to cadmium ions and reduces the availability and uptake of metal by plant roots [63, 67].

Since phytosiderophores exept iron bind to other heavy metals and transfer them into plants, this mechanism of phytosiderophores synthesis, available in cereals, can be used to clean soils contaminated by heavy metals. These compounds are able to form complexes and accelerate the absorption of iron and heavy metals that can compete with iron. It is expected that the release of phytosiderophores is the main adaptive response to accumulation of trace elements. When iron deficiency increases the activity of nicotineaminesinthase (NAS), catalyzing the formation of nicotineamine - a phytosiderophores precursor, particularly mugineic acid, in cereals increases too [50]. According to the ability to release phytosiderophores, Fe-deficiency plants are located in the following order: barley > wheat > oats > rye > corn > sorghum [68, 69]. The chemical structures of selected components differ among species and even varieties. Study of biosynthesis in vivo and in vitro of mugineic, dioxymugineic and avenic acids showed that L-methionine is a precursor of mugineic acid as well as 2-dioxymugineic and avenic acids [21, 68, 69].

At present the study of genetic regulation of phytosiderophores synthesis is worked out. The research to identify the gene-encoding enzymes that catalyze synthesis of mugineic acid is being conducted [21, 70]. It is contemplated that

transgenic plants could secrete phytosiderophores in the rhizosphere and absorb heavy metals in large quantities. It was found that in the roots of a hyperaccumulator *Arabidopsis hallieri*, the gene AhNAS2 is highly and constitutively expressed and this could play a definite role in Zn tolerance and accumulation [71]. In the roots of *Arabidopsis halleri,* a twofold increased content of NA, probably, is linked to the constitutive expression of AhNAS2 gene. At the expression of AhNAS2 cDNA in a zinc-sensitive *Schizosaccharomyces pombe* strain the tolerance to zinc is increased [71]. It was reported that the overexpression of TcNAS in *A. thaliana* transgenic plants also confers Ni resistance [72], supporting the idea that NA could play some role in metal tolerance and hyperaccumulation [56].

### *6.1.4 Possible Mechanisms of Heavy Metals Hypertolerance and Hyperaccumulation in Plants*

To identify mechanisms of hypertolerance and hyperaccumulation of heavy metals in plants is a necessary step in the development of phytoremediation. The researchers suggest that the increase in concentration of metal-binding proteins or peptides in plant cells can increase the ability to bind metals and plant tolerance. Detoxification processes can be specific or nonspecific. It depends on whether the synthesis of binding heavy metals compounds is induced or they are formed in a cell constitutively. To specific mechanisms belong for metal binding the sinthesis of cysteine-rich proteins - metallothioneins, synthesized in animal cells and plant organisms in response to heavy metals. Metallothioneins, metal-binding proteins, got their name due to the high metal content, which can reach 20 % of the molecular weight [73]. Metal-binding proteins are commonly synthesized in small quantities. Their content in the cell is increased rapidly if they are affected by heavy metals and is decreased in the case of reducing their concentration in the nutrient substrate [74, 75]. Sulfur is present in metallothioneins usually in the form of thiolate and its content generally is about 10–13 %[76].

Moreover, the increased concentrations of heavy metals in living organisms not only stimulate the synthesis of metallothioneins, but assist to bind these proteins to metals. Nevertheless, both the processes of synthesis of metal-binding proteins and the synthesis of heat shock proteins are integral responses of a cell to the effect of many cell's stressful agents. According to recommendations of the Committee on the Nomenclature of Metallothionein in the 2nd International Congress on Metallothionein and other low molecular weight metal-binding proteins (Zurich 1985), any polypeptide which is similar in structure and function to the mammalian metallothionein may be considered to be in the group of these compounds. Metallothioneins are divided into three classes based on the chemical structures of molecules [77]. The first class (MT1) — metal-binding proteins in vertebrates. The molecule of MT1 in a metal-binding domain contains 20 cysteine residues, the location of which is always constant for this class.

The second class (MT2) — polypeptides that are similar in structure to the MT1, but do not have such a conservative position of cysteine residues. They are common for invertebrates, plants, fungi, cyanobacteria, and other prokaryotes, algae, and yeast. The third class (MT3)—phytochelatins (PC), kadastins, glutamylpeptides, i.e., polypeptides of some algae, higher plants and fungi containing-γ-glutamyl cystein residues, and differ from other metallothioneins by enzymatic method of their synthesis [74]. All MT I (mammals) and MT II are composed of one polypeptide chain containing 60 amino acid residues (sometimes 25 and 70). The number of Cys therein is up to 30 %. Often there is such sequence as Cys-X-Cys, where X is anyone different from Cys the amino acid. The metallothioneins often contain thw basic amino acid like Lys, rarely Arg. Metallothioneins of higher plants and algae (MT 3) are composed of two or more amino- and polypeptides, including primarily cysteine, γ-glutamic acid, and glycine. The most common sequence is Cys-γ-Glu-Cys.

In most metallothioneins, all Cys residues are deprotonated and are able to bind heavy metals in the ratio 3 ligands to one metal ion. Glutamine (Glu) performs a definite role in the mechanism of resistance. This compound restores the metallothionein molecule, oxidized by superoxide anion radicals [78]. Glutamine is involved in the synthesis of phytochelatins in the cells of higher plants [79]. The specificity of this group of plant metallothioneins is a large gap, the length of which is about 40 amino acid residues including the aromatic acid residues, separating it into two metal-binding domains. The length of the gap of other groups of metallothioneins is less than ten amino acid residues and contains no aromatic amino acids [80]. Stimulation of metallothionein synthesis of the corresponding class is dependent on many factors. The main ones are the properties of heavy metal and its concentration, ionic environment, and specific features of the plant.

Various metal ions stimulate the synthesis of metallothionein not to the same extent. Metals such as Ca, Al, Na, Mg, and U do not induce MT [13, 81]. Elevated levels of metal-binding peptides in the cell under the effect of Fe and Cs are obviously observed in certain cases, which depend on the type of a plant and the concentration of heavy metals [82]. Predominantly, Cd, Zn, Cu, Hg, Au, Ag, Co, Ni, Pb induce a metallothionein synthesis [83]. However, the effectiveness of the activation of the MT synthesis is different. For example, according to some data, the synthesis of MT1 and MT2 is already stimulated by Cd at concentration of about $10^{-7}$ M, whereas to obtain the same effect the concentration of Zn exceeded $3 \times 10^{-4}$ M [74, 76, 84]. The same metal also affects the formation of metal-binding proteins differently. For example, Cd induces the MT2 synthesis some time after the beginning of the MT3 synthesis [80]. The formation of metallothioneins shows the dependence on the ionic environment. It was established, that the synthesis of CUP1 in *Saccharomyces cerevisia*e, inducible by Cu, when cultured yeast, in a medium contained ions of other heavy metals is largely reduced. At the same time, to inhibit the synthesis of CUP1, the content of $Co^{2+}$, $Ni^{2+}$, $Zn^{2+}$ should be higher than that of $Cd^{2+}$ and $Mn^{2+}$ [85].

The plants can form metallothioneins of several classes and the synthesis of metallothioneins is carried out in different organs. For example, in *Arabidopsis* were found metal binding proteins of all known types [86], and in *Silene vulgaris*

L. - MT2 only [80, 87]. Under the effect of Cu in *Arabidopsis,* MT2 is synthesized in trichomes and MT1 — in leaves, roots, and flowers [86]. Differences in the expression level of metal-binding proteins in *Silene vulgaris* populations were associated with not similar tolerance to copper [87]. The most widespread plants metallothioneins are metallothioneins of the third class (MT3) —phytochelatins (PC) found in almost all species of plants, as well as some fungi and invertebrates [75, 77, 78, 88]. Therefore, the investigation of genetic and molecular basis of metal detoxification in plants by this group of metal-binding peptides is of greatest interest. First, PCs were found by researchers in the *Schizosaccharomyces pombe* [85], in cell culture of *Rauvolfia serpentina* [89]. Nowadays, there is a considerable progress in understanding the molecular mechanisms of synthesis and functioning of MT3 [74].

*Structure of phytochelatins* (*PC*). Phytochelatins are compounds of general formula (γ-Glu-Cys)*n*-Gly, where *n* is equal to 11 maximum, but usually varies from two to five [90]. On this basis, MT3 are divided into two groups — with low and high molecular weights [88]. The terminal amino acid in the MT3 structure can vary: in addition to glycine (PC), it can be serine (hydroxymethyl-PC), β-alanine (homo-PC), and also glutamine (Glu) and cysteine (Cys) [90]. The ratio of PC and their derivatives is dependent on the plant species as well as on the ratio of metals in the soil or nutrient solution [75]. For example, the resistance to Cd of *Oriza sativa* L. is provided by hydroxymethyl-PC [91]. Thiol-peptide level and proteomic changes in response to cadmium toxicity in *Oryza sativa* L. roots [92], in *Vigna angularis,* are only provided by homo-phytochelatins [76].

*Biosynthesis of PC*. There are many mechanisms that regulate the synthesis of phytochelatins, which includes several stages [74, 75, 77, 93]. For example, in *Brassica juncea* L., the synthesis of phytochelatins is preceded by a series of stages, the initial one is the reaction between cysteine and glutathione. This process is regulated by genes involved in the transport and sulfur assimilation and the biosynthesis of glutathione. In *Arabidopsis thaliana* L., biosynthesis of PC begins with metal-activating transcription of genes encoding glutathione reductase (GR), and the enzymes involved in biosynthesis of glutathione are γ-glutamylcysteine synthetase (γ-Glu-Cys-synthetase) and glutathione sinthetase (GS) [77]. Glutathione is the main substrate for PC formation and the key enzyme with crucial activity in the process of biosynthesis is PC-synthase. In *Triticum aestivum* L., the synthesis of PC from glutathione can be carried out without intermediate steps. This process is catalyzed by PC-synthase [74, 90].

The specific activator of this enzyme is mainly Cd, but some other heavy metals can provide this role as well. In descending order of their specificity, they can be arranged as the following: Ag, Bi, Pb, Zn, Co, Hg, Au [84]. Previously, it was thought that only free metal ions are able to activate PC-synthase and subsequent PC synthesis. Now it is known that anions $(AsO_4)^{3-}$, $AsO^{2-}$ [88], phosphate anions [94], and jasmonic acid can participate in this process [95]. It is also has been shown [96] that heavy metal-thiolates, glutathionates, and heavy metal complexes with low molecular weight PC are active substrates for the synthesis of MT3, being either a catalyst or a substrate. Thus, the formation of MT3 is activated when exposed to a

large number of heavy metals. However, only the Cd detoxification mechanism can be considered universal: more than 90 % of the $Cd^{2+}$ ions penetrating into the cells of studied 200 species of three taxa (*Bryophyta*, *Pteridophyta*, *Spermatophyta*) are associated with phytochelatins synthesis [76, 87, 97].

*The role of PC in the detoxification of metal ions.* A number of researches clearly demonstrated that MT3 are involved in the detoxification of heavy metals in plants, although there are some other hypotheses about the role of PC [76]. Metal detoxification mechanism by PC includes a number of steps: (1) PC-synthase activation by metals; (2) the complex formation of MT3 with metals; (3) the complex transfer to the vacuole [97]. Moreover, it is considered that low molecular weight MT3 transport Cd to the vacuole where it accumulates in the form of a complex with high molecular weight MT3 or organic acids [98]. Any violation of at least one stage of detoxification leads to lowering the plant tolerance to heavy metals [76]. For example, either damage of PS synthase gene or glutathione synthase gene leads to hypersensitivity of organisms to Cd [76, 79]. Conversely, overexpression of these genes increases a metal plant tolerance, as it was demonstrated in cell cultures of *Lycopersicon esculentum* [99] and *Saccharomyces cerevisiae* [100]. Changes in the activity of enzymes involved in the metabolism of sulfur [88] and the reduce of sulfur containing amino acid - Cys [101], necessary for the synthesis of glutathione also affect the tolerance of plants to Cd.

With the growth of polymerization degree, the affinity of MT3 to metal ion [102] is increased and the efficiency of detoxification is raised. It is proved that the tolerance to heavy metals increases with the increase of the degree of MT3 polymerization and with saturation of the coordination valence with sulfide anions, i.e., with the formation of additional bonds to $S^{2-}$ [76]. In turn, the increase in the proportion of molecules with high degree of polymerization occurs with the increase of Cd concentration and the time of the exposure [98]. The formation of the complexes is largely dependent on the pH of the solution. In the acidic medium, metal is replaced by hydrogen [102] and hence the efficiency of detoxification of heavy metals decreases. It is important to mention that the activation of MT3 synthesis is already observed within a few minutes after the treatment of plants with heavy metals [103], but the highest concentration of peptides in the tissues is revealed only after a certain time after the start of exposure [99].

Some authors suggest that phytochelatins play no significant role in the hypertolerance of plants to heavy metals. Although in cell culture the expression of metallothioneins [89] or phytochelatins [77] increased plant tolerance to Cd, the transfer of genes responsible for the synthesis of metallothioneins in higher plants had no effect on the accumulation of metal ions. While testing plants hyperaccumulators, there were no changes in the concentration of phytochelatins which suggests that hypertolerance to Cd and Zn is provided not by phytochelatin synthesis [104]. The evidence for the certain role of phytochelatins is that there was found correlation between their presence and normal rate of tolerance to the metals. The mutation leading to inability to produce phytochelatins resulted *Arabidopsis thaliana* L. in being hypersensitive to Cd [105]. It was found that high phytochelatins content correlates with the ability of plants to transport Cd to aerial organs. The alternative

point of view is that phytochelatins supply plants with normal resistance to metal excess in the environment. For plants with normal resistance (*A. thaliana*), the synthesis of phytochelatins is definitely necessary if there is an excess of metals in the environment, while for hypertolerance of plants-hyperaccumulators PCs unlikely play any role [106].

To determine the limiting factors of heavy metals accumulation and to obtain tolerant transgenic plants with enhanced ability to accumulate heavy metals, the gene of *Escherichia coligshii*, encoding the synthesis of γ-glutathione synthetase (GS), was activated in the cytosol of Indian mustard [107]. Transgenic plants significantly more than wild species accumulated metal: the concentration of Cd in the above ground organs was higher by 25 %. However, these plants showed an enhanced tolerance to Cd in the phase of seedlings and mature stage. The accumulation of Cd and the tolerance to Cd was correlated with the level of *gshii* gene expression. Cadmium-treated plants contained a larger amount of glutathione, phytochelatins, thiols, S, and Ca as compared to the wild type. It was assumed that the enzyme (GS) at Cd presence is a limiting factor for the biosynthesis of glutathione and phytochelatins. The use of the method of overexpression of GS is a promising strategy to obtain plants with superabilities necessary to phytoremediation.

The reduced glutathione GSH plays an important role in the protection of plants against various stresses. Glutathione is not only a substrate for glutathione-*S*-transferase, neutralizing the potentially toxic xenobiotics [108], but also a reducing agent of dehydro ascorbate [109]. Moreover, GSH is a precursor of phytochelatins. Phytochelatins contain a high percentage of Cys-sulfhydryl residues that bind and isolate ions into stable complexes and are induced by metals such as Cd in all tested plants [110]. Glutathione is synthesized from its constituent amino acids in two consequences. ATP-dependent reaction is catalyzed by *γ-Glutamyl-Cys-Synthetas*e (GCS) and by *γ-glutathione synthetas*e (GS), respectively. *Phytochelatine synthase* sequentially catalyzes an elongation of (γ-Glu-Cys)*n* by the transfer of γ-Glu-Cys-group to glutathione or phytochelatins [111]. A manipulation by the expression of enzymes involved in the synthesis of glutathione and phytochelatins can be an excellent approach for the improvement of plant resistance to heavy metals. *Phytochelatin synthase* enzyme can not be a limiting factor for the synthesis of phytochelatins due to their constitutive expression in plants [112] and the activation at the presence of metals. The genes encoding enzymes involved in the synthesis of glutathione are more promising in this regard. The limiting step for the synthesis of glutathione in the absence of metal is a reaction catalyzed by γ-Glutamyl-Cys-Synthetase since the activity of this enzyme is regulated by glutathione feedback and depends on the availability of Cys. Overexpression of the gene *gshi* of *E. coli* encoding γ-Glutamyl-Cys-Synthetase increased glutathione levels in poplar [109]. Furthermore, the expression in tomato of γ-GCS can restore the tolerance of glutathione-deficient mutant of *Arabidopsis cad 2*. However, the overexpression of this gene did not increase tolerance to Cd in wild-type of *Arabidopsi*s.

Normally, the GS is not a limiting factor as a glutathione content does not change much due to the low concentration of phytochelatins. Overexpression of the gene *gshii* of *E. coli*, coding GS, did not increase the level of glutathione in poplars [113]. Nevertheless, the presence of heavy metals affects the regulation of the biosynthesis of glutathione which is substantially altered. Heavy metals activate phytochelatins synthase and thereby induce biosynthesis of phytochelatins resulting in reduced glutathione levels [114]. Successively as a feedback, glutathione removes the inhibition of γ-Glutamyl-Cys-Glutathione synthetase. Moreover, the expression of γ-glutamyl-cys-synthetase can be increased by heavy metals. It was demonstrated that Cd increases the gene transcription of γ-Glutamyl-Cys-synthetase and deactivates the GS. There is a decrease of glutathione and its accumulation is inhibited by γ-Glutamyl-Cys by the reducing of GS activity [115]. The corn roots exposure in the presence of Cd caused the decrease of glutathione and γ-glutamyl accumulation of cysteine by reducing the activity of GS. Therefore, GS can become a limiting factor for the biosynthesis of glutathione and phytochelatins [116]. The overexpression of *gshii* gene can increase the content of glutathione and phytochelatins synthesis (Fig. 6.3).

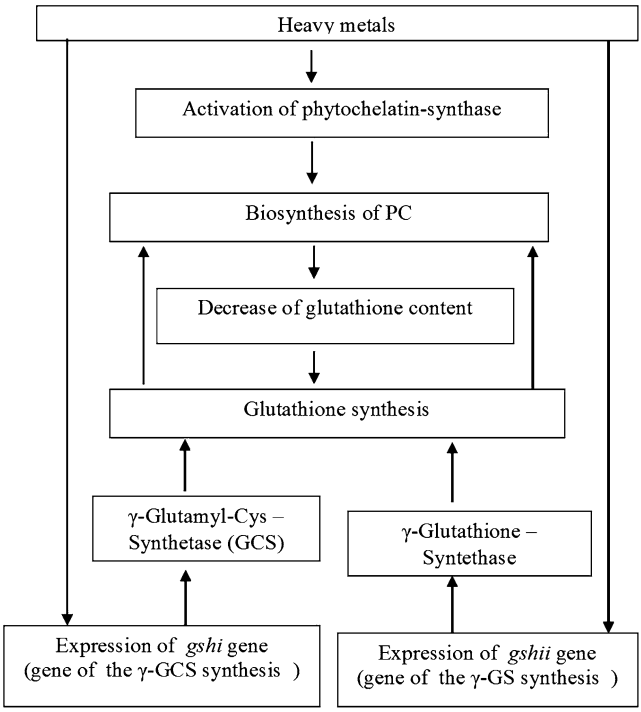


**Fig. 6.3** The scheme of regulation of phytochelatines synthesis in plants

As a result of overexpression of *E. coli* gene *gshii* in Indian mustard, there was an increase of glutathione and phytochelatins content. Cd accumulation and the tolerance of plants also increased. In the Cd-treated roots of wild-type plants glutathione content was three times lower than in the control plants by increasing the synthesis of phytochelatins in transgenic plants compared to wild type. In the tissues of transgenic plants, the glutathione content was similar in the treated and untreated plants, while the level of phytochelatins in roots and aerial parts of transgenic plants was two times higher than that in the wild type. Since the roots are the main organ of PS synthesis, there was a decrease of glutathione in the wild type in the roots, but not in aboveground organs. Cadmium increased the thiol groups content in the roots of transgenic plants by ten times, and only three times in the aerial parts, which is the best explanation that the tolerance of transgenic plants is a result of increased PS synthesis.

The high levels of glutathione in the roots of transgenic plants produce a greater resistance to cadmium. Cd-PC form complexes with sulfide groups in vacuoles. It is believed that the plant tolerance to metal can be limited by the availability of sulfur for Cys and by sulfides synthesis [117]. The level of total sulfur was higher in aboveground organs. Cadmium significantly reduced the concentration of calcium in wild and transgenic plants, but overexpression of the GS gene decreased the rate of decline in Ca in the aerial parts. In the roots Ca content are not much different in the transgenic plants and in the wild type. Cd is a calcium channel blocker, cadmium interferes with binding of Ca to calmodulin, the protein which regulates the activity of many enzymes and cellular processes [118]. The increased levels of Cd-bound peptides in transgenic plants may reduce the effect of cadmium on interaction with calcium.

Transgenic plants has accumulated more Cd in the aboveground organs than wild type plants. The Cd translocation from the roots to the aerial parts of the xylem transpiration stream was provided by transpiration flow [119]. The more Cd binds phytochelatins stored in vacuoles in transgenic plants, the smaller are destroyed vital biochemical and physiological processes. This leads to the leaf surface increase, thus to greater accumulation of Cd (as a result of increasing the transpiration). Transgenic plants absorbed more cadmium due to the less damage of the root surface. The water absorption is the primary mechanism for increasing the movement of Cd in the plant [119]. High level of phytochelatins in the roots of transgenic plants reduces the negative effect of Cd on water absorption. Thus, regulation of gluthatione synthesis promotes an accumulation of heavy metals and increases the tolerance of transgenic plants. Transgenic plants allow to increase the efficiency of the phytoextraction of heavy metals from contaminated soils. The manipulation of gene expression of glutathione synthesis, may be one of the promising approaches to increase phytoextraction of heavy metals and metal tolerans of plants.

### 6.1.5 The "Induced" Phytoremediation

For toxic metals, such as lead, main restricting factor is a limited solubility and absorption by plant roots. One of the ways to induce a solubility of lead is the lowering of pH. A strong acidification of soils mobilizes Pb below the root zone. To improve a phytoextraction of heavy metals can be used synthetic chelators. These components are associated with the lead and remain soluble in the metal chelate complexes available for plants and transport within them. The chelating agents, such as EDTA HEDTA EDDS, DTPA, EDDHA, EGTA, and others, were tested for their ability to dissolve the metal components and to increase the absorption by plants [26, 120, 121]. EDTA is a powerful relatively biostable and extractable chemical reagent that is widely used for phytoremediation of soils [122]. The addition of EDTA (ethylene diamine tetra acetic acid) is a technique of extraction and is intended for immobilization of heavy metals as an important aspect of managing leaching of metals from soils [123–126]. This method is a relatively cheap and environmentally friendly technology [127].

Indian mustard exposed to lead (Pb) and EDTA in a hydroponic medium accumulated up to 1 % of the dry biomass. Other synthetic chelators as HEDTA (*N*-(2-hydroxyethyl)ethylendiaminetriacetic acid) applied at a concentration of 2.0 g/kg in soil containing 2.5 g/kg Pb increased accumulation of lead by aerial organs of Indian mustard from 40 to 10,600 g/kg. The accumulation of increasing amounts of Pb is toxic and can cause death of plants. Therefore, the authors recommended the use of chelates after maximum accumulation of biomass by plants. Immediately in the optimum time at the maximum phytoextraction level (after 1 week treatment) the plants should be removed to minimize the loss of biomass from the toxic effect of the metal [128]. Blaylock et al. [129] suggests that the addition of chelates is possible for the other metals too. EDTA also stimulated Cd-, Ni-, Cu-, and Zn-phytoextraction. The ability of these compounds to chelate metals facilitates a phytoextraction due to the high affinity to metals. For example, EGTA (ethylene glycol-bis(2-aminoethylether)-*N,N,N′,N′*-tetraacetic acid) has high affinity to Cd, but does not bind Zn. EDTA, HEDTA, DTPA (diethylenetriaminepentaacetate) are selective for Zn.

The addition of EDTA at concentration of 100 mM into the cultivation medium of *Sesbania drummondii* increased the amount of Pb by 16 % in comparison to the control. The use of EDTA increased an extraction of Cd up to 1140 mg/kg, and the use of ammonium sulfate did not affect the phytoextraction [130]. Application of NTA (nitrilotriacetate) and an elemental sulfur S increased the mobility of Zn, Cd, Cu in the soil that caused the increase of its accumulation in the aerial plant organs in 2–3 times. The addition of chelating agents (0.5 and 2 g/kg EDTA), 0.5 g/kg DTPA (diethylene triaminopentaacetate) and 0.5 g/kg of NTA (nitrilotriacetate)), to the soils, where poplar trees were grown, caused the increase of Cd absorption. The authors note that it was necessary to select the optimal concentration of chelating agents and the optimal time of removing of plants [131]. The chelating agents such as EDTA and metal cations increase the solubility and thus bioavailability of metals

for plants. The positive effect of EDTA on phytoextraction of metals is accompanied by negative effects. The disadvantage of the use of the synthetic chelating agents is their non-selectivity. They extract a large number of metals including Ca and Mg, which are essential for plant growth [126].

The natural organic chelators are more suitable for phytoremediation. There are the widespread natural organic chelators in the soils, physiologically active compounds that the soil organic matter consists of and which determine its fertility. Humic acids are the dark-colored high molecular substances whose structure is not fully established. The maximum amount of humic acids is contained in the black earth (10 %). The structure of humic acids is defined by the presence of low condensed and substituted aromatic rings bound with sites of non-aromatic character. The molecules of humic acids include carboxyl and carbonyl groups, alcoholic and phenolic hydroxyls, sometimes methoxyl groups [24]. The most important function of humic acids in biosphere is their ability to protect and preserve soils and vegetation biotics, better to resist drought and water logging, and to bind firmly many radionuclides, detergents, pesticides, heavy metals. Humic acids are able to convert them into inactive forms, which over time break down into non-toxic compounds and thus remove them from the sphere of direct contact with living organisms, soil and groundwater, and atmosphere. The different chemical nature of the functional groups in the humic acids determine their high sorption capacity for radionuclides, pesticides, and heavy metals [132].

It has been found that Cd are mainly associated with low molecular weight fractions of humic acids (<1000 D), whereas Pb binds to high molecular weight fraction of humic acids (10,000 D). Their complexes with low molecular weight compounds have higher value of stability constant, more easily transported across cellular membranes than the complexes with high molecular substances that can cause a greater bioavailability of cadmium in the presence of humic acids [133]. Thus, to improve processes of phytoextraction, an optimization of agronomic practices used in phytoremediation technologies is required. The use of fertilizers is necessary for the greater accumulation of plants biomass, thus for the increase of extractable metals amount. Uptake of metals by plants may be limited by the low solubility of metals in the soil. This requires the use of synthetic chelators, which increase the mobility of metals in the soil.

It was shown that the humic acids increased the bioavailability of cadmium and concentration of Cd in the shoots of *Nicotiana tabacum* [134]. The authors suggested that the reason for this reduction of pH results from greater availability of cadmium. Another idea is to consider that plants can take cadmium complexes with fragments of humic acids, which are the result of microbiological degradation or self-dissociation. Humic acids have a positive effect on phytoextraction of other heavy metals such as Zn, Cu, Ni, and Pb [135]. The application of humic acids increased significantly the concentrations of metals in the shoots and plant uptake of metals, but the plant growth was declined. Thus, humic acids can be used in phytoremediation as an alternative way to increase a phytoextraction of heavy metals and remove them from the soils. Hence, to improve the processes of phytoextraction, the optimization of the agronomic practices used in phytoremediation

technologies is required. The use of fertilizers is necessary for the greatest accumulation of plant biomass to increase the amount of extractable metals. Uptake of metals by plants may be limited by the low solubility of metals in the soil. Therefore, the application of synthetic and natural organic chelating agents is the best way for successful phytoremediation technology.

### 6.1.6 *The Use of Energy Crops for Phytoremediation of Contaminated Soils*

Phytoextraction is a kind of phytoremediation technology which has a long duration. Therefore, for the productive use of the contaminated soils, the economically viable and socially acceptable method of purification of contaminated land must be implemented. Industrial crops and "energy" crops which have a phytoextraction potential may be the candidates for biofuel production [120]. The use of the "energy" crops for phytoextraction of heavy metals is possible, on the one hand, to reduce the level of pollution and, on the other hand, to increase the productive value of the contaminated soil. The best candidates are sunflower plants (*Helianthus annus* L.), castor bean (*Ricinus communis* L.) [136], and white mustard (*Sinapis alba* L.) [137]. High-yielding plant species as *Helianthus annuus* L. have the potential to extract large amounts of trace metals by the aboveground biomass if they reach a sufficient concentration in the tissues. Nevertheless, a low bioavailability of some metals limits translocation of them to aerial parts. The addition of EDTA or citric acid increased the concentration of heavy metals in the tissues of plants. When these plants were grown on calcareous soils contaminated by Cu, Pb, Zn, and Cd and treated with EDTA (0.1,1, 3,5, 7 and 10 mM/kg) and citric acid (0.01, 0.05, 0.25, 0.442, and 0.5 M kg), the concentration of metals in the tissues and the removal of heavy metals from the soils were increased [138].

Sunflower can be used for phytoextraction of arsenic (As). Oxidative status of As may be different. Pentavalentarsenate ($AsO^{4-}$) is the most persistent and prevalent in well-aerated soils, that is why arsenic contamination of soils is a big problem. Arsenates and phosphates ($PO^{4-}$) are chemically similar and therefore can compete for the binding sites in the soil. Thus, the addition of phosphates can increase the content in the soil solution of arsenate as a consequence of phosphate substitution of arsenate on specific anion exchange sites in soil. This will increase the bioavailability of arsenic to plant roots. Phosphate fertilizers directly increase the accumulation of As in plant by stimulation of phosphate-absorbing mechanism. The preliminary study showed that sunflower (*Helianthus annuus* L.) may be a candidate for the phytoextraction of arsenic by the addition of phosphorus as a mobilizing agent [139].

In the comparative study of the activity of metal-accumulating plants *Helianthus annuus*, *Nicotiana tabacum,* and *Vetiveria zizanioides* on hydroponic medium containing Pb $(NO_3)_2$ in concentrations of 0.25 and 2.5 mM Pb with and without chelating agents (EDTA or DTPA), it was found that the addition of chelators increased the absorption of Pb. Lead accumulated in the leaves and stems with the highest content in the leaves. After 4 weeks, the lead content was increased in

23-fold in aerial organs of *H. annuus* and *N. tabacum*. Higher concentration of Pb (2.5 m MEDTA) leads to its increase in tissues during 4 weeks compared to the medium containing DTPA. Pb-accumulation potential of *H. annuus* was higher than that of *N. tabacum* and *V. zizanioides*, which was determined by bioconcentration potential (171, 70, and 88 kg/ha, respectively). The largest amount of Pb was found in the roots, stems, and leaves of *H. annuus* (2668, 843, and 3611 mg/g, respectively) grown at 2.5 m MEDTA [140].

Among the species *Brassica juncea* (L.), *Brassica nigra* (L.), *Raphanus sativ*us L., *Helianthus annuus* L, and *Ipomea triloba* L., it was found that sunflower accumulates Pb in most amounts [141]. In the studies on the accumulation of metals in plants *Echinochloa crusgalli*, *Helianthus annuus*, *Abutilon avicennae,* and *Aeschynomene indica*, grown on soils contaminated by cadmium (Cd), lead (Pb), and 2,4,6-trinitrotoluenom (TNT), it was found that the concentration of Pb was high in *A. avicennae*and *H. annuus.* The total removal of Cd from the soil was the highest in *E. crusgalli* (50.1 %), than in *H. annuus* (41.3 %) [142].

The study of phytoextraction potential of rape seed (*Brassica napus* L.) and sunflower (*Helianthus annuus* L.) in the presence of 3 mM DTPA/kg of soil showed that maximum concentration of Pb and Zn in above the ground parts were 234.6 and 1364.4 mg/kg, respectively. Sunflower showed the greatest phytoextraction potential compared to rape [143]. These results proved that *H. annuus* is the best candidate for its use as a hyperaccumulator and that it has the potential to be used for remediation of contaminated soils. These data suggest the use of chelating agents to enhance the phytoextraction by plants with high biomass of poorly soluble complexes of heavy metals.

Another kind of oil-bearing plants, from which castor oil is extracted, is known as a castor bean (*Ricinus communis* L.), researchers also determined as hyperaccumulator. Plants of castor beans (*Ricinus communis* L.), growing in hydroponic medium at concentrations of Pb 0, 100, 200, and 400 μM/L, showed their hyperaccumulation ability. According to I. Raskin et al. [144], castor beans may be considered as hyperaccumulators of Pb plants accumulating 1.0 g/kg [21]. Castor plants accumulated in hydroponic environment — from 10.54 to 24.61 g Pb/kg of soil [145]. The use of chelating agents such as EDTA might enhance the translocation of lead to the shoots.

Other researchers in the study of cadmium and lead phytoextraction by sunflower plants (*Helianthus annuus* L.) and castor bean (*Ricinus communis* L.) found that these species are capable of accumulating metals in a large amount [146]. Experiments with *Ricinus communis* L., which were grown on soil contaminated with lubricants (1–6 % oil/soil) containing Ni and Mn, showed the highest concentration of metals in the leaves. At concentration of lubricating oils of 2 % and more, the content of Pb was the highest in the leaves. In that study Mn, Ni and Pb were most strongly accumulated in leaves and vanadium—in roots of *R. communis* [147]. According to the literatute white mustard also has the potential to accumulate heavy metals. In the study of phytoextraction potential of 14 plant species in the presence of 5 mM Pb/kg of soil EDTA increased the proportions of phytoavailable Pb, Zn and Cd. Their absorption was increased in 48 times in white mustard (*Sinapis alba*), 4.6

times in radish (*Raphanus sativus oleiformis*), and 3.3-fold in amaranth (*Amaranthus* spp.). In mustard, the concentration of Pb was equal to 479.71; Zn— 524.68; Cd — 7.93 mg/kg, respectively, and phytoextraction potential were 1.32; 1.44 and 0.022 kg/ha, respectively [148]. Thus, the need to develop phytoremediation technologies using "energy" crops is due to the following: (a) these species accumulate large biomass; (b) the use of chelating agents enhances phytoextraction of metals which have low bioavailability and intensifies their translocation to their aerial parts.

### *6.1.7 Transgenic Plants for Phytoremediation*

The efficiency of phytoremediation processes is largely determined by the ability of plants to absorb and accumulate toxicants in the cell structures. Progress related to phytoremediation of environment polluted by organic toxicants by its scale far exceeds similar processes associated with the assimilation of inorganic toxicants and radionuclides. This is due to long-term selection of suitable plants for organic toxicants, adapted to the specific soil and climatic zone, high productivity, ability to accumulate large biomass, availability of relevant physiological (capacity for transpiration) and morphological (developed root system) performance, adaptation to field conditions, the presence of the relevant enzyme systems, and others. The above-mentioned features and possibly some others cause accumulation and sequestration of heavy metals, i.e., it is determined by their phytoremediation potential of plants [149].

The ideal plant species for phytoremediation is a plant with a high biomass and with high phytoextraction ability. There are already exist a number of plant-derived transformants with enhanced ability to accumulate heavy metals in intracellular structures (predominantly in vacuoles), in the intercellular space, and to conjugate endogenous compounds with toxicants. In this direction, the study is being intensively developed in many countries of the world. More suitable for genetic engineering are plants like Indian mustard (*Brassica juncea*), poplar (*Popul*us spp.), yellow poplar (*Liriodendron tulipifera*), and cordgrass (*Spartina* spp.). The gene construct of the large biomass phytoremediation species may be transformed to the model plant species, like *A. thaliana*. This plant has a short life cycle and high seed production and it is very suitable to test it in a short time [150]. There are two possible strategies in genetic engineering to create the plants with high phytoremediation potential. One of them is to increase the biomass productivity of species that are good accumulators; another strategy is to enhance tolerance to heavy metals and metal accumulation capacity.

It is possible to introduce the genes responsible for the above-mentioned features into the plant from any other organism. The first large-scale field trials were conducted in USA. The most significant work on the production of recombinant plants were carried out in different directions. It is believed that phytoremediation can be commercialized very quickly if the absorption capacity of the plants hyperaccumulators as *T. caerulescens* is transformed into a highly productive plant as Indian mustard (*Brassica juncea*) and corn (*Zea mays*). Biotechnology methods has been successfully applied to manipulate the processes of absorption and tolerance

to heavy metals in several species. For example, in tobacco plants (*Nicotiana tabacum*) the tolerance to heavy metals was increased after expression of genes of metallothioneins and other metal-binding proteins [151].

An effective application of biotechnology methods for environmental restoration is a bioengineering of plants capable of volatilizing mercury from soils contaminated by methylmercury. Methylmercury is a powerful neotoxin synthesized on mercury-contaminated soils. For detoxifying the toxin were used transgenic plants of *Arabidopsis* or tobacco where the genes *mer B* and *mer A* were transformed. In these modified plants, *mer B* catalyzed the protonolysis of carbon-mercury associated with the generation of $Hg^{2+}$ in less mobile mercury. Consistently, *mer A* turns Hg (II) to $Hg^0$, a volatile element, which is released into the atmosphere. *Mer A-Mer B* double-transgenics were obtained by crossing *Mer A* and *Mer B* transgenics [152]. *Mer B* plants were tenfold more tolerant to organic mercury than wild-type plants; *Mer A-Mer B* plants were 50-fold more tolerant. When supplied with organic mercury, *Mer A-Mer B* double transgenics volatilized elemental mercury, whereas single transgenics and wild-type plants did not. Thus, *Mer* A-Mer B plants were able to convert organic mercury to elemental mercury, which was released in volatile form. The same *Mer A/Mer B* gene constructs were used to create mercury-volatilizing plants from tobacco plants and yellow poplar [153, 154]. They showed enhanced tolerance to mercury [155]. These experiments demonstrated that plant-expressed with *Mer B* (*organo mercurial lyase* under plant promoter control) can be used for the degradation of methyl mercury and subsequently for removing mercury through extraction. Despite the benefits of biotechnology, little is known about the genetics of plants hyperaccumulators. In particular, the heritability of such mechanisms as the mechanisms of metal transport and accumulation by plants and plant resistance to metals are to be better understood.

R. Chaney [39] suggested using conventional breeding approaches for improving phytoremediation processes and the possibility of combining field tests on metal tolerance and uptake of heavy metals in a highly biomass productive plant. For example, E.R. Brewer et al. [156] generated somatic hybrids between *T. caerulescens* (Zn-hyperaccumulators) and *Brassica napus* (canola), obtaining by hybrid selection for Zn-tolerance. The obtained hybrids have a large biomass and were hypertolerant to zinc. Among the large variety of plants, poplar plants (*Populus*), perspective for phytoremediation deserves a special attention because of their strong root system, which has a large absorption capacity. Multiple genetic engineering modifications of this plant convince the appropriateness of practical use of a number of obtained transgenic forms. One of such work is dedicated to the enrichment of the poplar genome of a bacterial gene coding the synthesis of γ-glutamylcysteine-synthetase (γ-ESC), which is a key enzyme in the biosynthesis of glutathione. The authors obtained several transgenic clones. Glutathione-*S*-transferase is widely distributed in plants, an enzyme which participates in normal metabolic processes in plant cells and in plant protection from stressful situations. In creating of transgenic plants for phytoremediation the target is a gene of this enzyme [157]. Overview of genetically engineering work conducted in this area indicates that in many cases in transgenic plants there is a significant increase the detoxification ability of plants.

This is evidenced by the fact that some transgenic plants have an increased ability to assimilate organic toxic compounds and heavy metals absorption capacity.

### *6.1.8 Utilization of Biomass*

Phytoextraction is a multiple process of planting of hyperaccumulators on contaminated soil, after which the concentration of metal in soil falls to an acceptable level. The ability of the plant to reduce the concentration of metals in soil, depending on the metal absorption, the biomass production plays an important role in the phytoremediation process. Theoretically, the level of removal of metals from the soil can be calculated by determining the concentration of the metal in plants multiplied by the value of the biomass and it is necessary to compare this value with the decrease in the concentration of metals in the soil. Many factors impede this process. One of the obstacles to the commercialization of phytoextraction is the removal of contaminated plant material. After the cycle of plant development, the plant biomass is removed from the field, which leads to the accumulation of large quantities of hazardous waste. This contaminated biomass should be a certain way buried or disposed of properly so that it represents no risk to the environment.

Biomass contains carbon, hydrogen, and oxygen. The basic components of any biomass is lignin, hemicellulose, cellulose, mineral substances, and ashes. They have high moisture and volatile components. The percentage of these components varies in the plants species. The dry weight of *Brassica juncea* at induced phytoextraction of lead reach up to 6 t 1 ha with 10,000–15,000 mg/kg of metal in dry weight [158]. The processing of huge amounts of this kind of waste is a problem and therefore there is a strong need to reduce the volume of the contaminated biomass [159]. Therefore, after removing, a composting and sealing of biomass are necessary [27]. During composting, the formation of soluble organic compounds occurs, which increases the solubility of the metals (Pb). Studies of some researchers [160] have shown that composting can significantly reduce the amount of harvested biomass, but plant biomass contaminated by metals should be treated. One of the traditional and necessary ways to use biomass in phytoremediation is a thermochemical conversion process.

If phytoextraction is accompanied by high biomass production, it is advantageous to use it commercially as a source of energy and ash formed after combustion can be used as bio-ore [161]. It is a basic principle of phytomining [162]. The process of phytomining can bring huge profits by extracting heavy metals from the ash. The process of combustion and gasification are the most important components for production of electricity and heat. Energy production from biomass during combustion or gasification can help to make the process of phytoextraction more cost-effective. The thermochemical conversion of energy promotes the use of the biomass by the best way because it cannot be used as animal feed and fertilizer. The biomass combustion process must take place under controlled conditions; the volume at the same time should be reduced up to 2–5 %, and the ash can be disposed off properly

[161]. *Gasification* is the process by which biomass is subjected to a number of chemical changes to produce clean fuel and gas. This mixture of gases is called *pyrogas* that can be burned to produce heat and electricity.

The gasification process of biomass includes *drying*, *burning*, *thermal decomposition* (pyrolysis), and *gasification* [163]. It is possible to use the co-firing of biomass with coal plant [160]. Such incineration reduces the weight of lead-contaminated plant biomass for more than 90 %. This makes it possible to recover lead from fly [16]. Further experiments should focus on the development of combustion systems and methods for processing of various metals from the ash. This process destroys organic matter. Metals are extracted in the form of oxides. Considering other technologies for the utilization, this method is environmentally friendly. *Pyrolysis* is a new method for processing municipal waste [164], which can also be used for the contaminated plant material. *Pyrolysis* decomposes material under anaerobic conditions with no emissions into the atmosphere. The final product is the liquid pyrolytic oil and coke; heavy metals remain in the coke from which the metals can be recovered.

### 6.1.9 Advantages and Disadvantages of Technology Phytoremediation

According to estimates in the literature, the cost of simply removing 50 cm of contaminated soil and dumping of conventional methods is $960,000 per hectare. This does not include the cost of transportation, sorting revegetation of excavated layer. In contrast, for the cleaning of the same soil, biological methods will cost from

**Table 6.2** Main factors, limiting the success of phytoremediation of contaminated soils

| Biological restrictions | Adjustable and other restrictions |
|---|---|
| 1. The weak resistance of plants to contaminants | 1. The lack of data on the cost and implementation of phytoremediation in a certain area |
| 2. Low translocation of contaminants from the roots to the aerial organs | 2. Lack of the technology knowledge |
| 3. The small size of the plants used for phytoremediation. The main problem of phytoremediation is that hyperaccumulators are small biomasses of leaves and small dimensions The level of recovery of heavy metals from the soil depends on the biomass and concentration of metals in the aerial part | 3. Distribution of contaminated plant waste |
| | 4. The risk of contamination of the food chain |
| | 5. The pollutants are below the root zone |
| | 6. Processing time is long. Removal rate may take 15–20 years, depending on the initial concentration of the metal in soil and the depth of the contaminated soil |
| | 7. Pollutants are in biologically unavailable form. Low bioavailability of some metals (Pb). Metals are associated with inorganic components and soluble metal complexes in the soils are available for plants |
| | 8. Lack of plant species suitable for phytoremediation |

Adapted from Lasat MM, The use of plants for the removal of toxic metals from contaminated soils. Environmental protection agency, New York, 2001 [165]

$144,000 to $240,000 per hectare [165]. But phytoremediation technology has both advantages and disadvantages (Table 6.2).

Thus, phytoremediation technology is the safest and most effective and economically advantageous method for purifying contaminated soil. However, to develop the technology for phytoremediation of specific areas, there is a strong need in individual approach associated with the peculiarities of the soil, plants and species specificity, and type of pollution. For effective development of phytoremediation, each element should be considered separately. It is necessary to apply agronomic approach taking into account the physical and chemical properties of the metal, soil, and genetic properties of plants.

## 6.2 The Possibility of Using Grass Species of Kazakhstan Flora for Phytoremediation

East Kazakhstan region is the largest mining and metallurgical industry center. The territory of the East Kazakhstan region stores about one billion tons of solid waste as a result of strong pressure of human activity [166]. The wastes of industrial production form new man-made landscapes. They become a source of intense dust, polluting the environment and posing a threat to human health and biodiversity of the region [167]. Considerable part of the land in Eastern Kazakhstan is contaminated with trace metals, mainly lead (Pb) and zinc (Zn), since metal smelters and metallurgical enterprises are located in this area of the country. Smelting and mining processes are the point sources of pollution and contamination causing environmental problems. Heavy metals from the air, soils, and water affect plants and ecosystems. The soil of Eastern Kazakhstan region is most polluted by Zn, Cd, Pb, Cu as a result of prolonged activity of metallurgic plants of East Kazakhstan [168]. An important feature of heavy metals is that they belong to a class of non-specific substances which are “normally” present in the biosphere, in contrast to specific pollutants such as pesticides. Another difference from other contaminants is that heavy metals do not apply the concept of “self-cleaning”.

At present in Kazakhstan, the ability of wild species to accumulate heavy metals in plants growing in contaminated areas is poorly studied. In this regard, the study of metal accumulation activity of natural species in Kazakhstan is particularly relevant and timely and the use of suitable species for phytoremediation of contaminated soils is the most promising direction. Grasses are tolerant to heavy metals and have played a considerable role in the use of phytostabilization [169–173]. There are a lot of mine soils and estuarine sediments that are successfully phytostabilized against erosion by grasses [174, 175]. The thick adventitious roots, unique root morphology [171], and high bioproductivity make grasses [171] suitable for using in phytostabilization. Moreover, grasses are often associated with mycorrhizal and endophytic fungi [176, 177]. Application of grasses together with legume plants improves in situ stabilization of chemical waste [178, 179]. The study of accumulation of heavy metals by plants widely spread in the contaminated areas in Kazakhstan and the selection of tolerant and capable to accumulate heavy metals plant species

are necessary steps for the development of phytoremediation technology in Kazakhstan. The aim of this work was the screening of wild plant species, growing on the contaminated area around the metallurgical plants of Eastern Kazakhstan, of their metal-accumulating ability, and test these grass species on artificially contaminated soils and hydroponic conditions.

## *6.2.1 Materials and Methods*

The wild grass species of the family *Poaceae*: cocksfoot (*Dactylis glomerata* L.), a fire inermis (Bromus inermis L.), white bent (*Agrostis alba* L.), timothy grass (*Phleum pratense* L.), couch (*Agropyron repens* L.) green foxtail widely spread around metallurgic plants in East Kazakhstan, were identified. Samples of soils and plants were collected from the territory of Zinc and Lead Plants (Center) and 500 m from the plants to the North, South, East, and West of the enterprises and analyzed on heavy metals content. Soil from roots was removed gently and mechanically without washing. Washing roots may result in uneven loss of certain trace metals [180]. All samples were air-dried for 4 days and divided into roots and shoots. Then they were dried at 105 °C for 3 h, ground, and analyzed.

### 6.2.1.1 Experiments with Artificial Contamination of Soils

Grass species (*Agropyron repens*, *Dactylis glomerata*, *Phleum pratense,* and *Setaria viridis*) widely spread around metallurgic plants in Eastern Kazakhstan were identified by local floras. The seeds of *Agrostis alba* did not germinate on these soils. The seeds were sown in test pots 1 m × 1 m in size spiked with the following salts: $ZnSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$, $(CuSO_4)_2 \cdot 5H_2O$, $CdSO_4$ in May. The final concentrations of the spiked soils were (in mg/kg): Zn — 1000, Pb — 1000, Cu — 100, Cd — 100. Grass species (*Agropyron repens*, *Dactylis glomerata*, *Phleum pratense,* and *Setaria viridis*) widely spread around metallurgic plants in Eastern Kazakhstan were identified by local floras. The seeds of *Agrostis alba* were sown, but they did not germinate on these soils. Seeds were collected in August from 25 to 30 plants. Seeds were sown in test pots 1 m × 1 m in size spiked with the following salts: $ZnSO_4 \cdot 7H_2O$, $Pb(NO_3)_2$, $(CuSO_4)_2 \cdot 5H_2O$, $CdSO_4$ in May. The final concentrations of the spiked soils were (in mg/kg): Zn — 1000, Pb — 1000, Cu — 100, Cd — 100.

There were three replicates for each treatment. The seeds were placed at a depth of 2–3 cm, with an inter-row distance of 5–7 cm. Grass seedlings were collected in August with roots. The contents of trace metals in shoots and roots were 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 [181]. 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, washed filter, and added deionized water up to final volume to 50 mL. The process proceeded to the analysis of samples, using the appropriate SOP.

*Hydroponic experiments:* Seeds of *Agropyron repens* (*A. repens*), *Agrostis alba* (*A. alba*), *Bromus inermis* (*B. inermis*), *Setaria viridis* (*S. viridis*), and *Pleum pratense* (*P. pratense*) were collected in August from fields of the Altay Botanic Garden. The seeds of *Dactylis glomerata* (*D. glomerata*) were not viable and they were not used in hydroponic experiments. Seeds were stored in a dark room at 22–24 °C; before sowing, the seeds were stored for 20 days at 4–6 °C [182]. The seeds were sterilized with 16 % $H_2O_2$ followed by three rinses in distilled water, 5 min for each rinse. Seeds were germinated on water-moistened filter paper at 25 °C in a dark room for 7 days. Afterwards, the seedlings were placed in plastic containers (20 × 30 cm) filled with Hoagland's 1/4 strength (macro- and microelements) medium [183]. After 7 days, 30 seedlings were transferred to medium containing various concentrations of Pb (450, 900 mg/L) and Zn (350, 700 mg/L) in the forms of Pb $(NO_3)_2$ and $ZnSO_4$. Control plants were grown on 1/4-strength Hoagland's without metals. The experiment was carried out in a controlled environment room under the following conditions: 14-h photoperiod with a light intensity of 400 μmol photons $m^{-2}$ $s^{-1}$; 22 °C: 18 °C day: night temperature; relative humidity, 60 %. There were three replicates for each treatment. Plants were harvested 6 days after treatments. Shoots and roots were separated, oven dried at 80 °C for 48 h, and dry weights were recorded. The contents of trace metals in shoots and roots were determined as described next.

#### 6.2.1.2 Analysis on Heavy Metals (Pb, Zn) Content

Plant samples (0.5 g) were digested in a mixture of 5 mL of 50 % $HNO_3$ and 0.5 mL HCl at 95 ± 5C according to standards for operation procedures [181]. Samples were transferred to a digestion block (section) at ±5 °C, closed within glass and heated without bringing to the boil for 10–15 min. Then they were cooled and 5 mL of concentrated $HNO_3$ was added, placed into a digestion block at 95 ± 5 °C, closed within glass, and heated without bringing to the boil for 30 min until brown fumes disappeared. Then the samples were cooled and 2 mL of water and 3 mL of $H_2O_2$ were added; heating was continued until the volume was reduced to about 5 mL, removed from digestion blocks, allowed to cool, then filtered; the filter was washed and deionized water was added up to final volume to 50 mL. Samples were analyzed using the appropriate SOP. The concentration of metals in plants and soils was measured by atomic absorption spectrophotometry using an installed Winlab A Analyst 300 (Perkin Elmer, Germany) [184] with an installed and aligned HCL/EDL lamp. HCL lamps were stabilized/aligned for 25-min, EDL lamps for 45 min; operating

pressure ~0.7 kgf/cm$^2$ for acetylene, and 2.8–3.0 kgf/cm$^2$ for compressed air. Following calibration, samples were analyzed.

#### 6.2.1.3 Statistical Analysis of Data

In field experiments, the samples for measurement of trace metal contents were taken from three test pots for each treatment. The data of pot experiments were analyzed statistically using two-way ANOVA with species and treatments as main effects for shoot and root biomass and concentration of metals in plant parts. LSD was calculated using the following equation: LSD $0.05 = t0.05 \sqrt{(2.MSError/n)}$ was used to differentiate the means. All values were expressed as the mean of three measurements for each treatment. Values represent means ± standard error (SE). In hydroponic experiments, the samples for measurement of trace metal contents were taken separately from three plastic containers (three replicates) for each treatment. All values are expressed as the mean of three measurements for each treatment. The data were analyzed statistically using two-way ANOVA with species and treatments as main effects for shoot root biomass and concentration of metals in plant parts. LSD was calculated as above to differentiate the means. Values represent means ± SE.

### *6.2.2 Results and Discussion*

#### 6.2.2.1 Contamination of Soils Around Metallurgic Plants in East Kazakhstan

The first step of the study was determination of heavy metal content in soils around metallurgic plants of East Kazakhstan. Soil samples were taken at a distance of 500 m to the North, South, East, and West from the territory of enterprises. The soils around Zinc plant (ZP) and Lead plant (LP) differed on the content of heavy metals.

**Table 6.3** Soil content of heavy metals around metallurgic plants

| Regions | Cd | Pb | Cu | Zn |
|---|---|---|---|---|
| Zinc Plant, South | 539.1 ± 17.8 | 5990.4 ± 215.6 | 5616.0 ± 179.7 | 129,792.3 ± 467.2 |
| Zinc Plant, West | 11.3 ± 0.4 | 12.3 ± 0.5 | 43.2 ± 1.5 | 355.7 ± 13.9 |
| Zinc Plant, North | 120.0 ± 4.5 | 1645.3 ± 64.2 | 1749.3 ± 69.9 | 22,990.8 ± 804.6 |
| Zinc Plant, East | 9.3 ± 0.29 | 195.8 ± 7.0 | 373.8 ± 14.6 | 2898.3 ± 101.4 |
| Zinc Plant, Center | 83.5 ± 3.0 | 932.8 ± 30.8 | 645.7 ± 23.2 | 17,881.9 ± 679.5 |
| Lead Plant, South | 35.8 ± 1.1 | 3046,0 ± 109,6 | 449.2 ± 14.8 | 3893.0 ± 120.6 |
| Lead Plant, West | 11.0 ± 0.3 | 1308.9 ± 45.8 | 159.8 ± 5.3 | 1598.7 ± 57.6 |
| Lead Plant, North | 22,4 ± 0,7 | 4769.2 ± 175.4 | 1073.7 ± 38.1 | 22,972.4 ± 748.8 |
| Lead Plant, East | 20.0 ± 0.5 | 1723.9 ± 65.5 | 1079.3 ± 41.0 | 5796.5 ± 226.0 |
| Lead Plant, Center | 134.9 ± 4.7 | 12,672.4 ± 430.7 | 1519.1 ± 51.6 | 22,986.2 ± 750.1 |

Soils around metallurgical plants had high concentrations of lead (Pb) (12.3–12,672.4 mg/kg) and zinc (Zn) (355.7–129,792.3 mg/kg) [185] (Table 6.3).

The content of Zn and Pb was much higher than that of Cd and Cu in the soil. The territory of LP (Center) was the most polluted by Pb; the soil to the West of the ZP was the least polluted site by Pb. On the content of Pb, the sites adjacent to the metallurgic plants can be arranged in the following order: LP, "Center" > ZP, South > LP, North > LP, South > LP, East > ZP, North > LP, West > ZP, "Center" > ZP, East > ZP, West. In soils to the South of the ZP was found the highest content of Zn. The least Zn content was found in the soils to the West of the ZP. The sites with the content of Zn can be arranged in the following order: ZP, South > ZP, North > LP, "center" > LP, North > ZP, "Center" > LP, East > LP, South > ZP, East > LP, West > ZP, West. Thus, the most polluted by heavy metals areas were located to the South and to the North of the ZP and in the territory of Zinc and Lead Plants (center). Minimal contaminated by heavy metals areas were found to the West and East from the ZP and LP. A Zn hyperaccumulator is defined as a plant that contains >10,000 mg/kg Zn dry wt, whereas a Pb hyperaccumulator contains >1000 mg/kg Pb dry wt. The mean of Zn and Pb concentrations in non-accumulating plants growing on contaminated soils is expected to be <1000 for Zn and <100 for Pb [43].

#### 6.2.2.2 Contamination of Plants, Growing Around Metallurgic Factories, by Lead and Zinc

These were collected and identified plant species, growing around Zinc and Lead Plants in East Kazakhstan (Ridder) at a distance of 500 m from the metallurgic factories. *Dactylis glomerata* L., *Bromus inermis* L., *Agropyron repens* L., *Agrostis alba* L., and *Phleum pratense* L. are the most common plant species growing in this area. The study of heavy metals content in the parts of plants growing around the metallurgical plants showed that all collected and identified grass species growing around Zn and Pb manufacturing plants in Eastern Kazakhstan accumulated Zn and Pb in great amounts, mainly in the roots [185]. Our data showed that the content of Pb and Zn in these species was much higher than the means defined for non-accumulators growing on contaminated soils. These plant species which were growing on highly contaminated soils (total soil Pb—12,672.4 mg/kg) around metallurgic plants in Eastern Kazakhstan accumulated Pb: *D. glomerata*—up to 3760.0 mg/kg in shoots and 6715.9 mg/kg in roots, *B. inermis*—up to 709.1 mg/kg in shoots and 6787.8 mg/kg in roots, *A. repens*—up to 287.0 3 mg/kg in shoots and 3982.8 mg/kg in roots, *A. alba*—up to 339.7 mg/kg in shoots and 2496.0 mg/kg in roots, and *P. pretense*—up to 419.6 mg/kg in shoots and 4789.4 mg/kg in roots (Table 6.4).

In the shoots of all species, content of Pb was relatively low compared with that in the roots. *Agropyron repens* L. had a low level of Pb accumulation in the aboveground parts at low- and medium-polluted soils (Table 6.4). In the shoots of *Agropyron repens,* concentration of Pb increased on soils with high content of metal in the soil, but it was lower than in other species.

**Table 6.4** Lead concentration in plants, growing around metallurgical enterprises in East Kazakhstan

| Plant, parts | *Dactylis glomerata* | *Bromus inermis* | *Agropyron repens* | *Agrostis alba* | *Phleum pratense* |
|---|---|---|---|---|---|
| Zinc Plant, West. Total Soil Pb—12.3 mg/kg | | | | | |
| Shoots | 26.2±0.9 | 249.0±8.9 | 44.9±1.67 | 26.2±1.1 | 10.80±0.39 |
| Roots | 109.8±4.2 | 164.7±6.9 | 36.7±1.4 | 171.8±6.5 | 119.8±4.3 |
| Zinc Plant, East. Total soil Pb—195.8 mg/kg | | | | | |
| Shoots | 92.4±3.2 | 27.90±0.89 | 56.3±2.2 | 146.7±5.4 | – |
| Roots | 159.7±6.1 | 99.8±3.7 | 69.3±2.5 | 870.0±36.5 | – |
| Zinc Plant, Center Total soil Pb—932.8 mg/kg | | | | | |
| Shoots | 256.9±9.2 | 190.0±5.7 | 179.8±5.8 | 191.7±6.0 | 68.3±2.7 |
| Roots | 269.7±8.36 | 140.0±4.9 | 270.5±8.4 | 121.7±3.8 | 83.6±2.8 |
| Lead Plant, West Total soil Pb—1308.9 mg/kg | | | | | |
| Shoots | 372.6±13.4 | – | 107.7±3.9 | 243.8±8.8 | 51.8±1.7 |
| Roots | 3719.2±141.3 | – | 309.8±11.8 | 254.0±9.1 | 239.7±8.9 |
| Zinc Plant, North Total soil Pb—1645.3 mg/kg | | | | | |
| Shoots | 176.0±5.0 | 581.0±20.9 | 83.1±2.8 | 118.8±4.3 | 69.9±2.9 |
| Roots | 384.0±15.7 | 1897.0±75.8 | 149.8±5.8 | 87.5±3.4 | 149.0±5.4 |
| Lead Plant, East Total soil Pb—1723.9 mg/kg | | | | | |
| Shoots | 146.0±5.5 | 96.1±3.8 | 101.8±3.6 | 69.9±2.3 | 8.2±0.3 |
| Roots | 567.7±117.6 | 1545.9±19.7 | 2735.0±15.1 | 525.0±11.9 | 399.2±40.7 |
| Lead Plant, South Total soil Pb—3046.0 mg/kg | | | | | |
| Shoots | 387.7±13.9 | 162.8±6.1 | 233.5±9.8 | 99.9±3.7 | 354.5±12.1 |
| Roots | 2164.3±69.2 | 1339.1±52.2 | 1331.8±59.0 | 1527.8±58.0 | 3137.5±11.9 |
| Lead Plant, North Total soil Pb—4769.2 mg/kg | | | | | |
| Shoots | 279.8±9.8 | 98.8±3.6 | 100.1±3.7 | 304.6±9.7 | 539.7±22.7 |
| Roots | 2800.0±117.6 | 546.3±19.7 | 399.1±15.1 | 2776.6±119.3 | 2917.6±113.7 |
| Zinc Plant, South Total soil Pb—5990.4 mg/kg | | | | | |
| Shoots | 85.1±0.3 | 291.6±8.0 | 125.0±4.4 | 452.0±15.8 | 8.5±0.4 |
| Roots | 97.5±3.12 | 101.8±3.6 | 2125.3±72.2 | 988.0±30.5 | 308.1±9.9 |
| Lead Plant, Center Total soil Pb—12,672.4 mg/kg | | | | | |
| Shoots | 3760.0±146.8 | 709.1±7.1 | 287.6±9.2 | 339.7±12.5 | 419.6±14.2 |
| Roots | 6715.9±255.0 | 6787.8±237.5 | 3982.8±151.0 | 2496.0±82.0 | 4789.4±181.9 |

– The species are not found in this area

It should be noted that in the aerial parts of this species were found relatively low levels of Pb as compared to roots even at the highest metal concentrations in the soil, indicating that there is an active protective function of the distribution of root system. With the increase of concentration of Pb in the soil, metal content in the plants roots steadily climbs up, which indicates that at low concentrations in the environment the selective permeability of cell membranes of roots prevents free penetration of metals into root cells, and at high concentrations, there occurs the breach in the barrier function of membrane permeability which drastically increases

**Table 6.5** Shoot/root ratio for Pb

| Soil content of Pb, mg/kg | *Agropyron repens* | *Dactylis glomerata* | *Bromus inermis* | *Agrostis alba* | *Phleum pratense* |
|---|---|---|---|---|---|
| ZP, West—12.3 | 1.2 | 0.24 | 1.5 | 0.15 | 0.09 |
| ZP, East—195.8 | 0.81 | 0.58 | 0.3 | 0.17 | – |
| ZP, Center—932.8 | 0.66 | 0.95 | 1.36 | 1.58 | 0.82 |
| LP, West—1308.9 | 0.35 | 0.1 | – | 0.96 | 0.22 |
| ZP, North—1645.3 | 0.55 | 0.46 | 0.3 | 0.60 | 0.47 |
| LP, East—1723.9 | 0.04 | 0.26 | 0.06 | 0.13 | 0.02 |
| LP, south—3046.0 | 0.18 | 0.18 | 0.05 | 0.07 | 0.11 |
| LP, North—4769.2 | 0.25 | 0,1 | 0.18 | 0.11 | 0.18 |
| ZP, South—5990.4 | 0.06 | 0.87 | 2.86 | 0.46 | 0.03 |
| LP, Center—12,672.4 | 0.07 | 0.56 | 0.10 | 0.14 | 0.09 |

– The species are not found in this area

the concentration of metal in the roots. For example, when the concentration in soil was 932.8 mg/kg (ZP, "Center"), the concentration of this metal in the roots of the species remained relatively low (270.5 mg/kg). With increasing Pb concentration in the soil in the roots of this plant, lead content increased, reaching 982.8 mg/kg on the soils with maximum content of Pb (12,672.4 mg/kg, LP, "Center"). To assess the degree of removal of heavy metal by shoots, it is necessary to calculate the ratio of heavy metal content in the shoots and in the roots (shoot/root ratio), i.e., rate of translocation of the metal. Usually for hyperaccumulators used for phytoextraction of heavy metals, the mean of ratio is greater than one.

The ratio of the metal content in aerial parts and its content in the roots, i.e., translocation rate of the metal, was calculated. The coefficient of Pb translocation (shoot/root ratio) of *A. repens* was <1 in almost all studied areas (Table 6.5).

For *D. glomerata,* the concentration of Pb in the roots sharply increases up to 3179.2 mg/kg (soil concentration Pb—1308.9 mg/kg) in the site to the West of the LP This is a manifestation of pronounced protective function of roots, which concentrate heavy metals and limit their translocation to the photosynthetic and reproductive plant parts. Only in the most contaminated site in the center of LP, where concentration of Pb in the soil equals to 12,672.4 mg/kg, the content of lead in the shoots rises sharply (Table 6.4), probably due to strong air pollution.

In *B. inermis,* Pb content in the roots begin to rise sharply at concentrations in the soil above 1645.3 mg/kg to the North of the ZP and reaches more than 6000 mg/kg at a maximum soil concentration (12,672.4 mg/kg of soil) (Table 6.4). To the South of the LP in the roots of *A. alba* and P. *pratense,* Pb content increased significantly compared to areas with relatively low lead content in the soil. In all investigated species, lead is accumulated predominantly in the roots. In these species, shoot/root ratio of Pb was lower than that in all studied pots (Table 6.5). For *D. glomerata,* this value was close to 1 (0.95) in the center of ZP. In the center of LP, *D. glomerata* accumulated up to 3760.0 mg/kg, which exceeds the threshold concentration for

Pb-hyperaccumulators (1000 mg/kg) in the shoots, but the shoot/root ratio of Pb was less than one. For *B. inermis* and *A. alba,* the factor of translocation in the center of ZP was >1, and for other species, this value was close to one, probably due to strong air pollution.

In highly contaminated soils (LP, Center), the content of Pb of the studied species was equal to 0.03–0.4 %, in roots—0.2–0.7 % of dry weight. The highest percentage of Pb was in the aboveground parts of *D. glomerata* (0.4 %) and in the roots of *D. glomerata* and *B. inermis* (0.7 %). Lead content in the roots of plants in highly contaminated soils in the center of LP (12,672.4 mg/kg Pb in the soil) can be

**Table 6.6** Zinc concentration in plants, growing around metallurgical enterprises in Eastern Kazakhstan, mg/kg

| Plant parts | *Dactylis glomerata* | *Bromus inermis* | *Agropyron repens* | *Agrostis alba* | *Phleum pratense* |
|---|---|---|---|---|---|
| Zinc Plant, West. Total Soil Zn—355.7 mg/kg | | | | | |
| Shoots | 181.8 ± 6.4 | 249.5 ± 9.0 | 226.7 ± 7.5 | 195.9 ± 6.9 | 162.7 ± 5.7 |
| Roots | 1547.2 ± 49.5 | 3095.0 ± 117.6 | 522.6 ± 19.9 | 1688.3 ± 60.7 | 1028.7 ± 36.0 |
| Lead Plant, West Total Soil Zn—1598,7 mg/kg | | | | | |
| Shoots | 299.7 ± 10.4 | – | 126.7 ± 4.6 | 319.7 ± 11.8 | 265.4 ± 7.7 |
| Roots | 6300.0 ± 239.4 | – | 499.7 ± 19.5 | 909.6 ± 34.5 | 359.6 ± 14.0 |
| Zinc Plant, East. Total soil—2898.3 mg/kg | | | | | |
| Shoots | 510.7 ± 18.3 | 199.7 ± 6.9 | 247.6 ± 8.9 | 828.6 ± 29.8 | – |
| Roots | 1647.0 ± 59.2 | 948.8 ± 33.2 | 1166.9 ± 45.5 | 4789.5 ± 167.6 | – |
| Lead Plant, South Total soil Zn—3893.0 mg/kg | | | | | |
| Shoots | 729.4 ± 25.5 | 379.7 ± 14.4 | 409.3 ± 14.3 | 138.8 ± 4.9 | 355.6 ± 12.8 |
| Roots | 5126.6 ± 179.4 | 2578.4 ± 92.8 | 1815.6 ± 74.7 | 2516.5 ± 95.6 | 2697.8 ± 105.2 |
| Lead Plant, East Total soil Zn—5796.5 mg/kg | | | | | |
| Shoots | 169.7 ± 6.1 | 303.7 ± 12.4 | 161.7 ± 4.14 | 679.7 ± 25.8 | 192.9 ± 7.3 |
| Roots | 1139.3 ± 36.4 | 2942.3 ± 105.9 | 1647.0 ± 67.5 | 1989.6 ± 65.6 | 968.0 ± 34.8 |
| Zinc Plant, Center Total soil Zn—17,881.9 mg/kg | | | | | |
| Shoots | 1499.4 ± 63.2 | 1300.0 ± 49.4 | 1298.9 ± 46.7 | 1498.2 ± 47.9 | 1399.4 ± 47.6 |
| Roots | 2697.3 ± 105.1 | 1600.0 ± 57.6 | 3194.2 ± 108.6 | 2195.3 ± 79.1 | 1747.5 ± 102.3 |
| Lead Plant, North Total soil Zn—22,972.4 mg/kg | | | | | |
| Shoots | 519.8 ± 20.7 | 309.4 ± 11.8 | 379.9 ± 14.1 | 595.3 ± 23.8 | 1049.6 ± 37.8 |
| Roots | 3539.3 ± 120.3 | 2197.4 ± 79.1 | 1147.5 ± 43.6 | 9288.8 ± 325.1 | 5095.9 ± 43.8 |
| Lead Plant, Center Total soil Zn—22,986.2 mg/kg | | | | | |
| Shoots | 1350.0 ± 48.6 | 271.7 ± 9.8 | 709.1 ± 23.4 | 439.6 ± 15.4 | 599.5 ± 22.7 |
| Roots | 1948.8 ± 70.2 | 2894.8 ± 104.2 | 3992.8 ± 139.7 | 4238.9 ± 135.6 | 5936.9 ± 189.9 |
| Zinc Plant, North Total soil Zn—22,990.8 mg/kg | | | | | |
| Shoots | 101.0 ± 3.6 | 4472.8 ± 147.6 | 698.7 ± 25.1 | 1068.9 ± 38.4 | 453.5 ± 15.4 |
| Roots | 4588.9 ± 165.2 | 6981.8 ± 251.3 | 2177.4 ± 82.7 | 1516.0 ± 57.6 | 2160.0 ± 84.2 |
| Zinc Plant, South Total soil Zn—129,792.3 mg/kg | | | | | |
| Shoots | 1498.2 ± 56.9 | 1498.2 ± 53.9 | 2743.9 ± 104.2 | 4900.0 ± 186.2 | 997.0 ± 35.9 |
| Roots | 2300.0 ± 89.7 | 2896.5 ± 107.2 | 29,934.1 ± 1047.6 | 14,820.3 ± 533.5 | 10,383.3 ± 394.6 |

– The species are not found in this area

arranged as follows: *B. inermis*≥*D. glomerata*>*P. pratense*>*A. repens*>*A. alba*. The content of lead in the aerial parts decreased in the following order: *D. glomerata*>*B. inermis*>*Ph. pretense*>*Ag. alba*>*A. repens*.

These species accumulated Zn in great amounts too: *D. glomerata*—up to 1498.2 mg/kg in shoots and 2300.0 mg/kg in roots, *B. inermis*—up to 1498.2 mg/kg in shoots and 2896.5 mg/kg in roots, *A. repens*—up to 2743.9 mg/kg in shoots and 29,934.1 mg/kg in roots, *Ag. alba*—up to 4900.0 mg/kg in shoots and 14,820.3 mg/kg in roots, *P. pratense*—up to 997.6 mg/kg in shoots and 10,383.3 mg/kg in roots (Table 6.6). The tested plant species have accumulated Zn predominantly in the roots (Tables 6.5 and 6.6). Coefficient of Zn translocation from roots to shoots of plant species was lower than one (Table 6.6). *A. repens* accumulated the highest amount of this metal in the roots as compared to other species on the soil with high concentration of Zn (ZP, South). The concentrations of Zn in the roots of *A. repens*, *A. alba,* and *P. pratense* to the South of ZP (29,934.1, 14,820.3, and 10,383.3 mg/kg, respectively) were significantly higher than those of other species.

Bioconcentration factor of Zn in less contaminated soil was more than one; in highly contaminated soils—less than one. In highly contaminated soils to the South of ZP (129,792.3 mg/kg), concentrations of zinc in grass species can be arranged as follows: in the roots—*A. repens*>*A. alba*>*P. pretense*>*B. inermis*>*D. glomerata*; in the shoots—*A. alba*>*A. repens*>*B. inermis*>*D. glomerata*>*P. pratense*. In this area, the percent of Zn for *P. pratense* was equal to 1 %, *A. alba*—1.4 %, *A. repens*—3 %. The analysis of the experimental data showed that wild grass species, growing around metallurgical plants of East Kazakhstan, accumulate significant amounts of lead and zinc mainly in the roots. It is known that for hyperaccumulators, the thresholds of concentrations of metals absorption have the following values (mg/kg): Zn—10,000, Pb and Cu—1000, Cd—100; the ratio of the metal content in aerial parts of its content in the roots must be greater than one [40]. The studied plant species do not belong to hyperaccumulators because the concentration of heavy metals in plant parts is lower than the thresholds for hyperaccumulators.

**Table 6.7** Shoot/root ratio for Zn

| Soil content of Zn, mg/kg | *Agropyron repens* | *Dactylis glomerata* | *Bromus inermis* | *Agrostis alba* | *Phleum pratense* |
|---|---|---|---|---|---|
| ZP, West—355.7 | 0.43 | 0.12 | 0.08 | 0.12 | 0.16 |
| LP, West—1598.7 | 0.25 | 0.05 | – | 0.35 | 0.74 |
| ZP, East—2898.3 | 0.21 | 0.31 | 0.21 | 0.17 | – |
| LP, South—3893.0 | 0.22 | 0.14 | 0.15 | 0.06 | 0.14 |
| LP, East—5796.5 | 0.1 | 0.15 | 0.1 | 0.34 | 0.20 |
| ZP, Center—17,881.9 | 0.4 | 0.56 | 0.19 | 0.68 | 0.08 |
| LP, North—22,972.4 | 0.33 | 0.14 | 0.64 | 0.06 | 0.21 |
| LP, Center—22,986.2 | 0.18 | 0.7 | 0.09 | 0.1 | 0.1 |
| ZP, North—22,990.8 | 0.32 | 0.22 | 0.6 | 0.7 | 0.2 |
| ZP, South—129,792.3 | 0.09 | 0.65 | 0.5 | 0.33 | 0.1 |

– The species are not found in this area

Analyzing the data, it should be noted that the Pb content in the aboveground parts was lower than one. But for some species, the shoot/root ratio was greater than one: *A. repens*—1.5 (ZP, West), *A. alba*—1.6 (ZP, "Center"), *B. inermis*—1.36 (ZP, "Center") and 2.9 (ZP, South) (Table 6.7). But the fact that it is not a natural phenomenon suggests that such data do not exist in other areas and it is likely the result of atmospheric pollution, rather than metal translocation from roots to aboveground parts.

Thus, there were found no plants in full compliance with the definition hyperaccumulator of heavy metals. But according to the literature, content of Zn and Pb in nonaccumulating plants in the contaminated soil must be less than 1000 mg/kg for Zn and less than 100 mg/kg for Pb [40]. In this case, Pb and Zn contents for the studied species were much greater than for nonaccumulating plants on the contaminated soil. Pb concentration values for *D. glomerata*, *B. inermis*, and *A. alba* were much higher than 100 mg/kg (the threshold concentration for Pb nonaccumulators) and the concentration of Zn was greater than 1000 mg/kg (the threshold concentration of Zn for nonaccumulators) for all species in the highly contaminated soil. Although these species are not concentrated in the aboveground organs, they accumulate metals in significant amounts in the roots. Thus, the study of wild grass species of flora in Kazakhstan, widely distributed in the contaminated area around metallurgical plants, showed that the investigated species accumulated heavy metals in amounts close to the generally accepted threshold concentration for plants-hyperaccumulators, but they are not fully consistent with this status.

The mean Pb concentration in plant parts of *A. alba*, *B. inermis*, and *D. glomerata* was much higher than 100 mg/kg and the mean Zn concentration for all species on highly contaminated soils was >1000 mg/kg. To estimate the effect of heavy metals on growth parameters and metal-accumulating ability in a comparative aspect, these species were chosen for further studies, including the screening of

**Table 6.8** Root and shoot biomass of wild grass species, g/м$^2$

| Variants | *Agropyron repens* | *Dactylis glomerata* | *Phleum pratense* | *Setaria viridis* |
|---|---|---|---|---|
| Shoots[a] | | | | |
| Zn—1000 mg/kg | 305.0±13.1 | 345.0±15.8 | 0 | 150.0±5.5 |
| Pb—1000 mg/kg | 395.0±15.5 | 350.0±13.1 | 800.0±41.2 | 280.0±1.0 |
| Cu—100 mg/kg | 425.0±17.2 | 340.0±16.0 | 655.0±30.2 | 115.0±4.0 |
| Cd—100 mg/kg | 440.0±20.2 | 320.0±11.0 | 570.0±21.1 | 245.0±9.8 |
| Roots[b] | | | | |
| Zn—1000 mg/kg | 605.0±19.2 | 16.0±0.55 | 0 | 40.0±1.2 |
| Pb—1000 mg/kg | 1060.0±4.2 | 39.0±1.3 | 50.0±2.2 | 90.0±3.0 |
| Cu—100 mg/kg | 840.0±4.3 | 40.0±1.9 | 45.0±1.9 | 25.0±0.7 |
| Cd—100 mg/kg | 1050.0±38.0 | 30.0±1.4 | 27.0±0.8 | 45.0±1.8 |

[a]The differences across species and variants are not significant: $P>0.05$ ($P=0.22$, $P=0.14$ for shoots and roots, respectively)

[b]The differences across species are significant $P<0.05$ ($P=1.46E-06$), across variants are not significant—$P>0.05$ ($P=0.26$)

these species for their ability to accumulate heavy metals in hydroponic conditions and their applicability for removal of trace metals from soils spiked with metals. The ability of wild grass species to accumulate trace metals in field conditions was studied by sowing their seed on artificially contaminated soils.

### *6.2.3 Experiments on Artificially Contaminated Soils*

#### 6.2.3.1 Effect of Heavy Metals on Plant Biomass

The shoot and root biomass of these wild grass species was compared to assess the abundance of trace metals in the soils. The root biomass of plant species from 1 $m^2$ of each heavy metal treatment increased in the following order (Table 6.8): Zn—*A. repens* > *S. viridis* > *D. glomerata* > *P. pretense* ($p < 0.05$) Pb—*A. repens* > *S. viridis* > *P. pratense* > *D. glomerata* ($p < 0.01$); Cu—*A. repens* > *P. pratense* > *D. glomerata* > *S. viridis* ($p < 0.05$); Cd—*A. repens* > *S. viridis* > *D. glomerata* ≥ *P. pratense*; in this treatment, the differences between species were significant ($p < 0.01$), with the exception of *D. glomerata* vs. *S. viridis* ($p > 0.05$).

Shoot biomass of plant species from 1 $m^2$ for each treatment increased in the following order: Zn—*D. glomerata* ≥ *A. repens* > *S. viridis* > *P. pratense*; the differences between species were significant ($p < 0.01$) with the exception of *D. glomerata* vs. *A. repens* ($p > 0.05$); Pb—*P. pretense* > *A. repens* ≥ *D. glomerata* > *S. viridis* ($p < 0.05$); the difference between *A. repens* and *D. glomerata* was not significant ($P > 0.05$); Cu—*P. pratense* > *A. repens* ≥ *D. glomerata* > *S. viridis*; $P < 0.01$ between all species with the exception of *A. repens* vs. *D. glomerata* ($P > 0.05$); Cd—*P. pratense* > *A. repens* > *D. glomerata* > *S. viridis* ($P < 0.05$ between all species). The mean shoot biomass of *P. pratense* was highest among all species in the presence of Cu, Cd, and Pb, and the shoot and root biomass of *S. viridis* was the lowest among all grasses. In the presence of Zn *D. glomerata* had the highest biomass, *P. pratense* the lowest. *A. repens* accumulated the highest root biomass in all treatments compared with other species.

Thus, *D. glomerata* and *A. repens* were tolerant to all four metals. The shoot biomass of these species was relatively high (Table 6.8). *S. viridis* was tolerant to Pb and sensitive to Zn, Cu, and Cd. *P. pratense* was relatively sensitive to all metals. *A. repens*. A comparison of shoot biomass in all treatments showed that, among metals, Zn was distinguishable by a stronger negative effect on biomass (305 g/$m^2$). In the presence of other metals (Pb, Cu, Cd), this species produced an approximately equal biomass (395, 425, 440 g/$m^2$, respectively, $p > 0.05$). The greatest root biomass was observed in the treatments with Pb and Cd and the lowest in the treatment with Zn.

*P. pretense.* Zn fully inhibited the growth of *P. pratense*. In the presence of Pb, the mean shoot biomass was highest, and in the presence of Cu and Cd, the biomass was almost equal. The mean shoot biomass in different treatments decreased in the following order (g/$m^2$): Pb (800.0) > Cu (655.0) ≥ Cd (570.0); root biomass decreased in the following order (g/$m^2$): Pb (50.0) ≥ Cu (45.0) > Cd (27.0) ($p < 0.01$) with the exception of Pb vs. Cu ($p > 0.05$).

**Table 6.9** Shoot/root ratio of trace metals content

| Species | *Agropyron repens* | *Dactylis glomerata* | *Phleum pratense* | *Setaria viridis* |
|---|---|---|---|---|
| Zn | 0.87 | 0.39 | 0 | 0.48 |
| Pb | 0.28 | 0.1 | 0.03 | 0.08 |
| Cu | 0.53 | 0.16 | 0.17 | 0.12 |
| Cd | 0.45 | 0.22 | 0.07 | 0.01 |
| The differences across species and metals are significant—$P<0.05$ ($P=0.046$, $P=0.005$ for shoots and roots, respectively) | | | | |

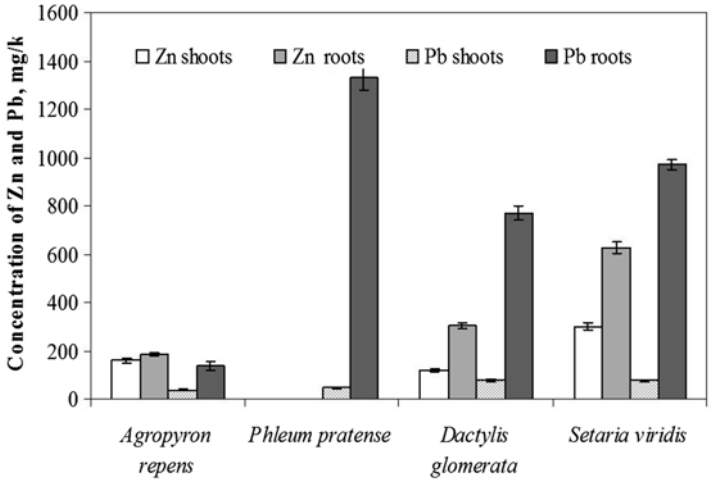


**Fig. 6.4** Zinc and lead content in the plant parts of wild grass species. Differences between species according to two-way ANOVA test are significant at $P<0.05$ ($P=1.98E-32$; 7.63E-24 for shoots and roots, respectively). LSD for roots—1067 and for shoots—210 at $P=0.95$. Values represent mean ± Standard Error (SE)

*D. glomerata*. In all treatments the shoot biomass was approximately equal (g/m$^2$): Pb (350.0) ≥ Zn (345.0) ≥ Cu (340.0) ≥ Cd (320.0) ($p>0.05$). The root biomass had the highest means in the presence of Pb and Cu. The mean root biomass decreased in the following order (g/m$^2$): Cu (40.0) ≥ Pb (39.0) > Cd (30.0) > Zn (16.0) ($p<0.01$), with the exception of Cu vs. Pb ($P>0.05$).

*S. viridis*. The shoot biomass decreased in the following order (g/m$^2$): Pb (280.0) > Cd (245.0) > Zn (150.0) > Cu (115.0) ($p<0.01$); root biomass (g/m$^2$): Pb (90.0) > Cd (45.0) ≥ Zn(40.0) > Cu (25.0) ($p<0.001$) with the exception of Zn vs. Cd ($P>0.05$).

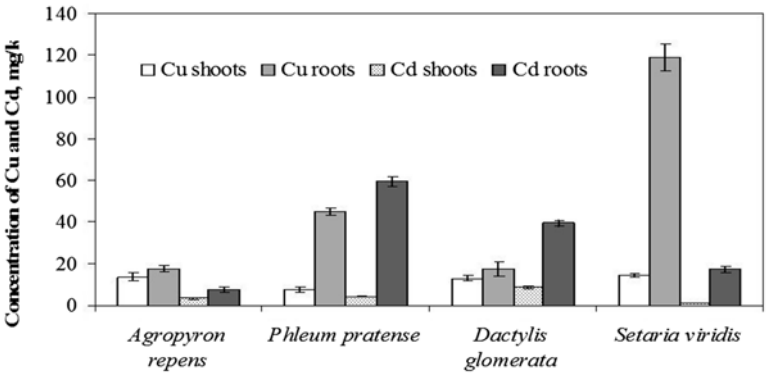


**Fig. 6.5** Copper and cadmium content in plant parts of wild grass species. Differences between species according to two-way ANOVA test are significant at $P<0.01$ ($P=6.6E-35$; 2.09E-37 for shoots and roots, respectively). LSD for roots—105 and for shoots—9 at $P=0.95$. Values represent mean ± Standard Error (SE)

### 6.2.3.2 Content of Heavy Metals in Plant Parts of Wild Grass Species

The content of heavy metals in plant parts was determined. Different plant parts accumulated high concentrations of metals. The shoot/root ratio was <1 for all species (Table 6.9). *S. viridis* accumulated the highest amount of Zn in shoots and roots, and Pb in shoots (Fig. 6.4). Cu was most accumulated in the roots. Cd was least accumulated by this species than by other species (Fig. 6.5). The shoot/root ratio was <1 for all metals. The lowest shoot/root ratio was for Pb. The concentration of metals in the shoots decreased in the following order (mg/kg): Zn (299.1) > Pb (76.9) > Cu (14.3) > Cd (0.2); in the roots: Pb (971.2) > Zn (627.4) > Cu (119.1) > Cd (17.3).

*P. pratense* was the most sensitive grass to the presence of Zn in the soil. This plant did not germinate in Zn-contaminated soil. *P. pratense* accumulated Pb in the roots >1000 ppm, i.e., 1330 mg/kg. Cd accumulated by the roots of *P. pratense* was the highest among all the grass species assessed (59.4 mg/kg) (Fig. 6.5). The shoots of *P. pretense* accumulated the least Cu compared with the other heavy metals (Fig. 6.5). The shoot/root ratio for all metals was <1 and was the lowest among all species. The concentration of metals in the shoots decreased in the following order (mg/kg): Pb (45.3) > Cu (7.5) > Cd (4.2); in the roots: Pb (1330.0) > Cd (59.4) > Cu (44.9).

*D. glomerata* accumulated a considerable amount of Pb in the roots. In the shoots, the concentration of Zn was lowest among all species (Fig. 6.5). The concentration of metals in the shoots of *D. glomerata* decreased in the following order (mg/kg): Zn (118.4) > Pb (79.3) > Cu (13.0) > Cd (8.7); in the roots: Pb (770.4) > Zn (304.1) > Cd (39.4) > Cu (17.5) (Figs. 6.4 and 6.5). *A. repens* accumulated the least amount of Pb in the shoots and Cd in the roots. The shoot/root ratio was <1. The

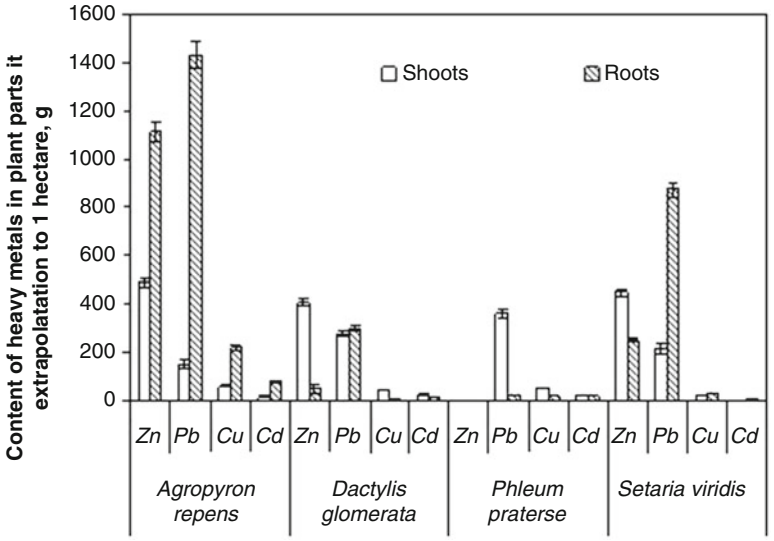


**Fig. 6.6** Content of trace metals in plant biomass in extrapolation to 1 ha. Differences between species and metals are according to two-way ANOVA test significant at $P<0.05$ ($P=8.63E-16$; 5.14E-19 for shoots and roots, respectively). LSD for Pb—1314 and for Zn—1608 at $P=0.95$. Values represent mean ± Standard Error (SE)

concentration of metals in the shoots of *A. repens* decreased in the following order (mg/kg): Zn (159.7)>Pb (38.2)>Cu (13.7)>Cd (3.4); in the roots: Zn (184.1)>Pb(135.2)>Cu (25.8)>Cd (7.5) (Fig. 6.4). Thus, the highest Zn concentration was observed in the roots and shoots of *S. viridis*. Pb was greatly accumulated in the roots of *P. pratense*, *S. viridis,* and *D. glomerata*. The shoot/root ratio was <1 for all species (Table 6.9).

The removal efficiency of trace metals by roots and shoots of plants was calculated using the means of plant part biomass and the concentration of trace metals in plant parts according to equation: g/ha = Concentrations of metals (g/kg) × yield (kg/$m^2$) × 10,000; preliminarily, the mean of metal concentrations in mg/kg was converted into g/kg, yield in g/$m^2$ into kg/$m^2$, and the coefficient 10,000 is necessary to convert 1 $m^2$ into 1 ha. The removal of Zn by shoots extrapolated into 1 ha increased in the following order (g/ha): *A. repens* (487.1)>*S.viridis* (448.7)>*D. glomerata* (408.5); by the roots: *A. repens* (1113.8)>*S. viridis* (251.4)>*D. glomerata* (48.7) (Fig. 6.6).

Thus, the shoots and roots of *A. repens* removed the most Zn, whereas the shoots and roots of *D. glomerata* the least. The roots of *A. repens* removed the most Zn and the roots of *D. glomerata* the least. The removal of Pb decreased in the following order (g/ha): by shoots: *P. pratense* (362.4)>*S. viridis* (215.3)>*D. glomerata* (277.6)>*A. repens* (150.1); by roots: *A. repens* (1433.0)>*S. viridis* (874.1)>*P. pratense* (665.0)>*D. glomerata* (300.5) (Fig. 6.6). *Ph. pratense* was removed the

most amounts of Pb by the shoots, *A. repens*—the lowest one. *A. repens* removed the most amounts of Pb by the roots, *D. glomerata*—the lowest ones. As for removal rates of Cu by the shoots, the species were disposed in the following order (g/ha): *A. repens* (58.2)>*P. pratense* (49.1)>*D. glomerata* (44.2)>*S. viridis* (16.5); by the roots—*A. repens* (216.7)>*S. viridis* (29.8)>*P. pratense* (20.2)>*D. glomerata* (7.0) (Fig. 6.6). The shoots of *A. repens* had the most removal rate of Cu, and the shoots of *S. viridis*—the lowest one. *A. repens* had the highest removal rate by roots and *D. glomerata*—the lowest one.

The removal of Cd by the shoots extrapolated to 1 ha decreased in the following order (g/ha): *D. glomerata* (27.8)>*P. pratense* (23.9)>*A. repens* (15.0)>*S. viridis* (0.5); roots—*A. repens* (78.8)>*P. pratense* (16.0)>*D. glomerata* (11.8)>*S. viridis* (7.8) (Fig. 6.6). Thus, the most removal rate of cadmium was observed in the shoots of *D. glomerata* and in the roots of *A. repens*. In general, all analyzed species were tolerant to trace metals. They accumulated varied amounts of trace metals mainly in the roots. All these species can be used for phytoremediation of contaminated soils, particularly for phytostabilization, due their ability to accumulate trace metals in the roots. The following species such as *D. glomerata*, *B. inermis*, *A. repens*, *A. alba,* and *P. pratense* accumulated Zn and Pb in great amounts mainly in the roots from heavily contaminated soils. Therefore, these species can be the candidates for using them in *phytostabilization*.

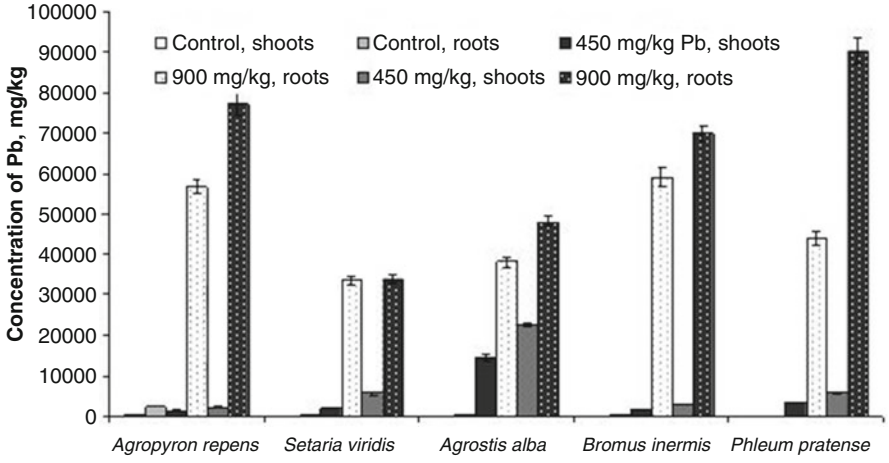


**Fig. 6.7** Lead content in plant parts. Differences between treatments according to two-way ANOVA test are significant at $P<0.05$, ($P=5.33E-12$; 5.15E-19 for shoots and roots, respectively). LSD for roots—14,425 and for shoots—5000 at $P=0.95$. Values represent mean ± Standard Error (SE)

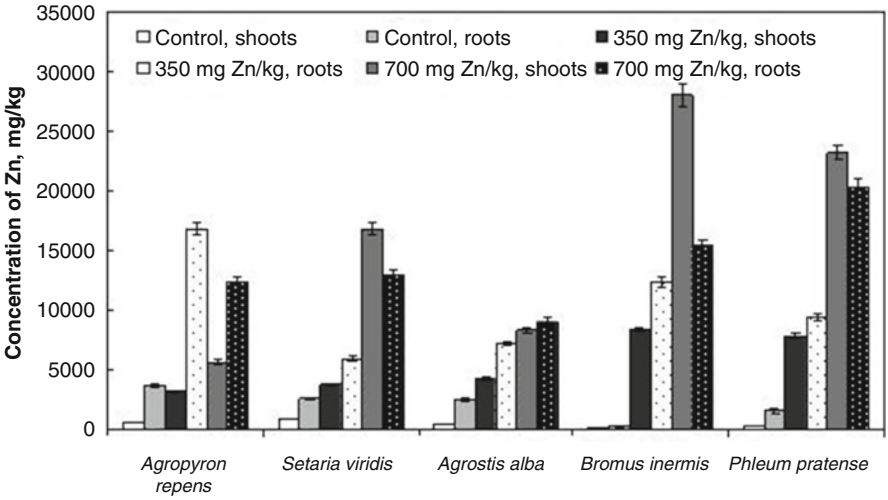


**Fig. 6.8** Zinc content in plant parts. Differences between treatments according to two-way ANOVA test are significant at $P<0.05$ ($P=1.261E-37$; 2.03E-34 for shoots and roots, respectively). LSD for roots—4477 and for shoots—6469 at $P=0.95$. Values represent mean ± Standard Error (SE)

### *6.2.4 Hydroponic Experiments*

The metal accumulation ability of grass species in hydroponic conditions at extremely high concentrations of zinc and lead (450, 900 mg/L of Pb and 350, 700 mg/L of Zn) was studied. The analysis of trace metals in plant parts has shown the highest level of lead concentration in the roots (Fig. 6.7). The highest level of lead at 900 mg/L was accumulated in the roots of the species: *P. pratense* (90,281.9 mg/kg), *A. repens* (77,137.9 mg/kg) and *B. inermis* (69,991.8 mg/kg).

The lowest level of lead was found in the roots of *S. viridis* (33,974.6 mg/kg). The shoot/root ratio for all species at the concentration 900 mg Pb/L was <1 and decreased in the following order: *A. alba* (0.47)>*S. viridis* (0.17)>*P. pratense* (0.06)>*B. inermis* (0.04)>*A. repens* (0.027). At concentration 900 mg Pb/L, the content of lead in shoots decreased in the following order: *A. alba*>*P. pretense*>*S. viridis*>*B. inermis*>*A. repens*; in roots—*P. pretense*>*A. repens*>*B. inermis*>*A. alba*. The highest level of Pb was detected in the shoots of *A. alba* (22,670.0 mg/kg) and the lowest in the shoots of *A. repens* (2091.3 mg/kg). The study of Zn content in plant parts has shown that all species except *A. repens* accumulated Zn mainly in the shoots (700 mg Zn/L). Shoot/root ratio of Zn for all species except of *A. alba* and *A. repens* was >1. *A. alba* accumulated approximately equal amount of Zn in both roots and shoots, whereas *A. repens* accumulated mainly in the roots (Fig. 6.8).

The shoot/root ratio decreased in the following order: *B. inermis* (1.8)>*S. viridis* (1.3)>*P. pratense* (1.14)>*A. alba* (0.92)>*A. repens* (0.46). The highest level of Zn

was detected at 700 mg Zn/L concentration in shoots and roots of *B. inermis* and *P. pratense*, the lowest in *A. repens* and *A. alba*. Concentration of Zn in shoots was decreased in the following order: *B. inermis* > *P. pratense* > *S. viridis* > *A. alba* > *A. repens*; in roots: *P. pratense* > *B. inermis* > *S. viridis* ≥ *A. repens* > *A. alba. P. pratense* and *B. inermis* were distinguishable by high accumulation of Zn in the shoots and roots. Thus, in hydroponic conditions, Pb at high concentrations was accumulated mainly by the roots for all species and the shoot/root ratio was <1. At a high concentration of Zn, it was accumulated mainly by the shoots except those of *A. repens*, whereas *A. alba* accumulated Zn in approximately equal amounts in both roots and shoots. *P. pretense* and *B. inermis* were distinguishable from the other species by accumulating Zn in their shoots to a high level. These species also accumulated a large amount of Pb in the roots.

As mentioned above, there are several types of phytoremediation of soils contaminated with heavy metals. Plants-hyperaccumulators, which mainly accumulate heavy metals in the aboveground parts, are mainly used for phytoextraction of heavy metals from the soil. *Phytotabilization* is a type of phytoremediation and is defined as a technology, which aims to immobilize heavy metals in the root zone and prevent the distribution of metals in the soil profile. *For phytostabilization,* it is very important for the property of plants to reduce the transport of heavy metals to aboveground parts, to avoid the promotion of metals through the food chain [186, 187]. Herbaceous plants are usually resistant to trace amounts of metals and play an important role in phytoremediation [171, 172, 188]. They have well-developed adventitious roots, the unique morphology of the root system [171], the high biological productivity [171], and therefore, have an additional advantage for use in phytostabilization. The tested plant species *D. glomerata*, *B. inermis*, *A. repens*, *Ag. Alba,* and *Ph. pratense* accumulated Zn and Pb in high concentrations mainly in the roots in highly contaminated soils. *A. repens* is known as a plant that can be used for stabilization of Pb in soil [189]. These species are the most suitable candidates for their use for phytostabilization of metals on the contaminated area.

### 6.2.5 *Effect of Humic Acids on Bioavailability of Cadmium and Lead*

Humic acids are the widespread nature of physiologically active compounds; they are in the soil organic matter and determine its fertility. The structure is defined by the presence of humic acids slightly condensed and substituted aromatic rings and carboxylic, alcoholic and phenolic groups [190–192], and therefore, they have an important role in the transport, bioavailability, and solubility of heavy metals. *Agropyron repense* L. plants were grown in the soils on the pots (1 $m^2$ for each variant) where the following concentrations of metals in the form of $Pb(NO_3)_2$ and $CdSO_4$ were added: 250 mg Cd/kg and 1000 mg Pb/kg. After 7 days humic acids were added. As a source of humic acid (HA) “Potassium humate” was used (LTD “Kairat and Co”), which contain 8 g/L of humic acids. According to the instruction,

**Table 6.10** Cadmium concentration in plant parts of *Agropyron repens* L

| Variants | Shoots, мг/кг | % to control | Roots, mg/kg | % to control |
|---|---|---|---|---|
| Control (-Cd; -HA) | 1.2±0.02 | 100 | 3.2±0.06 | 100 |
| HA1 (2.5 g/m$^2$) | 1.4±0.04 | 117 | 1.6±0.03 | 50 |
| HA2 (5.0 g/m$^2$) | 0.9±0.007 | 75 | 1.38±0.02 | 43 |
| Cd (250 mg/kg) | 6.6±0.17 | 550 | 200.7±4.81 | 6272 |
| Cd+HA1 | 5.8±0.13 | 483 | 325.6±9.11 | 10,175 |
| Cd+HA2 | 14.0±0.29 | 1167 | 421.0±7.578 | 13,156 |

**Table 6.11** Lead concentration in plant parts of *Agropyron repens* L.

| Variants | Shoots, мг/кг | % to control | Roots, mg/kg | % to control |
|---|---|---|---|---|
| Control (-Pb; -HA) | 10.8±0.28 | 100 | 43.2±0.99 | 100 |
| HA1 (2.5 g/m$^2$) | 8.2±0.18 | 76 | 21.2±0.55 | 49 |
| HA2 (5.0 g/m$^2$) | 8.5±0.17 | 79 | 30.2±0.69 | 70 |
| Pb (1000 mg/kg) | 12.2±0.34 | 113 | 2518.5±52.8 | 5830 |
| Pb+HA1 | 200.0±4.8 | 1852 | 4900.5±132.3 | 11,344 |
| Pb+HA2 | 252.6±6.82 | 2339 | 1942.8±40.79 | 4497 |

the drug of HA was dissolved for obtaining necessary quantities of HA. The following quantities of HA were added to the soil: 12.5 g/m$^2$ (HA1) and 25 g/m$^2$ (HA2). Then the seeds of *Agropyron repense* L. were planted in the soil.

There were nine variants: control (without metals and humic acids), HA1 (12.5 g/m$^2$), HA2 (5.0 g/m$^2$), Cd (250.0 mg/kg), Cd+HA1 (250.0 mg/kg Cd+12.5 g/m$^2$ HA), Cd+HA2 (250.0 mg/kg Cd+5.0 g/m$^2$ HA), Pb (1000.0 mg/kg), Pb+HA1 (1000.0 mg/kg + 12.5 g/m$^2$ HA), Pb+HA2 (1000.0 mg/kg +5.0 g/m$^2$ HA). After 1 month, plants were collected for analysis. The content of cadmium in aboveground organs in variant with HA2 slightly reduced as compared to control variant. It was possibly due to chelation with humic acids trace amounts of heavy metals in soil or increased green biomass in the presence of HA (in this case, the concentration of metals is reduced by dilution and quantity of metals per unit weight is decreased). Concentration of Cd in the shoots and, especially, in the roots in variant Cd+HA2 increased significantly as compared to variants with Cd, but without HA (Table 6.10).

In variant (Cd+HA1), cadmium content in roots was 1.6 times more than in variant without HA Cd (250 mg/kg), while in variant (Cd+HA2) concentration of Cd in roots was more than two times. Concentration of Cd in the shoots in variant (Cd+HA1) was significantly lower than in the variant without HA (Cd (250 mg/kg)), and at higher concentration of HA (Cd+HA2) the cadmium content was more than two times as compared to variant without HA (Cd (250 mg/kg)). This indicates that a certain concentration of humic acids may increase the uptake of cadmium by roots and translocate it to the shoots. The similar results were obtained by previous

studies. Humic acids increased the cadmium concentration in the shoots of *Nicotiana tabacum* from 30.9 to 39.9 mg/kg [134]. Humic acids decrease pH value, which promote higher heavy metals availability. Another reason of high bioavailability of heavy metals in the presence of humic acids is that plants may take up cadmium complexes with humic acid fragments, which result from microbiological degradation. The addition of humic acids reduces the lead content in the organs of plants grown without added metal (HA1 and HA2) as compared to control plants (Table 6.11).

In the presence of lead in the soil without humic acid (Pb (1000 mg/kg)), the content of this metal increased sharply in the roots and in the aerial organs was slightly higher than the control (13 %). The addition of humic acids in an amount of 2.5 g/m$^2$ (Pb + HA1) greatly increased the lead content in the aerial parts (16.4 times) and roots (1.9 times). Interestingly, the increasing amounts of HA in the soil (Pb + HA2) reduced the concentration of lead in the roots in 1.3 times as compared to variant Pb (1000 mg/kg), and in the shoots lead content in this variant was increased in 20.7 times as compared to variant without HA (Pb (1000 mg/kg)). Possibly that the Pb concentration in roots decreased in the roots is reduced by its translocation to aerial parts. According to other authors, the addition of humic acids to soil increased the Zn, Cu, Pb, and Cd content of tobacco plants from sludge-polluted soil. Similar results were obtained by previous studies [135, 193]. It has been found that Cd are mainly associated with low molecular weight fractions of humic acids (<1000 D), whereas Pb binds to high molecular weight fraction of humic acids (10,000 D). Their complexes with low molecular weight compounds have higher value of stability constant, more easily transported across cellular membranes than the complexes with high molecular substances that can cause a greater bioavailability of cadmium in the presence of humic acids [133].

## 6.3 Conclusion

Phytoremediation — the use of plants for the extraction of organic and inorganic contaminants from soil and waste water — attracted the attention of many researchers in recent years. Compared with the physical and chemical methods of purification of polluted soil from heavy metals, the method of cleanining by plants is less expensive, more efficient, and safe. The screening of grass species widely spread around metallurgic plants of East Kazakhstan in hydroponic and field conditions has shown that almost all studied grass species accumulate trace metals mainly in the roots in great amounts. The following wild grass species such as *D. glomerata*, *B. inermis*, *A. repens*, *A. alba,* and *P. pratense* can be used for phytostabilization. The experiments with addition of humic acids for phytoextraction of metals showed the possibility of applying of different chelators of trace metals, like EDTA, humic acids, etc., to enhance the removal efficiency of metals by plants from soils [194–196].

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