

Original Article

Phytoremediation as a way to clean technogenically polluted areas of Kazakhstan

Fitorremediação como forma de limpar áreas tecnologicamente poluídas do Cazaquistão

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Abstract

One of the most serious problems worldwide is heavy metal (HM) pollution. HMs can have a toxic effect on human health and thus cause serious diseases. To date, several methods have been used to clean environments contaminated by HMs, but most of them are expensive, and it is difficult to achieve the desired result. Phytoremediation is currently an effective and affordable processing solution used to clean and remove HMs from the environment. This review article discusses in detail the technology of phytoremediation and mechanisms of HM absorption. In addition, methods are described using genetic engineering of various plants to enhance the resistance and accumulation of HMs. Thus, phytoremediation technology can become an additional aid to traditional methods of purification.

Keywords: heavy metals, Kazakhstan, phytoremediation, genetic engineering.

Resumo

Um dos problemas mais graves em todo o mundo é a poluição por metais pesados (HMs). Os HMs podem ter um efeito tóxico na saúde humana e, assim, causar doenças graves. Até o momento, vários métodos têm sido utilizados para limpar ambientes contaminados por HMs, mas a maioria deles é cara, sendo difícil alcançar o resultado desejado. A fitorremediação é, atualmente, uma solução de processamento eficaz e acessível usada para limpar e remover HMs do ambiente. Este artigo de revisão discute em detalhes a tecnologia de fitorremediação e os mecanismos de absorção de HMs. Além disso, são descritos métodos que utilizam a engenharia genética de várias plantas para aumentar a resistência e o acúmulo de HMs. Assim, a tecnologia de fitorremediação pode se tornar uma ajuda adicional aos métodos tradicionais de purificação.

Palavras-chave: metais pesados, Cazaquistão, fitorremediação, engenharia genética.

1. Introduction

In recent years, more and more attention has been paid to the global problem of environmental pollution with heavy metals (HMs) and persistent organic pollutants. Due to growing industrialization and urbanization, the content of HMs in the environment has increased significantly over the past few decades, which has caused serious concern worldwide (Suman et al., 2018). HMs are a group of metal elements with a density greater than 5 g/cm³ and an atomic mass exceeding the mass of calcium (MW = 40) (Prieto et al., 2018). Most HMs are extremely toxic. They have a half-life of more than 20 years and are very stable by nature (Asati et al., 2016; Kapoor and Singh, 2021) and so are not decomposed by any biological or physical process but are stored in the soil, which poses a long-term threat to the environment (Suman et al., 2018). In nature, HMs are divided into two categories: essential and nonessential. Metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), nickel (Ni), manganese (Mn), and zinc (Zn) are essential for

the physiological and biochemical processes of living organisms and are essential metals; however, they can become toxic if present in excess. Cadmium (Cd), mercury (Hg), and lead (Pb) are nonessential HMs that are extremely deadly to living organisms (Yan et al., 2020). HMs accumulate in the soil and cause serious health problems in humans, plants, and livestock. The pathways of HMs mainly occur through agricultural products, with accumulation in the human body, thereby posing a serious threat to health (Tchounwou et al., 2012). Therefore, it is necessary to find and take measures to eliminate the ingress of HMs into the soil, atmosphere, and aquatic environment. There have been a number of studies devoted to technologies for removing HMs from soil (Dhaliwal et al., 2020; Hashim et al., 2011; Yao et al., 2012), but only a few of them are effective (Liu et al., 2018). To date, various mechanical or physicochemical methods have been developed, based on burning and washing of the soil, excavation, electric fields, etc. (Liu et al., 2018).

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However, there are limitations to such approaches: high cost, inefficiency at low metal concentrations, and irreversible changes in the physicochemical and biological properties of soils, which lead to deterioration of the soil ecosystem (Sidhu, 2016). Therefore, there is a need to develop effective and environmentally safe technologies for the restoration of soils contaminated with HMs. Phytoremediation technology, which, unlike traditional methods, is inexpensive, environmentally friendly, and generally available, is one of the most promising new methods for restoring the environment. Unlike physical and chemical treatments that irreversibly alter soil properties, phytoremediation generally improves the physical, chemical, and biological qualities of contaminated soils (Liu et al., 2018). In this review, we consider the main mechanisms of the absorption and movement of HMs in plants and characterize HM pollution in large industrial cities of Kazakhstan. Moreover, we provide an overview of current developments in the field of phytoremediation, including the use of genetic engineering to increase the productivity of plants for the accumulation of HMs.

2. HM pollution in large industrial cities of Kazakhstan

In Kazakhstan, as in other parts of the world, there has been an increase in urbanization and industry development processes, which has had negative effects on ecosystems (Kenessary et al., 2019). More than one-quarter of the Republic's territory is unsuitable due to tests at military ranges, industrial enterprises that leave behind toxic industrial emissions after their activities, and agriculture that uses tons of poisonous chemicals to control weeds and insects (Almaganbetov and Grigoruk, 2008; Nurzhanova et al., 2010).

Kazakhstan has a territory of 2,724,900 km², making it the ninth largest country in the world, about the same size as western Europe. Desertification of large areas is accompanied by soil pollution, surface and groundwater flooding, and a decrease in total regional biological capacity (Aiman et al., 2018). Most of Kazakhstan was affected by the activities of the nuclear test site, which was located on the territory of three regions: the East Kazakhstan, Karaganda, and Pavlodar regions (Grosche et al., 2015). Finding effective methods to restore anthropogenically contaminated soils is an important task in Kazakhstan. The relevance of the problem is related to soil pollution with HMs, particularly in the vicinity of metallurgical works (Alimbaev et al., 2020). The pollution of air, soil, plants, and water by HMs near large industrial centers has become one of the most acute environmental problems (Woszczyk et al., 2018).

Most of the waste comes from mining and processing in the Aktobe, Pavlodar, Karaganda, East Kazakhstan, Zhambyl, West Kazakhstan, and Atyrau regions, which are engaged in the extraction of coal and ferrous metals. The land of the East Kazakhstan region is contaminated with a combination of Cu, Zn, Cd, Pb, and arsenic (As). As shown by Zhumalipov (2011), the study of the behavior and distribution of HMs in all environments, especially

on the basis of snow cover monitoring, is of great interest for the territory of northern Kazakhstan. Soil pollution in the Karaganda region is caused by waste from the coal mining and metallurgical industries. Metals are contained in most types of industrial, energy, and motor vehicle emissions to the atmosphere and are indicators of the anthropogenic impact of these emissions on the environment. In this region (Karaganda), there is the greatest accumulation of metals such as Pb, Cu, and As (Zhumalipov, 2011). A large quantity of HMs in the East Kazakhstan region, exceeding the maximum permissible concentration (MPC), has entered the soil as a result of emissions from mining and the metallurgical zinc plant. Citywide chemical contaminants are tin (Sn) and Pb; common are Cu, Zn, Cd; and prevalent locally are Cr, molybdenum (Mo), Ni, Mn, Co, bismuth (Bi), As, and strontium (Sr). In the degraded chernozems of the studied territory, the content of lead is 9.7–2,545.6 mg/kg; zinc, 120–58,000 mg/kg; cadmium, 0.4–56.4 mg/kg; and copper, 0.3–881.8 mg/kg (Sarkulova 2019). The large-scale development and production of hydrocarbon raw materials are mainly conducted in western Kazakhstan, which covers five regions: Aktobe, Atyrau, West Kazakhstan, Mangystau, and Kyzyl-Orda. Currently, more than 50 million tons of oil and 100 billion m³ of gas are extracted from the subsoil annually, and their production volumes are increasing every year. Along with the extraction and transport of oil and gas, there is a tendency of increased environmental pollution due to waste from the oil industry. A study of the soil cover in different fields in Atyrau showed that the impact of oil and petroleum products leads to changes in the physicochemical and chemical properties of the soil. In oil-contaminated soils, nitrate nitrogen accumulation is 1.5–2.0-fold less, while the pH of the soil and the intensity of the enzyme activity in the soil decrease. Thus, oil entering the soil leads to significant, sometimes irreversible changes, while undesirable natural processes such as soil erosion, deflation, and cryogenesis are intensified (Diarov, 2003). Powerful human-made pollutants are mainly released from South Kazakhstan. The cities of Shymkent and Kentau are part of an area of increased HM contamination of the soil. The main concern in the city of Shymkent is the former Shymkent lead plant, which actively produced lead and zinc during the Soviet era (Kazorina, 2019). To date, the property of this plant has been confiscated, the enterprise is idle, and the amount of waste produced is 1,000,800 m³—a dump of lead sludge in the form of a mountain (the popular name is “Lead Mountain”) (Wikipedia, 2021). Despite the decision of the authorities to close the metallurgical plant, there has been a huge impact in terms of environmental pollution, affecting the health of residents, especially children. Therefore, there is a need to develop additional techniques to supplement the traditional methods of cleaning HMs. Thus, the activities of the mining, oil, and metallurgical industries are the main cause of severe land pollution in the central, eastern, and western parts of Kazakhstan (Nugumanova et al., 2017).

3. Toxicity of HMs

HM pollution is one of the most serious environmental problems worldwide, as it has become a source of health risks and causes serious diseases (Briggs, 2003; Landrigan and Fuller, 2015; Pandey and Singh, 2019). The accumulation of high concentrations of HMs can have a detrimental effect on the environment. Moreover, when used by a person for a long time, water and agricultural products containing HMs can cause diseases of the gastrointestinal tract, cardiovascular and nervous systems, as well as chronic kidney failure. It is known that exposure to HMs can lead to fatal outcomes (Hajeb et al., 2014; Järup, 2003; Kumar and Gayathri, 2009; Kyzas et al., 2018; Peng et al., 2017; Shyam et al., 2013).

According to the World Health Organization (WHO), according to the degree of environmental impact, when exceeding the maximum permissible concentrations, among all pollutants, dangerous chemicals are HMs, such as As, Cd, Cr, Cu, Zn, Co, Pb, antimony (Sb), Bi, Hg, Ni, Sn (Tchounwou et al., 2012; WHO 2021). In biological processes, HMs such as Fe, Cu, Zn, Ni, Mo, and boron (B) are important requirements for the functioning of all living organisms (plants, animals, and humans), but are harmful when their concentrations exceed acceptable concentrations (Morkunas et al., 2018).

Most HM pollution is caused by industrial wastewater, gas emissions, extraction of minerals, nonferrous metal smelting, weathering of rocks and minerals, and erosion processes (Ali et al., 2016; Krishnamurti et al., 2005; Masindi and Muedi, 2018; Vallero, 2014). HMs not only pollute the soil but also affect the production and quality of food (Hajeb et al., 2014; Morgan, 1999; Rai et al., 2019). Some HMs are toxic to plants even at very low concentrations, while other HMs can accumulate in plant tissues, without obvious side effects or reduced yields (Shah et al., 2010; Yadav, 2009). Growing plants in regions contaminated with HMs leads to changes in their metabolic, physiological, and biochemical processes, as well as to the accumulation of metals, further reducing plant growth and biomass (Amari et al., 2017; Chibuike and Obiora, 2014). Today, several methods are used to clean soil contaminated by HMs. The main methods are environmental methods and physicochemical methods (Kapahi and Sachdeva, 2019; Mosa et al., 2016; Rulkens et al., 1995; Shah and Daverey, 2020). The use of physical and chemical methods of soil cleaning, namely, leaching, chemical oxidation, and reduction, often leads to the accumulation of secondary pollutants and requires additional manipulations related to the removal of contaminated soil cover and subsequent waste collection.

4. Manipulation to clean up the environment

4.1. Environmental manipulation

The environmental method includes the removal of the top layer of soil in contaminated areas and replacing it with fresh, noncontaminated soil layers. Subsequently, the contaminated soil is disposed of in specially designated locations (Barakat, 2011; Kurniawan et al., 2006).

This method also includes soil washing, which minimizes the level of HM contamination in heavily polluted soil. This process can be applied using cleaning solutions such as surfactants, chelating agents, organic acids, and co-solvents. The flushing fluid containing surfactants is washed in bioreactors with an inert substrate. The use of strong acids can destroy the crystalline structure of the soil, so natural and low-molecular-weight acids such as oxalic, fumaric, acetic, formic, and lactic acids are used in this process. However, this method is costly and requires proper treatment after the use of detergent solutions (Rulkens et al., 1995; Wang et al., 2005). In addition, the type of soil is equally important, since soil washing is more effective in sandy compared to clay types. With a clay-type soil, there is a problem with the separation of solid and liquid for flushing. In addition, physicochemical cleaning methods are based on the use of special reagents that are not always safe for the environment (Sharma et al., 2018).

4.2. Biological manipulation

Biological remediation is a set of water and soil purification methods using microorganisms, algae, and plants (Bertan et al., 2020; Freitas et al., 2018; Kapahi and Sachdeva, 2019). Recently, bioremediation with the help of microorganisms and plants, has become widespread for the purification of HMs in the environment. The main advantage of bioremediation technology is associated with the ability of organisms to metabolize large amounts of organic matter (Chibuike and Obiora, 2014). The greatest efficiency of bioremediation of microorganisms is observed when using several strains compared to one. Kang et al. (2016) investigated the combined effect of bacterial mixtures (*Viridibacillus arenosi* B-21, *Sporosarcina soli* B-22, *Enterobacter cloacae* KJ-46, and *E. cloacae* KJ-47) on the bioremediation of Cd, Cu, and Pb in contaminated soils. They noted that bacterial mixtures showed high resistance and efficiency in the purification of HMs in comparison with cultures with a single strain (Kang et al. 2016).

Algae have a high rate of absorption of HMs. *Rhodophyta* (red algae) are effective biosorbents of HMs because their structures consist of large amounts of amorphous matrix polysaccharides, which can bind metal. It was shown that the algae *Chlorella vulgaris*, *Cladophora crispata*, *Anabaena* sp., and *Synechococcus* sp. could absorb Cr through their cell walls. The blue-green alga *Phormidium laminosum* is capable of accumulating Cu, Fe, Ni, and Zn. Green algae are more efficient at absorbing the metals Cr, aluminum (Al), and Fe in contrast to brown and red algae (Kang et al., 2016). The main disadvantages of microorganisms and algae are their applicability being mainly for the purification of wastewater, oil, and oil products. One of the most promising areas of bioremediation techniques is phytoremediation technology, which uses living plants. Phytoremediation plants, unlike microorganisms and algae, can absorb toxic HMs (Lee, 2013).

4.2.1. Phytoremediation

Phytoremediation implies the use of "green and living" plants to remove contaminants from the environment and can be used in addition to the known methods of cleaning the soil of excess HMs (Andereazza et al., 2015; Garbisu et al., 2002).

Phytoremediation technology is considered economically profitable, efficient, and ecological because it is based on the use of metalaccumulating plants to remove toxic metals, including radionuclides, as well as for petroleum hydrocarbons, pesticides (Nurzhanova et al., 2013; Silva et al., 2015), explosive or toxic gases, organic pollutants, and several industrial byproducts (Wan et al., 2016; Zodrow 1999). Therefore, it is necessary to use hyperaccumulating plants that can absorb high levels of pollution (Macnair 2003; Verbruggen et al., 2009). The main advantage of this technology is that the plant mass can be easily collected and burned, and the resulting ashes can be buried or used as secondary raw materials (Shrestha et al., 2019).

Thus, when plants are used to extract HMs using phytoremediation technology, the following mechanisms are used: phytoextraction, phytostabilization, rhizofiltration, and phytovolatilization (Freitas et al., 2018; Naz et al., 2022; Rezania et al., 2016; Shackira and Puthur, 2019; Suman et al., 2018; Verma et al., 2006). Figure 1 shows the various mechanisms involved in the phytoremediation of HMs.

4.2.2. Phytoextraction

This refers to the use of plants capable of extracting HMs in contaminated areas using the root system and concentrating them in the aboveground biomass. This phytoremediation method is especially useful for removing metals from soil. It also provides the ability to extract metals by burning plants, a process called phytomining (Suman et al., 2018).

4.2.3. Phytostabilization

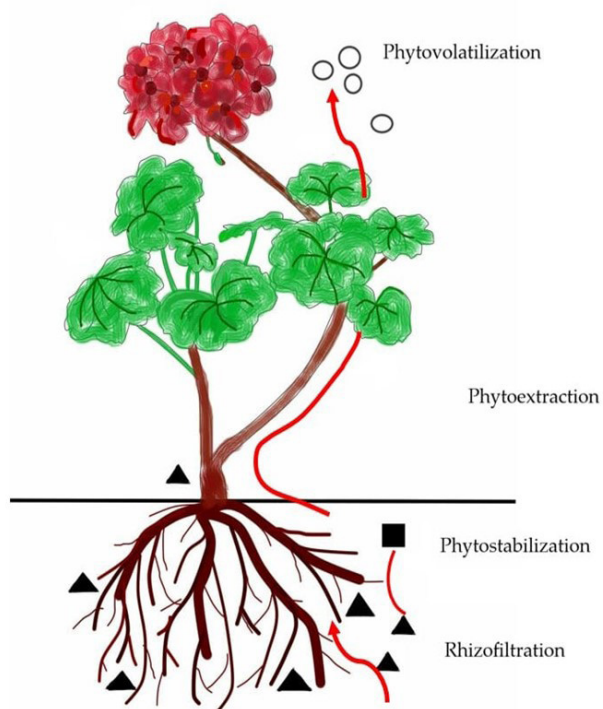
This refers to using plants to immobilize pollutants in the soil, which can reduce the concentrations of pollutants in the environment, thereby preventing the further migration of pollutants in the soil or groundwater (Shackira and Puthur, 2019). This method also includes the uptake of contaminants by the roots and adsorption to the root surface. Moreover, it involves the production of biochemicals by the plant, which are then released into the soil or groundwater around the roots. Biochemicals have the ability to isolate, precipitate, or otherwise immobilize nearby contaminants (Mang and Ntushelo, 2019).

4.2.4. Rhizofiltration

This refers to the use of the root system of plants to absorb sediment and concentrate pollutants from surface water and wastewater. For rhizofiltration, the root system of plants must be sufficiently developed, and plants must have fast-growing roots capable of removing toxic metals from solution. This technology is especially effective for treating surface and groundwater containing relatively low concentrations of toxic metals (Verma et al., 2006).

4.2.5. Phytovolatilization

This refers to the use of plants to extract pollutants from water, which are then dispersed into the atmosphere by evaporation from the leaf surfaces. This process is capable of absorbing pollutants into plants and releasing pollutants



▲ – Rhizofiltration, ■ – Phytostabilization, ○ – Phytovolatilization.

Figure 1. Various mechanisms involved in phytoremediation of HMs.

into the atmosphere. While using phytovolatilization, unlike other phytoremediation technologies, it is impossible to control the migration of contaminants that enter the environment during phytoevaporation. Therefore, phytovolatilization is the most controversial phytoremediation technology (Rezania et al., 2016).

The limiting factor of phytoremediation is the low rate of metal purification and insufficient metabolism of autotrophic plants, which lack the enzyme exchange mechanism that is necessary to achieve complete purification, thereby limiting the progress of phytoremediation (Chaudhry et al., 2002; Favas et al., 2014). This leads to the accumulation of toxic metabolites in plant tissues, which can enter the environment and the food chain. Genetic engineering of plants has the potential to overcome these problems (Fasani et al., 2018; Kärenlampi et al., 2000; Kumar et al., 2017).

4.3. Genetic manipulation

The use of genetic engineering for phytoremediation is considered a promising area in the application of model plants expressing genes that enhance resistance to HMs and their accumulation (Yan et al., 2020; Zhang et al., 2006). In a study by Gisbert et al. (2003), the wheat gene *TaPCS1*, which codes for phytochelatin synthase, was inserted in the plant *Nicotiana glauca* using genetic engineering. Phytochelatin synthase is an enzyme that binds metal ions (Gisbert et al., 2003). The use of this gene gave plants increased resistance to Pb and Cd. The authors analyzed various lead concentrations: 0, 0.4, 0.8, and 1.2 mM. As a result, root growth significantly improved and leaves were much larger and greener in transformed plants at the 0.8 mM lead concentration. Higher lead tolerance was achieved in a range of lead concentrations up to 1 mM. All plant lines containing the gene *TaPCS1* had better developed leaf and root systems compared to wild-type plants. Subsequently, the transgenic plants were grown in soil containing 1,572 mg/kg of lead. As a result, they accumulated twice as much Pb and Cd as nonmodified wild plants (Gisbert et al., 2003).

In another study, Bhuiyan et al. (2011) obtained transgenic plants of *B. juncea* with the *AtATM3* gene inserted that belongs to a family of ATP-binding cassette transporters (ABC) localized in the mitochondrial membrane of *Arabidopsis thaliana*. Overexpression of this gene not only improved the tolerance of the transgenic plants to Cd and Pb, but also increased metal transport to shoots by 1.5–2.5-fold compared to the wild type, thereby improving the potential for phytoremediation (Bhuiyan et al., 2011).

In addition, there were studies on enhancing polyamine synthesis. Polyamines play an important role in reducing cellular oxidative stress. For that reason, a transgenic pear, *Pyrus communis* L. (Ballad), with the overexpression of the apple spermidine synthase gene *MdSPDS1* was produced. After exposure to HMs (cadmium and zinc), the transgenic plants responded with enhanced synthesis and accumulation of polyamines compared to wild-type plants. Favorable outcomes were associated with the antioxidant activity of spermidine and its ability to bind free metal ions (Wen et al., 2010).

Under stress conditions, plants produce and accumulate various products of metabolism, including amino acids, such as proline and phenolic compounds (Díaz et al., 2001; Grace and Logan, 2000; Sakihama and Yamasaki, 2002). Many researchers have studied the accumulation of free proline as a response to oxidative and biotic stresses under the conditions of high salinity, drought, intensive light, ultraviolet radiation, and HMs (Choudhary et al., 2005; Fabro et al., 2004; Haudecoeur et al., 2009; Yang et al., 2009). Not only does proline participate in protein synthesis, but it also positively correlates with stress resistance in plants. Proline supports osmotic pressure and reduces the loss of electrolytes through membrane stabilization, protection from oxidative stress, and reduction of reactive oxygen species (ROS) (Hayat et al., 2012; Xu et al., 2009).

A strong correlation between the cellular proline content and HM concentration was demonstrated in hyperaccumulating artichoke plants (*Cynara scolymus* L.) (Tripathi and Gaur, 2004). Gohari and co-authors showed that proline concentration in rapeseed roots (*Brassica napus* L.) increased when the plants were exposed to high lead concentrations (100–400 µM) (Gohari et al., 2012). However, proline accumulation in aboveground plant organs was not as noticeable as that in roots. The accumulation of proline in roots rather than in shoots is common in studies on different plants exposed to Pb and Cd, including *Brassica juncea* L. (Favas et al., 2014).

In another study on sal tree shoots (*Shorea robusta*), it was established that cadmium, lead, and arsenic were strong proline inducers (Pant et al., 2011). Kumar and Gayathri (2009) showed that Cu is a stronger proline inducer than zinc in wheat germ (Kumar and Gayathri, 2009). Rastgoo and Alemzadeh (2011) studied the influence of equal amounts of HMs, such as Cd, Co, Pb, and silver (Ag), on gouan plants (*Aeluropus littoralis*) (Rastgoo and Alemzadeh, 2011). The study showed that the highest proline accumulation was achieved when the plants were treated with Cd. Zengin and Kirbag (2007) showed the strong influence of Hg, Cd, Cu, Pb on the amount of proline in sunflower shoots (*Helianthus annuus* L.) (Zengin and Kirbag, 2007). All of these studies show that proline accumulation depends on the concentration of the metal and its specific features.

4.3.1. Metallothioneins

Metallothioneins (MT) are low molecular weight (5–10 kDa) metal-binding proteins with high cysteine content, widely spread in living organisms (Bundy et al., 2014; Leszczyszyn et al., 2013). Plants produce metal-chelating proteins, such as MT, to overcome the toxic effects of HMs. However, only a limited number of transgenic plant species have been studied for high tolerance to HMs using various cloned MT genes (Eapen and D'Souza, 2005).

A MT gene *IIIMt2a* was successfully identified in an iris plant (*Iris lactea*) (93). Further insertion of this gene into the genome of *Arabidopsis* has led to a higher tolerance in transgenic plants exposed to Cd and Cu (Gu et al., 2014, 2015). Tolerance to HMs is apparently connected to the decreased production of ROS, which indicates a highly efficient antioxidant defence system.

Such mechanisms have led to an increase of HM tolerance in transgenic tobacco with the insertion of *SbMt2* gene from *Salicornia* spp. (for example *Salicornia brachiata*) (Chaturvedi et al., 2014). The transgenic plants were able to maintain cellular homeostasis through ROS detoxification. Furthermore, it was established that the overexpression of *SbMt2* enhances zinc translocation in shoots, which in turn may be a good sign of the increase in stress resistance and the effectiveness of phytoremediation using genetic engineering (Chaturvedi et al., 2014). Table 1 presents different genes used in transgenic plants.

5. Increasing bioavailability of HMs

HMs in soil are not always available for bioaccumulation. Only a small part of the total content of HMs in the soil

is in a soluble and light form for absorption by plants (Petruzzelli et al., 2020). The bioavailability of HMs is influenced by a number of factors, including soil properties, the environment, and plant characteristics (Kim et al., 2015). A peculiarity of plants is that they can use different strategies to increase the bioavailability of HMs. For example, carbon-emitting compounds from the root system of plants have the ability to acidify the rhizosphere, reducing the pH of the soil. The pH change (natural or anthropogenic) appears to be the most important factor influencing the mobility of metals. The pH reduction contributes to the mobility of HMs, in particular by dissolving metal salts or breaking down the retention phase. At a higher pH, retention occurs and the solubility of HMs decreases (Lone et al., 2008). In addition, a number of sources have reported the availability of microorganisms in the rhizosphere, which significantly

Table 1. Different genes used in transgenic plants.

Gene	Origin	Target plant species	Effect*	References
OASTL		Tobacco	Resistance of cadmium (Cd) up to 300 mM, Selenium (Se)- 250 mM, nickel (Ni) up to 500 mM higher biomass is produced	(Kawashima et al., 2004)
TaPCS1	Wheat	Tobacco	High tolerance to lead (Pb) (1 mM) and cadmium (Cd) (50 mM)	(Gisbert et al., 2003)
TaPCS1	<i>Triticum aestivum</i>	<i>N. glauca</i>	Good root formation on medium containing 800 µM of lead (Pb) and 50 µM of cadmium (Cd)	(Martinez et al., 2006)
YCF1	Yeast	<i>Arabidopsis</i> , poplar	Increases resistance to lead (Pb) and cadmium (Cd) and increases the accumulative capacity in lead (Pb) and cadmium (Cd) vacuoles in transgenic plants	(Song et al., 2003)
ZntA	<i>E. coli</i>	<i>Arabidopsis</i>	Resistance of the transgenic plant to lead (Pb) 0.7 mM and cadmium (Cd) 70 µM	(Lee et al., 2003)
ABCC1	<i>A. thaliana</i>	<i>A. thaliana</i>	Overexpression of AtABCC1 increases the accumulation of cadmium (Cd)	(Park et al., 2012)
MTP3	<i>A. thaliana</i>	<i>A. thaliana</i>	Overexpression of the MTP3 gene increases the accumulation of zinc (Zn) in roots and leaves	(Arrivault et al., 2006)
NRAMP1	<i>O. sativa</i>	<i>A. thaliana</i>	Expression of the OsNRAMP1 gene in <i>Arabidopsis</i> increases tolerance and accumulation of arsenic (As) and cadmium (Cd), increases the accumulation of Iron (Fe) and Manganese (Mn) in shoots, increases the genes expression of the AtABCC1, AtABCC2, and AtHMA4	(Tiwari et al., 2014)
AtPCS1	<i>A. thaliana</i>	<i>B. juncea</i>	Increase in the root of the medium-containing cadmium (Cd) in the concentration of 100 µM and arsenic (As) 500 µM	(Gasic and Korban, 2007)
HvNAS1	<i>Hordeum vulgare</i>	<i>Arabidopsis</i>	Gives increased resistance to high concentrations of metals, in particular to nickel (Ni)	(Kim et al., 2005)
NAAT	Barley	Rice	Transgenic plants grew better on iron-deficient soils	(Takahashi et al., 2001)

increases the availability of HMs and absorption by plants (Wu et al., 2006). These microorganisms include several strains of *Bacillus* and *Pseudomonas*, which increased Cd accumulation in *Brassica juncea* seedlings (Salt et al., 1995). Another widely considered strategy for increasing the bioavailability of HMs is chelating agents. Chelating agents increase the diffusion of metals in a soil solution and keep them in plant-available forms by generating larger, less reactive ions, increasing the concentration of these larger chelated ions in solution, and reducing the ability of free ions to react with the soil (Olaniran et al., 2013). In practice, various chelating agents are used, including synthetic and organic chelating agents. Synthetic chelating agents such as ethylenediaminetetraacetic acid (EDTA), ethylene glycol tetraacetic acid (EGTA), and diethylene-triaminepentaacetic acid (DTPA) can effectively increase the bioavailability of HMs and promote plant uptake (Gupta et al., 2008). However, the poor biodegradability of synthetic chelating agents leads to their persistence in the soil, which raises serious concerns about metal leaching and harmful effects on the environment (Olaniran et al., 2013). Alternatively, it has been shown that organic chelating agents such as citric acid, malic acid, acetic acid, and oxalic acid effectively form HM complexes and increase the bioavailability of HMs (Sarwar et al., 2017). These organic chelators are of natural origin and readily biodegrade in the soil, which means they may pose less of a risk to the environment than synthetic chelators (Dolev et al., 2020); therefore, it is more promising to use organic chelating agents for chelated phytoextraction.

6. Conclusions

In this review, we discussed in detail the methods and technologies of cleaning soil contaminated with HMs. It is widely known that Kazakhstan is polluted with human-made waste, especially HMs, which poses a great danger to the ecological health of the region. In this regard, the search for highly effective technologies for cleaning contaminated areas is relevant. It is known that phytoremediation is one of the key elements of cleaning technogenically contaminated areas and is used in combination with physicochemical methods. Phytoremediation technology appears to be a less destructive, more economical, and more environmentally friendly cleaning technology. The use of HM hyperaccumulators is the simplest approach to phytoremediation, and hundreds of hyperaccumulator plants have been identified to date. However, phytoremediation using these natural hyperaccumulators still has some limitations, as it is time-consuming to clean soil contaminated with HMs, especially in areas with moderate to severe contamination. This may partly be due to the slow growth and low biomass production of these hyperaccumulators. Thus, increasing plant productivity is an important step in developing highly efficient phytoremediation techniques. With the development of genetics, the ability of plants that accumulate and tolerate HMs and are used in phytoremediation can be significantly improved.

In addition, chelating agents and microorganisms can be used either to increase the bioavailability of HMs, thereby contributing to the accumulation of HMs in plants, or to improve soil health and further stimulate plant growth and adaptability. In practice, a single approach is insufficient for effective cleaning of soil contaminated with HMs. A combination of different approaches, including genetic engineering, microbial, and chelation approaches, is important for effective and comprehensive phytoremediation in the future.

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