A comparison of technologies for remediation of heavy metal contaminated soils

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ABSTRACT

Soil contamination with persistent and potentially (eco)toxic heavy metal(loid)s is ubiquitous around the globe. Concentration of these heavy metal(loid)s in soil has increased drastically over the last three decades, thus posing risk to the environment and human health. Some technologies have long been in use to remediate the hazardous heavy metal(loid)s. Conventional remediation methods for heavy metal(loid)s are generally based on physical, chemical and biological approaches, which may be used in combination with one another to clean-up heavy metal(loid) contaminated soils to an acceptable and safe level. This review summarizes the soil contamination by heavy metal(loid)s at a global scale, accumulation of heavy metal(loid)s in vegetables to toxic levels and their regulatory guidelines in soil. In this review, we also elucidate and compare the pool of available technologies that are currently being applied for remediation of heavy metal(loid) contaminated soils, as well as the economic aspect of soil remediation for different techniques. This review article includes an assessment of the contemporary status of technology deployment and recommendations for future remediation research. Finally, the molecular and genetic basis of heavy metal(loid) (hyper)accumulation and tolerance in microbes and plants is also discussed. It is proposed that for effective and economic remediation of soil, a better understanding of remediation procedures and the various options available at the different stages of remediation is highly necessary.

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1. Introduction

Heavy metal(loid)s refer to a group of toxic elements which are both biologically and industrially important. The widespread contamination of soil with heavy metal(loid)s represents currently one of the most severe environmental problems that can seriously affect environmental quality and human health. Heavy metal(loid)s are released into the soils by both natural and anthropogenic sources. Although environmental contamination of heavy metal(loid)s started in ancient times, the problem provoked after the industrial revolution owing to dramatic increase in the use of heavy metal(loid)s in various modern technologies. The current worldwide mine production of heavy metal(loid)s is considerably huge (Shahid et al., 2015a). Heavy metal(loid)s commonly present in soils include nickel (Ni), lead (Pb), cadmium (Cd), arsenic (As), chromium (Cr), copper (Cu), cobalt (Co), zinc (Zn), manganese (Mn), aluminum (Al) and mercury (Hg). Among these heavy metal(loid)s, As, Pb, Cd and Hg are included in the top 20 Hazardous Substances of the Agency for Toxic Substances and Disease Registry (ATSDR, 2012) and the United States Environmental Protection Agency (US EPA).

Excessive build-up of heavy metal(loid)s in agricultural soils results in increased heavy metal(loid) uptake by food crops and vegetables, which in turn may induce serious health risks to human beings (Xiong et al., 2016a; Pierart et al., 2015). Heavy metal(loid)s are reported to cause several disorders in humans including cardiovascular diseases, cancer, cognitive impairment, chronic anemia, damage of kidneys, nervous system, brain, skin, and bones (Jarup, 2003). Owing to potential toxic effects associated with heavy metal(loid) exposure, there is a global concern to comply that the heavy metal(loid) content of agricultural soil and the crops cultivated on these soils do not exceed the allowable regulatory limits. Moreover, people are becoming more aware of the inferences of heavy metal(loid) contaminated soils on human and environmental health, resulting in the improvement and development of technologies for clean-up of heavy metal(loid) contaminated sites.

Contrasting to organic contaminants, heavy metal(loid)s are somewhat unique by the fact that they are highly resistant to either biologically or chemically induced degradation. Therefore, total heavy metal(loid) contents of soil persist for a long time after being introduced into the soil. For example, Pb has a soil persistence period of 150–5000 years, and has been reported to persist in soil for >150 years after sludge application (Nandakumar et al., 1995). Similarly, Cd has a

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biological half-life of >18 years (Förstner, 1995). Soil being most essential component of ecological system is highly contaminated worldwide by heavy metal (loid)s. Excessive accumulation of heavy metal (loid)s in the soils may cause deterioration of the soil ecosystem and create other environmental issues. Soil physico-chemical properties such as pH, electrical conductivity, cation exchange capacity, soil mineralogy, microbial and biological conditions and presence of soil inorganic and organic ligands greatly influence the bioavailable and mobile heavy metal(loid)s in soil (Shahid et al., 2012a, 2012b, 2012c, 2012d; Minnikova et al., 2016). Numerous studies have reported heavy metal (loid)s build up in soil and associated risks to soil fertility/quality and biochemical activities (enzymes and microbes). Heavy metal(loid)s at the higher concentration are known to influence the microbial population of soil and their associated activities which may directly affect soil fertility (Minnikova et al., 2016). Pattnaik and Equeenuddin (2016) reported that soil enzyme activities are significantly negatively correlated with metal contents of Ni, Cu, Cr, Co, Mn and Zn. The decrease in the activities of enzymes follows the following order: urease > acid phosphatase ≥ dehydrogenase > β-glucosidase ≥ alkaline phosphatase. Minnikova et al. (2016) showed that the integrated biological index correlates with the total technogenic pollution index and soil contamination with heavy metals.

Therefore, it is imperative to deploy innovative and site-specific remediation technologies which could feasibly and efficiently remediate heavy metal(loid) contaminated soils. Numerous soil remediation techniques have been developed during the last two decades (Verbruggen et al., 2009; Murtaza et al., 2014; Sabir et al., 2015). These techniques aim to reduce the total and/or bioavailable fractions of heavy metal(loid)s in soils and their subsequent accumulation in the food-chain (Bhargava et al., 2012). Conventional techniques to remediate heavy metal(loid)s from contaminated soils are based on physical, chemical and biological methods (Fig. 1), which may be used in combination with one another to remediate contaminated sites. Despite high efficiency, majority of these techniques are costly, environmentally-destructive and time consuming. Financial and technical implications and complexities have made soil clean-up a challenging task. Practical implementation of these conventional soil remediation methods faces several shortcomings and may implicate some level of hazard. This review discusses and compares different technologies nowadays available for heavy metal(loid) remediation in term of mechanisms involved, advantages, limitations, applicability and cost effectiveness. This review can be highly useful for the owners of industrial sites polluted by long term historical pollution, farmers with soils currently polluted with metal(loid)s and interested to improve the quality of their products or urban gardeners who wish to improve the quality of cultivated lands.

2. Heavy metal(loid)s soil pollution: a global dilemma

Soils are a main and terminal sink for heavy metal(loid)s released into the environment by anthropogenic activities (Table 1). Various countries confronted with heavy metal(loid) soil contamination differ substantially in awareness of the problem and in strategies and technologies to tackle it (Baldantoni et al., 2016; Myoung Soo Ko et al., 2015). It is reported that >10 million contaminated sites exist worldwide, with >50% of the sites contaminated with heavy metal(loid)s (He et al., 2015). Majority of these heavy metal(loid) contaminated sites exist in developed countries i.e., the United States of America (USA), Australia, Germany, Sweden and China owing to their increased use in industrial processes (Foucault et al., 2013; Goix et al., 2014). In the USA, around 600,000 ha area (especially brown field sites) has been contaminated
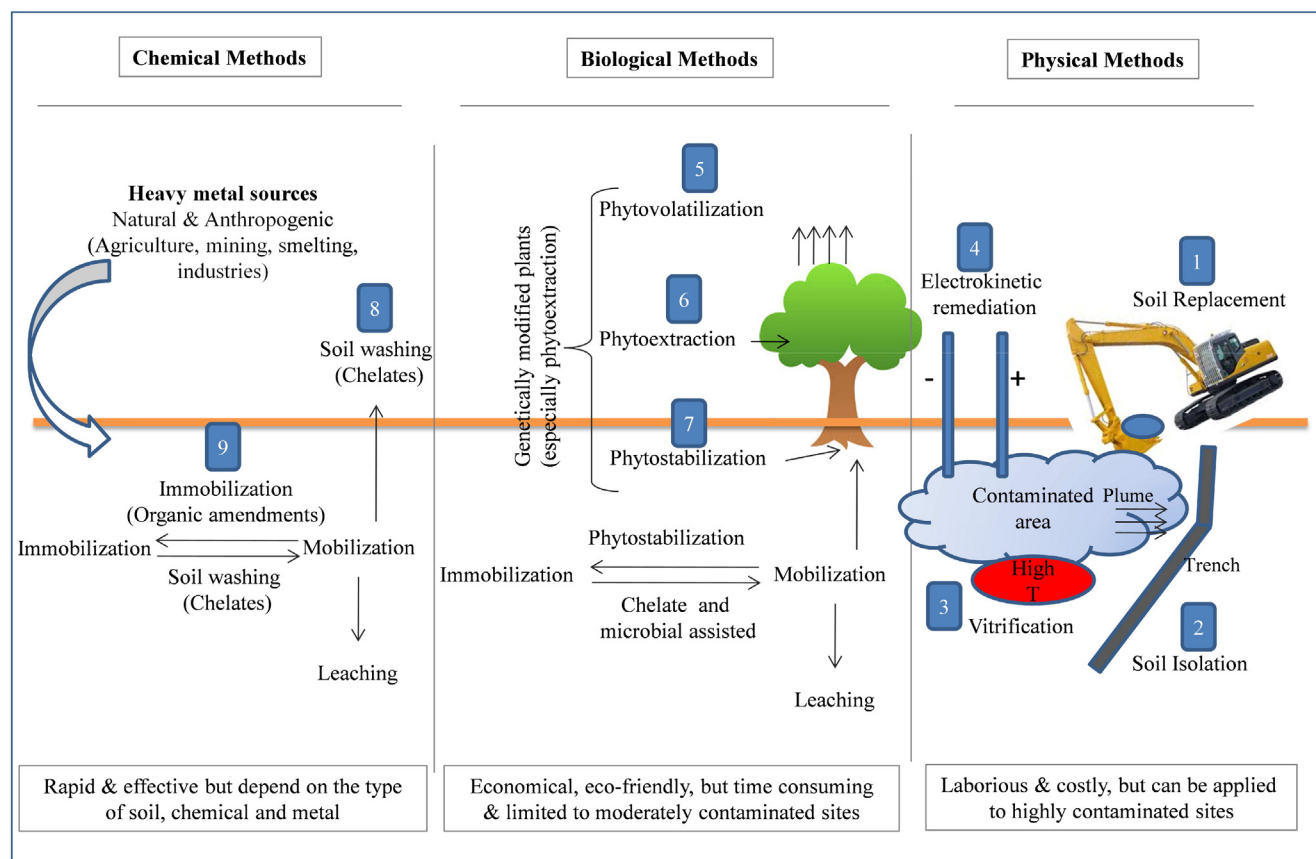

Fig. 1. Comparison of different soil clean-up methods. Soil remediation methods can be broadly divided into three categories: physical, chemical and biological. Physical remediation methods include (1) soil replacement, (2) soil isolation, (3) vitrification, and (4) electrokinetic; biological methods generally include (5) phytostabilization, (6) phytoevaporation and (7) phytoextraction, and chemical methods contain (8) immobilization and (9) soil washing. However, biological and chemical methods can be applied jointly depending on the type of metal, soil, plant and chemical reagent. Moreover, the effectiveness of different phytoremediation techniques can be enhanced by microbial-, chelate- and genetic-assisted remediation.

Table 1
Examples of some heavy metal(loid)s polluted soils worldwide exceeding permissible limits.

Heavy metal	Concentration in soil (mg/kg)	Maximum allowable limit[a]	Fold-higher than allowable limit	Study area	References
Cd	42	3	14.0	Southern Italy	Baldantoni et al., 2016
	19		6.4	India	Tiwari et al., 2011
	16		5.4	Switzerland	Quezada-Hinojosa et al., 2015
	14		4.7	Mexico	Torres et al., 2012
	14		4.6	China	Shi et al., 2015
Pb	4500	100	45.0	China	Luo et al., 2011
	1988		19.9	China	Niu et al., 2015
	711		7.1	UK	Nabulo et al., 2011
	452		4.5	Uganda	Nabulo et al., 2012
	302		3.0	Brazil	Carvalho et al., 2014
As	7490	20	374.5	Spain	Beesley et al., 2014
	4357		217.9	Italy	Marabottini et al., 2013
	354		17.7	China	Wei et al., 2015
	131		6.6	Korea	Myoung Soo Ko et al., 2015
	64		3.2	Bolivia	Acosta et al., 2015
Zn	3833	300	12.8	China	Niu et al., 2015
	370		1.2	Nigeria	Obiora et al., 2016
	1168		3.9	Germany	Shaheen et al., 2014
	905		3.0	Portugal	Anjos et al., 2012
	393		1.3	–	Kwon et al., 2015
Ni	2603	50	52.1	Mexico	Torres et al., 2012
	373		7.5	Spain	Arenas-Lago et al., 2016
	201		4.0	Zimbabwe	Mapanda et al., 2007
	200		4.0	Turkey	Avci and Deveci, 2013
	153		3.1	China	Wang et al., 2015
Cu	35,582	100	355.8	Mexico	Torres et al., 2012
	19,581		195.8	Australia	Sacristán et al., 2016
	448		4.5	China	Wang et al., 2015
	235		2.4	Portugal	Anjos et al., 2011
Cr	4309	100	43.1	Spain	Arenas-Lago et al., 2016
	590		5.9	China	Xu et al., 2014
	418		4.2	Greece	Panagopoulos et al., 2015
	224		2.2	Germany	Shaheen et al., 2014

[a] World Health Organization (WHO), Food and Agricultural Organization (FAO).

with heavy metal(loid)s. The US EPA has designated >50,000 priority heavy metal(loid) polluted sites awaiting urgent remediation in the USA alone (Ensley, 2000). Gorospe (2012) analysed 16 different heavy metal(loid)s in soil samples collected from 91 vegetable gardens in San Francisco, USA. They observed that majority (>75%) of the gardens surpassed the California Human Health Screening Level for Cd (84%), As (74%) and Pb (62%). Similarly, several heavy metal(loid) contaminated agricultural sites situated near mining areas have been reported in different countries of Europe (Foucault et al., 2013; Goix et al., 2014). According to the European Environmental Agency (EEA, 2007), there exist >3 million potentially polluting activity sites in Europe and about 250,000 heavy metal(loid)s polluted sites in the EEA member countries. More than 80,000 sites have been cleaned-up in Europe during the last 30 years (EEA, 2007). Approximately 0.5 million sites in Europe are highly contaminated and need remediation on priority basis. It is estimated that the total number of polluted sites needing remediation may increase by >50% by 2025 (EEA, 2007). Tóth et al. (2016) conducted the first harmonized topsoil sampling from approximately 22,000 locations and reported that an estimated 6.24% or 137,000 km² needs local assessment and remediation action.

In addition to these already recorded polluted sites, around three million sites in Europe are possibly polluted, based on the information about potentially polluting activities on site (Jensen et al., 2009). Poland and Greece each reported >10,000 contaminated sites, while Portugal and Ireland each reported >10,000 contaminated land areas (Perez, 2012). In France, 11,400 agricultural soil samples were analysed for Pb contamination and it was observed that about 1% of soil samples exceeded the French limit values for Pb (100 mg kg⁻¹ dry weight soil) (Mench and Baize, 2004). According to The French Ministry of Ecology, Sustainable Development and Energy, there are roughly 5759 polluted or potentially polluted sites in France, heavy metal(loid)s being the most prevalent pollutants (60.13%) in these sites (Agnello et al., 2015). BASOL (Base de données sur les sites et sols pollués), an inventory of polluted sites calling for action by the authorities, reported about 4300 polluted sites in France. The Campine area (700 km²) in the Netherlands and Belgium is contaminated by atmospheric deposition of Pb, Zn and Cd (Meers et al., 2010). In Germany, soil pollution by high levels of heavy metal(loid) contamination has taken out of food production about 10,000 ha of agricultural land (Lewandowski et al., 2006). A survey backed by the European Commission has estimated that society's loss due to soil contamination is about 17.3 billion euros per year.

The situation of soil contamination by heavy metal(loid)s is more worse in China. Approximately 4 mha of arable land (2.9% of China's arable lands) has been polluted moderately or severely by pollutants as reported jointly by the Ministry of Land Resources of China and the Ministry of Environmental Protection of China (Bulletin on National Survey of Soil Contamination). It is reported that >20,000,000 acres of farmland (25% of total arable farmland area) in China has been contaminated by heavy metal(loid)s such as Pb, Cd, Cr, Sn and Zn. Heavy metal(loid)s pollution causes 10,000,000 tons loss of crop output every year in China (Hongbo et al., 2011). According to the Ministry of Environmental Protection and Ministry of Land and Resources (China, 2014), the overstandard rates of Pb, Cd, Cu and Zn in China were 0.9%, 2.1%, 7.0%, and 1.5%, respectively. In China, heavy metal(loid)s in 16.1% farmland soils have exceeded the environmental quality standard for soil. For agricultural soils, >19.4% sites exceeded the environmental quality standard. High levels of heavy metal(loid)s in soil are also reported in less developed countries such as Pakistan, India, Bangladesh etc. In less developed countries, the major source of heavy metal(loid)s in soil is the use of untreated waste water (city as well as industrial) for crop irrigation. Several studies in Pakistan and India reported heavy metal(loid)s build up in soil as a result of crop irrigation by untreated waste water (Khan et al., 2015). In Pakistan, approximately 30% of wastewater is directly used for crop irrigation of 32,500 ha in Pakistan, while 64% of wastewater is directly discharged into water bodies without any primary treatment (Ensink et al., 2004).

About, 26% of the vegetables cultivated in Pakistan are irrigated with untreated wastewater (Ensink et al., 2004).

3. Sources of heavy metal(loid)s in soil

Heavy metal(loid)s occur naturally in Earth crust and are released into soil by various human activities, which have resulted in high heavy metal(loid) contents in soil to toxic levels. The most common heavy metal(loid)s present in polluted soils are in order of Pb, Cr, As, Zn, Cd, Cu and Hg. Natural processes also contributes towards heavy metal(loid) contamination of soils.

3.1. Natural sources

Heavy metal(loid)s are found naturally in soils resulting from weathering of underlying bedrock. These are generally mined from ores following mineral processing processes (UNEP/GPA, 2004; Shakoor et al., 2015). In rocks, heavy metal(loid)s are found in different chemical forms as ores from which these metals are recovered as minerals (Fuge et al., 1991). In ore forms, heavy metal(loid)s generally occur as sulfides of Pb, Co, Fe, As, Pb-Zn, Ag and Ni, and oxides of Se, Al, Mn and Sb. These metals are therefore generally recovered/mined from soils as oxide and sulfide ores. In soils, generally the sulfides of metals (such as As, Hg, Pb, Cd) occur naturally together with sulfides of Cu, (chalcopyrite, $CuFeS_2$) and Fe (pyrite, FeS_2). Therefore, mostly these heavy metal(loid)s are obtained as part of exhaust fumes in pyro metallurgical processes or as by-products of several hydro metallurgical processes after mining. For example, Cd is mainly obtained as by-product of the Zn refining process, owing to co-occurrence of Cd with the Zn ore sphalerite. Every year, significant amount of heavy metal(loid)s is thus redistributed from the contaminated aquifer of Earth's crust to different compartments of environment i.e., water, air and soil. Therefore, soils originating from parent material having an elevated metal concentration in soil bedrocks show high metal concentration naturally (Pourrut et al., 2011a, 2011b).

For example, high Pb concentration (up to 140 mg kg^{-1}) was detected in remote site soils of Swiss National Park, situated far away from major industrial areas and traffic routes above sea level at an altitude of 2400 m (Bernd et al., 2001). Similarly, in Mendip region soils of Great Britain, high levels of Pb, Zn and Cd were found because of high concentrations of these metals in bedrocks (Fuge et al., 1991). High As levels are reported in soils and waters of Bangladesh, Pakistan and India owing to presence of As-containing rocks in parent material of these areas (Shakoor et al., 2015, 2016).

3.2. Anthropogenic sources

Anthropogenic sources of soil contamination by heavy metal(loid)s include: refining and mining of ores, pesticides, batteries, paper industries, tanneries, fertilizer industries, solid wastes disposal including sewage sludge, wastewater irrigation and vehicular exhaust (Niazi et al., 2011, 2015; Shahid et al., 2015a). Generally, the metal(loid)s are released into the environment during processing and mining activities. Heavy metal(loid)s are released both in compound (inorganic and organic) and elemental forms. In some cases, metals emitted from these processes continue to build-up in the soil and other environmental compartments even long after these activities have ended. Peplow (1999) stated that mining of rigid rocks generally takes 5–15 years for complete depletion of minerals, but soil contamination by metals persists for several years after termination of the mining processes. Similarly, Metaleurop located in north of France was one of the largest European smelter plants for >100 years (1884–2004). Dust particles emitted from this smelter have contaminated the nearby agricultural field with high contents of Pb, Zn, Cd and As surrounding an area of 40 km^2 (Shahid et al., 2013a). Ross (1994) divided the anthropogenic sources of metal(loid)s contamination into five groups:

1. Agriculture (Zn, As, Pb, Cd, Cu, selenium (Se) and uranium (U))
2. Metalliferous mining and smelting (Cd, Pb, As and Hg)
3. Industry (Cd, Hg, As, Cr, Cu, Co, Ni and Zn)
4. Waste disposal (As, Pb, Cu, Cd, Cr, Zn and Hg)
5. Atmospheric deposition (As, Pb, Cr, Hg, Cu, Cd and U)

4. Soil heavy metal(loid)s pollution, food-chain contamination and human health

Consumption of food contaminated with heavy metal(loid)s is considered to be the major pathway (>90%) of human exposure compared to dermal contact or inhalation (Mombo et al., 2015; Xiong et al., 2016b). Soil is the direct path way for the contamination of heavy metal(loid)s in vegetables and crops via root uptake (Pierart et al., 2015). Vegetables and crops cultivated on heavy metal(loid)s polluted sites can take up heavy metal(loid)s (if pollutant bioavailability is high) greater than the maximum permissible limits (MPLs) and consequently may induce serious public health implications (Xiong et al., 2014). Increased accumulation of heavy metal(loid)s by vegetables higher than the MPLs has been reported in many parts of the world (Table 2). Excessive heavy metal(loid)s concentration in plant tissue is capable of inducing various physiological, morphological and biochemical toxic effects (Shahid et al., 2014a). Heavy metal(loid)s induce plant toxicity by disrupting nutrient and water uptake and transport, altering nitrogen metabolism, disrupting the activity of ATPase, reducing photosynthesis, interfering with plant growth, dysfunctioning plant photosynthetic machinery in chloroplasts and causing stomatal closure (Shahid et al., 2014b). Heavy metal(loid)s may also cause invisible symptoms of plant injury such as browning of roots, necrosis, chlorosis and leaf rolling (Pourrut et al., 2013). At the cellular level, excessive heavy metal(loid)s exposure can cause enhanced production of reactive oxygen species (ROS), alteration of cell cycles and division and chromosomal aberrations (Shahid et al., 2014a, 2015b).

Long-term use of heavy metal(loid)s contaminated vegetables/crops can cause continuous buildup of toxic metals in the kidney and liver of humans causing disorders in the physico-biochemical processes (Jarup, 2003). Owing to their high persistence and non-degradable nature, heavy metal(loid)s have the potential of biomagnification and bioaccumulation, thus inducing more exposure for some organisms than their level in the environment. Heavy metal(loid)s are even capable to induce toxic effects to living organisms including human beings at very low levels due to the absence of proper defense mechanism to mitigate the toxic effects of these metals and to remove them from the body. Consumption of heavy metal(loid)s contaminated vegetables can cause depletion of nutrients in the human body that causes many problems in humans, intrauterine growth retardation, disabilities with malnutrition, impaired psycho-social faculties, upper gastrointestinal cancer and immunological defenses (Hediji et al., 2015). Heavy metal(loid)s can induce oxidative stress by overproduction of ROS, which can destroy cell's inherent defense system and can cause cell damage or death (Shahid et al., 2014a). Moreover, heavy metal(loid)s can substitute essential metals in enzymes, thus disrupting their functioning (Pourrut et al., 2011a, 2011b). Heavy metal(loid)s (Pb and Cd) are capable to induce carcinogenesis, teratogenesis and mutagenesis; high Pb and Cd concentrations in edible plant parts were attributed to occurrence of upper gastrointestinal cancer (Jarup, 2003). Moreover, Pb is also reported to cause improper hemoglobin synthesis, renal and tumor infection, elevated blood pressure and dysfunctioning of reproductive system (Pourrut et al., 2011a, 2011b). Therefore, much attention is given worldwide to food safety and risk assessment.

5. Remediation of heavy metal-contaminated soils

Once metals are introduced into and contaminate the environment, they may persist for long time depending on the type of metal and soil. The remediation processes used for clean-up of heavy metal(loid)s contaminated sites may be in-situ or ex-situ, on-site or off-site, and

Table 2
Accumulation of heavy metal(loid)s in edible parts exceeding permissible limits of vegetables and crops when cultivated on heavy metal(loid)s polluted soils.

Heavy metals	Vegetables	Concentration in soil (mg/kg)	Concentration in plants edible parts (mg/kg)	[a]Maximum allowable limit	Fold-higher than allowable limit	References
Cd	*Lactuca sativa*	1.3	130	0.2	650	Pereira et al., 2011
	Solaum lycopersicum	11.24	13		65	Hediji et al., 2015
	Agaricus bisporus	–	10		50	Schlecht and Säumel, 2015
	Cynosurus cristatus	0.2	9.0		45	Quezada-Hinojosa et al., 2015
	Brassica napus	1	6.0		30	Wu et al., 2015
Pb	*Daucus carota*	0.01	390	1	390	Carvalho et al., 2015
	Solanum aethiopicum	452	144		144	Nabulo et al., 2012
	Brassica oleracea	2.58	49		49	Perveen et al., 2012
	Lactuca sativa	–	28		28	Kang et al., 2015
	Spinacia oleracea	66.78	20		20	Khan et al., 2013
As	*Nicotina glauca*	14,660	92	0.15	613	Santos-Jallath et al., 2012
	Lactuca sativa	5.83	14		96	Caporale et al., 2014
	Oryza sativa	–	1.3		8	Smith et al., 2008
Zn	*Nicotina glauca*	507	1985	50	40	Santos-Jallath et al., 2012
	Brassica juncea	190	201		4.0	Mapanda et al., 2007
	Zea mays	80	148		3.0	Avci and Deveci, 2013
	Lactuca sativa	–	118		2.4	Bosiacki and Tyksiñski, 2009
	Spinacia oleracea	124	84		1.7	Naser et al., 2012
Ni	*Lactuca sativa*	1.11	48	0.2	238	Perveen et al., 2012
	Solanum lycopersicum	1.11	43		215	Perveen et al., 2012
	Portulaca oleracea	–	36		181	Renna et al., 2015
	Diplotaxis tenuifolia	–	35		175	Renna et al., 2015
	Cupressus sempervirens	11.3	7.0		35	Farahat and Linderholm, 2015
Cu	*Solanum lycopersicum*	–	202	10	20	Liu et al., 2006
	Coriandrum sativum	–	48		5	Gupta et al., 2013
	Zea mays	41	47		5	Avci and Deveci, 2013
	Agaricus bisporus	–	36		4	Liu et al., 2015
	Apium graveolens	46.85	11		1	Chao et al., 2007
Cr	*Solanum aethiopicum*	256	65	1	65	Nabulo et al., 2012
	Brassica oleracea	12.78	24		24	Tiwari et al., 2011
	Capsicum	1.11	17		17	Perveen et al., 2012
	Sinapis	1.11	13		13	Perveen et al., 2012
	Coriandrum sativum	1.11	13		13	Perveen et al., 2012
Mn	*Allium cepa*	573	585	500	1.17	Chiroma et al., 2014
	Lactuca sativa	619	512		1.02	Chiroma et al., 2014

[a] EU European Union Standards (2006); FAO WHO/FAO 2007.

biological, physical and chemical (Fig. 1). These techniques are often used in combination with each other for more economical and efficient remediation of a contaminated site.

5.1. Physical remediation

5.1.1. Soil replacement

Soil replacement of contaminated soil refers to replacing or partly replacing contaminated soil by non-contaminated soil. Prior to 1984, excavation, off-site disposal and soil replacement were the most commonly method for cleaning-up contaminated sites. Soil replacement method dilutes the concentration of heavy metal(loid)s in soil, and in turns increases soil functionality (Yao et al., 2012). The replaced soil is generally treated to remove heavy metal(loid)s or in some cases dumped in other places. Soil replacement can also be carried out by (i) soil spading and (ii) new soil importing. In soil spading, contaminated site is dug deeply and the heavy metal(loid)s are spread into the deep sites, thus achieving the aim of metal diluting. New soil importing refers to adding clean soil into the heavy metal(loid) polluted soil. The added soil is either covered in the surface or mixed to make the metal concentration decreasing. Douay et al. (2008) carried out soil replacement remediation project in three kitchen gardens near Metaleurop Nord smelter situated in the North of France having high concentrations of Pb and Cd in the topsoils (up to 3300 and 24 mg kg^{-1} respectively). Under this study, they delineated a surface area of about 50–100 m^2 for each garden, removed the contaminated soil and replaced it with a non-contaminated one. After soil replacement, cultivation of vegetables (during 2003 to 2005) showed a clear improvement of vegetables and soil quality (Douay et al., 2008). Soil replacement method can effectively isolate the contaminated soil and ecosystem, thus minimizing its effect on the environment. However, this technique is costly due to high labor work, and is appropriate for heavily contaminated soils with small area. Costs for bulk excavation, transportation over short distance, and disposal vary from $270 to $460 per ton. Costs for long distance transport of excavated soil may be substantially higher. Moreover, this technique may not be applicable to agricultural sites because there is a risk of loss of soil fertility.

5.1.2. Soil isolation

Soil isolation means to separate the heavy metal(loid) contaminated soil from the uncontaminated soil, but for complete remediation it still needs other auxiliary engineering measures (Zheng and Wang, 2002). Isolation technologies are generally designed to prevent off-site movement of heavy metal(loid)s and other contaminants by restricting them within a specified area (Zhu et al., 2012). Soil isolation technology is used to avoid further contamination of groundwater by heavy metal(loid)s when other remediation methods are not economically or physically feasible. In some cases, contaminated sites are isolated temporarily in order to avoid transport during site assessment and remediation.

Subsurface barriers are commonly used to separate contaminated water and soil by restricting the flow of ground and/or surface water at a contaminated site. Subsurface barriers restrict the flow of contaminated groundwater through the uncontaminated site as well as the flow of uncontaminated groundwater from the contaminated site (Rumer and Ryan, 1995). Vertical subsurface barriers restrict the lateral movement of groundwater. These vertical barriers can be installed downstream, upstream, or completely surrounding the site. Vertical barriers are often installed in combination with a capping system to restrict infiltration of uncontaminated surface water. These barriers cannot be installed to deep soil and are often limited to around 30 ft. In order to effectively isolate the contaminated part of soil, the barrier should be continuous with low-permeability layer. This is mostly achieved by establishing a layer of low-permeable material such as clay below the contaminated region of soil (Rumer and Ryan, 1995). Various materials are used for sub-surface barriers, which include sheet piles, grout curtains and slurry walls.

5.1.3. Vitrification

The mobility of heavy metal(loid)s inside soil can be reduced by applying high temperature treatment at the contaminated site (Mallampati et al., 2015) that leads to the formation of vitreous material. During vitrification, some metal species (such as Hg) may be volatilized under high temperature that must be collected for further disposal or treatment. Vitrification is not considered a classical metal remediation technique. Vitrification is comparatively easy to apply compared to other physical remediation methods. Vitrification can be applied to majority of soils contaminated with inorganic (heavy metal(loid)s) and organic contaminants. During in situ vitrification, electric current is passed through the soil by vertically inserting an array of electrodes into the contaminated area. However, dry soil may not provide enough conductance for vitrification. Recently, Dellisanti (2016) carried out an in-field Joule heating vitrification of tons of Zn and Pb rich ceramic waste by heating up to about 1850 °C. They reported that the vitrification method was greatly efficient to clean-up tons of heavy metal(loid)s contaminated waste materials and can be applied for cleaning huge volumes of soil. Temperature during vitrification plays a key role in the immobilization of heavy metal(loid)s in soil samples. For example, Navarro et al. (2013) carried out vitrification of waste from Ag-Pb mines in Spain using solar technology. They showed that vitrification caused immobilization of Zn, Mn, Fe, Cu and Ni at 1350 °C, whereas Zn, Ni, Mn and Cu were mobilized at 1050 °C.

Vitrification can be performed both in situ and ex situ, although in situ method is preferred due to comparatively low cost and energy requirements. Ex situ vitrification processes contains various stages such as excavation, mixing, pretreatment, melting feeding, and casting of the melted product (Dellisanti, 2016). Ex situ vitrification requires high energy for melting, and is therefore costly. In some cases, the vitrified material is mixed with additives such as clay, native soil or sand to prepare a product with certain characteristics, which may improve the effectiveness of this technology. The vitrified material can be recycled and used as reusable materials, aggregate and clean fill (Smith et al., 1995). The main limitation of in situ vitrification is the potential of the soil to melt so that current can pass through it. Moreover, soil having high alkali content (1.4 wt%) may not be good conductor of current

(Buelt and Thompson, 1992). Therefore, in situ vitrification can be performed only under wet soil with low alkali content. This technique can be applied for small scale remediation of heavy metal(loid) polluted sites. Under field conditions or at large scale, this technique can be highly expensive.

5.1.4. Electrokinetic remediation

Soil electrokinetic remediation is a new and cost effective physical method for the remediation of heavy metal(loid)s. Soil electrokinetic remediation operates on the principle that the electric field gradient of suitable intensity is established on two sides of the electrolytic tank containing saturated contaminated soil. Heavy metal(loid)s present in the soil are separated via electrophoresis, electric seepage or electro-migration, and thus decrease the contamination (Yao et al., 2012). Rosestolato et al. (2015) remediated approximately 400 kg of soil using electrokinetic remediation and removed about 60% of total Hg from contaminated soil in <3 months. Electrokinetic remediation method is also used in combination with other techniques/processes such as electrokinetic-microbe joint remediation (Yu et al., 2009), electrokinetic-chemical joint remediation (Vocciante et al., 2016), electrokinetic-oxidation/reduction joint remediation (Yang et al., 2015), coupled electrokinetic phytoremediation (Mao et al., 2016), electrokinetics coupled with electrospun polyacrylonitrile nanofiber membrane (Peng et al., 2015), and electrokinetic remediation conjugated with permeable reactive barrier (Rosestolato et al., 2015).

The removal of heavy metal(loid)s having poor conductivity (e.g. sulfides) or present in metallic form (e.g. Hg) requires preliminary dissolution. In such cases, use of an appropriate electrolyte (distilled water, organic acids or synthetic chelates) may increase removal efficiency of electrokinetic remediation method (Iannelli et al., 2015). However, the removal efficiency varies with the type of chemical used (anolyte) and metal remediated (Vocciante et al., 2016). For example, Lee et al. (2016) used KH_2PO_4 as an anolyte and showed that removal efficiencies increased by >50% for As species and ~20% for Cu species. However, removal of the Pb and Zn was relatively inefficient (<20%). Rozas and Castellote (2012) analysed the effectiveness of different experimental variables (type of electrolyte, applied level, constants of precipitation (pKs) and chelation equilibriums (log β), initial and final pH of the catholyte and anolyte, and zeta potential) for different electrolyte solutions (distilled water, humic acid, acetic acid, citric acid and ethylene diamine tetraacetic acid (EDTA)). They reported that the zeta potential, pH of the cathodic solution and quelation ability influence the efficiency of electrokinetic remediation. Suzuki et al. (2014) also reported enhanced Pb and Cd remediation efficiencies by adding ethylene diamine disuccinate (EDDS). Mao et al. (2016) used coupled electrokinetic phytoremediation and showed that electro-kinetic field lowered soil pH to around 1.5 and enhanced dissolution of Pb, As and Cs, thus increasing their overall solubility and bioavailability.

However, electrokinetic remediation method operates well for soils having low permeability (Hanson et al., 1992). Electrokinetic remediation method is economically effective because it is easy to install and operate (Virkutyte et al., 2002), moreover, electrokinetic remediation does not abolish the original nature of the soil (Page and Page, 2002). However, the main limiting factor for direct electrokinetic remediation is fluctuation in soil pH because it cannot maintain soil pH value. In some cases, soil pH is controlled by adding buffer solutions in cathode and anode, using complexant or ion exchange membrane (Wang et al., 2007).

5.2. Chemical remediation

5.2.1. Immobilization techniques

Immobilization refers to decrease in metal mobility, bioavailability and bioaccessibility of heavy metal(loid)s in soil by adding immobilizing agents to the contaminated soils. Heavy metal(loid)s can be immobilized in soil by complexation, precipitation and adsorption

reactions. These processes cause redistribution of heavy metal(loid)s from soil solution to solid particles, thus limiting their transport and bio-availability in soil. Heavy metal(loid)s immobilization in soil is generally carried out by using organic and inorganic amendment to soils (Shahid et al., 2014c; Austruy et al., 2014; Ashraf et al., 2016). The most commonly amendments include cement, clay, zeolites, phosphates, minerals, microbes and organic amendments (Sun et al., 2016). Recent research also highlighted the potential of low-cost industrial residues such as termitaria (Anoduadi et al., 2009), industrial eggshell (Soares et al., 2015) and red mud (Smičiklas et al., 2014) for immobilization of heavy metal(loid)s in contaminated soils.

It is well known that the organic amendments can immobilize heavy metal(loid)s in soil through adsorption reactions or forming stable complexes (Shahid et al., 2014d; Sabir et al., 2015). Over time, effect of organic amendments on bioavailability of metals varies due to decomposition of organic matter. However, the immobilizing effect of organic amendments on heavy metal(loid)s at the initial stage of their application is important for remediation of contaminated sites. The major organic amendments used for heavy metal(loid)s immobilization include animal manures and biosolids. Biosolids, treated and stabilized solid organic residual by-products, generally contain heavy metal(loid)s, although advances in the sewage and wastewater treatment technologies are successfully reducing heavy metal(loid)s level in biosolids. Several studies have reported the negative effect of biosolid application as a source of heavy metal(loid)s contamination in the soil (Cele and Maboeta, 2016); however, some studies have reported the advantageous impact of organic amendments as adsorbent for stabilization of heavy metal(loid)s in the soil (Venegas et al., 2015; Shakoor et al., 2015).

Manure byproducts containing low levels of metal(loid) are nowadays used for metal immobilization in soils (Venegas et al., 2015). Khan et al. (2015) reported that the use of farm yard manure (FYM) was highly effective in immobilizing Fe, Cr, Ni, Mn, and Pb, while di-ammonium phosphate (DAP) was more effective for stabilizing Cu, Cd, and Zn in soil. Organic amendments usually increase organic contents of soil (Bolan et al., 2014). Organic amendments usually contain cellulose and lignin as the main constituents. Other components are extractives, hemicellulose, proteins, lipids, starches, simple sugars, hydrocarbons, and many other compounds that contain a number of functional groups such as carbonyl, phenolic, acetamido groups, amido, amino, structural polysaccharides and esters (Niazi et al., 2016a) having high affinity for metal complexation. Metals are known to form complexes (both soluble and insoluble) with organic component in soils (Shahid et al., 2014e). Formation of metal-organic matter complexes apparently depends on the type and nature of the organic matter (Shahid et al., 2014e). Organic constituents of soil have a high affinity for metal(loid)s due to high binding constants of metal ions with phenolic hydroxyl and carboxylic (COOH) groups of dissolved organic matter (Shahid et al., 2012a). Immobilization of heavy metal(loid)s by organic amendments can be due to increase in soil pH by preventing sulfide oxidation/hydrolysis (Walker et al., 2004). In addition to formation of stable complexes with metal ions, organic amendments can reduce metal bioavailability by increasing in surface charge (Gadd, 2000).

Biomaterials have been greatly used in recent years to immobilize heavy metal(loid)s in soil due to their easy availability and low-cost. Among biomaterials, the use of biochar has received significant attention to immobilize heavy metal(loid)s in soil. Biochar is a carbon rich, porous, purpose-produced charcoal manufactured during the pyrolysis of organic residues such as municipal waste, animal wastes, wood, crop residues, and biosolids. Several studies revealed that addition of biochars to soil greatly enhanced the sorption of heavy metal(loid)s and significantly reduced their mobility and phytoavailability (Al-Wabel et al., 2015; Puga et al., 2015). Addition of biochar to soil changes chemical, physical, and biological properties of soil. Changes in the soil properties especially increase of pH can cause their precipitation and thereby heavy metals immobilization in soils.

5.2.2. Encapsulation

Among the remedial approaches, immobilization of toxic metal solutions is an effective method to reduce hazardous material and their subsequent safe disposal as a landfill by encapsulating them in manageable solid blocks (Ucaroglu and Talinli, 2012). Encapsulation involves the mixing of the contaminated soils with other products, such as concrete, lime, or asphalt. The contaminated soil becomes immobile and thus prevents contamination of the surrounding materials. A number of binding materials are used in solid blocks formation but cement is preferred because of its easy availability, versatility, and cost-effectiveness (Pandey et al., 2012). Ordinary Portland Cement (OPC) has been commonly used as a good metal retainer, and in the last few years, alternative binders are receiving growing attention. For instance, encapsulation of hazardous materials with calcium aluminate cement (CAC) has been examined to be highly efficient and effective (Navarro et al., 2013). A variant of a CAC matrix is phosphate-bonded CAC, which is found by acid-base reaction between acidic phosphate solutions and CAC as the base reactant (Sugama and Carciello, 1995). These phosphate amended CAC have been applied and investigated as potential cementing materials for encapsulation of radioactive wastes (Swift et al., 2013) and to prevent the aluminum containing nuclear wastes from corrosion (Kinoshita et al., 2013).

The leaching of organic materials may be prevented effectively by encapsulation (He and Chen, 2014). In encapsulation, various immobilization agents, e.g., polyvinylalcohol, chitosan, alginate, agar, polyacrylamide and polyurethanes are used. However, encapsulation by lime and concrete has been used simultaneously in the effective treatment of oil contaminated and heavy metal(loid)s soil, whereas asphalt encapsulation used for hydrocarbons contaminated soils. The major drawback of these methods is an instant market for the final product, otherwise the end result will be random patches of asphalt and concrete (Mulligan et al., 2001). This limitation has led to the development of a silica based encapsulation remediation technology. The presence of different carboxylic groups in Algin make it an excellent encapsulating agent as well as an efficient sorbent for metal ions (Kuang et al., 2015). These reactive groups may interact with metal cations via chelation, and also contribute in gelling biopolymer. Algin encapsulated polyethylenimine (PEI)-derivatives microparticles were tested for recovering Cd(II), Zn(II), and Cu(II) ions from simple and complex synthetic solutions (Bertagnolli et al., 2016).

Metallic nanoparticles (NPs) of Fe (Park et al., 2000), Co (Tripp et al., 2002) and Ni (Hou and Gao, 2003) are of great interest due to their unusual magnetic, optical, and electrochemical properties (Hou and Gao, 2003) as well as chemical catalytic activities (Sun and Xia, 2002) resulting from their fine size and high specific surface area (Rashid et al., 2016). Carbon-encapsulated magnetic NPs were justified as a promising candidate for efficient removal of heavy metal(loid) from wastewater (Zhang et al., 2010). Sun et al. (2006) have reported the removal of methyl orange by carbon-encapsulated magnetic NPs. Application of encapsulated nanoparticles for removal of heavy metal is very limited. Magnetic NPs with regular shape and narrow size distribution are necessary to maintain the dispersion stability of NPs in the wastewater. Additionally, introduction of external magnetic fields will greatly enhance mobility of magnetic NPs, thereby facilitating recycling of NPs used for treatment of waste water containing toxic heavy metal(loid)s (Zhang et al., 2010).

5.2.3. Soil washing

Soil washing refers to removal of heavy metal(loid)s from soil using various reagents and extractants (Guo et al., 2016; Park and Son, 2016) that can leach the heavy metal(loid)s from the soil. Recently, use of suitable extractants for leaching heavy metal(loid)s from contaminated soils have proven as an alternate to some of the conventional techniques for the clean-up of contaminated soils. During soil washing, the contaminated soil is dug out and mixed with a suitable extractant solution depending on the type of metal and soil. The extractant solution and soil

are mixed thoroughly for a specified time. Through the precipitation, ions exchange, chelation or adsorption, the heavy metal(loid)s in soil are transferred from soil to liquid phase, and then separated from the leachate (Ferraro et al., 2015). The separated soil that fulfills regulatory criteria can be backfilled to original site. Use of soil washing for the remediation of heavy metal(loid) contaminated sites is frequently used because it completely removes the metals from soil. Moreover, soil washing is a rapid method which can meets specific criteria without any long-term liability (Park and Son, 2016). Owing to its high efficiency, soil washing is considered one of the most cost-effective soil remediation technologies.

A number of reagents have been used to mobilize and remove heavy metal(loid)s from soil including synthetic chelating agents (EDTA, EDDS), organic acids, humic substances, surfactants and cyclodextrins (Shahid et al., 2014a; Kulikowska et al., 2015). These reagents used for soil washing have been established on a case-by-case basis and their application and efficiency vary with the type of heavy metal(loid) and site. It is well-known that the exchange/extraction/solubilization of heavy metal(loid)s during soil washing depend on soil and metal type (Liao et al., 2015). The efficiency of soil washing depends on the ability of extractant to dissolve the heavy metal(loid)s in soils. Therefore, extractants which can dissolve high levels of metals would be appropriate for soil cleaning. Among the extractants, synthetic chelates such as EDTA and EDDS are considered the most effective and suitable for soil washing because these chelates can form stable complexes with most of the heavy metal(loid)s in the wide pH range (Saifullah et al., 2015). For the removal of cationic metals, EDTA is known as the most effective synthetic chelating agent but not in the anionic metals (Udovic and Lestan, 2010).

Other soil washing chemicals include high concentration salt chloride solution such as iron (III) chloride and calcium chloride (Makino et al., 2008). Makino et al. (2006) stated that $FeCl_3$ was highly effective for soil washing of Cd contaminated paddy soils because of cost effectiveness, its Cd extraction efficiency and lower environmental impact. Rinsing steps and repeated washing are conducted to improve the removal efficiency of heavy metal(loid)s which can reduce the consumption of washing agents and the washing costs (Torres et al., 2012). Similarly, combined use of different chelators also improves heavy metal(loid)s washing efficiency especially for multi-metal contaminated soils (Guo et al., 2016). Some authors used more than one washing chelators for sequential extraction/washing of heavy metal(loid)s contaminated soil. For example, Wei et al. (2016) reported that phosphoric-oxalic acid-Na_2EDTA order based soil washing enhanced heavy metal(loid)s removal efficiency by 41.9% for As and 89.6% for Cd.

5.3. Biological remediation

Bioremediation is one of the most viable options to rectify and re-establish the natural condition of soil considered detrimental to environmental health. Bioremediation makes use of microorganisms/plants to detoxify or remove heavy metal(loid)s from the soil. Bioremediation is cost-effective, non-invasive and provides a permanent solution.

5.3.1. Phytoremediation

Phytoremediation, also known as botanoremediation, vegetative remediation, green remediation or agroremediation, comprises technologies that make use of plants to remediate and revegetate contaminated sites. The concept of using metal-accumulating plants to clean up heavy metal(loid)s contaminated sites was first presented in 1983, but the practice of cleaning soil by plants has been executed for the last 300 years. Phytoremediation is considered environmental friendly, attractive, aesthetically pleasing, non-invasive, energy efficient, and cost-effective technology to clean up the sites with low-to-moderate levels of heavy metal(loid)s (Sabir et al., 2015). Phytoremediation can be used effectively in combination with several other traditional remediation techniques as a finishing step to the remedial process. The efficiency of phytoremediation depends on numerous plant and soil factors such as the physico-chemical properties of the soil, bioavailability of metals in soil, microbial and plant exudates, and the capacity of living organisms to uptake, accumulate, sequester, translocate and detoxify metals. The term phytoremediation includes several techniques and applications which differ greatly in the process/mechanism by which plants can immobilize, remove, or degrade metals. Phytoremediation is generally categorized into phytostabilization, phytoevaporation and phytoextraction are based on different uptake mechanisms.

5.3.1.1. Phytovolatilization. During phytovolatilization, heavy metal(loid)s are taken up from the soil and converted into less toxic vapours, which are then released into the atmosphere through transpiration process of the plants. In phytovolatilization, metals are assimilated into volatile organic compounds which are then released into atmosphere as biomolecules (Marques et al., 2009). Some heavy metal(loid)s such as Se, Hg and As may exist as gaseous species in the environment. Some plant species such as *Arabidopsis thaliana*, *Brassica juncea* and *Chara canescens* are capable to uptake heavy metal(loid)s and transfer them to gaseous species inside the plant followed by their release into the atmosphere (Verbruggen et al., 2009). Arsenic was effectively volatilized in the form of As compounds (arsenite and arsenate) in the frond of *Pteris vittata* (Sakakibara et al., 2010). Phytovolatilization has been used mainly for removing Hg by converting it into gaseous Hg, which is the comparatively less toxic (Ghosh and Singh, 2005). *Brassica juncea* and *Arabidopsis thaliana* can grow under high Se level and are capable to volatilize Se. Plant-induced volatilization of Se includes conversion of inorganic Se into the organic selenomethionine and selenoaminoacids selenocysteine followed by biomethylated to form dimethylselenide or dimethyldiselenide, which are volatile and can be secreted to the atmosphere (Bañuelos and Mayland, 2000).

Conversion of heavy metal(loid)s into gaseous/volatilized form inside the plants is via specific mechanisms, which are generally governed inside plants by some specific enzymes or genes. There are very less number of naturally occurring plants capable of converting metals into volatilize form. Phytovolatilization technique therefore generally makes use of genetically modified plants to enhance ability of plants to volatilize metals. *Arabidopsis thaliana* and *N. tabacum* have been genetically engineered (Meagher, 2000) with mercuric reductase, a gene which can volatilize Hg (Meagher, 2000). In order to enhance Hg phytovolatilization by plants, Hg reductase genes has been inserted from bacterial into plants (Rugh et al., 1998). Transgenic plants genetically engineered with bacterial genes (merA and merB) can volatilize almost 100–1000 times more Hg than wild-type plants (Rugh et al., 1996).

A gene which can encode sterol methyl transferases (SMT) enzyme was introduced into Arabidopsis and *Brassica juncea* from *Astragulus bisculatus* (Neuhieral et al., 1999). Over expression of SMT enzyme in *Brassica juncea* and Arabidopsis enhanced Se accumulation, tolerance and volatilization (Neuhieral et al., 1999). It is reported that volatile Se compounds (dimethylselenide) are almost 500–600 fold less toxic compared to inorganic Se forms usually present in the soil (Deesouza et al., 2000). Cystathionine Gamma-Synthase (CGS) is an enzyme that is reported to play a key role in heavy metal(loid) volatilization. Enhanced production of CGS in transgenic Brassica enhanced volatilization of Se, and the CGS transgenic plants were more tolerant to selenite than wild type (Van Huysen et al., 2003). The transgenic Brassica overexpressing CGS contained 50–70% lower Se level in roots and 20–40% lower in shoots than in wild type (Van Huysen et al., 2003). Encoding and expression of As(III)-S-adenosylmethionine methyltransferase (arsM) gene in an As-sensitive strain of *E. coli* resulted in the biosynthesis of several volatilized forms of As (Qin et al., 2006).

However, the practical application of heavy metal(loid)s phytovolatilization for soil remediation seems questionable. This is because, in phytovolatilization, once the metal has been volatilized, no one has control over its fate in the atmosphere. Some studies reported

that after being released into atmosphere, volatile compounds are diluted and dispersed in the atmosphere and thus induce very minute or no environmental hazard (Meagher, 2000). Moreover, phytovolatilization involves little erosion and no disposal of contaminated plant biomass with negligible site disturbance (Rugh et al., 2000).

5.3.1.2. Phytostabilization. Phytostabilization is the use of plants to decrease the bioavailability and mobility of heavy metal(loid)s in soils due to their stabilization with the help of plants (Sylvain et al., 2016). Phytostabilization of metals does not reduce the concentration of heavy metal(loid)s present in contaminated soil but prohibits their off-site movement. Phytostabilization aims to restrict heavy metal(loid)s in the vadose zone of plants through accumulation by roots or precipitation within the rhizosphere (Bolan et al., 2011). Therefore, unlike other methods of phytoremediation, phytostabilization does not remediate the contaminated soils but reduces the contamination of nearby media/area. Phytostabilization is generally used for soils where phytoextraction is not desirable or possible. Furthermore, phytostabilization can also be used at sites where technical or regulatory limitations interrupt with the selection and implementation of the most appropriate remediation techniques. For example, in order to limit off-site heavy metal(loid)s movement from barren contaminated site, phytostabilization can be a useful option. Plants can prohibit movement of metals through several methods: reduced leaching through upward water flow generated by plant transpiration, reduced soil erosion due to stabilization of soil by plant roots and decrease in runoff due to above-ground vegetation. Phytostabilization does not produce contaminated secondary waste that needs further management. Phytostabilization is helpful in achieving ecosystem restoration because it increases soil fertility. However, since the heavy metal(loid)s are stabilized within soil, the site needs regular monitoring to make sure that the optimal stabilizing conditions are retained (Bolan et al., 2011). Phytostabilization may raise some issues under highly contaminated soils. In such cases, cultivation of plant species tolerant to metal contamination and adapted to the local environmental conditions is advantageous.

Metal excluder plants accumulate high levels of heavy metal(loid)s from soil into their roots with limited transport to their aerial parts (Ali et al., 2013). These plants have a little potential for metal extraction but are highly efficient for phytostabilization purposes (Ali et al., 2013). Among the hyperaccumulator plants, the most effective for phytostabilization are able to (i) restrict heavy metal(loid) leaching by reducing water percolation through the soil matrix, (ii) inhibit soil erosion and movement of heavy metal(loid)s to other areas and (iii) prevent direct contact with heavy metal(loid) contaminated soil (Bolan et al., 2011). Plants generally cause heavy metal(loid)s immobilization in polluted soils by inducing changes in their rhizosphere, which has discrete physico-bio-chemical conditions (Abbas et al., 2016). For example, Cd forms complexes with sulfide (de Knecht et al., 1994) and Pb is precipitated as phosphate (Cotter-Howells and Caporn, 1996) in the root zone of *Silene vulgaris* and *Agrostis capillaris*, respectively. During last 2–3 decades, numerous plant species, especially the agricultural grasses have been effectively used in phytostabilization. Willows (Salix spp.) are considered ideal plants for both phytoextraction and phytostabilization owing to their elevated tolerance to heavy metal(loid)s, high territorial expansion and intense evapotranspiration (Sylvain et al., 2016). Some plant species such as Festuca spp. and Agrostis spp. are most commonly used for phytostabilization of Pb, Zn and Cu polluted soils in Europe (Galende et al., 2014).

Phytostabilization can also be used in combination with other remediation approaches such as use of soil microorganisms and organic amendments to enhance heavy metal(loid)s immobilization in soil. Soil microorganisms are reported to increase root metal contents via increased growth as well as the heavy metal(loid)s immobilization in soil (Rajkumar et al., 2013). Moreover, addition of compost significantly enhances microbial diversity via long-lasting buffering-effect on pH, and

consequently allowing plants to germinate and accumulate more heavy metal(loid)s in roots (Valentín-Vargas et al., 2014). Organic and inorganic amendments (natural and synthetic) also facilitate plant-induced immobilization of heavy metal(loid)s in soil (Parra et al., 2016). Lee et al. (2014) evaluated the effect of four different amendments (furnace slag, bottom ash, bone mill, and red mud) as immobilizing agents in conjugation with *Miscanthus sinensis* and *Pteridium aquilinum* in aided phytostabilization of heavy metal(loid)s contaminated soil. Application of amendments significantly reduced soluble and extractable heavy metal(loid)s fractions (up to 99%) in soil in the presence of *Pteridium aquilinum* and *Miscanthus sinensis*. The phytostabilized sites require continuous monitoring to make sure that the stabilizing condition is sustained. Soil amendment used to reduce heavy metal(loid)s mobility in soil may need to be occasionally reapplied to retain immobilizing conditions (Bolan et al., 2011).

5.3.1.3. Phytoextraction. Phytoextraction involves clean-up of heavy metal(loid)s from soil by means of plant uptake. This solar-driven technique is based on the capability of the plant roots to uptake, translocate, and concentrate heavy metal(loid)s from soil to the aboveground harvestable plant parts. In this way, phytoextraction results in the decrease of contaminated mass (soil). During phytoextraction, heavy metal(loid)s are transferred from soil to plant biomass. Plant biomass is comparatively very easy to recycle, dispose, treat or oxidize compared to soil. Phytoextraction guarantees a permanent removal of metal(loid)s from the contaminated sites. However, phytoextraction is suitable to those sites which are polluted by low-moderate levels of metals, because most plant species are not able to sustain in heavily polluted sites (Rascio and Navari-Izzo, 2011; Sabir et al., 2015). For example, phytoextraction technique can be used for Pb remediation by *Brassica juncea* for sites whose Pb level is <1500 mg/kg (Blaylock and Huang, 2000).

Hyperaccumulators are plants species capable to accumulate heavy metal(loid)s in their shoot tissues (without visible toxicity symptoms) to levels far above those present in the soil or in non-accumulating plant species (Arshad et al., 2008; Shahid et al., 2012b; Ali et al., 2013). Plant species which can be effectively used for phytoextraction must have (i) high metal-accumulation capability in their above ground parts, (ii) tolerance to high concentration of metals, (iii) ability to grow fast with high biomass and (iv) profuse root system. There are no defined standards for hyperaccumulator plants. However, different researchers or research groups have defined hyperaccumulators. Brooks et al. (1977) were the first to use the term "hyperaccumulator" for plants which can accumulate Ni > 1000 mg kg^{-1} dry weigh (0.1%). Hyperaccumulator plant species are capable to accumulate 100–500 fold higher metals in shoots with no effect on the yield as compared common nonaccumulator plants (Bhargava et al., 2012; Mahar et al., 2016; Sheoran et al., 2016). Storage and accumulation requirements of hyperaccumulator plant species are different for different metals. Plant species which accumulate >100 mg/kg Cd and Se (on dry weight basis), >1000 mg/kg Cu, Ni, As, and Pb or >10,000 mg/kg Mn and Zn in their aerial plant parts when grown on heavy metal(loid) contaminated soils are called hyperaccumulator plants (Mahar et al., 2016). According to Verbruggen et al. (2009), concentration criterion (% in leaf dry matter) for hyperaccumulator plants is Cd ≥ 0.01, Pb ≥ 0.1, Co ≥ 0.1, Sb ≥ 0.1, Cu ≥ 0.1, Ni ≥ 0.1, Mn ≥ 1.0 and Zn ≥ 1.0. Table 3 presents some hyperaccumulator plants which can accumulate high levels of these metal(loid)s (many fold higher than the minimum threshold level of hyperaccumulators).

Currently, >450 hyperaccumulating plant species of 45 families fulfilling the criteria of being hyperaccumulator are known which represents <0.2% of all angiosperms, the majority of them being Ni hyperaccumulators (75%) (Verbruggen et al., 2009). These plant families mainly included Brassicaceae, Asteraceae, Violaceae, Fabaceae, Caryophyllaceae, Euphorbiaceae, Lamiaceae, Flacourtaceae and Poaceae (Bhargava et al., 2012; Bolan et al., 2014). Among these

Table 3
Some examples of hyperaccumulator plants accumulating high levels of heavy metal(loid)s in their above ground parts.

Vegetables	Heavy metals	Concentration in plant aerial parts (mg/kg)	Threshold level for hyper-accumulator	Type of remediation	Fold-higher than threshold level	Reference
Prosopis laevigata	Cd	8176	100	Phytoextraction	81.8	Buendía-González et al., 2010
Arabidopsis halleri		5722		Phytoextraction	57.2	Küpper et al., 2000
Thlaspi caerulescens		5000		Phytoextraction	50.0	Koptsik, 2014
Thlaspi caerulescens		3000		Phytoextraction	30.0	Sheoran et al., 2009
Viola principis		1201		Phytoextraction	12.0	Wan et al., 2016
Potentilla griffithii		852		Phytoextraction	8.5	Hu et al., 2009
Solanum nigrum		387		Phytoextraction	3.9	Sun et al., 2008
Lonicera japonica		286		Phytoextraction	2.9	Liu et al., 2009
Thlaspi caerulescens		263		Phytoextraction	2.6	Lombi et al., 2001
Eleocharis acicularis		239		Phytoextraction	2.4	Sakakibara et al., 2011
Deschampsia cespitosa		236		Phytoextraction	2.4	Kucharski et al., 2005
Solanum photeinocarpum		158		Phytoextraction	1.6	Zhang et al., 2011
Phyllanthus serpentinus	Ni	38,100	1000	Phytoextraction	38.1	Chaney et al., 2010
Alyssum murale		22,800		Phytoextraction	22.8	Chaney et al., 2008
Alyssum corsicum		18,100		Phytoextraction	18.1	Li et al., 2003
Berkheya coddii		18,000		Phytoextraction	18.0	Mesjasz-Przybyłowics et al., 2004
Thlaspi caerulescens		16,200		Phytoextraction	16.2	Koptsik, 2014
Salvinia minima		16,600		Phytoextraction	16.6	Fuentes et al., 2014
Alyssum murale		15,000		Phytoextraction	15.0	Li et al., 2003
Alyssum serpyllifolium		10,000		Phytoextraction	10.0	Prasad, 2005
Isatis pinnatiloba		1441		Phytoextraction	1.4	Altinozlu et al., 2012
Arrhenatherum elatius	Pb	24,000	1000	Phytoextraction	24.0	Deram et al., 2000
Brassica juncea		10,300		Phytoextraction	10.3	Koptsik, 2014
Brassica nigra		9400		Phytoextraction	9.4	Koptsik, 2014
Pelargonium (Atomic)		7000		Phytoextraction	7.0	Arshad et al., 2008
Helianthus annuus		5600		Phytoextraction	5.6	Koptsik, 2014
Pelargonium (Clorinda)		5000		Phytoextraction	5.0	Arshad et al., 2008
Pelargonium (Attar)		4000		Phytoextraction	4.0	Arshad et al., 2008
Viola principis		2350		Phytoextraction	2.4	Wan et al., 2016
Euphorbia cheiradenia		1138		Phytoextraction	1.1	Chehregani and Malayeri, 2007
Pteris vittate	As	23,700	1000	Phytoextraction	23.7	Ma et al., 2001
Pteris vittate		13,800		Phytoextraction	13.8	Tu et al., 2002
Pteris vittata		8331		Phytoextraction	8.3	Kalve et al., 2011
Pteris vittata		6017		Phytoextraction	6.0	Han et al., 2016
Pteris vittata		4106		Phytoextraction	4.1	Wan et al., 2016
Pteris ryukyuensis		3647		Phytoextraction	3.6	Srivastava et al., 2006
Pteris quadriaurita		2900		Phytoextraction	2.9	Srivastava et al., 2006
Corrigiola telephiifolia		2110		Microbial assisted phytoextraction	2.1	Garcia-Salgado et al., 2012
Pteris biaurita		2000		Phytoextraction	2.0	Srivastava et al., 2006
Pteris cretica		1800		Phytoextraction	1.8	Srivastava et al., 2006
Eleocharis acicularis		1470		Phytoextraction	1.5	Sakakibara et al., 2011
Arabidopsis halleri	Zn	32,000	10,000	Phytoextraction	3.2	Zhao et al., 2000
Potentilla griffithii		11,400		Phytoextraction	1.1	Hu et al., 2009
Eleocharis acicularis	Cu	20,200	1000	Phytoextraction	20.2	Sakakibara et al., 2011
Aeolanthus biformifolius		13,700		Phytoextraction	13.7	Chaney et al., 2010
Pteris vittata	Cr	20,675	1000	Phytoextraction	20.7	Kalve et al., 2011
Prosopis laevigata		5461		Phytoextraction	5.5	Buendía-González et al., 2010

hyperaccumulating plant species, there are about 20 As hyperaccumulators (fern species) (Niazi, 2011; Niazi et al., 2016b), 1 Cd hyperaccumulator, 14 Pb hyperaccumulator, 30 Co hyperaccumulators, 34 Cu hyperaccumulators, >320 Ni hyperaccumulators, 30 Se hyperaccumulator, 11 Zn hyperaccumulator, and 10 Mn hyperaccumulator (Ghosh and Singh, 2005). Most of the hyperaccumulator plant species can hyperaccumulate and hypertolerate only one metal. However, some plant species are capable

to accumulate and tolerate more than one metal at high levels. The best known multi-metal hyperaccumulator plant species is *Thlaspi caerulescens* (Ali et al., 2013), which can hyperaccumulate Ni, Cd, Pb, and Zn; *Thlaspi ochroleucum* and *Thlaspi goesingense* can hyperaccumulate Zn and Ni; and *Thlaspi rotundifolium* can hyperaccumulate Zn, Ni and Pb (Keller and Hammer, 2004; Vogel-Mikuš et al., 2006). Some other plants species belonging to the Brassicacea, such as *Brassica juncea*, *Brassica napus*, *Leguminosae milkvetch*, *Astragalus racemosus* and serpentine-endemic shrub Alyssum are also known to accumulate more than one metal(loid) at high concentrations (Kotrba et al., 2009). More recently *Sedum alfredii* (Crassulaceae) has gained increased attention, as multi-metal hyperaccumulator for Zn, Pb and Cd with Zn level reaching <2% of shoot weight (Xiong et al., 2011).

Phytoextraction allows soils clean-up over a large scale with an uneven pollution pattern. The advantages of phytoextraction compared to other classical remediation methods are (i) highly economical, (ii) less disruptive to the soil and environment, (iii) no need of disposal sites, (iv) high public acceptance, (v) no excavation or transport of contaminated media, and (vi) feasible for multi-metal contaminated sites (Sheoran et al., 2016; Mahar et al., 2016). The disadvantages of phytoextraction are as follow (i) depends on the growing conditions essential for plants and microorganisms, (ii) large-scale tasks need considerable experience and expertise to agricultural equipment, (iii) depends on plant tolerance to metal(loid)s, (vi) relatively long time period required to completely remediate the sites. The potential of hyperaccumulator plant species to uptake and accumulate heavy metal(loid)s is of great significance. However, in depth mechanisms/processes involved in metal accumulation are so far less studied, and the physiological roles are not fully developed. Recent development in physiological processes of hyperaccumulation revealed mechanisms involved in metal hyperaccumulation. The mechanisms of heavy metal(loid)s hyperaccumulation include biochemical and biophysical processes. Essential pre-requisites of successful phytoextraction include increased metal uptake and translocation to aerial parts, enhanced loading of metals into xylem, detoxification of metals within plant (Bhargava et al., 2012; Sheoran et al., 2016).

Physiological studies revealed that enhanced metal xylem loading and transfer to the aerial plant parts is mediated by carrier proteins (Ali et al., 2013; Shahid et al., 2016). Several studies have recently revealed that the transporters proteins like CDF (cation diffusion facilitator), ZIP [zinc-regulated transporter proteins (ZRTP), Iron-regulated transporter proteins (IRTP)], HMA (heavy metal(loid) ATPase) and Nramp (natural resistance and macrophage protein) are associated with the enhanced transfer of heavy metal(loid)s to aerial tissues in hyperaccumulator plant species (Ovečka and Takáč, 2014; Shahid et al., 2016). These carrier proteins are generally found in intracellular or plasma membranes. Several proteins of ATP family (AtATM3 or AtADPR12), ZIP family (TcZNT1, ZIP4, ZIP6, ZIP7, ZIP9, ZIP10 etc), Nramp family (NRAMP1, NRAMP3, NRAMP 5), DMT family (IRT1), and CDF transporters (MTP1, MTP8, MPT11 etc.) were identified in *Anemone halleri* and *Thlaspi caerulescens* as being involved in metal uptake, vacuolar sequestration, xylem loading and enhanced translocation of metals (Verbruggen et al., 2009). A role of human divalent metal transporter, DMT1 in the transport of Pb via a pH-dependent process has been also evidenced (Bressler et al., 2004). IRT1 has been shown to transport Cd and Zn in different hyperaccumulator plant species (Connolly et al., 2002). Similarly, overexpression of AtMT2b in tobacco resulted in increased As accumulation in shoot tissues (Grispen et al., 2009). AtHMA3 (At4g30120) (a member of the P-type ATPase superfamily), is reported to be involved in Zn loading into the xylem of *Anemone halleri*.

Heavy metal(loid)s are capable to induce a range of biochemical, morphological, physiological disorders (Shahid et al., 2015a). Heavy metal(loid)s induced generation of ROS is one of the earliest response of plants to metal toxicity (Pourrut et al., 2008; Shahid et al., 2012c).

Overproduction of ROS inside plant cells can induce oxidation and alteration of cellular proteins, amino acids, membrane lipids and DNA (Shahid et al., 2011; Adrees et al., 2015). Hyperaccumulator plant species adopt diverse strategies to avoid cellular injury and maintain their growth in metal-contaminated soil. One of the tolerance mechanisms adopted by plants constitutes various antioxidants to combat increased production of ROS. These enzymes include such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (POD), and glutathione reductase (GR), as well as antioxidants such as proline, cysteine, ascorbic acid, glutathione (GSH) and non-protein thiol (Shahid et al., 2013b). Several previous physiological studies showed that these enzymes and proteins can transform ROS into less-toxic products, thus limiting tissue dysfunction and cell injury (Shahid et al., 2014f, 2014g; Adrees et al., 2015). Plant species differing in metal tolerance show varying behaviour of certain enzymes under metal stress. Shahid et al. (2014a) also observed activation of antioxidative enzymes, including APX, CAT, SOD and under Pb stress in *Vicia faba*. Zouari et al. (2016) reported a significant increase in enzymatic (SOD, APX, GR) activities in the date palm when exposed to Cd.

Hypertolerance is considered the key aspect that makes hyperaccumulation possible. Vacuolar sequestration of heavy metal(loid)s is an important aspect in hyperaccumulator plant species towards metal homeostasis (Shahid et al., 2014a; Adrees et al., 2015). Hyperaccumulator plants are capable to sequester and/or bind metals to vacuole to prohibit their otherwise toxic effects. In plants, some natural chelates such as phytochelatins (PCs), GSH and metallothioneins (MTs) are characterised as the best metal-chelating ligands for sequestration of metal(loid)s to vacuoles, and are main part of the metal detoxification system in plants (Ali et al., 2013; Shahid et al., 2016; Adrees et al., 2015). Heavy metal(loid)s complexation with MTs, GSH and PCs is an effective way to neutralise the toxic effects of metals, most likely because the metal-PC complexes are transferred and sequestered in the cell vacuole (Yadav, 2010). Phytochelatins can form mercaptide bonds with various metals and play an important role in their detoxification in plants (Shukla et al., 2013). Generally, the formation of PC–metal complex takes place in cytosol, which is then sequestrated in the vacuole (Shahid et al., 2016; Török et al., 2015), thereby reducing the concentration of ROS in the cytosol. The induction of phytochelatin synthase (PCS) genes in plants has shown enhanced heavy metal(loid) stress tolerance by regulating PCs production (Eapen and D'Souza, 2005; Zhang et al., 2013). Synthesis of PC is catalyzed by the PCS. The expression of PCS genes in transgenic *N. tabacum* increases Cd uptake and tolerance (Wawrzyński et al., 2006). In contrast, transgenic *Arabidopsis thaliana* plants that lack PCS were hypersensitive to Hg and Cd (Memon and Schröder, 2009).

5.3.1.4. Phytoextraction by genetically modified plants. It is well known that several genes are involved in metal uptake, root-shoot translocation, and sequestration in vacuole. These genes can be transferred into candidate plants for improved phytoremediation traits using genetic engineering. Use of genetic engineering in plant sciences is also becoming very popular in modern era to introduce a desirable trait from one to other plant species (Eapen and D'Souza, 2005; Clark and Pazdernik, 2015). For phytoextraction, using this technique, plants can be engineered

- to adjust their rhizosphere to increase mobility of target metal,
- to modify metal speciation within plant system for better root-to-shoot translocation,
- to enhanced metal tolerance,
- to transfer metals into less toxic forms through binding with organic acids and thiol-rich chelators,
- to sequester heavy metal(loid)s in vacuoles.

The introduction of a gene in a specific species depends on the objective of mutation. For example, phytostabilization requires plant

engineering resulting in enhanced metal uptake and improved ability to sequester them in roots with limited translocation to aerial parts. In case of phytoextraction where increased uptake with enhanced translocation to areal parts is required can be achieved by genetically introducing genes responsible of (1) increasing metal absorption in root cells; (2) reducing root metal sequestration; (3) enhancing metal xylem loading and transferring them to shoots for storage in epidermal and mesophyll cells. Several key steps have recently been identified at the molecular level indicating the possibility of engineering metal hyperaccumulation in plants. Multi-fold increase in absorption, xylem loading, translocation to aerial shoot along with enhanced tolerance have been reported in bioengineered plants as compared to wild types, for some metal pollutants e.g. As in *Arabidopsis thaliana* (Guo et al., 2012), Zn in *Lactuca sativa* and *Brassica oleracea* (Barrameda-Medina et al., 2014), Pb in *Nicotiana glauca* (Gisbert et al., 2003) and Cd in *N. tabacum* (Khoudi et al., 2013).

According to Eapen and D'Souza (2005) and Marques et al. (2009), the possible areas of genetic manipulation are: phytochelatins (for enhanced tolerance and sequestration of metals in vacuoles), metallotioneins (for metal hypertolerance), phytosiderophores (for enhanced growth, e.g., nicotianamine aminotransferase), oxidative stress mechanisms (for enhanced heavy metal(loid)s tolerance such as glutathione-S-transferase and peroxidase), ferritin (for enhanced root-shoot metal transfer, example is iron-binding protein ferritin), metal transporters (to improve xylem loading and translocation such as Zn transporter-ZAT gene), metabolic pathways (for metal hypertolerance, e.g., ars C and γ-ECS genes), and phytohormones synthesis (for enhanced growth and biomass production such as giberellin). The genetic engineering of *A. thaliana* by an iron uptake ZIP transporter gene IRT1 renders Fe deficiency in these plants. Similarly, the high expression of HMA4 in *Anemone halleri*, encoding a P1B-type ATPase, has been reported to enhance root-to-shoot transport of Zn and Cd (Wong and Cobbett, 2009). Ma et al. (2008) showed that Lsi2 mutation, gene responsible for arsenite loading into the xylem, caused almost 50% decrease in As accumulation in rice shoot.

However, the most common strategy involves in genetic engineering is the induction of proteins responsible for metal detoxification (MTs, PC and GSH) for genetic manipulations (Eapen and D'Souza, 2005). The induction of genes like GSH1 and GSH2, PCS, glyoxalases (glyoxalaseI and II), ATP sulfurylase (APS), cystathionine synthase (CTS), GR and serine acetyltransferase (SAT) have shown enhanced heavy metal(loid) stress tolerance by regulating GSH and PCs production (Zhu et al., 2009; Zhang et al., 2013). Increased expression of PCS has been endeavored to enhance heavy metal(loid)s plant tolerance. For example, Hu et al. (2016) reported that overexpression of OsLEA4 in transgenic rice plants conferred enhanced tolerance to heavy metal(loid)s stress compared with the wild type rice plants. The induction of a modified bacterial GSH1 gene (S1ptTECS) in *A. thaliana* resulted in Hg tolerance. The expression of SAT, GSH1 and PCS genes in transgenic tobacco (*N. tabacum*) have increased Cd uptake in roots (Wawrzyński et al., 2006). Similarly, in the cytosol or chloroplast of *Populus canescens*, the overexpression of a bacterial GSH1 gene caused increased GSH level and tolerance to heavy metal(loid)s stress (Bittsánszkya et al., 2005). The lack of response in heavy metal(loid)s accumulation and tolerance could be due to the fact that PC synthesis is also governed by GSH production. Guo et al. (2008) showed that the overexpressing GSH1 and AtPCS1 in *Arabidopsis thaliana*, which encode g-glutamylcysteine synthetase (g-ECS) enhanced As accumulation and tolerance. Kumar et al. (2013) reported that GSH gene encoding in the Arabidopsis caused significantly enhanced heavy metal(loid)s accumulation and tolerance than the wild-type. Similarly, GSH1 and GSH2 genes encoding respectively in *Escherichia coli* and *Brassica juncea* resulted in higher concentrations of PCs, γGSH, and total non-protein thiols in mutant plants (Zhu et al., 1999), which increased tolerance to Cd. The increased tolerance and accumulation of Cd and As has been reported by simultaneous overexpression of AtPCS1 and GSH1 genes in mutant plants. Overexpression of PCS due to mutation may sometime causes hypersensitivity

to heavy metal(loid)s in some plants. For example, overexpression of AtPCS1 in transgenic Arabidopsis caused 12–25 fold higher accumulation of AtPCS1 mRNA, and enhanced PCs production 1.3–2.1 fold compared to wild-type plants (Lee et al., 2003). Similarly, transgenic Brassica and Nicotiana plants with enhanced GSH and PC levels due to overexpression of cysteine synthase, or APS–adenosine triphosphate sulphurylase showed higher Cd accumulation and tolerance than the wild-type plants (Verkleij et al., 2009).

5.3.1.5. Chelate assisted phytoremediation. The process of phytoextraction is often more time consuming than other physiochemical technologies, and it is usually limited by the low metal availability, uptake and translocation and plant biomass (Bhargava et al., 2012; Sheoran et al., 2016). Despite high levels in soil, most of the heavy metal(loid)s are sparingly soluble and available in soil (Mahar et al., 2016). Heavy metal(loid)s immobilization and binding with different soil constituents can significantly limit the potential for soil phytoextraction. For >10 years, chelate-assisted remediation of metals from contaminated soils has gained significant consideration as an economical alternative to conventional soil remediation techniques. Potential chelating agents include elemental sulfur, ammonium fertilizers, low molecular weight organic acids (LMWOAs), EDDS, EDTA, nitrilo triacetic acid (NTA), hydroxyethylene diamine triacetic acid (HEDTA) and humic substances (Shahid et al., 2012d; Saifullah et al., 2015). These chelating agents enhance the phytoextraction of Pb, Cd, Cu, Zn and Ni (Evangelou et al., 2007). For example, HEDTA increased Pb concentration in soil solution by 1000 times (Saifullah et al., 2009).

Application of chelates to soils modifies the main way of heavy metal(loid) uptake by the plants from the symplast to the apoplast. It is reported that chelating agent form metal-chelate complexes which are taken up by the plant, mainly through a passive apoplastic pathway (Zhao et al., 2010; Shahid et al., 2014c). Synthetic chelates are well known to assist the movement of metals towards plant root by releasing metals from soil solids to soil solution, thus enhancing metal phytoavailability. Owing to their neutral charge, metal-chelate complexes do not make bonding with negatively charged cell wall constituents such as polysaccharides or carboxyl groups of rhizoderm (Shahid et al., 2012a). Metal-chelate complexes are reported to be readily uptaken by majority of the plant species, especially, accumulator plant species (Blaylock et al., 1997; Saifullah et al., 2009). Several previous studies showed significant increase in plant accumulation of Pb, Cd, Ni, Cu, and Zn from contaminated soil in the presence of synthetic chelates (Usman and Mohamed, 2009). The effect of synthetic chelates on heavy metal(loid) uptake and accumulation depends on the type of metal and plant, and varies from no to >200-times higher accumulations (Saifullah et al., 2009; Shahid et al., 2012a).

Among the chelating agents, EDTA is considered the most effective and tested mobilizing amendment for the remediation of metals especially Pb (Shahid et al., 2014c). EDTA is known to enhance heavy metal(loid)s uptake from the soil to >1% of shoot dry biomass (Shahid et al., 2014c). Several factors are involved in EDTA-assisted enhanced uptake of heavy metal(loid)s by plants which include (i) enhanced phytoavailable metal concentration in soil, (ii) increased movement of metal-EDTA complex towards plant root, (iii) destruction of physiological barriers in roots by EDTA, (iv) limited binding between metal-EDTA complex and the negatively charged cell walls, and (v) enhanced xylem loading and transfer of metal-EDTA complex towards aerial parts (Saifullah et al., 2009; Shahid et al., 2014e). Recently, Cay et al. (2016) reported EDTA enhanced Cd phytoextaction by four ornamental plant species such as *Lonicera japonica*, *Althaea rosea*, *Dahlia hybrida* and *Salvia virgata* growing in the natural vegetation of Black Sea Region-Turkey.

Despite the potential effectiveness of chelate-assisted phytoremediation, some concerns have been voiced regarding the use of chelating agents such as EDTA owing to low decomposition, possible contamination of the ground water, adverse effects on the soil microorganisms (Lestan et al., 2008; Cay et al., 2016). Owing to high persistence

and related environmental issues associated with the use of EDTA, numerous other alternative chelating agents have been recommended by various authors. Of these, EDDS and NTA have been proposed as promising and environmental friendly mobilizing agents (Saifullah et al., 2015). Application of EDDS can reduce leaching of metals, but not fully prevented, compared to EDTA (Saifullah et al., 2015). For example, Marques et al. (2007) reported that the addition of EDTA or EDDS significantly increased water extractable Zn concentration up to 4.0- and 3.1-fold, respectively. Furthermore, LMWOAs such as tartaric, acetic, malic, citric and oxalic acids can also be used for heavy metal(loid) phytoextraction as an alternative to persistent synthetic chelates (Shahid et al., 2012a). LMWOAs, being natural root exudates, have very low persistence in soil which varies with the type of soil, root exudates and the microbial activity of rhizosphere (Abbas et al., 2015). LMWOAs are reported to enhance dissolution of heavy metal(loid)s in soil and uptake by high biomass accumulating plant species (Evangelou et al., 2007; Shahid et al., 2012a). Root exudates from *Zea mays*, *Nicotiana rustica* and *N. tabacum* increased extractable concentration of Cu, Cd and Mn (Mench and Martin, 1991). Chelate-assisted phytoextraction of heavy metal(loid)s from soil has not gained considerable acceptance because of its high leaching risk, relatively low efficiency and high cost.

5.3.2. Microbial assisted phytoremediation

As mentioned earlier, phytoextraction is usually limited by the low metal availability, uptake and translocation and biomass. Like chelate-assisted phytoextraction, microbial-assisted phytoextraction of heavy metal(loid)s also represents a promising method for contaminated soil remediation (Rajkumar et al., 2012). Microbial remediation refers to using microorganisms to induce the absorption, precipitation, oxidation and reduction of heavy metal(loid)s in the soil. Soil microbial association helps plants to grow well even under metal stress conditions. It is well established that plant growth-promoting and heavy metal(loid)s-resistant soil microorganisms can protect plants from the noxious effects of heavy metal(loid)s, or even increase metal uptake by hyperaccumulator plants (Weyens et al., 2009a, 2009b). Microorganisms-assisted remediation of heavy metal(loid)s can be via several mechanisms such as biosorption, intracellular accumulation, enzyme-catalyzed transformation, bioleaching and biomineralization, redox reactions (Lloyd, 2002). Metal-resistant rhizobacteria are capable to stimulate growth and metal accumulation by plants through the production of various substances i.e., monocyclopropane-1-carboxylate (ACC) deaminase and siderophores, indole acetic acid (IAA) (Rajkumar et al., 2012). Moreover, metal-resistant rhizobacteria enhance metal tolerance of host plants by inducing thiol compounds (Courbot et al., 2004), superoxide dismutase (Vallino et al., 2009) or metallothionein (Ramesh et al., 2009). Inoculation of Burkholderia sp. (Z-90) enhanced heavy metal(loid)s removal efficiency in soil by 31% for Pb, 32% for As, 44% for Zn, 37% for Cd, 52% for Mn and 24% for Cu (Yang et al., 2016). Ma et al. (2015) reported that *Sedum plumbizincicola* significantly enhanced Cd uptake (43%), whereas Bacillus sp. (E1S2) enhanced the Zn accumulation (18%) in *Sedum plumbizincicola*. Moreover, microbial association causes several other beneficial effects on plant growth such as stomatal regulation, osmotic adjustment, and enhanced uptake of minerals (Compant et al., 2005).

Soil bacteria can enhance metal mobility and bioavailability in soil via several ways, such as lowering soil pH, producing plant growth promoting and metal-chelating compounds such as siderophores, organic acids, biosurfactants and by altering soil redox conditions (Ahemad, 2014; Ullah et al., 2015). Bacteria such as *Bacillus mucilaginosus* (K-solubilizer), *Bacillus megaterium* (P-solubilizer) and *Azotobacter chroococcum* (N-fixer bacteria) can lower soil pH value, possibly by secreting LMWOAs, thereby increasing the bioavailability of Zn, Pb and Cd (Chen et al., 2005). However, the effect of soil microorganisms on metal solubility and uptake by plants is not always similar (Ma et al., 2015). Soil microorganisms may increase or decrease metal uptake by

plants (Ahemad, 2014; Ma et al., 2015). Indeed, certain microbial processes can increase metal solubility and bioaccumulation, whereas other processes may cause metal immobilization, thus decreasing their phytouptake (Gadd, 2010). Saravanan et al. (2007) reported increased solubilization of Zn compounds by microorganisms by producing 5-ketogluconic acid. Braud et al. (2009) showed 113% enhanced exchangeable Pb fraction in soil with *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Abou-Shanab et al. (2006) reported *Microbacterium arabinogalactanolyticum* assisted 15-folds increase of extractable Ni concentration in the soil. *Paxillus involutus* enhanced the concentrations of extractable Zn, Cd, Pb and Cu respectively by 1.33, 1.22, 1.33 and 1.11 times compared to non-bioaugmented soil (Baum et al., 2006). Sheng et al. (2008) observed that Pb accumulation increased by 131% with *P. fluorescens* and 80% with Microbacterium sp. in the shoots of *B. napus*. Xiong et al. (2008) observed significant increase in the uptake of Zn, Cd, and Pb by *Sedum alfredii* by rhizosphere microorganisms.

6. Comparison of remediation techniques for their applicability and selection

There exist several important factors which can affect the selection and applicability of available soil clean-up technologies. These factors include: (i) cost involved, (ii) time required, (iii) effectiveness under high metal(loid)s contamination, (iv) general acceptance and commercial availability, (v) long-term effectiveness, and (vi) applicability to multi-metal contaminated sites (Table 4).

6.1. Economic considerations of remediation techniques

Management of soil pollution is a major economic challenge worldwide. In addition to societal and environmental acceptability aspects, cost involved is the key factor determining the success and practical application of remediation technology in the field conditions. Studies regarding economic aspect of soil remediation with heavy metal(loid)s are scarce. Generally, various factors contribute to the cost involved for remediation of polluted site including those related to contaminated site (such as type and depth of soil, depth of groundwater, possible migration pathways and purpose of remediation), the heavy metal(loid)s (such as type of metal, and concentration of metal in soil) and the remediation technology (various technologies have different requirements and cost of action).

Physical remediation methods generally require large amount of manpower and material resources. Excavation is generally the most expensive remediation approach when large amount of soil has to be removed or disposed. Chemical remediation (immobilization and soil washing) is a cost-effective technique compared to physical remediation methods. The amount of chemicals required in heavy metal(loid) immobilization or soil washing is generally not very high due to comparatively low applied doses of chemical extractants or immobilizing agents such as EDTA. However, the cost of chemical remediation of soil varies with the type of metal and soil as well as that of chemical extractants or immobilizing agents. For example, soil washing is easy for Cd than Pb due to immobile nature and high affinity of Pb for soil constituents. Similarly, soil washing requires more chemicals for clayey soil than sandy soils due to strong binding of metals in clayey soils. In case of extractants or immobilizing agents, applied levels/concentrations of synthetic chelates such as EDTA are 10–50 times higher than natural ligands LMWOAs or humic substances (Shahid et al., 2012a).

Bioremediation is relatively economical compared to physical and chemical remediation techniques. All the types of bioremediation generally harnesses natural processes and treat the metal contaminated sites in place without any excavation or physical removal, thereby reducing the cost of site clean-up. Besides, in some cases bioremediation can remove or contain heavy metal(loid)s without any human involvement and this natural attenuation results in considerable cost savings.

Table 4
Comparison of different soil clean-up methods.

Techniques	Process involved	Advantages	Limitations	Applicability	Acceptance	Multi-metal sites	Time required
Physical remediation							
Soil replacement	Excavating contaminated soil and replacing by non-contaminated soil	Can effectively isolate HM from contaminated site. Effective for highly contaminated soils.	Large in working volume, costly, production of dangerous waste and negative effect on soil	Small scale but long term	Very low: limited to highly contaminated soils	Effective	Comparatively very less
Soil isolation	Isolating the contaminated soil from the uncontaminated soil using subsurface barriers	Prevent off-site transport of heavy metals, Effective for highly contaminated soils.	Costly, soil clean up still needs further engineering measures, effectiveness varies with the type of subsurface barrier	Small scale, and short to long term	Very low: limited to highly contaminated soils	Effective	Comparatively very less
Vitrification	Reduction in metal bioavailability by forming vitreous material using high-temperature	Easy application, applicable to variety of contaminants	High cost due to energy requirement	Small scale but long term	Very low	Effective	Comparatively very less
Electrokinetic remediation	Removal of HM from soil via electrophoresis, or electro-migration by applying DC-voltage	Easy application, economically effective, do not destroy the nature of soil	Requires soil with low permeability, pH needs to be controlled	Small scale but long term	Very low	Effective	Comparatively very less
Chemical remediation							
Immobilization	Reduction in metal mobility and bioavailability by applying immobilizing amendment, and forming stable and immobile complexes via adsorption	Fast and easy applicability, relatively low costs, covers a broad spectrum of inorganic pollutants	Temporary solution and permanent monitoring is necessary	Small to medium scale and short-term	High public acceptability	Can be effective. Depends on the type of soil, metal and immobilizing amendment	Less to medium
Soil washing	Removal of heavy metals from soil by extractants (organic or inorganic) and forming stable and mobile complexes	Cost-effective, completely removes metals, meets specific criteria and reduces long-term liability	Washing extractants may cause environmental issue, effectiveness varies with soil, metal and extractant type	Small scale but can be long term	Medium to high public acceptability	Can be effective. Depends on the type of soil, metals and mobilizing amendment	Less to medium
Biological remediation							
Phytovolatilization	Heavy metal uptake by plants from soil and release in vapour form to atmosphere	Economical and less disruptive	Restricted to volatiable metals, may cause other environmental issues, no control after metal release to atmosphere	Small to medium scale and long-term	Low-medium public acceptability	No	Very high
Phytostabilization	Use of plants to decrease metal bioavailability and mobility in soils via sequestration in plant roots	Economical, less disruptive	Temporary solution, effectiveness varies with soil, plant and metal type	Small to medium scale and short-term	Medium public acceptability	Very low	Very high
Phytoextraction	Use of hyperaccumulator plants to uptake, translocate, and concentrate heavy metals from soil to the aboveground harvestable plant parts	Highly economical, ecofriendly, less disruptive	Effectiveness depends on growing conditions, tolerance of the plant, bioavailability of metals in soil. Metal accumulator plants are generally very less in number	Large-scale and long-term	Highest public acceptability	Generally very low except for some plants	Very high
Chelate assisted phytoextraction	Use of organic and inorganic ligands to enhance phytoextraction capacity of plants	Low time of remediation, enhance metal uptake and translocation	Costly, can be disruptive, effective for low-moderately contaminated soils, groundwater contamination risk	Small to medium scale and long-term, low to moderate levels of metal	Very high public acceptability	Generally very low but more effective than phytoextraction alone	Very high but less than phytoextraction alone
Microbial assisted phytoextraction	Use of microorganisms to enhance phytoextraction capacity of plants	Economical, low time of remediation, enhance plant growth and metal uptake and translocation	Depends on microorganism, soil, plant and metal type	Large-scale and long-term	Very high public acceptability	Generally very low but more effective than phytoextraction	Very high but less than phytoextraction alone

For example, since phytoremediation can operate with minimal maintenance after its establishment in the field, therefore phytoremediation costs almost ten-fold less than engineering-based methods (Marques et al., 2009). Likewise, post-clean-up cost is very low for bioremediation methods because these methods causes minimum site disturbance compared with conventional physical clean-up methods.

Blaylock et al. (1997) estimated cost for remediating one acre of Pb contaminated soil. They reported that the cost of phytoremediation

(€140–230) is 50–65% less compared to conventional treatments such as excavation and landfill (€460). According to the US EPA (2004), phytoremediation costs about US$25-US$100 per ton of soil as compared to vitrification 300–500 US$/ton and flushing 75–210 US$/ton. Schnoor (1997) compared the cost of different remediation techniques for heavy metal(loid) contaminated sites, and reported phytoremediation to be the most cost effective (US$10–35) than in situ bioremediation (US$50–150), stabilization/solidification (US$240–340), soil venting (US$20–220), solvent extraction (US$360–440), soil washing (US$80–200) and incineration (US$200–1500). Cunningham and Ow (1996) showed that the estimated 30-years cost for cleaning-up 12-acre of Pb contaminated soil was €185,000 for phytoextraction, €555,000 for soil capping, €5,833,000 for soil washing and €11,100,000 for excavating and disposal. Salt et al. (1995) reported that to remediate one acre of sandy loam soil upto a depth of 50 cm, phytoextraction costs (€55,000 to €92,500) almost 4–7 times than soil excavation (€370,000).

Phytoextration of heavy metal(loid) contaminated soil in combination with the use of chelating agents can further reduce the cost of phytoremediation by enhancing heavy metal(loid) plant uptake and translocation upto 200-fold. Chaney et al. (2002) reported that phytoextration of Pb contaminated soil using EDTA (10 mmol EDTA kg^{-1}) would cost approximately US$30000 ha^{-1} to attain Pb level of 10 g kg^{-1} dry weight. EDTA reduces the cost of remediation because field scale application of EDTA is not very high (2.5 to 10 mM) (Shahid et al., 2012b). Moreover, other costs of heavy metal(loid) remediation such as designing, maintenance, installation and operation remain same as for phytoextraction without EDTA application. The extent to which cheating agents reduces the cost of phytoremediation varies with soil, metal and plant type (Shahid et al., 2014c). This is because effect of different chelating agents on heavy metal(loid) mobility, bioavailability, uptake and translocation varies with soil, metal and plant type. Application of chelating agents can also minimize the time required for remediation and thereby reduces the cost of remediation. Similar to chelate-assisted, microbial-assisted phytoextraction or use of genetically engineered plants can further reduce cost of remediation by enhancing metal accumulation and decreasing remediation time. Moreover, operational costs remain the same as for phytoextraction alone.

6.2. Time required for different remediation techniques

Physical remediation methods require minimum time among different remediation technologies available. Indeed, physical remediation involves either replacement or removal of soil or installation of barriers. Therefore, physical remediation methods are considered most effective with respect to the time required for complete remediation of polluted sites. Chemical remediation techniques are also fast but their effectiveness varies with metal, soil and chemical type. Certain chemicals can immobilize or washout great quantities of metals compared to others but the effect is greatly dependent on soil conditions especially soil pH.

In case of phytoremediation, major limitation for cleanup of heavy-metal-polluted soils is the long time period required to completely remediate the sites (Bhargava et al., 2012). Several factors influence the time required for complete or targeted remediation of a contaminated sites. These factors include metal levels found in the contaminated soil, heavy metal(loid) mobility in soil, translocation to aerial parts, target metal final concentrations, plant biomass and rates of plant growth (Shahid et al., 2012a). Time required for complete remediation of contaminated sites may also vary with the type of soil, plant and metal as well as the soil depth of remediation required (Arshad et al., 2008; Rajkumar et al., 2012). Despite high levels in soil, most of the heavy metal(loid)s are only low-soluble and phytoavailable (Rascio and Navari-Izzo, 2011). Moreover, majority of the hyperaccumulators are slow growing with low plant biomass and therefore require long time period to completely remediate the soil. Similarly, the objective of

remediation also affects the time required for remediation due to variation in geochemical background values of heavy metal(loid)s fixed by regulations of a country.

Chelates- and microbial-assisted as well as use of transgenic plant species can greatly reduce the time required for complete remediation of contaminated site. For example, several studies showed that application of EDTA can enhance bioaccumulation index of metal(loid)s in plants by a factor of 200 times. Nevertheless, it is not true for all the metals and plants or under all plants growth conditions. Shahid et al. (2012b) modelled the phytoremediation efficiency of pelargonium cultivated on a multi-metal contaminated site (Pb, As, Cd, Zn and Cu levels in the bulk soil were 39,250, 1060, 706, 3995 and 2085 mg kg^{-1} DW respectively). They showed that complete remediation of this site may take >35 years at an annual removal of 2.7% by pelargonium. They estimated that EDTA can further minimize this time to 7 years, if the metal(loid) accumulation by pelargonium is enhanced by a factor of 5-times. Nevertheless, the decrease in time required for complete clean-up of heavy metal(loid) contaminated soil might be affected by the potential toxicity of EDTA and/or metal-EDTA complexes to plants and microorganisms, thereby decreasing plant biomass and, consequently, reduced metal(loid)s bioaccumulation. It means, assisted phytoextration needs proper management.

6.3. Applicability of different remediation techniques to high metal and multi-metal contamination

During physical remediation methods, the entire contaminated soil is either replaced or isolated, therefore this method can be applied to soils polluted with more than one heavy metal(loid)s at high level. Application of physical remediation is considered to be most effective for highly contaminated sites containing different kinds of pollutants. In case of chemical remediation methods (immobilization and washing), effectiveness to clean-up soils containing high levels of more than one heavy metal(loid)s depends on the types of metals, soils and chemical extractants. Some extractants such as EDTA has high affinity for several metals under wide range of soil conditions. Therefore, soil washing using EDTA can be effective for high metal and multi-metal contaminated sites. On the other hand, efficiency of certain chemical extractants such as LMWOAs varies greatly with soil conditions (especially with soil pH) and metal type. Therefore these extractants cannot be applied to high metal and multi-metal contaminated sites. Similarly, heavy metal(loid) immobilization using chemical reagent may not be feasible for multi-metal contaminated sites as there are very rare reagents having immobilization capacity for all kinds of metals.

Bioremediation can be applied to multi-metal contaminated soils because some hyperaccumulators can grow and accumulate high levels of various metals. However, majority of the hyperaccumulator plants generally uptake only a specific metal, and are not appropriate to be applied in the field under multi-metal contaminated soil conditions. Bioremediation is generally restricted to low or moderately contaminated sites because majority of the plants and microorganisms cannot survive under severe heavy metal(loid) levels in soil. Phytoextraction is effective only for soils containing easily available heavy metal(loid)s. Some heavy metal(loid)s, such as Cr and Pb are highly immobile in soils, and their phytoextraction is often limited. The efficiency of phytoextraction is also limited by the depth accessible for plant root growth (generally 30–90 cm). However, the application of chelating agents may enhance the ability of phytoextraction to remediate multi-metal contaminated sites due to increased heavy metal(loid) bioavailability is soil, and accumulation as well as translocation inside plants.

6.4. Long-term effectiveness of different remediation techniques

Physical remediation methods (soil replacement and electrokinetic remediation) can completely remove heavy metal(loid)s from contaminated soil. Therefore, there is very little long-term liability using these

physical remediation methods. These methods can be highly effective in term of long-term effectiveness due to complete removal of heavy metal(loid)s. However, soil isolation, subsurface barriers and vitrification do not have long-term effectiveness, because under these remediation techniques, the contaminants are still in the site and need continuous maintenance/conservation. Chemical remediation methods (immobilization and washing) are not highly effective for long period of times owing to different limitations associated with these methods. For example, heavy metal(loid) immobilization is only a short-term solution because the metals are still retained in the soil. The immobilized heavy metal(loid)s may become active (mobile) when soil physicochemical properties change, therefore, these remediation techniques require permanent monitoring. Chemical remediation has high invasivity to the environment and danger of the release of additional contaminants to the environment.

Phytoextraction has long-term effectiveness because the contaminants are removed from the site and there is no need of disposal sites. All the types of bioremediation generally harnesses natural processes and treat the metal contaminated sites in place without any excavation or physical removal. However, storage, treatment and placement of contaminated plant biomass may affect long-term effectiveness of phytoremediation. One of the most safe and economical approaches of utilizing the contaminated plant biomass is its use as a source of energy. Combustion and gasification are important approaches used for producing electric and thermal energies from contaminated plant biomass. Another operational method is thermochemical processing of contaminated plant biomass on pyrolysis. Similarly, ashing of plant biomass can produce bio-ores especially after the phytomining of precious metals.

Phytostabilization of metals does not reduce the concentration of the contaminants but the contaminants are left in place. Therefore, the site requires regular monitoring to ensure that the optimal stabilizing conditions are sustained. The conversion of heavy metal(loid)s to volatile form and subsequent release into the atmosphere by plants during phytovolatilization is restricted by the fact that the process does not fix the contaminant completely; only transfers pollutants from soil to atmosphere from where it can be redeposited.

Similarly, chelate-assisted remediation of heavy metal(loid)s can also cause off-site movement of heavy metal(loid)s (Shahid et al., 2014c). During EDTA-assisted solubilization of heavy metal(loid), plants can absorb only a limited fraction of mobilized metal and the remaining amount of heavy metal(loid)s are generally leached down (Saifullah et al., 2015). Therefore, it is highly necessary that application of chelating agents is limited to their lowest level for ecological and economic benefits. The key necessities under chelate-assisted remediation are optimal methods and applied levels as well as rapid degradation following their application. Presence of EDTA in soil can negatively affect soil enzymatic and microbial activities as well as soil fungi, and plants. EDTA is also reported to deteriorate the chemical properties and physical structure of soils. Some authors reported that split application of EDTA or in combination with other easily degradable organic ligands like NTA or EDDS can decrease metal leaching (Saifullah et al., 2009). Moreover, use of easily degradable organic ligands like NTA or EDDS can be better option for the remediation of metal polluted soil (Saifullah et al., 2015). Therefore, due to low degradability and long persistence in environment, chelate-assisted remediation does not have long-term effectiveness.

6.5. General acceptance/application of different remediation techniques

The application and acceptance of different remediation methods depends on various factors such as area of contamination, type and amount of contaminant, purpose of contamination etc. Physical remediation methods are generally applicable to small-scales contaminated sites such as residential areas or small sites near a factory etc. These methods also have high application to multi-metal contaminated sites.

Physical remediation methods are generally not applicable to agricultural areas owing to their destructive nature and lose of soil fertility. On the other hands, chemical and bioremediation methods can be applied to large areas (especially bioremediation) such as agricultural fields owing to their non-destructive nature. Moreover, bioremediation requires the same agronomic practices and is easy to apply to agricultural areas.

7. Future development for clean-up of heavy metal(loid)s polluted sites

Financial and technical implications and complexities have made soil clean-up a challenging task. Compared to physico-chemical methods, bioremediation of heavy metal(loid)s contaminated soils shows great advantage with respect to environmental safety, field-scale application, public acceptability and cost involved. Although, field-scale application of bioremediation faces several practical and technical limitations, but following proper management and advancement in this field, these limitations can be minimized. This is because studies regarding bioremediation of heavy metal(loid)s contaminated soils field are increasing and its mechanism is clear.

The first way to use bioremediation is developing more systematic use on low-contaminated industrial or urban sites to reduce soil metal content and promote fertility. A second way is the use of bioremediation on the low-contaminated sites once the highly-contaminated soils have been excavated. The third approach can be the use of transgenic technology to improve bioremediation efficiency and effectiveness as well as field-scale application. Through the introduction of foreign resistant genes, the possibility to create an ideal plant species for clean-up of heavy metal(loid)s contaminated soil is feasible. Several researchers have proposed that establishing ideal crop hyperaccumulator in the future can be an ideal choice due to its feasibility and applicability in the field of which current emphasis is scarce. By mean of genetic engineering, ability of a plant to accumulate, translocate and detoxify heavy metal(loid)s can be significantly enhanced. An ideal transgenic plant species for phytoremediation must has ability to produce high biomass, appropriately competitive in harsh climatic conditions, good phytoremediation capacity, has a widespread and branched root system, capable to mobilize, absorb, transfer and sequester metals preferably in the aboveground parts, easy to harvest, and is suitable for genetic transformation. However, no plant is known to date that meets all these criteria. Establishing an ideal plant species for hyperaccumulation and hypertolerance is not possible until the availability of complete hyperaccumulator genome sequences. Our understanding of hyperaccumulator genome sequences and evolution could be greatly improved by merging ecological and molecular genomics. One of the important research avenues is the pursuit for signatures of latest adaptive evolutions across candidate genes for metal hypertolerance or hyperaccumulation. Using genetic engineering, several heavy metal(loid)s resistant genes responsible for enhanced expression of specific protein have been introduced successfully into plant cells. Due to over expression of specific protein and natural chelators (MTs, PCs and organic acid) in transgenic plants, their ability of heavy metal(loid)s accumulation, translocation in xylem and tolerance is improved. The different processes/mechanisms introduced in plants via transgenic technology includes excretion of certain transporter proteins, binding and transportation of specific metal ions and volatilization of heavy metal(loid)s by encoding special oxidoreductase such as MerA and MerB.

In addition to the use of transgenic plants, plant-microbes associations, in many cases, are highly efficient in absorbing, accumulating, translocation and tolerating heavy metal(loid)s because of their capacity to produce various substances (IAA, ACC etc.). Therefore, finding and establishing an appropriate kind of symbiotic bacteria using genetic engineering can be highly effective in bioremediation. Another major advantage of microbial association can be the production of some

necessary nutrients and even plant growth hormones by microorganisms. Moreover, despite environmental concerns associated with chelate-assisted bioremediation, several studies reported/suggested techniques to overcome environmental concerns of synthetic chelates such as split application, combined application, application at proper stage of plant growth, sub-irrigation drainage systems and application to loamy soil. These management practices of using chelate-assisted remediation can be jointly applied with transgenic plants and smicrobes assisted heavy metal(loid)s remediation. Besides, the role of agronomic practices/strategies needs to be explored for increasing the biomass and thus phytoextraction potential of natural hyperaccumulators. Similarly, there is also need to pursuit the role of plant growth regulators (indolebutyric acid, cytokinins, gibberellic acid, naphthylacetic acid and indole-3-acetic acid) to increase the biomass potential of hyperaccumulating plants. Therefore, establishing an optimum soil + plant + microbes combination using transgenic technology can be a promising way in the future development. However, for judicial and environmentally safe application of optimum plant + soil + microbes combination, a full understanding of important processes and factors related to metal and chelate type, soil physico-chemical properties, and plant and microbe species and association is necessary.

8. Conclusions and perspectives

Soil contamination by heavy metal(loid)s is a worldwide problem, therefore effective remediation approaches are necessary. A number of remediation techniques are used for effective remediation of contaminated sites. In this review we compared the effectiveness of different remediation techniques generally used to clean-up contaminated soils. Technical and financial implications have made soil remediation a complex and difficult task. Most traditional soil remediation techniques do not offer acceptable solutions for the cleanup of heavy metal(loid)s polluted sites. Physical remediation methods can completely remove heavy metal(loid)s from contaminated soil but are destructive in nature and highly costly. These methods can only be applied to small area of soils. Chemical remediation methods are fast, simple, easy to apply, high public acceptability and relatively economical. However, these remediation methods are not eco-friendly because they have limitations of releasing additional contaminants to the environment. Phytoextraction is safe, least destructive, eco-friendly and cost-efficient remediation technique which allows soils clean-up over a large scale. However, still fundamental and field-scale research is needed in this field. Effectiveness of phytoremediation depends on the growing conditions, plant tolerance to metal and solubility of metals in soil. The process of phytoextraction is often more time consuming and is effective for low-to-moderate levels of heavy metal(loid)s.

Phytoremediation can be used effectively in combination with several other traditional remediation techniques; chelate assisted, microbial assisted and transgenic plants. Chelate-induced remediation of heavy metal(loid)s by high biomass species is the most efficient technique due to enhanced metal solubilization, uptake and translocation. The metal-chelate complexes have relatively low biodegradability and induce negative influence to soil physico-chemical properties, soil biota and plant. Chelate-induced enhanced leaching of metals also checks its practical application in field. Soil microbial association helps plants to grow well, protect plants from the noxious effects of heavy metal(loid)s along with increased metal uptake by producing various substances. Use of genetically engineered plants equipped with useful remediation traits according to the requirements of contaminated sites can be highly effective tool to support this technology for on filed application. Transgenic approaches have successfully improved phytoextraction potential of hyperaccumulators mainly by the employment of metal transporters, and improved production of antioxidative enzymes and metal-detoxifying chelators. Moreover, judicial and environmentally safe application of chelating agents along with genetically engineered plants can further improve the effectiveness of this technique. In point of the fact, and since bioremediation generally addresses heterogeneous and multi-phasic environment (soils), effective bioremediation depends on an interdisciplinary approach that integrates the work of soil chemists, plant biologist, geneticists, microbiologists, and environmental engineers.

This review revealed that cost involved, time required, long-term effectiveness, general acceptability, applicability to high metal and multi-metal contaminated sites are the key factors that affect the applicability and selection of remediation technologies. The cost of heavy metal(loid) contaminated soil by phytoremediation can be minimized by gaining a better understanding of mechanisms/processes involved in remediation, and the various options available at different steps during remediation.

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