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Review

Soil Pollution and Plant Efficiency Indices for Phytoremediation of Heavy Metal(loid)s: Two-Decade Study (2002–2021)

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Abstract: This paper reviews research on phytoremediation (2002–2021), particularly for the estimation of plant efficiency and soil pollution indices, examining the extraction of metals from soil and plants growing under both artificial (spiked with specific metal) and natural conditions. From the analysis of >200 published experimental results, it was found that contamination factor and geo-accumulation index as well as translocation and bioconcentration factors are the most important soil pollution and plant efficiency metrices, respectively, which are gaining importance to assess the level of metal pollution and its transfer from soil to plant to find a better metal clean-up strategy for phytoremediation. To access the metal concentration, it was found that the most widely accepted extractants to dissolve and extract the metals from the soil and plant were HNO_3 and $HClO_4$ (mainly in 5:1; *v*/*v* or 4:1; *v*/*v*), which are used both in natural and artificial metal contamination studies. Moreover, plants such as *Pteris vittata*, *Monochoria korsakowi*, *Lolium perenne*, *Festuca rubra*, *Poa pratensis*, *Ricinus communis*, and *Siegesbeckia orientalis* can act as hyperaccumulators under both natural and artificial experiments and can be directly implemented into the fields without checking their further efficiency in phytoremediation.

Keywords: hyperaccumulator; natural and artificial contamination; bioconcentration factor; translocation factor; enrichment and contamination factor; geoaccumulation index; pollution load index; potential ecological risk index; metal extraction ratio; phytoextraction potential

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1. Introduction

An increase in metal concentration due to anthropogenic activities and natural processes results in water, air, and soil pollution. Heavy metals (HMs) are non-biodegradable, which easily mobilize and accumulate in the environment and thus pose risks to human health and its surroundings. In addition, HMs slowly contaminate varieties of land which can be used for commercial purposes such as agriculture, forestry, nursery, horticulture, etc. However, these metals and metalloids slowly enter the food chain and result in oxidative stress, enzyme disruption, chronic anemia, endocrine disruption, autoimmune and carcinogenic diseases, allergic dermatitis, etc. in humans [1–3].

For many years, research has been carried out to decontaminate or reduce the metal contamination by means of physical, chemical and biological methods. However, physical and chemical methods are costly and not environmentally safe, resulting in need for a new and safer technology called "phytoremediation" [1,4–7]. Phytoremediation is a biological method which found popularity in the late 1990s. It is one of the safest, eco-friendly and cost-effective technologies and helps to control the metal problem without creating adverse effects on the ecosystem [5,8].

From time to time, there has been comprehensive reviews reported every year on the progress of research on phytoremediation such as [2,9–14]. Most of the reviews were focused on the search of plants-hyperaccumulators and the mechanism involved in metal transfer from soil to plant. Effective research is ongoing in the world for the use of plant varieties which can help in the remediation of metal-contaminated sites. The research was focused on metal-tolerant, hyperaccumulator, accumulator or excluder plant species on naturally contaminated substrates or in artificially metal spiked soil to remove, stabilize or prevent the leaching of toxic metals in the environment [1,15,16]. Some of the plant efficiency factors that help to assess phytoremediation were also studied; however, they vary from one to another. The extractants used to study the bioavailable and total metal content in soil and plant are also quite variable, which creates strong confusion while choosing the right extractant for the recovery of metal from substrate. Moreover, no research reported all the various soil pollution indices and plant efficiency metrics along with various extractants in a single place to exactly understand the metal pollution level in soil and plant to implement the best methods for its remediation. To enhance the efficiency of phytoremediation, there is a great need to understand and integrate both plant metrics and soil factors. This approach will also give clear ideas to early career researchers, volunteers, and industrialists to study the specific parameters

We put forward the hypothesis that our review will form the scientific basis for the unification of methodological approaches to assess the behavior of metals in the soil–plant system and ensure terminological uniformity. In order to understand the various factors, which play a vital role in phytoremediation, a detailed study of the past two decades (2002–2021) of published articles is conducted to gather a depth of knowledge on soil factors and plant metrics, which must be considered while planning and executing a phytoremediation under artificial laboratory-based experiments and/or for naturally contaminated sites. In the present study, >200 high-quality research works on metal contamination (natural and artificial) were reviewed to understand whether the trend and concept of phytoremediation are going in an appropriate direction for the use of plant metrics and soil factors, the use of single or mixed extractants, sequential and total metal extracts in soil, and the total metal content in plants.

2. Phytoremediation

Phytoremediation is novel, sustainable, cost-effective, promising, solar-driven, eco-friendly technology used for the decontamination of metal-contaminated or enriched sites by removing, destroying or sequestering the hazardous metals using varieties of plant species growing naturally (in situ) or under controlled conditions (ex situ) [17–29]. The HMs are non-degradable and remain for a long time in the environment. The only possible and most effective method is to sequester them into the plant and use harvest to extract metals from plant parts [9,30,31]. However, the ability to accumulate HMs varies significantly between species and cultivars within a species [2,7,32]. This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air [31] and can be used for the removal of toxic metals from the biosphere [33–35]. Phytoremediation is highly applicable for the low to moderately metal contaminated very large fields where other physical and chemical methods are impracticable for a long period of time [6]. Phytoremediation is often referred as botanical bioremediation or green remediation also [9,36]. Out of the various techniques involved in remediation, phytoremediation was found to be least expensive (US$ 5–40/ton) as reported by [37,38].

The main types of phytoremediation involved in the removal of HMs from the contaminated site are:

- *Phytosequestration*: plants which accumulate metals mainly in their roots;
- *Phytoextraction*: plants which transfer metals from soil to the aerial part;

- *Phytodegradation*: plants which help in the biotransformation of pollutants inside them;
- *Phytovolatilization*: plants which help in the volatilization of metals from its leaves; and
- *Rhizoremediation*: exudates from plants which help the bacteria for the biodegradation of contaminants.

3. Experimental Studies Using Heavy Metal-Contaminated or Spiked Soil

Hundreds of papers in peer-reviewed journals were studied thoroughly to categorize the study pattern in the field of phytoremediation in metal-contaminated soil between 2002 and 2021. Many studies were carried out on the naturally contaminated or non-spiked metallic-ferrous waste; however, there were also numerous reports from artificially metal-spiked soil. Nevertheless, limited studies were performed together by both naturally and artificially contaminated soil to exactly understand the success of phytoremediation under lab and field conditions, which is reported in the present study.

3.1. Naturally/Non-Spiked Metal-Contaminated Substrate Studies

Non-spiked metal-contaminated soils are those substrates that do not involve any direct addition of metals in salt form from outside. These soils were mainly contaminated because of natural weathering lithology or industrial activities including mining. This kinds of soils are generally deficient in carbon (C) and nutrient content (N, P, K, Ca, Mg, Zn, Mn) as well as in biological agents (enzymes and microbes). Additionally, these soils are characterized by unfavorable physical properties (water-holding capacity, porosity, grain size, bulk density, etc.) with a variable range of multiple metals from moderate to very high concentration. The samples were normally collected from the contaminated sites and used for field, plot, greenhouse, and glasshouse-based studies. Some pot studies were also conducted in the field without providing controlled conditions. On the other hand, some were conducted in greenhouses or glasshouses under controlled conditions by providing optimum requirements using the natural substrates (soil) collected from the metal-contaminated field. It was found that vast research lies in the search and identification of naturally growing, colonized plants, herbs, shrubs, and trees, which are effective in metal accumulation, exclusion and hyperaccumulation and testing them on non-spiked metal-contaminated soil. Apart from these properties, researchers are also in search of high biomass plants which can improve the phytoremediation efficiency. A list of important research carried out for contaminated areas during the past two decades is presented in Table 1.

Table 1. List of metal(loid)s accumulating, excluding and hyperaccumulating plant species studied for the substrate remediation under non-spiked soil condition.

Experiment(s)	Metal(s) Studied	Concentration of Metal(s)	Type of Accumulation	Plant(s)	Remediation Status and Capacity	Type of Remediation	References
P	Cd, Cr, Cu, Ni, Pb, Zn	NS	NS	*Brassica juncea, B. napus, B. carinata, R. sativa*	*Brassica* species demonstrated a similar performance for Cd and Zn, whereas for other elements, the bioconcentration factor was very low	NS	[39]
F	Fe, Mn, Zn, Cu, Pb, Ni, Cd	NS	Excluder	*Blumea lacera,* *Avera aspera,* *Borrhevia repens,* *Cynodon dactylon*	*Cynodon dactylon* can be used for remediation; all studied plants are useful in in situ biostabilization	BS	[40]
F	Pb, Cu, Zn	NS	Accumulator	*Phyla nodiflora,* *Gentiana pennelliana,* *Cynodon dactylon,* *Bidens alba var. radiata,* *Rubus fruticosus, and 29 others*	*Phyla nodiflora* was the most efficient in accumulating Cu and Zn in its shoots, while *G. pennelliana* is a potential phytostabilizer (Pb, Cu and Zn)	PS, PR	[41]
F	Cu, Zn, Mn, Cr, Pd	NS	Accumulator	*Calotropis. procera,* *Sida acuta,* *Ricinus communis,* *Cassia fistula*	*Sida acuta and Cassia fistula* are suitable for decontamination of metals	PE	[42]
P	Cd, Zn	NS	Accumulator	*Salix caprea,* *S. fragilis,* *S. × smithiana,* *S. × dasyclados,* *Arabidopsis halleri*	*Salix × smithiana* suitable for PE	PE	[43]
F	Zn, Pb, Cd	NS	Accumulator	*Vertiveria zizanioides,* *Dianthus chinensis,* *Rumex K-1 (Rumex upatientia × R. timschmicus),* *R. crispus,* *R. acetosa,* *Viola baoshanensis,* *Sedum alfredii*	EDTA applied. Phytoextraction rates of *V. baoshanensis* and *S. alfredii* for Cd and Zn were 0.88% and 1.15%, respectively. *Rumex crispus* is best for Cd and Zn phytoextraction	PE	[44]

Table 1. *Cont.*

Experiment(s)	Metal(s) Studied	Concentration of Metal(s)	Type of Accumulation	Plant(s)	Remediation Status and Capacity	Type of Remediation	References
F	Zn, Cu, Pb, Ni	NS	NS	*Typha latifolia,* *Fimbristylis dichotoma,* *Amaranthus defluxes,* *Saccharum spontaneum,* *Cynodon dactylon*	*Typha latifolia* and *S. spontaneum* can be used for bioremediation: rhizofiltration for Zn, Cu, Pb, Ni and phytoextration for Mn	RF PE	[45]
F	Cr, Zn, Cd, Cu, Ni, Pb	NS	Excluder	*Stipa austroitalica,* *Dasypyrum villosum* *Carduus pycnocephalus,* *Silybum marianum,* *Sinapis arvensis*	*Carduus pycnocephalus, S. marianum* and *S. arvensis* act as metal excluder	PS	[46]
F	Cd, Zn, Pb	NS	Accumulator	*Populus tremala X P. tremuloides,* *Acer campestre,* *Acer pseudoplatanus,* *Alnus glutinosa,* *Betula pendula,* *Fraxinus excelsior,* *Prunus avium,* *Quercus robur,* *Salix caprea, and 8 others*	Salicaceae family can accumulate 950 mg Zn kg^{-1} DW	PE	[47]
F	Cu, Ni, Fe, Mn	NS	NS	*Triticum aestivum*	Citric acid and NH_4OAc are the good indicators of Cu availability	NS	[48]
F	Sr	NS	NS	*Euphorbia macrocleda,* *Verbascum cheirunthifolium,* *Astragalus gummifer*	Shoots of these plants are good bioaccumulators. *Astragalus gummifer* can be useful either for the cleaning of Sr from contaminated soils or for phytoremediation	PR	[49]
PL	Mn	NS	Excluder, Accumulator	*Equisetum hyemate,* *Telypteris kunthii,* *Cnidoscolus multilobus,* *Platanus mexicana,* *Solanum diversifolium,* *Asclepius curassavia,* *Pluchea sympitifolia*	Re-vegetate and stabilize Mn tailings: *E. hyemate* and *T. kunthii* are excluders (E) whereas *C. multilobus, P. mexicana, S. diversifolium, A. curassqavia, and P. sympitifolia* are accumulators (A)	PS	[50]

Table 1. *Cont.*

Experiment(s)	Metal(s) Studied	Concentration of Metal(s)	Type of Accumulation	Plant(s)	Remediation Status and Capacity	Type of Remediation	References
F	Fe, Mn, Zn, Cd, Cu, Pb, Cr, As	NS	Accumulator	*Spinacia oleracea, Raphanus sativus, Lycopersicon esculentum, Lepidium sativum, Peucedanum graveolens, Coriandrum sativum, Capsicum annum, Brassica oleracea var capitata, Solanum melongena, Hibiscus esculentus*	*Spinacia oleracea*, *L. esculentum*, *C. annum*, *B. oleracea var capitata*, *R. sativus* can accumulate As, Cd, Cr, Pb, Ni	NS	[51]
F	As, Fe, Mn, Cu, Co, Zn	NS	Hyperaccumulator	*Pteris vittata*	*Pteris vittata* is a hyperaccumulator of As and suitable for phytoremediation	PR	[52]
F	Fe, Zn, Pb and Mn	NS	Accumulator	*Scrophularia scoparia, Centaurea virgata, Echinophora platyloba, Scariola orientalis, Centaurea virgata, Cirsium congestum and 6 other species*	*Scrophularia scoparia* was the most suitable for the phytostabilization of Pb, *C. virgata*, *E. platyloba* and *S. orientalis* had the potential for the phytostabilization of Zn and *C. virgata* and *C. congestum* were the most efficient in the phytostabilization of Mn	PS	[53]
F	Fe, Cu, Pb, Mn, Ni, Zn, Cr, Cd	NS	Excluder	*Saccharum munja*	*Saccharum munja* is suitable for metal rehabilitation and stabilization	PS	[54]
F	Cu, Cd, Pb, Cr, Mn, Ni	NS	Accumulator	*Ipomea carnea*	NS	PR	[54]
F	As	NS	Accumulator	*Ranunculus trichophyllus, Ranunculus peltatus* subsp. *saniculifolius, Lemna minor, Azolla caroliniana, Juncus effusus, Callitriche lusitanica, Callitriche brutia Callitriche stagnalis, Fontinalis antipyretica*	The highest concentration of arsenic was found in: *C. lusitanica* (2346 mg kg^{-1} DW), *C. brutia* (523 mg kg^{-1} DW), *C. stagnalis* (354 mg kg^{-1} DW), *L. minor* (430 mg kg^{-1} DW), *A. caroliniana* (397 mg kg^{-1} DW), *R. trichophyllus* (354 mg kg^{-1} DW), and *F. antipyretica* (346 mg kg^{-1} DW). Callitriche family plants are accumulator.	PF	[55]

Table 1. *Cont.*

Experiment(s)	Metal(s) Studied	Concentration of Metal(s)	Type of Accumulation	Plant(s)	Remediation Status and Capacity	Type of Remediation	References
PL	Hg, Cd, As, Hg, Pb, Cr, Cu, Zn, Ni	NS	NS	*Brassica napus*	PE capacity is limited	PE	[56]
F	As, Fe, Mn, Pb, Zn	NS	Accumulator, Hyperaccumulator	*Plantago orbignyana*, *Lepidium bipinnatifidum*, *Sonchus oleraceus*, *Baccharis atifolia*	*Lepidium bipinnatifidum* is a phytoextractor, *P. orbignyana* is a Pb and Zn hyperaccumulator	PE	[57]
F	Cu, Fe, Pb, Zn	NS	Accumulator	*Taraxacum officinale*	Accumulates both in root and shoot	PE	[58]
F	Ni, Cu, Zn, Cd, Pb	NS	Accumulator	*Ricinnus communis*	*Ricinnus communis* is suitable for phytostabilization and revegetation	PS	[59]
F	Cr, Cu, Ni, Pb, Cd	NS	Accumulator	*Calotropis procera*, *Croton bonplandianum*, *Cyperus rotundus*, *Datura stramonium*, *Euphorbia hirta*, *Parthenium hysterophorus*, *Phyllanthus amarus*, *Sida cordifolia*, *Solanum nigrum*, *Solanum xanthocarpum*, *Spinacia oleracea*, *Tridax procumbens*	EF > 1 for all the weed suggests its use for the phytoremediation and restoration of land contaminated toxic metals	PR	[60]
F(GL), P	As	NS	Hyperaccumulator	*Pteris vittata*	NS	PE	[61]
F	As	NS	Accumulator	*Kandelia obovata*	NS	NS	[62]
F	Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, Zn	NS	Accumulator	*Tanacetum vulgare*	*Tanacetum vulgare* accumulates Cr and Fe in roots. Bioindicator of Cd, Mn, and Zn		[63]
F	Fe, Pb, As, Cu, Mn, Sb, Zn	NS	NS	*Rosmarinus officinalis*	Health risks related to ingestion of contaminated rosemary may be limited ADI for As, Cu, Pb and Sb	PS	[64]
P	Fe, Si, As, Cd, Pb	NS	Accumulator	*Thelypteris dentata*	Phytoremediation/revegetation	PR/RV	[65]

Table 1. *Cont.*

Experiment(s)	Metal(s) Studied	Concentration of Metal(s)	Type of Accumulation	Plant(s)	Remediation Status and Capacity	Type of Remediation	References
HP	Ni	16,600 mg Ni kg^{-1} DW	Hyperaccumulator	*Salvinia minima*	The plant species can be used to hyperaccumulate Ni in their tissues	PE	[66]
F	As, Pb	6017 mg As kg^{-1} DW, 499.5 mg Pb kg^{-1} DW	Hyperaccumulator (As), Accumulator (Pb)	*Pteris vittata*(As) *Pteris vittata (Pb)*	The plant species can tolerate high metals accumulation in the mining areas and can be used for phytoextraction purposes.	PE	[67]
	As, Pb, Cd	1032 mg As kg^{-1} DW, 2350 mg Pb kg^{-1} DW, 1201 mg Cd kg^{-1} DW	Hyperaccumulator (As), Accumulator (Pb, Cd)	*Viola principis*	Can hyperaccumulate As and accumulate a significant concentration of Pb and Cd		
P	As, B, Fe, Mn, Zn			*Ricinus communis*	Plant species have better efficiency to remove copper from the soil	PS	[68]
HP	Cd, Cr, Cu, Pb, Ni, Zn	NS	NS	*Centella asiatica*	Lower the toxic metals in the effluent in the range of: Cd (14–54%), Cr (2–43%), Cu (18–81%), Pb (35–90%), Ni (13–59%), and Zn (20–81%)	NS	[69]

F: Field; P: Pot; GH: Greenhouse; CT: Culture tubes; PL: Plot; PC: Pot culture; F(PC): Field (Pot culture); GL: Glasshouse; HP: Hydroponic; F(GL): Field glasshouse; CP: Culture plates; DW: Dry weight; NC: Natural contamination; AC: Artificial contamination; E: Excluder; A: Accumulator; H: Hyperaccumulator; RF: Rhizofiltration; PE: Phytoextraction; PS: Phytostabilization; BS: Biostabilization; PR: Phytoremediation; PF: Phytofiltration; BR: Bioremediation; RV: Revegetation; NS: not specified.

The major benefits for using naturally growing plants in phytoremediation of metal contaminated area are:

- High metal tolerance;
- High biomass;
- Possibility to tolerate climatic variability of that area;
- Ability to withstand in harsh conditions;
- Drought or wet resistance;
- Ability to withstand variable chemical properties;
- Ability to tolerate nutrient stress;
- Possibility to grow in poor physical conditions; and
- Ability to grow in presence of multiple metals.

3.2. Artificially Metal Contaminated Substrate Studies

Artificial metal contamination experiments are those investigations which were conducted by spiking the substrate with a specific amount of metal from an external source: mainly, the salt of metals. Different metals were being used constantly in fixed concentration so as to check the ability of the plant species for its growth and survival and success in phytoremediation. Most of the experiments include only single metal spiking [70–79]. However, some researchers had also performed multiple metal spiking experiments to find out new varieties of plants, herbs, shrubs and trees which can decontaminate the multi-metal contaminated sites [69,80–86]. High-quality research articles published in the past two decades were studied and evaluated, and the majority of the researchers had reported many plants which were able to tolerate and accumulate metals in their root part as well as in the aerial parts in high concentration. Some of the research carried out using the spiking of metals in soil during the last two decade is listed in Table 2.

3.3. Both Naturally and Artificially Metal Contaminated Substrate Studies

It is obvious that lab-based experiments sometimes fail when implemented in a natural situation, and thus, both the studies together could help to understand the plant behavior and suitability and survivability for clean-up of the metal-contaminated sites. However, it was found that naturally growing native plants of metal-contaminated sites are more efficient than non-native plants because of the above stated reasons (see Section 3.1). Some of the experiments carried out during the last decade by the researchers to check the efficiency of the same plant under both natural and artificial contamination, which are listed in Table 3. However, much research is needed for those plants that were tested in artificial conditions because these plants were grown under controlled conditions such as by keeping optimum light, moisture, temperature, and humidity. Field studies are further required to check the efficiency of laboratory-tested plants in naturally metal-contaminated fields where there is great competition and variability in biotic and abiotic conditions (temperature, moisture content, water availability, etc.). Keeping all the aspects in mind, it can be concluded that it is better to search native plant species that have high biomass, drought-resistant, and hyperaccumulation properties in roots with high commercial importance.

Table 2. List of competent plant species used to study the remediation potential under spiked metal contaminated condition.

Experiment(s)	Metal(loid)s Studied	Spiked/Metal Addition	Concentration of Metal(loid)s	Type of Accumulation	Plant(s) Studied	Remediation Status and Capacity	Type of Remediation	References
PC	Cr	$K_2Cr_2O_7$	0, 5, 10, 20, 50, 100 and 200 mg kg^{-1}	A	*Brassica juncea* *Brassica campestris* *Ipomea carnea* *Phragmytes karka* *Lantana camara* *Cassia tora*	Cr extraction was *I. carnea* > *D. innoxia* > *C. tora* > *P. karka* > *B. juncea* > *L. camara* > *B. campestris*. *B. juncea* and *B. campestris* are accumulators. *Ipomea carnea* and *P. karka* are useful in phytoextraction	PE	[87]
P	Hg	Coarse and fine silica	ns	A	*Brassica juncea*	Thiosulfate induced plant Hg accumulation	PE	[88]
PC	Cd	$Cd(NO_3)_2$	10, 20, 50, 100 and 200 mg kg^{-1}	NS	*Ipomea carnea*, *Brassica juncea*, *Dhatira innoxia*, *Phragmytes karka*	*Brassica juncea* accumulated maximum Cd; *I. carnea* followed by *D. innoxia* and *P. karka* were the most suitable species for the phytoextraction of cadmium from soil	PE	[70,89]
F(P)	Cd, Cu, Zn, Pb, and As	$CdCl_2 \cdot 2.5H_2O$; $Na_2HAsO_4 \cdot 7H_2O$	Cd |: 10, 25, 50 mg kg^{-1}; As: 50, 250 mg kg^{-1}	H	*Rorippa globosa*	As a Cd hyperaccumulator, Large amounts of Cd could accumulate in the shoots, and the TF and BF values are >1.0	PR	[90]
HP	Cd, Pb	$CdCl2 \cdot 2.5H_2O$, $Pb(NO_3)_2H_2O$	Cd: 0, 5, 10, 20 mg L^{-1} ; Pb: 50, 100, 200 mg L^{-1}	A	*Helianthus annuus*, *Brassica juncea*, *Medicago sativa*, *Ricinus communis*	*Helianthus annuus* showed better ability of accumulation than the others	PE	[81]
F(PC)	Cd, As	$CdCl_2 \cdot 2.5H_2O$, $Na_2HAsO_4 \cdot 7H_2O$	10, 25, and 50 mg kg^{-1}	H	*Solanum nigrum*	No reduction in plant height and shoot dry biomass was noted when the plants were grown at Cd concentration of 625 mg kg^{-1}	PR	[82]
PL	Cd, Cu, Pb, Zn	Sludge	5 mg Cd kg^{-1}, 385 mg Cu kg^{-1}, 180 mg Pb kg^{-1}, 1885 mg Zn kg^{-1}	NS	*Triticum aestivum*	NS	NS	[91]
PC (GH)	As, Cr, Zn	$Na_2HAsO_4 \cdot 7H_2O$, $K_2Cr_2O_7$ and $ZnSO_4 \cdot 7H_2O$	As, Cr: 0, 25, 50, 100, 250 and 500 mg kg^{-1}; Zn: 0, 500, 1000, 2000, 3000 and 4000 mg kg^{-1}	NS	*Jatropha curcas*	Application of organic amendment stabilizes the As, Cr and Zn and reduced their uptake in plant tissues. *Jatropha curcas* has the potential for the recovery and reclamation of a metalloid and metal-contaminated soil system	PS	[83]

Table 2. *Cont.*

Experiment(s)	Metal(loid)s Studied	Spiked/Metal Addition	Concentration of Metal(loid)s	Type of Accumulation	Plant(s) Studied	Remediation Status and Capacity	Type of Remediation	References
P(GH)	Cd	$CdCl_2 \cdot 2.5H_2O$	0, 50, 100 or 200 mg kg^{-1}	A	*Cannabis sativa, Linum usitatissimums, Arachis hypogaea and 5 others*	Good energy crop on contaminated soil, all are good Cd tolerant. *C. sativa*, *L. usitatissimums*, *A. hypogaea* are acculator.	PS	[71]
P	Cd	$CdCl_2 \cdot 2.5H_2O$	0, 8, 16, 24, 32, 50, and 100 mg kg^{-1}	H	*Bidens pillosa*	Has the potential for the phytoremediation of HMs-contaminated soils	PR	[72]
CP	Cr, Cr^3, Cr^{6+}	Tannery sludge; Cr^{3+} as $CrCl_3 \cdot 6H_2O$, Cr^{6+} as $K_2Cr_2O_7$	Cr: 0–8000 mg kg^{-1}; Cr^{3+}: 0–2000 and Cr^{6+}: 0–500 mg kg^{-1}.	NS	*Avena sativa, Sorgham bicolor, S. sadanense, Triticum aestivum*	Cr accumulated mostly in the roots but not in the shoots	NS	[73]
P	Cd	0 or 50 µM of $CdSO_4$	NS	A	*Poplar and Willow Clones*	Salicaceae clones are suitable phytoremediation. Willows had a far greater ability to tolerate Cd than poplars	PR	[74]
CT (50 days)	Cd, Cr	$CdCl_2 \cdot 2H_2O$, $K_2Cr_2O_7$	Cd(II): 0, 0.3, 0.65, 1.3, 2.2 mM; Cr (VI): 0, 0.5, 1, 2, 3.4 mM	H	*Prosopis laevigata*	Bioaccumulation factor greater than 100 for Cd and 24 for Cr. Hyperaccumulator of Cd(II) and Cr(VI)	PR	[84]
P	Cr	$K_2Cr_2O_7$		H	*Arabidopsis thaliana*	A significant increase in Cr accumulation (0.64–4.19 mg g^{-1}) DW, stem; and 0.77–1.1 mg Cr g^{-1}	PE	[75]
P	As	As_2O_3	0, 50, 100, 300, 600 and 1000 g L^{-1}	NS	*Arundo donax*	*Arundo donax* can be used for the remediation of arsenic-contaminated soils	PE, PV	[76]
P	Cu	$CuSO_4 \cdot 5H_2O$	100–400 mg kg^{-1}	A	*Salix jiangsuensis, S. babylonica*	Suitable for use in the phytoremediation of Cu-contaminated wetlands	PS	[77]
PC	Cu, Zn, Pb, Cr, Cd	$CuSO_4$ (60, 200, 300 mM), $ZnSO_4$ (200, 280, 350 mM), $Pb(NO_3)_2$(30, 90, 180 mM), $CrCl_3$ (60, 180, 360 mM) and $CdSO_4$ (20, 25 and 30 mM)	NS	A	*Amaranthus spinosus*	*Amaranthus spinosus* is a potential agent for accumulation and translocation	NS	[85]

Table 2. *Cont.*

Experiment(s)	Metal(loid)s Studied	Spiked/Metal Addition	Concentration of Metal(loid)s	Type of Accumulation	Plant(s) Studied	Remediation Status and Capacity	Type of Remediation	References
P(GH)	Cd	$CdCl_2 \cdot 2.5H_2O$	0, 25 mg kg^{-1}	NS	*Cannabis sativa*	Phytoremediation of Cd-contaminated soil	PR	[92]
P(GH)	Hg	$HgCl_2$	0.6, 5.5 mg kg^{-1}	NS	*Silene vulgaris*	*Silene vulgari* is good for phytostabilization	PS	[93]
GH	Cu, Zn, Cr		Low: 60 mg Cu kg^{-1}, 125 mg Zn kg^{-1}, 50 mg Cr kg^{-1}. High: 700 mg Cu kg^{-1}, 1050 mg Zn kg^{-1}, 116 mg Cr kg^{-1}	NS	*Sesbania virgata*	*Sesbania virgata* tolerated and stabilized high concentrations of Cu, Zn and Cr	PS	[86]
GH	Cr	$K_2Cr_2O_7$	0, 50 and 100 mg kg^{-1}	NS	*Zea mays*	Good for Cr and Co-contaminated soil	PR	[78]
HP	Hg	$HgCl_2$	2, 10, 50, 100, 200, 500, and 800 g L^{-1}		*Opuntia stricta, Aloe vera, Setcreasea purpurea, Chlorophytum comosum, Oxalis corniculata*	-	PE	[94]
P	Pb	$Pb(NO_3)_2$	500, 1000, 1500 to 2000 mg Pb kg^{-1} of soil	H	*Pelargonium hortorum, Mesembryanthemum criniflorum*	Out of 21 plant species, *P. hortorum* and *M. criniflorum*, accumulated more than 1000 mg Pb kg^{-1}	PE	[95]
P	Cr	NS	50, 100, 200 mg Cr kg^{-1}		*Sesbania sesban*	Good for Cd remediation	NS	[96]
P	Hg	$HgCl_2$	10, 50, 100, 500, 1000 mg Hg kg^{-1}	NS	*Brassica juncea*	The plant species showed good uptake of Hg up to the concentration level at 500 mg kg^{-1}	PR	[29]

F: Field; P: Pot; GH: Greenhouse; CT: Culture tubes; PL: Plot; PC: Pot culture; F(PC): Field (Pot culture); GL: Glasshouse; HP: Hydroponic; F(GL): Field glasshouse; CP: Culture plates; F(P): Field (Pot); DW: Dry weight; P(GH): Pot(Green house); NS: not specified; E: Excluder; A: Accumulator; H: Hyperaccumulator; PE: Phytoextraction; PS: Phytostabilization; PR: Phytoremediation; PV: Phytovolatilization.

Table 3. List of competent plant species used to study the remediation potential in both naturally and artificial metal-contaminated site/substrate.

Experiment(s)	Metal(loid)s	Spiked/Metal Addition	Metal(loid)s Concentration	Type of Accumulation	Plant(s) Studied	Remediation Status and Capacity	Type of Remediation	References
GH	As, Cd, Ni, Pb	Nitrate salts of Pb, Cd, Ni, Zn	50 or 200 mg kg^{-1} of each metal	H	*Pteris vittata*	-	PE	[97]
PL, P, HP	Pb	$Pb(NO_3)$	20, 24.1, 48.3, 96.5, 241.3, 482.6 μM Pb (0, 0.5, 1, 2, 5, 10 mg Pb^{2+})	A	*Monochoria korsakowi*	Useful in phytoremediation of co-contaminated metal	PE	[98]
P	Pb, Mn, Cu, Zn		80 mg Cu kg^{-1}, 146 mg Pb kg^{-1}, 408 mg Mn kg^{-1}, 148 mg Zn kg^{-1}	-	*Lolium perenne, Festuca rubra, Poa pratensis*	Phosphate addition increased exchangeable Mn fraction by 35%, and a combined application of amendments lowered the exchangeable Mn fraction by 50%	PS	[99]
P	Cd	-	2.8 mg P kg^{-1}	A	*Ricinus communis (21 varieties)*	Phytoremediation of DDTs/Cd co-contaminated soils	PR	[100]
F, P, PL	Cd, Zn, Pb	$CdCl_2.2.5H_2O$	0, 5, 10, 30, 60, 90, 120 and 150 mg Cd kg^{-1}	H	*Siegesbeckia orientalis*	*Siegesbeckia orientalis* is a Cd-accumulator with hyperaccumulating ability. In shoot: F: 117.48, P: 192.92, PL: 77.10 mg kg^{-1}, P: 0–150 mg kg^{-1}, PL: Cd, Pb, Zn and Cu concentrations were 28.44, 517.53, 1814.15 and 57.04 mg kg^{-1}, resp.	PR, PE	[101]
GH	Hg	$[Hg(NO_3)]_2$	1, 5, and 10 μg Hg g^{-1}	A	*Jatropha curcas*	*Jatropha curcas* is accumulator	A	[102]
P	Hg	$HgCl_2$	5, 10, 20, 50, 100, 200, 500 and 1000 mg Hg kg^{-1}	H	*Cyrtomium macrophyllum*	Promising plant species to remediate Hg from the soil	ns	[79]
HP	Hg	$HgCl_2$	2, 10, 50, 100, 200, 500, and 800 μg Hg L^{-1}		*Setcreasea purpurea, Chlorophytum comosum* and *Oxalis corniculata, Aloe vera, Opuntia stricta*	*Oxalis corniculata*: most suitable for transferring Hg at concentrations of less than 500 μg L^{-1}	ns	[94]
P	Hg	$HgCl_2$	10, 50, 100, 500, and 1000 mg Hg kg^{-1} soil	H	*Brassica juncea*	Plant showed better efficiency up to the concentration level of 500 mg Hg kg^{-1} soil	PE	[29]

F: Field; P: Pot; GH: Greenhouse; PL: Plot; GL/GH: Glasshouse; HP: Hydroponic; A: Accumulator; H: Hyperaccumulator; PE: Phytoextraction; PS: Phytostabilization; PR: Phytoremediation; ns: not specified.

4. Quantification of Soil Pollution Indices or Metrices

4.1. Enrichment Factor (EFs)

To search for the most likely source of origin of elements in soil, enrichment factors were calculated for individual elements over the average elemental composition of the tailing [103–112] (Equation (1)).

$$\text{Enrichment factor (EF)} = \frac{\left[(\text{M})_{\text{soil}} \times (\text{Al})_{\text{tailing}}\right]}{\left[(\text{Al})_{\text{soil}} \times (\text{M})_{\text{tailing}}\right]} \tag{1}$$

where $(\text{M})_{\text{soil}}$ and $(\text{Al})_{\text{soil}}$ are the concentrations of the studied element and Al in the soil, while $(\text{M})_{\text{tailing}}$ and $(\text{Al})_{\text{tailing}}$ are the mean concentrations of the element and Al, respectively, in the tailing. Here, aluminum is considered as the reference material because of its wider acceptance as a reference element.

Regional geochemical background values are constant and are recommended by Rubio et al. [113] for the assessment of enrichment of metal in contaminated soil. However, the levels of contamination vary with time and place [105,114], and background values are distinctly different among different soil types. For most HMs of environmental interest, concentrations in soil easily vary over two to three orders of magnitude depending on the parent materials [105]. There are different elements which were used as reference elements to study the contamination pattern. However, iron (Fe) and aluminum (Al) are widely used as the reference elements for geochemical normalization because of the following reasons [105,115]:

- Both are associated with fine solid surfaces;
- Its geochemistry is similar to that of many trace metals;
- Its natural concentration tends to be uniform.

Enrichment factors are distributed under five categorizes [111,116]:

- EF < 2: depletion to minimal enrichment;
- EF = 2–5: moderate enrichment;
- EF = 5–20: significant enrichment;
- EF = 20–40: very high enrichment;
- EF > 40: extremely high enrichment.

Due to its unitless dimension and relatively simple formula, it is universally accepted for the assessment of the degree of metal enrichment and source of anthropogenic origin caused by metal mining.

4.2. Contamination Factor (CF)

The contamination factor (CF) is the ratio obtained by dividing the concentration of each metal in the soil by the baseline or background value (concentration in unpolluted soil) (Equation (2)):

$$\text{Contamination factor}(\text{CF}) = \frac{[\text{C}]_{(\text{heavy metal})}}{[\text{C}]_{(\text{background})}} \tag{2}$$

where $[\text{C}]_{(\text{heavy metal})}$ is the concentration of each metal in contaminated soil and $[\text{C}]_{(\text{background})}$ is the concentration of each metal in non-contaminated or baseline or unpolluted soil [117].

The contamination levels may be classified based on their intensities on a scale ranging from 1 to 6 [105–107,109,114,115,117–119]:

- CF = 0: None;
- CF = 1: None to medium;
- CF = 2: Moderate;
- CF = 3: Moderate to strong;
- CF = 4: Strongly polluted;

- CF = 5: Strong to very strong;
- CF = 6: Very strong.

The highest number indicates that the metal concentration is 100 times greater than what would be expected in the crust.

4.3. Geoaccumulation Index (I_{geo})

Geoaccumulation indexes for the metals were determined using Muller's [114] expression (Equation (3)):

$$\text{Geoaccumulation indexe } (\mathrm{I_{geo}}) = \log_2\left[\frac{(\mathrm{Metal})_\mathrm{s}}{1.5\ (\mathrm{Metal})_\mathrm{b}}\right] \tag{3}$$

where $(\mathrm{Metal})_s$ is the concentration of metals examined in soil samples and $(\mathrm{Metal})_h$ is the geochemical background concentration of the metal. Factor 1.5 is the background matrix correction factor due to lithospheric effects [120,121].

The geoaccumulation index consists of seven grades or classes [106–109,111,114,115,119,122–125]:

- $I_{geo} \leq 0$: practically uncontaminated;
- $0 < I_{geo} < 1$: Uncontaminated to moderately contaminated;
- $0 < I_{geo} < 2$: Moderately contaminated;
- $2 < I_{geo} < 3$: Moderately to heavily contaminated;
- $3 < I_{geo} < 4$: Heavily contaminated;
- $4 < I_{geo} < 5$: Heavily to extremely contaminated;
- $5 < I_{geo}$: Extremely contaminated and can be a hundredfold greater than the geochemical background value.

4.4. Pollution Load Index (PLI)

It is the integrated index which combines all the HMs to one index and compares the status of pollution of various sites without considering the grain size, natural geochemical variability and changes of heavy metal/reference element ratios which are based on natural processes. This empirical index provides a simple, comparative means for assessing the level of HM pollution. PLI is calculated for the entire sampling site by taking the nth root of the product of the n CF [105–107,109,115,117–120,126,127] (Equation (4)):

$$\text{Pollution load index (PLI)} = (\mathrm{CF_1} \times \mathrm{CF_2} \times \mathrm{CF_3} \times \cdots \times \mathrm{CF_n})^{\frac{1}{n}} \tag{4}$$

where CF_n is the contamination factors of different elements.

- PLI < 0: Unpolluted;
- $0 < \mathrm{PLI} \leq 1$: Baseline levels of pollutant present;
- $1 < \mathrm{PLI} \leq 10$: Polluted;
- $10 < \mathrm{PLI} \leq 100$: Highly polluted;
- PLI > 100: Progressive deterioration of environment.

4.5. Risk Assessment Code (RAC)

Risk assessment code (RAC) is a classification system which includes an assessment of available reactive HMs in soil and calculated as the percentage of metals present in exchangeable and carbonate fraction [128,129]. It is the percentage of metal concentration extracted by acetic acid when used in 0.11 M concentration, which is scaled as [109,130]:

- RAC < 1: No risk;
- $1 < \mathrm{RAC} \leq 10$: Low risk;
- $11 < \mathrm{RAC} \leq 30$: Medium risk;
- $31 < \mathrm{RAC} \leq 50$: Very high risk.

This reactive or available metal gives the indication of potential risk to the ecosystem.

4.6. Potential Ecological Risk Index

The potential ecological risk index, proposed by Hakanson [131], was employed to evaluate the potential risk of HMs in the rhizospheric soil [56,125,132].

Based on an overall consideration of the toxicities and the differences in regional background values of HMs, this evaluation method could eliminate the influence of regional differences and embody the toxicities of HMs and their relative contributions. As a result, a comprehensive reflection of the potential of HMs' impact on the ecological environment was provided, which made it suitable for evaluating the pollution of HMs in a wide range of area. According to the literature [117,133,134], the toxic factors of Hg, Cd, As, Cu, Pb, Ni, Cr and Zn are 40, 30, 10, 5, 5, 5, 2 and 1, respectively.

According to this method, the potential ecological risk index (E_r^i) of individual heavy metal and the comprehensive potential risk index (RI) of several HMs could be calculated by the following Equation (5):

$$\text{Potential ecological risk index } \left(E_r^i\right) = \frac{[T_i] - [C_i]}{[C_{oi}]} \tag{5}$$

where C_i and C_{0i} are the measured and background concentrations of element i in soil, respectively, while T_i is the toxic factor of element i [121,125]. The potential ecological risk criteria were scaled as:

- $E_r^i < 40$: Low;
- $40 < E_r^i < 80$: Moderate;
- $80 < E_r^i < 160$: Considerable;
- $160 < E_r^i < 320$: High;
- $E_r^i > 320$: Very high.

4.7. Potential Risk Index (RI)

It is calculated as the sum of all the four risk factors for HMs in soils. Hakanson [131] had given the standardized heavy metal toxic factor by the order of level of heavy metal present in the soil (Cd > Pb = Cu > Zn) [56,135,136] (Equation (6)):

$$\text{Potential risk index (RI)} = \sum_{i=1}^{m}\left(E_r^i\right) \tag{6}$$

where E_r^i is the potential ecological risk index.

- RI < 150: Low;
- 150 < RI < 300: Moderate;
- 300 < RI < 600: Considerable;
- 600 < RI < 1200: High;
- $E_r^i \geqq 1200$: Very high.

5. Quantification of Plant Phytoremediation Efficiency Metrices

Different efficiency indices were being used in the past two decades in the field of phytoremediation of metal to study the plant–soil interaction, transport mechanism and accumulation pattern in plants. The different efficiency indices of phytoremediation which can be used by researchers to evaluate the actual status of remediation taking place in the implemented area are available. However, few important indices, i.e., translocation factor, bioconcentration and bioaccumulation factors become more popular to evaluate the efficiency of the plant species for the phytoremediation of metal-contaminated soil/sites. Moreover, from time to time, different names were used for the study of the same factor, which created chaos and misunderstanding in the field of phytoremediation, which is being studied here to resolve such problems for researchers. The present review includes different types of efficiency indices which were frequently used to evaluate the phytoremediation potential of plants in both natural and artificial (spiked) condition. However, translocation and bioconcentration/bioaccumulation factors and extracts used to calculate it are most widely studied by the researchers (between 2002 and 2021) which are exhaustively discussed in Tables 4 and 5.

5.1. Translocation Factor (TF)

The translocation factor, also termed as accumulation factor, uptake factor, and concentration factor, is an important index for evaluating the transfer potential of metals from soil to plant [3,137,138]. It is regularly used for both naturally colonized/growing and artificially grown/cultured plants under controlled conditions. Plants require metals for their proper growth and development and include different specific carriers and mechanisms for the transport of these metals from soil to plant [139]. Metals present in soil become available to plants in a bioavailable form, which easily become absorbed by the roots and transfer into the shoot through suitable carriers. However, the transportation of metals in plants varies from plants to plant and species to species and further depends on many other factors such as the age of the plant, climatic regime, nature of soil, soil chemistry, ecotype, etc. [45,54,140–142]. It seems that the transfer factors derived from different types and ranges of soil metal concentrations are not comparable. Efroymson et al. [143] estimated the uptake of inorganic contaminants in soil to the plants by using a single uptake factor, single-variable regression model and multiple regression models with soil. The present work involves the study of two-decade research papers to study the use of a bioavailable portion for the calculation of transfer factors. Although it is considered that the bioavailable portion of toxic metals is the basis of soil risk assessment of soil contaminants, there are still only a few reports that are using the bioavailable metal concentration for this purpose. In most of the cases, total or pseudo-total metal concentrations were used to calculate the translocation factor.

The translocation factor is the efficiency index of the plant species, which indicates the translocation of metals from the root part to the shoot part [41,45,46,49,53,57,61,63,65,91,141,144–146] and can be calculated as follows in Equation (7):

$$\text{Translocation factor (TF)} = \frac{[\mathrm{C}]_{(\text{shoot})}}{[\mathrm{C}]_{(\text{root})}} \tag{7}$$

where $C_{(\text{shoot})}$ indicates the metal concentration accumulated in the shoot part and $C_{(\text{root})}$ indicates the metal concentration accumulated in the root part.

The same factor was calculated by different researchers and denoted as the "*shoot*" part using different terms such as "*aerial part*" [39,51,62,98], stem [54,59,100,147], aboveground tissue part [50,56], and leaves [62,100,148]. Similarly, in the case of ferns, the term "frond" [65,97] and "cap" in case of mushroom [149] were also used to denote the shoot part. It was found that the main aim of all the researchers was to calculate the translocation of metals from root to shoot but not on the term used for the plant part, i.e., shoot, aerial part, tissue aboveground part, leaf, and stem.

The translocation factor can also be calculated in percentage (%) by using the following Equation (8) [2,74]:

$$\text{Translocation factor (TF)} = \frac{[\mathrm{C}]_{(\text{aerial part })}}{[\mathrm{C}]_{(\text{root})}} \times 100 \tag{8}$$

It is evaluated that a translocation factor > 1 for any plant shows its potential to phytoextract the metal from the root into the shoot, whereas TF < 1 indicates its phytostabilizing property. In low and moderately contaminated soils, the TF values were found to be >1. However, it does not imply the same for the highly metal-contaminated sites.

Transfer factor (Tf): It is the efficiency index of the plant species to accumulate metals from its surrounding substrate (soil/sediment) and can be calculated as follows in Equation (9) [42,50,57,62,97,98,150]:

$$\text{Transfer factor (Tf)} = \frac{[\mathrm{C}]_{(\text{plant})}}{[\mathrm{C}]_{(\text{substrate})}} \tag{9}$$

where $C_{(\text{plant})}$ is the concentration of metal in the whole plant, and $C_{(\text{substrate})}$ is the concentration of metal in the substrate (soil) in which it is growing.

Dynamic factor of metal translocation (TR_{dyn}): Baltrenaite et al. [151] have introduced a dynamic factor that helps to integrate information about metal concentration in different substrates and provide a comparison between control and treated soil. They are related both to internal (physiological) and external (ecological) factors.

To understand the actual transfer and accumulation of metals, Baltrenaite et al. [151] introduced a few formulas (Equation (10)), which include metal transfer assessment compared to control or non-contaminated soil:

$$TR_{(\text{dyn})} = \frac{TR_{\text{i, treated}}}{TR_{\text{i, control}}} = \frac{[C_{\text{i, v, treated}}] \times [C_{\text{i,r, control}}]}{[C_{\text{i,r, treated}}] \times [C_{\text{i,v, control}}]} \tag{10}$$

where $TR_{\text{i, treated}}$ is the translocation factor of metal i in trees on the treated site; $TR_{\text{i, control}}$ is the translocation factor of metal i in trees on the control site; $C_{\text{i,v, treated}}$ is the concentration of metal i in tree vegetative organs on the treated site, in mg per kg dry weight (DW); $C_{\text{i, r, treated}}$ is the concentration of metal i in tree roots on the treated site, in mg per kg DW; $C_{\text{i, r, control}}$ is the concentration of metal i in tree roots on the control site, in mg per kg DW; and $C_{\text{i, v, control}}$ is the concentration of metal i in tree vegetative organs on the control site, in mg per kg DW.

Table 4. List of plant and soil extractant, plant efficiency metrics to evaluate the remediation potential of naturally metal-contaminated site/substrate.

Metal(s)	**Plant Digest(s)**	**Soil Extractant(s)**	**Plant Efficiency Metrics**					**References**
			A = Aboveground Part/Root	**B = Aboveground Part/Substrate**	**C = Root/Substrate**	**D = Plant/Substrate**	**E = Tissue/Substrate**	
Cd, Cr, Cu, Ni, Pb, Zn	US EPA Method 3051 (1994)	US EPA Method 3051 (1994)	TF = Aerial/Plant	BCF = Shoots Soil	BCF = Roots/Soil	-	-	[39]
As, Cd, Ni, Pb, Zn	* HNO_3	* HNO_3	TF = Frond/Root biomass conc.	-	-	BF = Plant/Soil	-	[97]
Pb, Cu, Zn	$^{C\#}$USEPA Method 3050	$^{C\#}$USEPA Method 3050	TF = Shoot/Root	-		BCF= Plant root/Soil	-	[41]
Cu, Zn, Cd, Pb	HNO_3	quaregia	TF = Shoot/Root	-	BCF = Root/Soil	-	-	[152]
Zn, Cu, Pb, Ni	Conc. HNO_3 + $HClO_4$ (5:1)	DTPA ($^{C\#}$ Total; EDTA	TF = Shoot/Root	Bioaccumulation Coefficient = Shoot/DTPA in soil	Bioaccumulation Coefficient = Root/DTPA in soil	-	-	[45]
Pb	Acid digestion (Undefined)	ns	TF = Aerial/Root	-	-	BCF = Plant/Soil	-	[98]
Cd, Cu, Pb, Zn	Aquaregia 70% + 305 H_2O_2	DTPA Total-$^{C\#}$	TF = Shoot/Root	-	BCF= Root/Total soil	-	-	[91]
Cr, Zn, Cd, Cu, Ni, Pb	HNO_3:H_2O_2:HCl, 7:1:1	HNO_3:H_2O_2:HCl, 7:1:1; DTPA	TF = Total shoot/Total root	BF= Total shoot/Total soil	-	-	-	[46]
Cu, Ni, Fe, Mn	HNO_3 + $HClO_4$	HF + $HClO_4$ + HNO_3	TF = Other plant part/Root	-	-	-	BCF = Plant tissues rooted soil	[48]
Sr	HNO_3 then HCl + HNO_3+ H_2O, 1:1:1	HCl+HNO_3+H_20, 1:1:1	TLF = Shoot/Root	ECS = Enrichment coefficient for shoot	-	-	-	[49]
Pb, Mn	10 mL 1 M HCl	Sequential Extraction	TF = Shoot/Root	EF_s = Shoot/Soil	EF_r = Root/Soil	-	-	[99]
Mn	$^{C\#}$USEPA 3051, 1995	Sequential Extraction ($^{C\#}$); USEPA 3052, 1995	TF = Aboveground tissue part/roots	-	-	BCF = Whole plant DW/Soil	-	[50]
Cd	HNO_3:$HClO_4$, 5:1	HNO_3: HF: $HClO_4$, 5:1:1	TF_{leaf} = Leaf/Root; TF_{stem} = Leaf/Root	BCF = Leaf/Soil BCF = Stem/Soil	BCF = Root/Soil	-	-	[100]
Fe, Mn, Zn, Cd, Cu, Pb, Cr, As	HNO_3:$HClO_4$, 4:1	Aqua regia+HNO_3	TF = Aerial/Root	-	-	-	BCF = Plant tissue/Background soil conc. in agri. field	[51]

Table 4. *Cont.*

Metal(s)	Plant Digest(s)	Soil Extractant(s)	Plant Efficiency Metrics					References
			A = Aboveground Part/Root	**B = Aboveground Part/Substrate**	**C = Root/Substrate**	**D = Plant/Substrate**	**E = Tissue/Substrate**	
As, Fe, Mn, Cu, Co, Zn	HNO_3 and HCl, 5:1	Aqua regia; DTPA and TEA; Sequential extraction	- (because it was not possible to separate completely the roots of the plants)	BF = Bioaccumulation factor; BF = shoots (total DW)/tailings	-	-	-	[52]
Fe, Zn, Cu	Without any chemical treatment	Without any chemical treatment	TF = Cap/Stripe	-	-	BF = Mushroom/Substrate (soil)	-	[149]
Fe, Zn, Pb and Mn	Acid digestion (Undefined)	Acid digestion (Undefined)	TF = Shoot/Root	-	BCF = Root/Soil	-	-	[53]
Fe, Cu, Pb, Mn, Ni, Zn, Cr, Cd	HNO_3:$HClO_4$, 5:1	HNO_3:$HClO_4$, 5:1	TF = Total shoot/Total root	BAF = Shoot/Substrate	BAF= Root/Substrate	-		[54]
Cu, Cd, Pb, Cr, Mn, Ni	HNO_3:$HClO_4$, 5:1	HNO_3:$HClO_4$, 5:1	TF = Stem/root	-	-	-	BCF = Plant part/Substrate	[54]
Hg, Cd, As, Hg, Pb, Cr, Cu, Zn, Ni	HCl: HNO_3: $HClO_4$, HF	HCl: HNO_3: $HClO_4$, HF	TF = Aboveground tissue part/roots	-	-	-	BCF = Tissue/Rhizospheric soil	[56]
As, Fe, Mn, Pb, Zn	HNO_3 (65%) and H_2O_2 (30%), 5:1	$HNO_3+H_2O_2+HF_{conc.}+HCl+H_2O$, 9:1:3:2:1	TF = Shoot/Root	-	-	BF = Plant/Soil	-	[57]
Cu, Fe, Pb, Zn	C#	C#	TF = Shoot/Root	-	BCF = Root/Soil	-	-	[58]
Ni, Cu, Zn, Cd, Pb	HNO_3:$HClO_4$, 5:1	HNO_3:$HClO_4$, 5:1	TF = Stem/root	-	-	-	BCF = Plant part/Substrate	[59]
Cd, Zn, Pb, Cu	HNO_3:$HClO_4$, 4:1	HNO_3:HCl:$HClO_4$, 1:2:2	TF = Shoot/Root	BCF = Shoot/Soil	BCF = Root/Soil	-	-	[101]
Cr, Cu, Ni, Pb, Cd	HNO_3:$HClO_4$, 3:1	HNO_3:$HClO_4$, 3:1	TF = Shoot/Root	EF = Shoot/Contaminated soil	EF = Root/Contaminated soil	-	-	[60]
As	H_2SO_4/$HClO_4$ Natural plants: HNO_3: $HClO_4$, 17:3	H_2SO_4/$HClO_4$	TF = Shoot/Root	BCF = Aerial biomass concentration/Soil	-	-	-	[61]

Table 4. *Cont.*

Metal(s)	Plant Digest(s)	Soil Extractant(s)	Plant Efficiency Metrics					References
			A = Aboveground Part/Root	**B = Aboveground Part/Substrate**	**C = Root/Substrate**	**D = Plant/Substrate**	**E = Tissue/Substrate**	
As	HNO_3 then 30% H_2O_2	30%H_2O_2+9.6 M HCl	TF = Aerial (leaf or stem)/Root	-	-	BCF = Plant/Environment (soil)	-	[62]
Cd, Co, Cu, Cr, Fe, Mn, Ni, Pb, Zn	HNO_3 (65%)+$HClO_4$ (70%), 3:2	HNO_3 (65%)+$HClO_4$ (70%), 3:2	TF = Shoot/Root	-	BF = Root/Soil	-	-	[63]
Fe, Pb, As, Cu, Mn, Sb, Zn	HNO_3+HCl, 2:1	Aquaregia (1/3 HNO_3+2/3 HCl)	TF = Shoot/Root	BAF = Shoot/Soil	-	-	-	[64]
Fe, Si, As, Cd, Pb	HNO_3: $HClO_4$, 3:1	HNO_3: $HClO_4$	TF = Frond/Root biomass conc.	-	BAF = Root/Substrate	-	-	[65]
Hg	HNO_3: 30% H_2O_2	Aqua regia	TF = C_{Leaf}/C_{root}	BCF = $C_{leaf/root/stem}/C_{soil}$				[79]
Hg	HNO_3:H_2SO_4, 4:1 (*v*/*v*)	HCl:HNO_3, 3:1 (*v*/*v*)		BCF = C_{soot}/C_{soil}				[153]
As, B, Fe, Mn, Zn	HNO_3: $HClO_4$, 3:1	HNO_3:$HClO_4$, 5:1	TF = C_{shoot}/C_{root}	BCF = C_{soot}/C_{soil}				[68]
Hg	HNO_3 and 30% H_2O_2, 5:2	65% HNO_3	TF = C_{shoot}/C_{root}	BCF = C_{root}/C_{soil}				[29]
Cd	HNO_3: H_2O_2, 5:2	DTPA extraction	TF = C_{Leaf}/C_{root}	BCF = C_{root}/C_{soil}				[154]

A: Translocation/transfer factor; B: Bioconcentration/Bioaccumulation factor in shoot; C: Bioconcentration factor in root; D: Bioconcentration factor in plant; E: Tissue-specific bioconcentration factor; TF: Translocation factor; Tf: Transfer factor; EF = Enrichment factor; ne: not evaluated; c#: cross referenced; *: ratio not specified.

5.2. Bioconcentration Factor (BCF) or Bioaccumulation Factor (BF)

The bioconcentration factor or bioaccumulation factor is the efficiency index of the plant species to accumulate metals in its harvestable tissue part (root or shoot or leaf) from its surrounding substrate (soil/sediment) [39,100,101,132,145,152,155] and can be calculated as follows Equation (11):

$$\text{Bioconcentration (BCF) or Bioaccumulation factor (BF)} = \frac{[\text{C}]_{(\text{plant tissue or aerial part})}}{[\text{C}]_{(\text{substrate})}} \tag{11}$$

where $\text{C}_{(\text{plant tissue})}$ indicates the metal concentration accumulated in the plant tissue (shoot or root or leaf) and $\text{C}_{(\text{substrate})}$ indicates the metal concentrations accumulated in the substrate (soil/sediment).

Both the factors, bioconcentration factor and bioaccumulation factor, are rigorously used to calculate the ratio in shoot or in root concentration against substrate concentration [39,45,46,63–65]. However, few researchers had used "transfer factor" [155–157] or "enrichment factor" (EF) [99] in place of BCF or BF.

The bioconcentration factor or bioaccumulation factor (BF) can also be represented in percent according to the following Equation (12) [158,159]:

$$\text{Bioconcentration (BCF) or Bioaccumulation factor (BF) \%} = \frac{[\text{C}]_{(\text{plant tissue or aerial part})}}{[\text{C}]_{(\text{soil})}} \times 100 \tag{12}$$

where BF in %; $\text{C}_{(\text{plant tissue}) \text{ or aerial part}}$ is the metal concentration in plant tissue and $\text{C}_{(\text{soil})}$ is the metal concentration in soil.

It can be stated after reviewing the papers from the last decade that all the factors are different in terms of their name or notations (BCF, BF and EF); however, their purpose was the same, i.e., to calculate the ratio between the concentration of metal in plant tissue (root or shoot or leaves) and that in substrate (soil or sediment).

Dynamic factor of metal bioaccumulation (BA_{dyn}): Similar to the dynamic factor of metal translocation, another factor called the dynamic factor of bioaccumulation (BA_{dyn}) was calculated by comparing the metal concentration in soil and its accumulation in plants of the contaminated area to that of the metal concentration in soil and its accumulation in the plants of control soil using Equation (13) [151]:

$$\text{Bioaccumulation, } \text{BA}_{(\text{dyn})} = \frac{[\text{C}_{\text{i, tree, treated}}] \times [\text{C}_{\text{i, soil, control}}]}{[\text{C}_{\text{i, soil, treated}}] \times [\text{C}_{\text{i, tree, control}}]} \tag{13}$$

where $\text{C}_{\text{i, tree, treated}}$ is the concentration of metal i in tree (the whole plant) ash on the treated site; $\text{C}_{\text{i, soil, treated}}$ is the concentration of metal i in the treated soil; $\text{C}_{\text{i, soil, control}}$ is the concentration of metal i in the control soil; and $\text{C}_{\text{i, tree, control}}$ is the concentration of metal i in the control tree (the whole plant) ash. All values are in milligrams per kg DW.

5.3. Enrichment Factor (EF)

It is the ratio of metal concentration in plant of polluted or contaminated soil to that of the metal concentration in control soil plant [160] and calculated as shown in Equation (14):

$$\text{Enrichment factor (EF)} = \frac{[\text{C}]_{(\text{polluted})}}{[\text{C}]_{(\text{control})}} \tag{14}$$

where $\text{C}_{\text{polluted}}$ and $\text{C}_{\text{control}}$ are the metal concentration in the plant parts (roots, shoots) from the contaminated or polluted sampling soil and control or non-polluted soil.

5.4. Tolerance Index (TI)

The tolerance index (TI), also called as the growth ratio (GR) (reported by [84]), is an important factor to evaluate the efficiency of the plant to grow on metal-contaminated sites in respect to control soil and can be calculated on the basis of biomass [71,74,75,77,92,161] (Equation (15)):

$$\text{Tolerance Index (TI) or Growth ratio (GR)} = \frac{[\text{Biomass}]_{\text{treated or contaminated}}}{[\text{Biomass}]_{\text{control or non-contaminated}}} \quad (15)$$

where $[\text{Biomass}]_{\text{treated or contaminated}}$ is the biomass of the whole plant in treated or metal-contaminated soil; $[\text{Biomass}]_{\text{control or non-contaminated}}$ is the biomass of the whole plant in control or non-metal-contaminated soil.

However, different research studies have been carried out in the world to evaluate the efficiency of the plant tolerance in compared to non-contaminated soil (control) in respect to plant length, root length, shoot length, and this was calculated as [71,83,84] (Equation (16)):

$$\text{Tolerance Index (TI)} = \frac{[\text{Growth parameters}]_{\text{treated or metal contaminated}}}{[\text{Growth parameters}]_{\text{control or non-metal contaminated}}} \quad (16)$$

where [Growth parameter] can be plant length, root length or shoot length in treated or metal-contaminated soil and in control or non-metal-contaminated soil.

5.5. Metal Extraction Ratio (MER)

It is the ratio of metal accumulation in shoot to that of the soil. Metal extraction ratio (MER) is the efficiency assessment of the phytoextraction capability of plants, which is also known as the phytoextraction ratio and phytoextraction efficiency assessment and can be calculated as shown in Equation (17) [44,68,80,82,101,162,163]:

$$\text{Metal extraction ratio (MER)} = \frac{\left[(\text{C})_{\text{plant}} \times (\text{M})_{\text{plant}}\right]}{\left[(\text{C})_{\text{soil}} \times (\text{M})_{\text{rooted zone}}\right]} \times 100 \quad (17)$$

where $(\text{C})_{\text{plant}}$ is the metal concentration in the harvested component of the plant biomass, $(\text{M})_{\text{plant}}$ is the mass of the harvestable aboveground biomass produced in one harvest, $(\text{C})_{\text{soil}}$ is the metal concentration in the soil volume; and $(\text{M})_{\text{rooted zone}}$ is the mass of the soil volume rooted by the plant species.

5.6. Plant Effective Number (PEN)

The plant effective number (PEN) helps to evaluate and compare the ability of different plant species to phytoremediate metal-contaminated soil using hyperaccumulator plants. It is defined as the number of plants needed to extract 1 g of metal when the biomass of shoot and of total plant biomass is considered, as shown in Equation (18) [68,80,82,101,164,165]:

$$\text{Plant effective number (PEN)} = \frac{1}{\left[(\text{B})_{\text{Shoot or plant}} \times (\text{M})_{\text{Shoot or plant}}\right]} \quad (18)$$

where $(\text{B})_{\text{shoot or plant}}$ is the shoot or whole plant biomass; and $(\text{M})_{\text{shoot or plant}}$ is the metal concentration in the shoot or in the whole plant.

Table 5. List of plants and soil extractants as well as plant efficiency metrics to evaluate the remediation potential of artificially spiked contaminated site.

Metal(s)	**Plant Digest(s)**	**Plant Efficiency Metrics**					**References**
		A = Aboveground Part/Root	**B = Aerial Part/Substrate**	**C = Root/Substrate**	**D = Plant/Substrate**	**E = Tissue/Substrate**	
Cr	HNO_3:$HClO_4$, 3:1	T_i = Leaves/Root × 100	-	-	BCF = Plant tissue/Soil	-	[87]
Cd	HNO_3:$HClO_4$	Ti = Leaves/Root × 100	-	-	-	BCF = Plant tissues at harvest/Substrate	[70]
Cd, As	HNO_3:$HClO_4$, 3:1	TF = Shoot/Root	-	-	BCF = Plant/Soil	-	[90]
Cd, Pb	HNO_3	TF = Aerial/Root	-	BCF = Plant/Culture	-	-	[81]
Cd, As	HNO_3:$HClO_4$, 3:1	TF = Stem/Root	-	-	BF = Plant/Soil	-	[82]
As, Cr, Zn	HNO_3:$HClO_4$	TF = Leaf or Stem/Root	-	-	BCF = Whole Plant/Soil	-	[83]
Cd	HNO_3:$HClO_4$, 3:1	TF = Shoot/Root	BCF = Shoot/Soil	BCF = Root Soil	-	-	[71]
Cd	HNO_3:$HClO_4$	TF = Shoot/Root	-	-	BCF = Plant/Soil	-	[72]
Cr	HNO_3:HCl	BAF = Shoot/Root	-	BAF = Root/Soil	-	-	[73]
Cd	HNO_3:H_2SO_4, 6:2.5	Tf = Aerial part/Root	-	-	-	BCF = Harvested plant material/Solution	[74]
Cd, Cr	HNO_3	TF = Shoot/Root	BF = Shoot/Culture	-	-	-	[84]
Cr	HNO_3:30% H_2O_2	T_i = Leaves/Root × 100	-	-	-	BCF = Tissue/Soil	[75]
As	NS	TF = Shoot/Root	-	-	BA = Plant/Solution	-	[76]
Cu	HNO_3:$HClO_4$, 4:1	TF= Shoot/Root	-	-	BAF = Plant/Soil	-	[77]
Cu, Zn, Pb, Cr, Cd	HNO_3:$HClO_4$, 4:1	TF = Shoot/Root	-	-	BCF = Plant tissue/Soil	-	[85]
Cd	HNO_3:$HClO_4$, 3:1	TF = Shoot/Root	BCF = Shoot/Soil	BCF = Root/Soil	-	-	[92]
Hg	HCl:HNO_3	TF = Shoot/Root	BCF = Shoot/Soil	-	-	-	[93]
Cu, Zn, Cr	0.01 M $CaCl_2$	TF = Shoot/Root	-	-	-	BCF = Plant tissue/Soil	[86]
Cr	HNO_3 (30%)	TF = Shoot/Root	SCF = Shoot/Soil	RCF = Root/Soil	-	-	[78]
Hg	HNO_3 :30% H_2O_2	TF = Aerial part/Root	-	-	-	BCF = Root/Soil	[79]
Pb	HNO_3:$HClO_4$, 4:1	TF = Shoot/Root	-	-	-		[95]
Hg	HNO_3: 30% H_2O_2, 5:2	TF = Shoot/Root	-	-	-	BCF = Plant/Soil	[29]
Cd	HNO_3:$HClO_4$, 3:1	TF = Shoot/Root	-	-	-	BCF = Plant/Soil	[166]

A: Translocation/transfer factor; B: Bioconcentration/Bioaccumulation factor in shoot; C: Bioconcentration factor in root; D: Bioconcentration factor in plant; E: Tissue-specific bioconcentration factor; TF: translocation factor; Tf: Transfer factor; BA or BAF: Bioaccumulation factor; BCF: Bioconcentration factor; SCF: Shoot concentration factor; RCF: Root concentration factor; EF = Enrichment factor; T_i: Transportation Index.

5.7. Phytoremediation Factors

The phytoextraction efficiency of plants depends on the concentration of HMs accumulated in the dry aboveground biomass of the plants and the plant yields. The remediation factor (RF) [167–169] represents the percentage of an element removed by the plant dry aboveground biomass from the total element content in the soil during one cropping season and was calculated as follows (Equation (19)):

$$\text{Remediation factor (RF)} = \frac{\left[(\mathrm{C})_{\mathrm{plant}} \times (\mathrm{B})_{\mathrm{plant}}\right]}{\left[(\mathrm{C})_{\mathrm{soil}} \times (\mathrm{W})_{\mathrm{soil}}\right]} \times 100, \tag{19}$$

where $(\mathrm{C})_{\mathrm{plant}}$ is the metal content in plant dry aboveground biomass (mg kg^{-1}); $(\mathrm{B})_{\mathrm{plant}}$ is the plant dry aboveground biomass yield (g); $(\mathrm{C})_{\mathrm{soil}}$ is the total metal content in soil (mg kg^{-1}) and $(\mathrm{W})_{\mathrm{soil}}$ is the amount of soil in the pot (g).

Total metal uptake: Similarly, the effectiveness of the phytoextraction process (total metal uptake) for the phytoremediation of the metal-contaminated site can also be calculated by multiplying the number of plants growing and the remediation factor (Equation (20)) [170]:

$$\text{Total metal uptake (\%)} = \frac{\left[(\mathrm{C})_{\mathrm{plant}} \times (\mathrm{B})_{\mathrm{plant}}\right]}{\left[(\mathrm{C})_{\mathrm{soil}} \times (\mathrm{W})_{\mathrm{soil}}\right]} (\mathrm{N})_{\mathrm{plant}} \times 100, \tag{20}$$

where $(\mathrm{C})_{\mathrm{plant}}$ is the metal content in plant dry aboveground biomass (mg kg^{-1}); $(\mathrm{B})_{\mathrm{plant}}$ is the plant dry aboveground biomass yield (g); $(\mathrm{C})_{\mathrm{soil}}$ is the total metal content in soil (mg kg^{-1}); $(\mathrm{W})_{\mathrm{soil}}$ is the amount of soil in the pot (g) and $(\mathrm{N})_{\mathrm{plant}}$ is the number of plants.

Dynamic factor of phytoremediation: Another factor called the dynamic factor of phytoremediation ($\mathrm{FR_i}$) has been introduced by Baltrenaite et al. [151] to evaluate the phytoremediation capacity of the plants growing in contaminated or treated waste compared to control soil and was calculated as follows (Equation (21)):

$$\mathrm{FR_i} = \frac{[\mathrm{C_{i,\,tree}}] \times \mathrm{B}}{1000 \times [\mathrm{C_{i,\,Soil}}] \times \rho \times \mathrm{d}}, \tag{21}$$

where $\mathrm{FR_i}$ is the annual metal phytoremediation factor, in kg per ha; $\mathrm{C_{i,\,tree}}$ is the metal concentration in tree, in mg per kg; B is the annual tree increment, in kg per ha; $\mathrm{C_{i,\,soil}}$ is the metal concentration in a 40 cm soil layer, in mg per kg; ρ is the soil density, in grams per cubic cm; and d is the soil layer (depth), in cm.

5.8. Phytoextraction Potential (PP)

The phytoextraction potential (PP) is the total amount of HMs extracted per ha of soil in a single phytoextraction cycle [71,168,171]. It is calculated as follows (Equation (22)):

$$\text{Phytoextraction potential (PP)} = [\mathrm{C}]_{\mathrm{plant}} \times [\mathrm{B}]_{\mathrm{plant}}, \tag{22}$$

where $[\mathrm{C}]_{\mathrm{plant}}$ is the metal content in plant dry aboveground biomass (mg kg^{-1}) and $[\mathrm{B}]_{\mathrm{plant}}$ is the plant dry aboveground matter biomass yield (t ha^{-1}).

5.9. Removal Efficiency (RE)

It is the efficiency index of the plant to remove metal from a contaminated site and can be calculated as shown in Equation (23):

$$\text{Removal efficiency (RE \%)} = \frac{[\mathrm{C_o}] - [\mathrm{C_f}]}{[\mathrm{C_o}]} \times 100, \tag{23}$$

where $[C_o]$ is the initial metal concentration and $[C_f]$ is the final metal concentration in the soil after plantation.

6. Measurement of Metal Concentration in Soil and Plant

6.1. Measurement of Bioavailable and Total Metal Concentration in Soil/Substrate

Metals which are available to the plants from the soil/substrate are termed as "bioavailable". These metals can be extracted by using different extraction methods (using different extractants). Some of the widely used different acid or acid mixtures used by various researchers for total metal analysis in plants under natural and artificial contamination are depicted in Tables 4 and 5.

6.1.1. Single Extraction Methods

It is well documented that the total concentrations of metals in soil do not act as a good indicator of phytoavailability, or a good tool for potential risk assessment, due to the different and complex distribution patterns of metals among various chemical species or solid phases [56,172]. Several authors have used a single extraction method for the evaluation of the availability of metals in soils [173–175], while Tessier et al. [176] and Ure et al. [177] used sequential extraction methods for the evaluation of bioavailability of metals [178]. However, the sequential extraction methods were proposed for sediment, which are quite laborious and time consuming. Among single extraction methods, $CaCl_2$, DTPA, EDTA and CH_3COOH were the most widely used extractants [179–188]. DTPA (0.005 M) is suitable for calcareous soils, as it is buffered at a pH 7.3 and therefore prevents $CaCO_3$ from dissolution and releases occluded metals, especially Cd^{2+} and Zn^{2+} [173]. EDTA (0.01 M and 0.05 M) is a very good chelating agent, which can solubilize carbonate-occluded metals from soil [180]. The extraction with water is to simulate the metal distribution equilibrium of metals in soil pore water [185]. It has long been recognized that the soluble, exchangeable and loosely adsorbed metals are quite labile and hence more available for plants [189]. Therefore, in order to assess the environmental risk and the phytoavailability of metals, efforts should be concentrated on the measurements of these available fractions. However, different researchers had used various extractants in different concentrations, which restricts the comparison of data. It is now necessary to agree on a uniform method to obtain concrete and comparable results.

6.1.2. Sequential Extraction Method

In the sequential extraction procedure, metals which are exchangeable, carbonates bound, bound to Fe/Mn oxides, bound to organic matter and sulfides and residual are quantified [50,99,176,190,191]. However, several other researchers have also proposed and modified these methods but were not used consistently [192]. It was found that exchangeable and acid-soluble fractions are the main and more reactive form which is available to living organisms when originating from an anthropogenic source [109]. The main reagents used for sequential extractions are depicted in Table 6.

Table 6. Tessier's scheme for the sequential extraction of metals [67,176,193,194].

Metal Fractions	Reagents Used
Exchangeable	$MgCl_2$ 1 mol L^{-1} at pH 7.0
Carbonatic	CH_3COONa 1 mol L^{-1}/HOAc at pH 5.0
Oxides Fe/Mn	$NH_2OH.HCl$ 0.04 mol L^{-1} in 25% HOAc
Organic matter and sulfidic	H_2O_2 8.8 mol L^{-1}/HNO_3 + NH_4OAC 0.8 mol L^{-1}
Residual	HF/$HClO_4$

6.1.3. Total Metal (Digestion) Method

Five mineral acids, namely concentrated hydrochloric acid (HCl), nitric acid (HNO_3), sulfuric acid (H_2SO_4), perchloric acid ($HClO_4$) and hydrofluoric acid (HF), have been very widely used for the estimation of total metals or pseudo-total metals [195]. For the simultaneous extraction of the large number of metals, H_2SO_4 has one of the notable properties of dissolving silica. Thus, it can be used in conjugation with HNO_3, HCl or $HClO_4$ for the total decomposition of silicates [196,197]. Sometimes, HF is also used in conjugation with HNO_3, $HClO_4$ [129,185,198–200] or HCl [48,56,57,100,184,186,201] for the same purpose. The HNO_3 is also used separately [202] or either with HCl [126,203] or $HClO_4$ [63,65,182]. Such methods provide a high degree of metal extractability but do not dissolve silicates completely; they destroy organic matter, dissolve all precipitated and adsorbed metals, and leach out a certain amount of the metal from the silicate lattice. HF is used to break the silica matrix. Aqua regia (HNO_3: HCl; 1:3; v/v) and HNO_3 are weaker extracting agents than $HClO_4$. Aqua regia is a stronger oxidizing and extracting agent than HNO_3 as a result of the presence of nascent chlorine. HNO_3, aqua regia and $HClO_4$ have their strongest leaching effect when they are boiling. Especially, $HClO_4$ is a strong leaching, dehydrating and oxidizing agent only when it is hot and in concentrated form. The amount of metal extracted by $HClO_4$ depends on the type of mineral and organic matter content.

6.2. Measurement of Total Metal Concentrations in Plants

HNO_3 is often used for metal extraction from plant samples [48,180,204]. A binary acid mixture of HNO_3 and $HClO_4$, which is the most widely used extractant (4:1 or 5:1 on in 3:1; v/v), has been mainly used by the researchers for many years for the estimation and determination of metal concentrations in plants [51,54,100,182,185,202,205–207]. Sometimes, tertiary acid mixtures of HF, HNO_3 and $HClO_4$ [46,61,199,201,208] are also used for the same purpose. However, the use of HF is limited because of the lack of silica estimation in plant parts.

The two decades of research articles reviewed (as stated earlier) were categorized into two sections: (a) papers related to plants collected from a natural condition/habitat growing on natural substrate/soil in greenhouse, pot culture, field, plot, etc. without any artificial contamination and (b) papers related to experiments which include the use of artificial contaminants (spiked) for metal enrichment in soil. It was found that out of the 54 experimental papers, 35 belonging to natural contamination had used HNO_3 and $HClO_4$ acids as the main metal extractant from the plant. However, some other extractants were also used such as HCl, H_2O_2 and HF for the same purpose. When artificially contaminated experimental papers (19) were reviewed, it was found that majority (>50%) of the researchers had used HNO_3 and $HClO_4$ as the main plant metal extractant. It suggests that in most of the cases, whether belonging to natural or artificial contamination, these two metal extractants are self-sufficient to digest and extract the majority of the metals present in plant parts.

Similarly, in the case of soil (for a similar number of research papers), the metal extractants used were of wide variety. However, the main extractant used for natural metal-contaminated soil remains the same, i.e., HNO_3 and $HClO_4$ (in different ratios). Apart from HNO_3 and $HClO_4$, the other most suitable widely used extractant is aqua regia mixture. In case of artificial contaminated soil, metals are spiked with known concentration and were estimated only with HNO_3 and $HClO_4$ mixture.

7. Conclusions

The analysis and systematization of the large number of research articles published in the past two decades (2002–2021) allows us to identify the most reliable and representative indices, the use of which will provide a more adequate assessment of the accumulative strategy of plants and contribute to the choice of the more effective metal clean-up phytoremediation technologies. To provide new insight, the present review draws the following conclusions:

(a) Different soil pollution metrics such as contamination factor, geoaccumulation index, enrichment factor, pollution load index and potential risk indexes provide the opportunity to assess the soil metal pollution; however, the usage of the first two metrics is the most important for both artificial and naturally metal-contaminated sites before implementing phytoremediation strategies.

(b) Different plant efficiency metrics such as translocation factor, bioconcentration factor, phytoremediation factor, dynamic factor, metal extraction ratio, plant effective number, tolerance index, etc. can provide assessment and practical knowledge about the metal uptake, transfer, and its distribution in plants growing on artificial and natural contaminated sites. Among them, the most suitable are translocation and bioconcentration factors.

(c) Experiments performed under both natural and artificial contamination suggests some of the hyperaccumulators (*Pteris vittata*, *Monochoria korsakowi*, *Lolium perenne*, *Festuca rubra*, *Poa pratensis*, *Ricinus communis*, *Siegesbeckia orientalis*) identified in the present review provide further strength to the previous studies reported in the literature.

(d) The available results in this review of the literature indicate that the translocation and bioconcentration factors were the most important factors which can help to select suitable plants for the decontamination of metal and metalloid-contaminated sites.

(e) From the depth analysis of published results, it can be concluded that most widely accepted extractants to dissolve and extract the metals from the soil and plant are HNO_3 and $HClO_4$ (mainly in 5:1, v/v or 4:1, v/v). It is also important to report that for both natural and artificial contamination, we used the same acid to extract the metal from plant and soil.

Researchers are attempting to identify new and potential hyperaccumulators. However, it was found that most of the artificial experiments are conducted by spiking the substrate/soil material with a single metal that does not correspond to the natural conditions. More research is required to analyze the effect of mixture of metals on plants under artificial condition to provide a better strength of its hyperaccumulation property.

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