Understanding the Phytoremediation Mechanisms of Potentially Toxic Elements: A Proteomic Overview of Recent Advances

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Potentially toxic elements (PTEs) such as cadmium (Cd), lead (Pb), chromium (Cr), and arsenic (As), polluting the environment, pose a significant risk and cause a wide array of adverse changes in plant physiology. Above threshold accumulation of PTEs is alarming which makes them prone to ascend along the food chain, making their environmental prevention a critical intervention. On a global scale, current initiatives to remove the PTEs are costly and might lead to more pollution. An emerging technology that may help in the removal of PTEs is phytoremediation. Compared to traditional methods, phytoremediation is eco-friendly and less expensive. While many studies have reported several plants with high PTEs tolerance, uptake, and then storage capacity in their roots, stem, and leaves. However, the wide application of such a promising strategy still needs to be achieved, partly due to a poor understanding of the molecular mechanism at the proteome level controlling the phytoremediation process to optimize the plant’s performance. The present study aims to discuss the detailed mechanism and proteomic response, which play pivotal roles in the uptake of PTEs from the environment into the plant’s body, then scavenge/detoxify, and finally bioaccumulate the PTEs in different plant organs. In this review, the following aspects are highlighted as: (i) PTE’s stress and phytoremediation strategies adopted by plants and (ii) PTEs induced expressional changes in the plant proteome more specifically with arsenic, cadmium, copper, chromium, mercury, and lead with models describing the metal uptake and plant proteome response. Recently, interest in the comparative proteomics study of plants exposed to PTEs toxicity results in appreciable progress in this area. This article overviews the proteomics approach to elucidate the mechanisms underlying plant’s PTEs tolerance and bioaccumulation for optimized phytoremediation of polluted environments.
INTRODUCTION

The continued accumulation of potentially toxic elements (PTEs), including cadmium (Cd), lead (Pb), chromium (Cr) and arsenic (As), copper (Cu), mercury (Hg), nickel (Ni), and selenium (Se) in the environment poses a significant danger to human health and undermines global environmental sustainability efforts (Habiba et al., 2019; Rizvi et al., 2019, 2020; Alsafran et al., 2021). Anthropogenic activities due to rapid industrialization, especially from oil and gas producing industries, infrastructural development, mining, foundries, smelters, coal-burning power plants, and agricultural activities, are significant contributors that enhance the elements of hazardous pollutants in the soil (Al-Thani and Yasseen, 2020; Yan et al., 2020; Usman et al., 2020b). While this is the case, bioaccumulation strategies and tolerance to higher concentrations of PTEs, thus sequestering of PTEs can be varied among different plant species as they faced diverse pollutant sources and other environmental conditions. Generally, PTEs sequestration mechanisms in plant tissues include exclusion, stabilization, removal, and transfer to the various parts such as roots, shoots, and stems. Of these, the removal and translocation of the elements to plant's aerial parts, the process also known as “phytoextraction,” are known as the most efficient remediation strategy (Saleem et al., 2020a). Phytoextraction is inexpensive, the amount of waste material that must be disposed of is substantially decreased (up to 95%), and the disposal of hazardous material or biomass is not required (Usman et al., 2020b). Plant species demonstrating the capacity to remove and transfer PTEs to their aerial parts are categorized as metal hyperaccumulators (De Bellis and Aprile, 2020; Zhang et al., 2020; Usman et al., 2020b).

The PTEs are non-biodegradable and prone to ascend along food chains, making their environmental prevention a critical intervention (Wuana and Okieimen, 2011; Sharma and Pandey, 2014). Given the potential adverse effects of many remediation strategies, alternative technologies, including phytoremediation—the use of plants to remove PTEs from contaminated environments, are being explored for large-scale applications (Usman et al., 2019b). Phytoremediation is the direct use of living green plants and is an effective, cheap, non-invasive, and environmentally friendly technique used to transfer or stabilize all the toxic metals and environmental pollutants in polluted soil or ground water (Mosa et al., 2016). Phytoremediation is widely applicable for metal contaminated areas, with some long-term esthetic merits and it is famous due to its low cost and eco-friendly nature, so it is used on large-scale areas, with some long-term esthetic merits and it is famous due to its low cost and eco-friendly nature, so it is used on large-scale areas with high contents of toxics metals (Rascio and Navari-Izzo, 2011). Plants are sessile organisms, and therefore, could not escape from exposure to high concentrations of PTEs (Wiszniewska, 2021). However, several plant species (~450) are known to accumulate high concentrations of various PTEs (Rascio and Navari-Izzo, 2011). PTEs mainly enter plant systems from soil or water via passive or active transport. Following uptake that is facilitated by membrane-embedded ion channels, elemental ions translocate to aerial parts of plants (i.e., the stem and leaves) via xylem channels. In general, plants capable of accumulating PTEs in their tissues majorly bio-concentrate the elements in the root, followed by the stem, leaves, and in some species, even the seeds (Shamim, 2018; Dinu et al., 2020).

Biotechnologically, three main strategies are embarked upon to improve PTEs phytoextraction using different plants species: (i) utilizing the metal/metalloid transporters, (ii) enhancing metal/metalloid ligand production, and (iii) conversion of metal/metalloid into volatile and less detrimental forms (Mosa et al., 2016). The toxicity of PTEs primarily depends on various factors such as concentrations and chemical properties of toxic elements, their bioavailability, and plants’ developmental stage. When exposed to PTEs, plant’s basal tolerance mechanism becomes activated and enables them to cope with the stress (Gill et al., 2021). However, at elevated concentrations, these elements suppress the plant defense machinery and cause harmful effects to physiological processes, including photosynthesis, transpiration, and energy metabolism, thus reducing overall plant growth and development (Kumar et al., 2018; Gautam et al., 2020; Ahmad et al., 2020a; Usman et al., 2020a). Generally, PTEs stress symptoms on plants can be measured as it is similar to that of deficiency in essential nutrients that may be appeared in the forms of leaf necrosis, poor root development, and decreased fresh biomass (Usman et al., 2019a; Singh and Fulzele, 2021).

Recently, the “Omics” approaches emerge as valuable tools for understanding the changes in molecular mechanisms of plant’s response to the PTEs during phytoremediation (Meena et al., 2017; Raza et al., 2021). The traditional characterization methods relating to physiological and biochemical assays seem insufficient, and therefore, further investigation especially on the response of whole-genome proteome to PTE can be a promising approach to coping with the potential threats posed by PTEs (Xie et al., 2019; Kosakivska et al., 2021). These changes are not only limited to the expression pattern but also protein quality and quantity. Transcriptomic approaches are used to target transcriptional changes at the mRNA level (i.e., changes in gene expression), which may differ from changes at the protein level (i.e., translational modifications). In a true sense, the mRNA/protein ratio is a factor of mRNA transcription rate and protein stability (Reimegård et al., 2021).

To alleviate PTEs stress and restore cellular homeostasis, plants develop antioxidative capacity, sophisticated and highly efficient regulatory mechanisms to help tolerate the uptake, accumulation, translocation, and eventual detoxification (El-Amier et al., 2019; Alshali et al., 2020; Ahmad et al., 2020b; Bhat et al., 2021). To achieve this, the living system's functional molecules, the proteins, particularly metal chelators, transporters, and chaperones, play crucial roles in alleviating the negative impact of PTEs stress (Saleem et al., 2020b). Together, these proteins enable plants to tolerate PTEs, detoxify PTEs polluted environments and their system through binding, transport, and vacuolar sequestration (Peco et al., 2020; Dhir, 2021; Jogawat et al., 2021).

Proteins are crucial to regulating the cellular processes of plants; proteomics, comprising cellular protein roles, quantification, identification, the pattern of expression, modification, and interactions, all together provides an excellent strategy to assess
stress impact on them. Because of the central roles of proteins, researchers in this area need to prioritize studies focusing on proteomics to gain further insights into the mechanisms of PTEs tolerance and detoxification in plants to improve the efficiency of PTEs removal from contaminated soil or medium.

Recent progress in plant proteomics could be possible due to new technological advancements in protein separation, quantification, mass spectrometry (MS), and bioinformatics. Mass spectrometry is central to large-scale proteome analysis that enhances the resolution, sensitivity, and accuracy of proteins mass prediction (Cassidy et al., 2021). Due to these and the speed of analysis for large protein samples through released peptides after proteolytic digestion (bottom-up), shotgun proteomics is used to describe the process (Gutsch et al., 2019b). On the other hand, protein is partially digested to characterize co-existing post-translational modifications (PTMs; Sidoli et al., 2017). Following fractionation and tandem mass spectrometry (MS/MS) analysis, the bottom-up process indirectly measures proteins through tryptic digested peptides having amino acids approximately between 8 and 30 (8 > aa > 30). Proteins are inferred through identified peptides compared to MS/MS spectra previously generated from in-silico fragmented peptides in a protein database. Figure 1 shows a schematic representation of typical steps in PTEs phytoremediation studies involving the use of shotgun proteomics.

In contrast to the bottom-up approach (analysis of digested proteins), the proteomics of characterizing intact proteins is another strategy termed “top-down” (Figure 2). Proteomic research has made significant progress, especially on model plants, *Oryza sativa*, and *Arabidopsis thaliana*. Essential proteins, such as metal ion transporters, binding proteins, phytochelatins (PCs), and metallothioneins (MTs), are notable in aiding PTEs sequestration in plants. PCs are induced by phytochelatins synthase (PCS), which is triggered when metal ions are present. PCs (oligomers of glutathione) bind to toxic metals to form a significant part of the detoxification mechanism, while MTs are gene-encoded, small, and cysteine-rich proteins (Jorrin-Novó et al., 2019; Usman et al., 2020b).

Due to the exponential increase in the number of studies and publications in the proteomics of plant abiotic stress, including PTEs, it is almost impossible to provide an extensive summary in one review. For further references within the last decade, readers are referred to some of the recent reviews (Ahsan et al., 2009; Hossain and Komatsu, 2013; Cvjetko et al., 2014; Kosová et al., 2018; Raza et al., 2020; Kosakivska et al., 2021; Sytar et al., 2021). This review aims to provide a non-exhaustive overview of plant proteomics and highlights its importance in understanding PTEs tolerance, uptake, and detoxification mechanisms in plants during phytoremediation when grown in metal contaminated soil. To the best of our knowledge, this review is among the few articles focused on the plant proteomics of trace and heavy metals.

**PHYTOREMEDIATION**

A combinatorial strategy involved the physiological and chemical properties, and biological processes adopted by plant species

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**FIGURE 1** | A workflow illustrating the comparative proteomic methods that have been used to investigate the phytoremediation of potentially toxic elements. Classical in-gel proteomic methods include 1-D and 2-D polyacrylamide gel electrophoresis (PAGE) sometimes further developed by differential in-gel electrophoresis (Dige) using fluorescent tags like cyanine 2 or 3 or 5 (Cy2, Cy3, and Cy5). Gel-free methods are advanced and used to overcome limitations in-gel proteomics and to study the heavy metal detoxification and phytoremediation mechanisms. These include novel gel-free methods with protein labeling such as Stable Isotope Labeling with Amino acids in Cell culture (SILAC) and Isobaric Tags for Relative and Absolute Quantitation (iTRAQ) techniques followed by multi-dimensional chromatography (MupPit).
to clean up environmental pollutants (Baldwin et al., 2015; Hasegawa et al., 2016). Physical and chemical methods have several limitations such as non-economical, alterations in native soil flora, changes in the physicochemical properties of the soil, and need intensive labor (Shankar, 2017). PTEs are essentially immutable by any chemical or physical process short of nuclear fission and fusion, and thus, their remediation presents special scientific and technical problems. Because of this, new approaches for better treatment of PTEs polluted environment are essential. In this regard, the use of biological treatment strategies could be adopted that are cheaper and environmentally friendly. The promising one is phytoremediation which has gained increased attention in recent years since it is the most viable alternative. Phytoremediation takes advantage of plant ability to tolerate, accumulate, and translocate PTEs across their aerial tissues (Ludvíková and Griga, 2019). Phytoremediation is often referred to as “green remediation” or “botanical bioremediation” involving the use of plants to remove, transfer, or stabilize the PTEs (Figure 3) to clean up the environment and render the pollutants harmless (Suman et al., 2018; Adiloğlu et al., 2021). Moreover, this mechanism is a species-specific, effective, economical, eco-friendly, and scientifically accepted method. Generally, when there is an encounter with PTEs, plants activate their defense machinery by adopting one or several mechanisms simultaneously to safeguard themselves from unwanted physiological or molecular alterations induced by PTEs. Some of the most studied and common strategies are presented in Table 1.

Phytoremediation has a great potential for providing much-needed green technology. During phytoremediation, the plant’s selection for the remediation strategy to neutralize PTEs may differ; the strategies used could be removal, transfer, degradation, immobilization, etc. (Hasanuzzaman et al., 2018). While hundreds of plant species have been identified as potential phytoremediators, identifying suitable hyperaccumulators is still a challenge (de Castro Ribeiro et al., 2018). Previously, Yıldız and Terzi (2016) studied Brassica napus under Cr stress and noticed through 58 proteins spots in two-dimensional electrophoresis (2-DE) that 58 proteins were differentially regulated by Cr (VI) stress (+S/+Cr), S-deficiency (−S/−Cr), and combined stress (−S/+Cr). The translocation capacity of plants (the ability to take up and accumulate toxic metal from the root to shoot parts) is a critical parameter considered in evaluating phytoremediation potential in plants (Meng et al., 2017). A translocation factor of 1 or more suggests a plant’s ability
to transfer metals to its aerial parts adequately. Various methods covered under phytoremediation strategies are discussed briefly in the following sections.

**Phytoremediation Strategies**

Plants employ different strategies during phytoremediation. The type of elements, their chemical properties, and bioavailability play a crucial role in achieving PTEs remediation success (Usman et al., 2020b). The different phytoremediation strategies include phytoextraction, phytofiltration or Rhizofiltration, phytovolatilization, phytostabilization, and phytodegradation (Figure 3; Usman et al., 2018; Wei et al., 2021). Phytoextraction involves the use of plants to remove PTEs or organics from the soil by concentrating them in the harvestable parts (Kumar et al., 2017; Ali et al., 2018). PTEs accumulating plants are utilized to transport and concentrate contaminants (metals or organics) from the soil into the above-ground plant parts as shown in the example by Viana et al. (2021). Phytoextraction involves PTEs removal, accumulation, and translocation to plant’s aerial parts (Viana et al., 2021). Often, phytosequestration, photoabsorption, and phytoaccumulation are used to refer to the same process. Several studies have reported plants demonstrating PTEs phytoextraction capacity. Phytoextraction

![Diagram of phytoremediation strategies](image-url)
Phytoremediation is preferred over other techniques because toxic elements can be harvested from plant shoots in an extractable form (Jeyasundar et al., 2021). Several studies have reported different plants with varying capacities in the phytoextraction of PTEs. Examples are the Indian mustard, rapeseed, and sunflower plants (Shaheen and Rinklebe, 2015; Chowdhary et al., 2018; Surucu et al., 2020).

**Phytodetransformation or Phytofiltration**

Phytodetransformation or Phytofiltration involves the adsorption of PTEs via the root. It is a process most seen in aquatic plants (Meitei and Prasad, 2021). In rhizofiltration, plant roots are used to absorb and adsorb pollutants, mainly metals, from contaminated soils and aqueous waste streams. It is the removal of pollutants from metal-polluted soil/waters by precipitation, absorption, and accumulation into plant biomass (Mahajan and Kaushal, 2018). Phytodetransformation is essential because it prevents toxic elements transmission to different environmental components, including underground water (da Conceição Gomes et al., 2016; Meitei and Prasad, 2021). However, phytodetransformation is also demonstrated by terrestrial species, where metals are remediated with microbial bio-filter aid in the rhizosphere region (Wei et al., 2020). Previously, studies conducted on rhizofiltration by Yadav et al. (2011) in Carex pendula in Pb contaminated wastewater soil noticed that C. pendula accumulate a large amount of Pb in their roots and can be used to clean up the Pb contaminated environment in combination with proper biomass disposal alternatives.

**Phytostabilization**

Plants can reduce PTEs toxicity by converting them to a different form or changing their bioavailability. Thus, the bioavailability of PTEs in the environment is reduced using plant systems. Plants stabilize PTEs in soils, thus rendering them harmless, thereby reducing the risk of further environmental degradation by leaching of PTEs into the groundwater or by airborne spread. This is achieved by preventing surface runoff, erosion, and leaching (Yan et al., 2020). Phytostabilization is vital because it helps prevent PTEs transmission into the food chain. The element's chemical properties are some of the most critical determinants of whether potential plants can stabilize them (Hamidpour et al., 2020; Usman et al., 2020b). Although phytostabilization offers some advantages, it has limited use because metals are only temporarily immobilized and restricted, and therefore, unpopular compared to phytoextraction (Radziemska, 2018). It is commonly employed in emergencies for quick metal immobilization in plants' rhizosphere (Meng et al., 2017).

**Phytovolatilization**

It involves converting metal contaminants into a gaseous form that is eventually released into the atmosphere (Aweng et al., 2018). In this process, PTEs are only transferred to other parts of the environment and could still be redeposited into the soil following precipitation. For this reason, phytovolatilization is less popular, especially compared to phytoextraction and phytofiltration (Nikolić and Stevović, 2015; Bisht et al., 2020).

**THE MECHANISMS OF PTEs**

**TOLERANCE AND BIOACCUMULATION**

Plants with the enhanced potential of taking up PTEs and translocating them to their aerial parts are identified as metal hyperaccumulators, while those with limited metal translocation are known as non-hyperaccumulators (Maestri et al., 2010). Recently, the interest in proteomics studies of plant hyperaccumulators and their metal sequestration and detoxification mechanisms has increased (Visioli and Marmiroli, 2013; Kumar and Prasad, 2018; Raza et al., 2020). Proteomic studies of PTEs accumulators can make comparisons of differentially expressed proteins (DEPs) between different plant parts (Table 2). Many studies suggest that the hyperaccumulators including transporters and chelators showed enhanced DEPs compared to non-accumulating plants (Visioli and Marmiroli, 2013; Paape et al., 2016; Domka et al., 2020). During PTEs phytoremediation, plant tissues play essential roles.

The root is the first tissue to encounter metal stress, and therefore often witnesses dramatic proteomic changes. When comparing the root protein of two accessions, glycosyl hydrolase family 18 differed in abundance, affecting the plant's capacity to uptake metal; the variant that had a higher protein abundance had higher Ni and Cd accumulation (Lai et al., 2020; Raza et al., 2020). The proteome of a variety of plant species was studied, and several proteins that protect plants against various stresses, including oxidative, biotic, and abiotic stress conditions were identified (Fan et al., 2016; Goodin, 2018; Kumar et al., 2018). When comparing Thlaspi caerulescens proteomes that had variable tolerance to Cd and Zn, it was determined that the element's higher accumulation was due to the protein photosystem II (Paunov et al., 2018). Proteomic analysis of Sorghum bicolor has also shown that a total of 33 DEPs were found when plants were (Table 2) exposed to cadmium (Cd) stress (Roy et al., 2016). Examples of such proteins are glutathione S-transferase, ribulose bisphosphate carboxylase small chain, carbonic anhydrase, glyceraldehyde-3-phosphate dehydrogenase, and cytochrome P450, which are well characterized this far in historical literature. The less characterized contenders that were upregulated in S. bicolor include pentatricopeptide
TABLE 2 | Examples of PTEs phytoremediation studies involving the use of comparative proteomics from 2015 to date.

| PTEs | Plant species | Plant parts | PTEs concentration/ exposure time/media | Technology used | Key findings | References |
|------|---------------|-------------|----------------------------------------|-----------------|-------------|------------|
| As   | Artemisia annua L. | Shoot, Root | 100μm Na₂HAsO₄, 5H₂O 7-days/ | 2-DE PAGE, MALDI-TOF-MS | Upregulation of secondary metabolites-related genes enhances as tolerance. Biomass, carotenoid, flavonoids were enhanced, whereas total chlorophyll pigment was reduced under As treatment. | Kumari and Pandey-Rai, 2018 |
|      |                |             | Hoagland nutrient’s solution         |                 |             |            |
|      | Brassica napus | Leaves | 200 μmol/l – 1 NaAsO₃/7-days/ 50% Hoagland solution | LC-MS/MS, SEM, TOF-MS, qRT-PCR | Photosystem II (PSII) and photosystem I (PSI) proteins were upregulated. Secondary metabolites biosynthesis increased. | Farooq et al., 2021 |
|      | Oryza sativa L. | Leaves, Root | NaAsO₃: 25 μM/ 7 days/ modified Hewitt’s media | 2-DE, MALDI-TOF-TOF | The sulfur treatment alleviates As stress by forming disulfide linkage in proteins involved in glycolysis, TCA cycle, energy metabolism, and photosynthesis. | Dixit et al., 2015 |
|      | Populus deltoides cv. *zhonglin 2025* and euamericana cv. *1-45/51* | Leaves, Root | Na₂AsO₃: 12H₂O 50, 100μM/21 days/Hoagland’s nutrient solution | MALDI-TOF/TOF MS, 2-DE, RT-PCR | Overexpression of photosynthetic and antioxidative responsive proteins in As tolerant cultivar | Liu et al., 2017 |
| Cd   | Arabidopsis thaliana L. | Leaves, Root | 100μm CdCl₂/7-days/1/2 MS solid media | 2-DE, MALDI-TOF/TOF-MS | The natural accession Chernobyl-07 (One) has a higher Cd tolerance than normal accessions. This accession particularly changed the expression related to ROS protection and energy modulation proteins for obtaining tolerance. | Klimenko et al., 2019 |
|      | Brassica campestris | Root | 50μm CdCl₂/1-day/ hydroponic | 2-DE, MALDI-TOF/TOF-MS | Hydrogen gas (H₂) and nitric oxide (NO) enhance the antioxidant capabilities of B. campestris seedlings in response to Cd toxicity. | Su et al., 2019 |
|      | Brassica napus | Xylem sap | 10μm CdCl₂/3-days/ hydroponic | LC-MS/MS | Cd stress-induced the overexpression of stress response-related proteins. | Luo and Zhang, 2019 |
|      | Medicago sativa | Stem | 88.9μm CdSO₄/4-months/ potted soil | 2DE, MALDI-TOF/TOF-MS | Cd stress caused the differential expression of proteins involved in cell wall remodeling, defense response, carbohydrate metabolism, and promotion of the lignification process. | Gütsch et al., 2019a |
|      | Microsorum pteropus | Leaves, Root | 100, 250 and 500μm CdCl₂/7-days/hydroponic | 2-DE, MALDI-TOF/TOF-MS | Different protein expression patterns were observed involving related functions of energy metabolism and antioxidant activity in the root, cellular metabolism, protein metabolism, and photosynthesis in leaves. | Lan et al., 2018 |
|      | Sorghum bicolor | Shoot | 100 and 150μm CdCl₂/5-days/semi hydroponic | 2-DE, MALDI-TOF/TOF-MS | Cd stress inhibits carbon fixation, ATP production, and the regulation of protein synthesis. | Roy et al., 2016 |
| Cr   | Brassica napus L. | Leaves | 100 μM K₂Cr₂O₇/3-days/ hydroponic | 2-DE, MALDI-TOF/TOF-MS | Increased abundance of defense-related proteins such as antioxidant enzymes, molecular chaperones involved in scavenging the excess ROS, and refolding of misfolded proteins under Cr stress. | Yildiz and Terzi, 2016 |
|      | Callitriche cophocarpa | Shoot | 1 mm K₂Cr₂O₇/3-days/liquid MS medium | SDS-PAGE, 2DE, MS/MS | Quinone dehydrogenase, FQR1 (NAD(P)H) newly identified to act as a detoxification protein by protecting the cells against oxidative damage. | Kaszycyki et al., 2018 |
|      | Nicotiana tabacum | Shoot | 100 μM K₂Cr₂O₇/5-days/ hydroponic | 2DE, MALDI-TOF/TOF-MS | Twelve Cr-tolerance-associated proteins were identified. These include mitochondrial processing peptidase, dehydrogenases, superoxide dismutase, adenine phosphoribosyl transferase, and mitochondrial malate dehydrogenase proteins. | Bukhari et al., 2016 |
|      | Pteris alba | Leaves, Root | 146.7 – 261.5 mm Cr/4-years/waste landfill field | 2DE, Nano HPLC MS/MS | ROS scavenging proteins assist poplar threes long-term adaptation to Cr polluted environments. | Szuba and Lorenc-Pluziksa, 2018 |
| Cu   | Agrostis capillaris L. | Shoot | 1–50μm CuSO₄/90-days/ semi hydroponic | 2DE, LC-MS/MS | Overexpression of a Heat shock protein 70 (HSP70) may be pivotal for Cu tolerance by protecting protein metabolism. | Hego et al., 2016 |
|      | Hyoscyamus albus L. | Root | 0, 0.1, 1, 20, and 200 μm CuSO₄/7-days/cell culture | MALDI-QIT-TOF-MS | High Cu levels enhanced respiration activity and propagated H. albus roots through the activation of the energy supply and anabolism. Increased abundance of proteins involved in carbohydrate metabolism, de novo protein synthesis, cell division, and ATP synthesis, and decreased proteasome. | Sako et al., 2016 |
TABLE 2 | Continued

| PTEs       | Plant species   | Plant parts      | PTEs concentration/ exposure time/media | Technology used                          | Key findings                                                                                                                                                                                                 | References                  |
|------------|-----------------|------------------|----------------------------------------|-----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| Triticum aestivum L. | Root          | Leaves          | 100 μM CuSO₄/3-days/hydroponic         | 2D-GE, HPLC-Chip                        | Cu responsive network of 36 key proteins, most of which may be regulated by abscisic acid (ABA), ethylene, and jasmonic acid (JA). Exogenous JA application showed a protective effect against Cu stress and significantly increased glutathione S-transferase (GST) gene transcripts. | Li et al., 2013             |
| Hg         | Paspalum distichum L. | Root        | 1,115 μM Hg/60-days/contaminated soil in glass box | LC–MS/MS                                | Observed changes in the expression patterns of metal binding and transport proteins. Increased accumulation of photosynthesis and energy metabolism, related proteins.                                      | Ding et al., 2019           |
| Triticum aestivum L. | Root          | Shoot           | 25, 50, 100, 200 and 400 μM HgCl/3-days /hydroponic | 2D-GE, LC–MS/MS                         | 49 abscisic acid (ABA) potentially regulated Hg-responsive proteins identified. Exogenous ABA application conferred protection against Hg stress and increased peroxidase enzyme activities, suggesting that it may be an important factor in the Hg signaling pathway. | Kang et al., 2015           |
| Pb         | Cannabis sativa L. | Leaves          | Pb(NO₃)₂ 3 g/kg soil /40-days/Potted soil | LC–ESI-MS/MS, SWATH-MS                  | Adaptation to Pb stress by accelerating adenosine triphosphate (ATP) metabolism; enhancing respiration, light absorption, and light energy transfer; and eliminating reactive oxygen species. | Xia et al., 2019            |
| Chrysopogon zizanioides | Root        | Shoot           | Pb(NO₃)₂ 400 mg/l, 800 mg/l and 1,200 mg/l/10-days/hydroponic (half strength Hoagland solution) | LC–MS/MS                                | Increased levels of key metabolites including amino acids, organic acids, and coenzymes in response to Pb.                                                                                                   | Pidatala et al., 2018       |
| Raphanus sativus L. | Root          | Shoot           | 1,000 mg/L Pb(NO₃)₂/3-days/modified half-strength Hoagland nutrient solution | GC–MS                                   | Pb exposure altered metabolites and divergent expression of enzymes which are responsible for profound biochemical changes, including carbohydrate metabolism, energy metabolism, and glutathione metabolism. | Pang et al., 2015           |
| Glycine max L. | Nodules       |                | 107.8 μM PbCl₂ or 1.84 μM HgCl₂/60-days /potted peat, perlite, and vermiculite (1:1:1) | 2D-GE, MALDI-TOF MS/MS                  | Pb stress increased the abundance of defense, development, and repair-related proteins.                                                                                                                      | Baig et al., 2018           |
| Zea mays   | Root           |                 | 18,000 μM Pb (NO₃)₂/12, 24 and 48 h/semi hydroponic | NANO-LC–MS/MS                           | Upregulation of stress, redox, signaling, and transport proteins, while proteins related to nucleotide metabolism, amino acid metabolism, RNA, and protein metabolism were down-regulated. | Li et al., 2016             |
| Se         | Allium cepa L. | Root            | 10 mg/l Se Na₃SeO₄/10-days/Hoagland’s nutrient solution | Cap HPLC-ESI-QTOF-MS and MS/MS, nano LC–ESI-Q Orbitrap-MS and MS/MS | Different abundances of proteins involved in transcriptional regulation, protein folding/assembly, cell cycle, energy/carbohydrate metabolism, stress response, and antioxidant defense were identified in response to Se stress. | Karasinski et al., 2017     |
| Brasica oleracea L. | Florets      | Leaves          | 25 μM Na₃SeO₄/14-days/Hoagland solution | UPLC–MS/MS, qRT-PCR, LC–MS/MS           | Glucosinolate reduction in broccoli leaves and florets is associated with negative effects on precursor amino acids (methionine and phenylalanine), biosynthesis, and glucosinolate-biosynthetic-gene expression in response to Se supplementation. | Tian et al., 2018           |
| Capsicum annumum L. | Shoot       |                 | 100 ppm Na₂SeO₃/1-day | LC–MS/MS                                | Overexpression of heat shock and metabolism proteins. Others are involved in post-translational modification, protein turnover, chaperones, and protein processing in the endoplasmic reticulum. | Zhang et al., 2019           |
| Oryza sativa L. | Shoot        | Root            | 25 μM NaAsO₃ and 25 μM Na₂SeO₃/15-days/Hewitt nutrient medium | MALDI-TOF/TOF, qRT-PCR, Western blot     | Differentially expressed proteins altered the gene expression related to abiotic and biotic stresses and defense responses such as ROS homeostasis, photosynthesis, energy metabolism, and transport and signaling. | Chauhan et al., 2020        |

repeat-containing protein, Zn finger CCCH domain-containing protein 14, flavonoid 3',5' hydroxylase, aspartate aminotransferase 3 (chloroplastic), protein Brevis radix-like 1, bergaptol O-methyltransferase, and probable F-actin-capping protein subunit beta proteins under Cd stress (Roy et al., 2016). Physiologically, in S. bicolor plants, there is the suppression...
of carbon fixation, ATP production, and protein synthesis regulation in Cd-stressed plants (Roy et al., 2016). In fact, under 500 μM Cd stress, the fern *Microsorum pteropus* is capable of sequestering high amounts of cadmium in roots and dry matter of leaves (up to 4,000 mg/kg), while the water fern *Azolla*, widely seen in Asian rice fields, does not have the same capacity to phytoaccumulate Cd.

In a study performed on hemp cultivars (Xia et al., 2019), it was found that phytoremediation of Pb impacts the following key pathways: protein synthesis, transcription, transport, signal transduction, photosynthesis, energy metabolism, and protein storage, among other systems. Examples of proteins that are upregulated in Y1 cultivars of hemp include ones that optimize ATP generation using ATP synthase subunit a (P65758 and P56757), ATP synthase protein M125 (Q04613), ATP synthase protein YMF19 (P93303), nucleoside diphosphate kinase III (O49203), pyruvate kinase (PKE; Q94KE3, Q9FNN1, and Q9FM97), and adenylate kinase 5 (ADK; Q8VYL1; Xia et al., 2019). Therefore, making more chemical energy appears to be a favorable development when exposed to high Pb stress. In particular, the pyruvate kinase that mediates pyruvate production for the Kreb’s Cycle is a key protein that is upregulated. In the same cultivar (Y1) under Pb stress, the following proteins were upregulated for signal transduction and transport: Five water transport-related aquaporins (e.g., Q06611, P25818, and others), patellin (Q56Z12 and Q56Z59), mitochondrial dicarboxylate/tricarboxylate transporter DTC (Q9C5M0), mitochondrial phosphate carrier protein 3 (Q9FMU6), mitochondrial carnitine/acetylcholine carrier-like protein (Q93XM7), MD-2-related lipid recognition domain-containing protein/M domain-containing protein (F4J7G5), and ras-related protein RABA2a (O04486; Xia et al., 2019).

Aquaporins on the contemporary are not seen solely as water transporters but can transport ammonia, boron, carbon dioxide, silicon, urea, and even PTEs such as As (Mosa et al., 2016). An Aqual gene from *Populus trichocarpa*, which has a very high number of aquaporins in its proteome, when expressed in a Zn-sensitive strain of yeast, was able to confer Zn-resistance. Furthermore, Aqual protein product was observed to co-localize with AtTIP1, a well-known Arabidopsis vacuolar marker (Ariani et al., 2019). The contenders for phytoremediation that are DEPs come in large datasets that it is difficult to describe in detail covering all proteins in one review article. There were 63 and 372 differently expressed proteins (≥1.5) in the tolerant (BM) and susceptible (Y1) cultivars of industrial hemp (Xia et al., 2019). A collection of 5,838 proteins were quantified in Poplar plants to check up- or down-regulation of proteins that play a role in phytoremediation in solely “Cd stressed” and “Cd stress remediated with nitrogen” groups (Huang et al., 2020). In the study, the differentially expressed proteins were in the high double digits and hundreds. The following pathways were also upregulated (in the process category) in Cd + N (nitrogen) plants compared to the Cd only group: inositol metabolic process, polyol biosynthetic process, polyol metabolic process, alcohol biosynthetic process, monosaccharide metabolic process, hexose metabolic process, and phospholipid biosynthetic process showcasing that nitrogen has the potential to recover phyto-destructive events (Huang et al., 2020). Furthermore, in the same study, there was upregulation of the following candidate proteins at both the proteome and phosphoproteome levels: heat shock protein 70 (HSP70), 14–3–3 protein, peroxidase (POD), zinc finger protein (ZFP), ABC transporter protein, eukaryotic translation initiation factor (eIF), and splicing factor 3 B subunit 1-like (SF3B1). In fact, plant transport and absorption were optimized, with 11 binding proteins, seven transporter proteins, and five-storage proteins upregulated in the Cd + N treatment. The main transporters that were upregulated were ABC transporters, which represented 57.1% of total transporters that were upregulated in the Cd + N treatment (Huang et al., 2020).

Biotechnologically, three main strategies are embarked upon to improve the clean-up of PTEs (i) manipulating metal/metalloid transporters, (ii) enhancing metal/metalloid ligand production, and (iii) conversion of metal/metalloid into volatile and less detrimental forms (Mosa et al., 2016). For the first strategy, tinkering with aquaporins that are capable of As transport, as well as other metalloids, antimonite (SbIII), silicon (Si), and boron (B) can be one way forward. The As is known to be present in rice grains and contributes to As in the human body (Chowdhury et al., 2020). For the second strategy, cysteine-rich proteins such as metallothionein and glutathione S-transferase take precedence, and this is a well-researched area in phytoremediation (Mosa et al., 2016). For the third one, Se, which is an essential micronutrient that can have negative repercussions when consumed in excess, is seen as a contender for intervention to turn excess Se into volatile products, such as dimethyl selenide, that can be released into the air (Mosa et al., 2016).

Studies available to date report either the up- or down-regulation of a considerable number of proteins related to several cellular essential processes. A general observation cannot be made from these studies since the change in proteome profile may depend on many factors, including the type of metal, the concentration of metal, exposure duration, growth environment, and other biological or non-biological entities associated with the plant system. However, it can be suggested that the toxic outcome of PTEs lies in the profile of functional proteins subject to change by various parameters being major among them is the metal type/concentration. Some of the essential proteins/enzymes and their expression altered by PTEs in leaves and roots are presented in Figures 4, 5, respectively. Since there can be hundreds of proteins in a single type of plant tissue whose expression is changed by PTEs when comparative proteomics is performed, therefore, combining all under one umbrella is cumbersome. To understand the impact of specific PTE on a specific plant species, proteomic toxicity profiling of PTEs with respect to plant organs or tissue needs to be performed in future studies. Many hyperaccumulator species of Brassicaceae and Caryophyllaceae do not possess mycorrhizal networks in their roots. However, hyperaccumulator plants (for example, the genus *Thlaspi*) have been documented to possess mycorrhizae, although sparsely under field and experimental conditions (Ferrol et al., 2016). The
inverse—mycorrhizae as determined by spore counts or root colonization has been significantly lower in soils rich in PTEs than non-metal rich soils—appears to claim that PTEs can have a detrimental effect on mycorrhizal survival (Ferrol et al., 2016).

However, mycorrhizal fungi never disappear from the ambient soil, suggesting that they can reform symbioses with plant roots under opportunistic conditions. AM fungi are capable of resisting PTEs by collective means that include cell wall binding to metals, chelation by glomalin, increased efflux to the exterior while diminishing uptake, cytosolic chelation, compartmentalization in the vacuoles, and upregulating antioxidant responses (Ferrol et al., 2016).

The success of exogenous nitrogen application suggests that the application of nitrogen-fixers (diazotrophs such as Rhizobium and Azotobacter) as biofertilizers can be an option to remediate polluted soils as well promote the capacity of plants to remediate PTEs such as Cd. In fact, metal-resisting Rhizobia can alleviate PTEs stress by production of phytohormones, nitrogen fixation, phosphorus solubilization, ACC deaminase synthesis, and siderophores (Brígido and Glick, 2015). The opulence in phenotypic and genotypic Rhizobial diversity makes it essential to choose the correct elite strains which can remediate soils that are not conducive to plant growth, such as N-deficient degraded lands.

**CONCLUSION**

The PTEs accumulation in the environment above threshold levels poses a high risk to biota health and significantly undermines global environmental sustainability efforts. Phytoremediation has proven to be one of the most efficient strategies to remediate PTEs polluted sites. However, the large-scale application and commercial success of phytoremediation are still to be demonstrated, partly due to the limited understanding of the PTEs sequestration mechanisms. Although several successes were recorded, the evolution of plant proteomics provides further opportunity to sufficiently elucidate PTEs phytoremediation mechanisms.
particularly in known high metal accumulating plants. This comprehensive review has demonstrated the potential of several PTEs accumulating plants and the active involvement of their proteome specific to the internal and/or external stimuli of applied PTEs. Various advanced tools and techniques both gel dependent and gel-free methods including qRT-PCR, western blotting, 2D-GE, LC-MS/MS, and MALDI TOF MS/MS have recognized the association of specific PTE with the enhanced expression of resulting proteome. The alteration of proteome expression (up- or down-regulation) in response to applied PTEs such as Cd, Cr, or Hg depends on the intracellular concentration of accumulated PTE, plant species, and the phytoremediation strategy being deployed by the plant. However, the PTE’s concentration effectively mitigated by the plant species in a defined or local environment without reducing crop production still needs further investigation. The species-specific (both plant and PTE’s species) knowledge of plant proteome changes under different growth conditions and growth phases such as from vegetation to flowering to fruiting stage indeed requires further investigation. A better understanding of PTEs-proteome relation will provide obvious benefits like (i) sustainable and effective decontamination of PTEs polluted sites while maintaining the plant growth and crop production and (ii) protection of soil biodiversity and quality. With an enhanced mechanistic understanding of the process, studies focusing on the engineering of the existing mechanisms of a plant’s PTEs sequestration should be prioritized. This will enable the development of an increased number of transgenic plant species with enhanced PTEs tolerance, uptake, and detoxification capabilities.

**AUTHOR CONTRIBUTIONS**

KU and MA: conceptualization. KU and BA: software. KU, MA, and BA: formal analysis. MA and HA: resources and funding acquisition. KU: writing—original draft preparation. KU, MA, HA, MR, MS, and BA: writing—review and editing. MA, KU, and HA: project administration. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling Editor declared a past collaboration with the author MS at the time of the review.

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