Review

Potentially Toxic Elements: A Review on Their Soil Behavior and Plant Attenuation Mechanisms against Their Toxicity

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Abstract: The presence of potentially toxic elements (PTEs) can induce phytotoxicity and growth inhibition in plants. These elements are bioaccumulated and biomagnified in the food chain due to their high stability and resistance to biodegradation. The availability and mobility of PTEs in soil depend on certain physicochemical procedures. Many scientific studies on PTEs have provided valuable information about the processes, environmental fate, effects and remediation techniques. However, there is a need for gathering and presenting all up-to-the-date information concerning mechanisms and processes of PTE mobility in the soil-plant interface. More specifically, soil chemical reactions and processes need to be discussed under the light of PTE potential uptake by plants, as well as the physiological mechanisms at plant molecular level of PTE attenuation when plants are subjected to PTE stress. Thus, in this study we discuss the important soil processes that influence the bioavailability of PTEs for plant uptake. We also elucidate the mechanisms such as phytochelation and antioxidant defense through which plants can mitigate PTE toxicity, enhance their tolerance, and promote their survival in contaminated soils. Moreover, we discuss the major mechanisms of reactive oxygen species (ROS) production and the strategies for ROS scavenging which involve enzymes and non-enzymatic compounds that demonstrate antioxidant effects. In conclusion, this review provides a comprehensive understanding regarding PTE toxicity, utilization and transportability. It could be used by the scientific community and soil end-users towards a better understanding of the mechanisms that plants use to alleviate PTE toxicity, significantly affecting the potential use of plants in soil remediation programs and their capacity to grow in PTE-contaminated soils.

Keywords: heavy metal toxicity; reactive oxygen species (ROS); antioxidant mechanisms; stress tolerance; soil characteristics; phytoremediation

1. Introduction

Elements such as Fe, Mn, Mo, Ni, Zn and Cu are known to be essential for plant physiological functions and growth, and phytotoxic symptoms may emerge when their concentrations exceed critical levels. On the other hand, there are elements that are highly toxic without any known biological functions in plants and can be classified as metals (Cd, Pb, Ag, Sr, Cr), metalloids (such as As and Sb) and non-metals (e.g., Sb) [1]. These elements are found in elevated concentrations in land and water ecosystems due to anthropogenic processes [2]. Whether essential for plant nutrition or non-essential, all these elements can be potentially toxic (thus they are referred to as “potentially toxic elements”, PTEs) and can cause phytotoxic effects (inhibit plant growth, photosynthetic and respiratory functions, alter enzymatic activity, and affect cell membrane and DNA structure) when their concentration exceeds a certain threshold; this largely depends on the nature of the specific element, on plant species, and on soil physicochemical characteristics [3–5].
Symptoms that are commonly related to PTE toxicity include inhibition of N metabolism, reduced photosynthetic rate and water uptake, limited uptake (acquisition) of micro- and macronutrients, root growth inhibition, reduced biomass production and, consequently, when plants are exposed to severe PTE stress, plant necrosis [6]. It is known that PTEs tend to be bioaccumulated and biomagnified through the trophic levels of the food chain, due to their very high stability and lack of biodegradability [7–11]. However, there is a need to discuss under one roof all information regarding PTE behavior and their interaction with plants in a comprehensive manner: starting from their mobility and fate at soil level as affected by important soil physicochemical and biological features and their uptake in the soil-plant interface, leading to their behavior as affected by plant physiological attenuation mechanisms at molecular level. Thus in this review we aimed to contribute towards a better understanding of the PTE behavior in the soil-plant continuum by comprehensively discussing: (a) soil physicochemical features that affect the behavior of PTEs regarding their mobility and plant availability; and (b) plant physiological processes that function as attenuation mechanisms.

2. Availability of PTEs to Plants

The bioavailable fraction of PTEs is essential for assessing the associated risk and is defined by interconnected physiochemical and biological parameters of soil and the nature of the elements [12]. In soil conditions (i.e., pH at the range of 4 to 9 and Eh at $-0.3$ to $+0.9$ V), cationic PTEs are commonly found as divalent cations that can form bonds of varied strength, mainly with clay silicates, oxides and organic matter [13]. In addition, soil texture plays an important role in the bioavailability of PTEs to plants. Clay-rich soils have a negative surface charge which promotes the attraction and retention of cations including PTEs. Contrary to that, coarse-grained soils (e.g., sandy soils) have a lower cation exchange capacity (CEC), and as a result, show a lower retention capacity for PTEs, leading to their eventual leaching [14,15]. Furthermore, iron (Fe) and aluminum (Al) oxides are two prevalent soil hydrous oxides that are abundant in soil and have a high ability for PTEs sorption. These metal oxides have a binding capacity for cationic PTEs in stable complexes, thereby immobilizing them and minimizing their bioavailability for plant absorption [15]. Determining how these soil factors affect the bioavailability of PTEs is crucial for risk assessment and efficient environmental management. The main soil properties that influence the fate of PTEs and their availability are analyzed below.

2.1. The Role of Soil pH

Soil pH significantly affects PTE availability. With increasing pH values cationic element bioavailability declines, while anionic element bioavailability increases. Decreased bioavailability of cationic elements under high pH conditions is caused due to the increased electronegative surface charge non-permanent-charged soil colloids, i.e., organic matter, Al and Fe oxides, 1:1 clay minerals, as well as some 2:1 clays [14]. Also, increasing pH values result in the formation of organometallic complexes which, if stable enough and of sufficiently high molecular weight, remove PTEs from soil solution and render them unavailable to be absorbed by plant roots. Apart from that, PTE bioavailability may be decreased due to the formation of inorganic complexes, mainly with phosphates and carbonates, which are typically very insoluble [16]. Cationic element sorption onto soil colloidal surfaces varies with pH values, ranging from weak at acidic conditions to strong at neutral to alkaline pH. More specifically, transition from weak to strong retention (sorption edge) of cationic elements onto negative charge solid phases is noticed at pH values greater than 3–5 for the elements Cu and Pb, 4–6 for Co and Zn, while at pH values greater than 5–7 strong fixation is noticed for the elements Cd and Ni [15]. An overview of the soil parameters affecting PTE availability is shown in Figure 1.
2.2. Redox Potential

The redox potential of soils can significantly affect the toxicity and lability of PTEs in hydromorphic soils and in PTEs with multiple oxidation states [16]. High redox (positive Eh) values are typically found in well aerated soils, while in water-logged soils Eh values are low and when they prevail for a long time period (e.g., several decades), higher organic matter content compared to other soils under a similar pedo-climatic regime is commonly noticed [17]. However, there are somehow conflicting reports regarding the effect of soil redox on cationic element availability [18]. Some researchers report that the availability of Pb, Cd and Zn increases when the soil redox potential is low. The increase in bioavailability is attributed to the increased dissolution of Fe–Mn oxyhydroxides that result in the release of the previously retained cationic elements [19]. On the other hand, reduced availability of some PTEs has been reported under reducing conditions [18]. A possible mechanism for this finding is the formation of insoluble carbonate and sulphide complexes with metals such as Fe$^{2+}$, Cd$^{2+}$ and Pb$^{2+}$ [16]. This is possible due to the fact that reducing conditions induce the reduction of abundant elements in the soil solution such as $\text{SO}_4^{2-}$ to $\text{S}^-\text{I}$ or $\text{S}^-\text{II}$ species This process consumes one mol of solution H$^+$ per mol $\text{SO}_4^{2-}$ reduced to $\text{S}^-\text{I}$, and two mols of H$^+$ per mol $\text{SO}_4^{2-}$ reduced to $\text{S}^-\text{II}$, as shown in the following reactions:

$$\text{SO}_4^{2-} + H^+ \rightarrow \text{S}^-\text{I} + 1.75 \text{O}_2 + 0.5 \text{H}_2\text{O},$$  

also written as

$$\text{SO}_4^{2-} + 0.5 \text{H}_2\text{O} \rightarrow \text{S}^-\text{I} + 1.75 \text{O}_2 + \text{OH}^-.$$  

$$\text{SO}_4^{2-} + 2H^+ \rightarrow \text{H}_2\text{S} + 2\text{O}_2,$$  

also written as

$$\text{SO}_4^{2-} + 2 \text{H}_2\text{O} \rightarrow \text{H}_2\text{S} + 2\text{O}_2 + 2\text{OH}^-.$$  

In turn, the produced alkalinity stabilizes primary carbonate surfaces or causes the transformation of CO$_2$ to HCO$_3^-$ and the subsequent precipitation of the latter product with abundant exchangeable cations, mainly Ca$^{2+}$, to secondary CaCO$_3$.

Some elements (Cr, As, Sb, Se) can be found in multiple stable oxidation states, resulting in varying bioavailability and toxicity [20,21]. More specifically, Cr is found in soil in two stable oxidation states, Cr(III) (commonly found as ionic Cr$^{3+}$ or chromite (FeO$\text{Cr}_2\text{O}_3$)) and Cr(VI) (found in two forms, chromate (CrO$_4^{2-}$) and dichromate (Cr$_2$O$_7^{2-}$)), that vary markedly concerning their biogeochemical properties [22,23]. Hexavalent chromium (Cr(VI)) is highly soluble, bioavailable and more toxic compared to Cr(III) [24]. In general, the biochemical behavior of Cr species in soil can be affected by soil texture, pH, EC, organic matter characteristics, Fe and Mn oxides, soil moisture and microbial activity [21]. However, organic matter, sulphides and ferrous species are considered to be the most significant reductants of Cr(VI) to Cr(III) [1].

Arsenic can also be found in two stable oxidation states, the less toxic As(V), which is commonly found in soil under aerobic conditions, and the highly toxic As(III) usually found in reducing conditions. Arsenite (As$^{\text{III}}\text{O}_3^{3-}$) exhibits a 100-fold higher toxicity and solubility than arsenate (As$^{\text{V}}\text{O}_3^{5-}$) [20]. Overall, the mobility and bioavailability of oxyanion species can be affected by redox potential and pH [25]. Experimental data indicate that oxyanion sorption and bioavailability can be affected by the physiochemical characteristics of the soil.
of each element, pH values, nature of colloids, the type of Fe (hydr)oxides, and the organic acids found in soils [25,26]. Other PTEs found in soil as oxyanions are antimonate (SbO$_4^{2-}$), selenate (SeO$_4^{2-}$), borate (BO$_3^{3-}$) and tellurate (TeO$_4^{2-}$) [20]. However, As sorption onto organic matter is favored by the presence of amino and thiol groups. Experimental data indicate that increased As sorption is often the result of the bridging of Al and Fe hydrolytic species to organic matter functional groups and the formation of tertiary As complexes [1].

2.3. Inorganic Colloidal Surfaces

Secondary phyllosilicate minerals, as well as crystalline and amorphous oxides significantly affect PTE availability [27]. Clay mineral content in soils increases their ion exchange capacity and thus their ability to retain PTEs due to the silanol and aluminol oxygen functional groups that are found on all clay minerals [28]. The potential of clay minerals to retain trace elements depends on their cation exchange capacity (CEC) that is largely influenced by their type and origin which can largely be sorted in the following descending order (typical CEC values in parenthesis—units in cmol$_c$ kg$^{-1}$ mineral): vermiculitic 2:1 clays (150) > expandable bentonite-type 2:1 clays (90–100) > illitic non-expandable clays (20–40) > 1:1 non-reactive clays (less than 10) [14,28]. It is estimated that kaolinite (1:1 clay mineral) can sorb 6.8 to 12.6 mg Cd g$^{-1}$, while total Pb sorption capacity ranges from 7.75 to 13.32 mg g$^{-1}$. For montmorillonite (an expandable 2:1 clay mineral), sorption capacity for Cd may be 30.7 mg g$^{-1}$, while for Pb it ranges from 31.1 to 75.4 mg g$^{-1}$ [27]. The sorption of PTEs onto clays can be categorized as specific and non-specific. Non-specific sorption is relatively weak and occurs due to the electrostatic attraction of solution hydrated ions to permanently charged sites; this retention mechanism is due to the formation of what is known as outer-sphere complexes. On the other hand, specific sorption is relatively strong and PTEs are much more closely related to the colloidal surfaces due to the formation of inner-sphere complexes of non-hydrated ions with the colloidal negatively charged surfaces [28]. Sorption studies with kaolinite have shown that Pb retention onto clay particles can be largely attributed to specific sorption to its octahedral Al hydroxyl groups [29,30]. This metal is typically known to be specifically bound with any clay mineral. Nickel sorption onto clay particles is mediated by both specific and non-specific sorption mechanisms. As for Cd sorption, in low pH values it has been attributed to non-specific sorption mechanisms, while in higher pH values to specific [27].

Other colloidal soil surfaces typically include Fe, Al, and Mn oxides. They contribute significantly to the PTE retention due to their relatively high specific surface area when in amorphous form. The retention efficiency of PTEs onto the functional oxygen groups found on those mineral surfaces depends on the acidity constant ($pK_a$) of the functional groups and the physicochemical characteristics of the studied metal [28]. Experimental data indicate that Fe oxide amendment to soils can reduce the available fraction of PTEs significantly. In particular, Fe oxide application was found to have led to a significant reduction of the available Pb and Cd by 49% and 41%, respectively [27].

2.4. Organic Colloidal Surfaces

Soil organic matter (SOM), also of colloidal size, consists of biomolecules of varying decomposition degrees. More specifically, SOM includes (a) organic compounds that are susceptible to microbial and chemical decomposition, and (b) stable organic compounds resistant to biological and physicochemical oxidative factors [31]. In general, soil organic matter has high specific surface area that bears numerous functional groups [28], very high cation exchange capacity (typical overall value of ca. 200–300 cmol$_c$ kg$^{-1}$ material at neutral pH) and buffer capacity leading to increased PTE sorption [16]. More specifically, on organic matter surface numerous carboxylic, hydroxyl, phenolic, amine, thiol and other functional groups that may be deprotonated can be found and the resulting variable charge that these chemical groups bear can limit the availability of PTEs in soils [27,28].
Humic substances can be described as part of soil organic matter formed from the decomposition of organic matter and are characterized by high molecular weight, recalcitrant compounds whose stability is attributed to their complex and condensed structure, as well as to their interaction with soil minerals [32]. Dissolved organic matter (DOM) is derived from partly decomposed organic materials and from root exudates and can be described as the fraction of organic molecules (mainly fulvic acids and less than 10% of low molecular weight organic acids (mono-, di-, and tricarboxylic acids)) that can pass through a 0.45-µm filter [13]. DOM availability and mobility can either promote or limit the availability of PTEs. Availability of DOM-sorbed PTEs largely depends on the molecular weight of DOM: relatively high molecular weight DOM-PTE complexes are of limited availability, contrary to the low molecular weight DOM complexes. Also, the density of active groups and the composition of DOM can affect the availability and speciation of absorbed PTEs [33]. Experimental data indicate that phenolic and carboxylic groups of DOM are the main sites that cationic elements are sorbed [13]. Fulvic acids addition to soils was found to result in increased PTE availability, contrary to humic acids that significantly reduced it [34]. Moreover, calcium concentration in soil can promote DOM coagulation, reducing DOM solubility, leading to limited potential to bind cationic elements [35]. The extent that DOM will form complexes with cationic elements depends on the number of negatively charged groups (COO\(^{-}\) and Ph-O\(^{-}\)) [13,32], although in acidic conditions their effect is reduced [13]. Besides cation retention, soil clay minerals and organic matter can also effectively retain anion species (e.g., CrO\(_4^{2-}\), SeO\(_3^{2-}\) and AsO\(_4^{3-}\)) that are sorbed as inner- and outer-sphere complexes which effectively control plant availability and leaching [28]. Inner-sphere complexes are formed through covalent bonds, while outer-sphere complexes are formed through electrostatic attraction [26]. Furthermore, precipitation of oxyanions on soil mineral surfaces is noticed when multinuclear complexes are formed on the mineral surfaces [28].

2.5. Ageing of PTEs

Potentially trace element ageing can be described as the gradual process of element fixation to soil pools from where their transfer to the solution is difficult and thus their immobilization nearly non-reversible. This process may take months or even years; it is affected by a host of soil properties, most important of which are reactive colloids content (2:1 clays, amorphous oxides, organic matter) and pH [36]. Ageing was reported to be an important factor limiting the availability of Cu, Ni and Zn for time periods that lasted up to 24 months, while the availability of Cd and Pb was unaffected [13]. Experimental data show that Cd, Zn and Ni sorption during ageing can be attributed to the diffusion of elements to inaccessible interlayer niches in clay minerals. Also, carbonate precipitation and stabilization through the formation of double-layered hydroxides were reported, leading to decreased Cd, Zn and Ni availability [37]. For Cu, experimental data indicated that it was complexed mainly to organic matter and sorption to clay minerals was of secondary importance [37]. In the case of Cr(VI), the ageing process functions in a different way: Cr(VI) seems to be naturally attenuated in soil over time due to its reduction to the inert Cr(III) species, a process that may occur even within the time frame of a short pot experiment [38,39]; organic C is also an important factor of this process, as it functions as an electron donor, accelerating thus Cr(VI) reduction to Cr(III), while it oxidizes organic C (C\(_{org}\)) to CO\(_2\) [40].

Ageing of soil PTEs is a rather underrated attenuation process of PTE mobility, as it leads to the gradual diminishing of their toxic effects over time. However, there is a void in the knowledge derived from experimental evidence, because PTE ageing studies are usually conducted under controlled conditions where soils have been spiked with PTEs and ageing time is short compared to real natural processes [12].
3. Potential Toxic Elements in Plant Tissues

Soil bioavailable PTEs can enter plant cells using the symplastic pathway (active transport of PTEs through metal ion carriers, facilitated by the formation of PTE-organic compounds complexes) or through the apoplastic pathway (through diffusion) [41]. Plants have developed defense mechanisms to limit the effects of PTEs. Therefore, plant species that are characterized as excluders have developed mechanisms leading to PTE stress avoidance mainly through PTE detainment in root cells [14]. On the other hand, in plant species that are characterized as indicators, PTE concentrations in aerial plant parts reflect the concentrations of PTEs in soil (resulting to soil-to-plant transfer coefficient (TC) values of approximately 1.0) [42]. However, in indicator plant species, correlation between aerial tissues and soil is noticed for a limited number of PTEs [43]. The majority of plant species retain PTEs in the root tissues, with minimal PTE translocation to aerial parts. However, for a limited number of plant species that are characterized as metal hyperaccumulators significant translocation of PTEs from root to aerial tissues is noticed, leading to the accumulation of high concentrations in aerial tissues without severe effects on plant growth and physiology [20,43]. The main effects on plant physiological functions of various PTEs are shown in Table 1.

Table 1. PTEs effects in plant physiological functions [43]. Upward arrows indicate increase, while downward arrows decrease.

<table>
<thead>
<tr>
<th>Potentially Toxic Element</th>
<th>Effects in Plants</th>
<th>Biological Functions</th>
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<tbody>
<tr>
<td>As</td>
<td>Oxidative stress</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ Phytochelatine synthesis</td>
<td></td>
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<tr>
<td>Cd</td>
<td>↑ NO synthesis</td>
<td>Enzyme inactivation</td>
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<tr>
<td></td>
<td>↓ NO₃⁻ assimilation</td>
<td>↓ ATP synthesis</td>
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<tr>
<td></td>
<td>↓ chlorophyll synthesis</td>
<td>Membrane disfunction</td>
</tr>
<tr>
<td>Cr</td>
<td>Root cell membrane damage</td>
<td>Protein oxidation</td>
</tr>
<tr>
<td></td>
<td>↑ Reactive oxygen species (ROS) production</td>
<td>Lipid, DNA, RNA peroxidation</td>
</tr>
<tr>
<td>Cu</td>
<td>↑ oxidative stress</td>
<td></td>
</tr>
<tr>
<td>Pb</td>
<td>↓ nutrient and water uptake</td>
<td>Electron transport chains in mitochondria and chloroplasts</td>
</tr>
<tr>
<td></td>
<td>↑ oxidative stress</td>
<td>Interaction with -SH groups (S-Hg-S bridge formation)</td>
</tr>
<tr>
<td>Zn</td>
<td>Oxidative damage</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaf non typical growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mn and Cu deficiency</td>
<td></td>
</tr>
<tr>
<td>Hg (forms: ionic, methyl and phenyl forms)</td>
<td>Inhibition of enzymatic and non-enzymatic antioxidants</td>
<td></td>
</tr>
<tr>
<td></td>
<td>↑ ROS production</td>
<td></td>
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</table>

In hyperaccumulator species, PTE concentrations in aerial tissues may be 100 to 1000 times higher compared to the PTEs concentrations found in the non-hyperaccumulator plant species [44,45]. Overall, 450 plant species from 45 families (mainly found in the Asteraceae, Brassicaceae, Fabaceae, Euphorbiaceae, Lamiaceae and Scrophulariaceae families) have been characterized as hyperaccumulators [41]. Plant species such as *Thlaspi caerulescens* (Zn, Pb, Cd), *Alyssum* sp. (Ni), *Brassica juncea* (Cd, Cr, Zn), *Brassica napus* (Zn), *Brassica nigra* (Pb), *Helianthus annuus* (Cd, Cu, Pb, Zn), *Helianthus tuberosus* (Hg) and *Pteris* sp. (As, Cr, Cu) are commonly referred to as metal hyperaccumulators [43,44]. Overall, most of the hyperaccumulators are found in three plant families: Asteraceae, Phyllanthaceae, and Brassicaceae [4]. For a plant species to be characterized as hyperaccumulator the following criteria are taken into consideration: translocation factor (TF) values (ratio of aerial/root tissue PTE concentration) should be greater than 1.0, TC values of aerial tissues (ratio of aerial tissue/soil PTE concentration) greater than 1.0, and PTE concentrations in...
aerial plant tissues exceed 100 for Cd and Se, 1000 mg kg\(^{-1}\) for Co, Cr, Cu, Ni and Pb and 10,000 mg kg\(^{-1}\) for Zn and Mn \[41,43,44\]. Experimental data indicate that the ability of hyperaccumulator species to translocate PTEs from root to aerial tissues largely depends on the overexpression and upregulation of genes related to vacuole PTE sequestration, chelating agent synthesis, and transport proteins \[43\]. Every PTE has been associated with a different number of hyperaccumulator species, and most of these are able to hyperaccumulate one specific element. Particularly, for the element Ni 532 hyperaccumulator species have been identified (distributed in 130 genera), for Cu 53 (43 genera), for Se 41 (15 genera), for Pb 8 (8 genera), and for Cd 7 (7 genera) \[45\].

Upon entering plant cells, PTEs significantly affect plant homeostasis, due to cell membrane structural and functional impairment, and excessive reactive oxygen species production that impacts mainly chloroplast and mitochondrial structure and functions; moreover, a series of biochemical functions involved in protein synthesis are affected and negative impacts are exerted on the structure and functions of nucleic acids \[46\]. However, plants have developed several mechanisms to limit PTE toxicity such as organic acid production, glutathione (GSH), phytochelatins (PCs), metallothionines (MTs), enzymes that alleviate oxidative stress such as catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), heat shock proteins (HSPs), and the increased concentration of osmolytes such as proline (Pro) \[11,14,47\].

4. Mechanisms Involved in Limiting PTE Uptake from Plants

4.1. Excretion of Organic Compounds from Root Tissues

Organic substances that roots excrete can be categorized as high molecular weight substances such as proteins and polysaccharides, and as low molecular weight compounds such as organic acids, amino acids, phenolic compounds and sugars. Root excretions modify the physicochemical characteristics of the rhizosphere that lead to the reduction of PTE uptake from root tissues \[14\]. For instance, a reduced uptake rate has been noticed for the elements Cd, Pb, Al and Cu due to their complexation with low molecular weight organic acids (citric, oxalic, succinic, tartaric, and malic acid) and amino acids \[48,49\].

Also, phytosiderophore excretion from Poaceae, that are excreted under low Fe availability, may form phytosiderophore-complexes with Zn, Cu, Mn and Cd that limit their uptake from plants \[47,48,50,51\]. On the other hand, phytosiderophores, organic acids, phenolic substances and enzymes form complexes with essential for plant growth elements leading to their higher bioavailability \[47,52\].

4.1.1. Amino Acids

Amino acids proline and histidine can form complexes with metallic ions mainly found in plant vessels and cytoplasm. Furthermore, proline concentration in plant cells was found to have increased under PTE stress that led to limited reactive oxygen species production, increased glutathione efficiency, protein structure stability and contribute to cell osmoregulation \[53–55\].

4.1.2. Organic Acids

Low molecular organic acids (such as citric, oxalic and malic) can form complexes with PTEs. Organic acid-PTE complexes lead to increased PTE transport to the vacuole of plant cells that alleviates plant stress. Additionally, increased excretion of organic acids from root cells was noticed as a response to increased concentrations of Al, Ga, Zn, or Pb leading to reduced PTE bioavailability \[52,53,56\]. Contrary to these findings, root tissues of hyperaccumulator plant species have been found to release exudates (including organic acids) that can increase PTE bioavailability. Experimental results indicate that exogenous application of low molecular weight organic acids led to increased bioavailable concentrations of Pb, Cu and Cd that were attributed to the dissociation of soil organic matter amorphous oxides. On the other hand, the application of high molecular weight organic acid led to decreased bioavailable concentrations of Pb, Cu and Cd that was attributed to
the increase of amorphous iron oxides (Figure 2) [57]. Increased PTE availability due to exogenously applied organic acids can well be attributed to soil acidification. However, organic acids secreted from root cells are in the form of anions (combined with simultaneous K⁺ secretion) resulting in minimal pH disturbance. Furthermore, experimental data measuring the bioavailability of PTEs complexed with plant excreted organic acids have indicated that Pb-oxalate complexes were of extremely low solubility, Cd-oxalate complexes were characterized as partially soluble, and the complex formations resulted in lower Pb and Cd bioavailability [56].

Figure 2. Organic acids and their effects on potentially toxic element availability (information obtained from Yao et al. [57]). HMWOA: high molecular weight organic acid; LMWOA: low molecular weight organic acid. Bold arrows indicate the effects of processes induced by LMWOAs and HMWOAs.

4.2. Effect of Cell Wall and Cell Membrane Biomolecules on PTE Toxicity

4.2.1. PTE Efflux from Plants

Leaves of some plant species bear trichomes that are uni- or multicellular structures with high variability concerning the chemical substances that they store or excrete, while they show great morphological variability. In leaf trichomes, complexes of metals with secondary metabolites may be formed, resulting in reduced toxicity [6, 58–60]. For instance, when tobacco plants (Nicotiana tabacum) were exposed to high Cd concentrations, the number of trichomes in their leaves doubled and a significant excretion of crystals containing Cd, Ca, Mn, P, S or Mn was noticed [58, 61]. PTE translocation to leaf senescent leaves has also been found, leading to reduced PTE concentrations in plant tissues after leaf shedding [43].

4.2.2. PTE Transporters

In plant cells, extensive PTE transport from cytoplasm to the vacuoles is noticed aiming to prevent the disturbance of important biochemical processes occurring in the cytoplasm and in organelles such as mitochondria and chloroplasts [62]. Plants have evolved specific transporters in order to influence the absorption and movement of PTEs in plants’ cellular compartments. PTE transport into the vacuole is mediated from various proteinic transporters [47, 63]. The iron-regulated transporter (IRT) family has key role in the uptake of iron and other metal cations such as Cd, Zn and Mn [64]. Also, PTE transporters of the ZIP (zinc regulated transporter (ZRT) and IRT-like proteins) and heavy metal ATPases (HMAs) families mediate cation transport from root to aerial tissues in hyperaccumulating plant species [41]. HMAs are metal efflux proteins that remove PTEs from cells and are responsible for PTEs detoxification [65]. In hyperaccumulating plants, PTE ligands are formed with metallothioneins (MT), phytochelatins (PCs) and nicotinamides (NA), and these complexes can be transferred to aerial plant parts by specific transporters (most commonly through ABC and yellow stripe-like (YSL) transporters) [66]. Moreover, ATP-binding cassette transporters (ABC) are found on the vacuolar and plasma membrane. These transporters are involved in the uptake and translocation of PTEs using ATP hydrolysis [67]. Another group of transporters is the natural-resistance-associated
Agriculture 2023, 13, 1684

4.3. Biomolecules That Conjugate PTEs

When PTEs enter plant cells, a series of defense mechanisms are employed to counter the toxic effects to plant cells. Synthesis of various biomolecules (such as nicotinamide, glutathione, phytochelatins, metallothioneins and phenolic substances) takes place that conjugate PTEs resulting in PTE stress reduction [47].

4.3.1. Amino Acids and Heat Shock Proteins

Amino acids form complexes with PTEs which may lower their toxicity and contribute in the maintenance of the osmotic potential of plant cells. Conjugated metals can be isolated to plant cell vacuoles, thus protecting vital biochemical processes from PTEs toxicity [53,68]. Additionally, literature reports show that heat shock proteins (HSPs) protect the structure and the function of enzymes and proteins [6,9,10,69]. The contribution of the above-mentioned biomolecules in the alleviation of PTEs-induced stress varies depending on the plant species and its growth stage [6,69].

4.3.2. Glutathione

Glutathione is a tripeptide (γ-Glu-Cys-Gly) that greatly contributes to plant resistance to PTE stress. The enzyme glutathione reductase (GR) using reducing power from NADPH results in the transformation of the oxidized glutathione form (GSSG) to the reduced form of glutathione (GSH), keeping the ratio of the reduced form to the oxidized form of glutathione (GSH/GSSG) at high levels [53]. The minimized disruption of key metabolic functions by PTE toxicity is caused by the combination of glutathione’s capacity to form complexes with PTEs, along with their rapid transfer in cell vacuole and their high solubility [47]. Experimental data indicate that in Gossypium hirsutum growing under Pb stress, the addition of GSH at rates ranging from 50 to 500 µM led to increased activity of the antioxidant enzymes CAT, SOD, POD, GR and APX and the structure of chloroplast was not significantly affected [70]. Furthermore, increased glutathione concentrations were noticed for plant species Brassica juncea and Phragmites australis resulting in increased photosynthetic rates [11,70,71]. Moreover, glutathione (GSH) homeostasis largely depends on the enzyme glutathione peroxidase (GPX) that uses GSH as a reductant (GSSG is formed) leading to H_{2}O_{2} reduction [72].

4.3.3. Phytochelatins

Phytochelatins are low molecular weight compounds found in the cytoplasm; they contribute to plant resistance to a series of stress factors such as high concentrations of PTEs, high temperature, salinity and high levels of ultraviolet radiation [73]. Phytochelatins are polymeric peptidic compounds that are synthesized from 2 to 11 dipeptides of γ-Glu-Cys and the phytochelatin structure terminates with the addition of the amino acid Gly ((γ-Glu-Cys)_{n=2 to 11}-Gly). Alternatively, phytochelatin structure may be terminated using one of the amino acids serine (Ser), glutamic acid (Glu), glycine (Gln), alanine (Ala) or the terminal amino acid may be absent from the phytochelatin structure ((γ-Glu-Cys)_{n=2 to 11}). Phytochelatin synthesis is mediated from the enzyme phytochelatin synthase using glutathione molecules ((γ-Glu-Cys)-Gly) and increased phytochelatin synthesis in plant cells is noticed upon PTE stress [6,75–77]. Sulfhydryl and carboxylic groups found in the phytochelatin structure result in the effective complexation of PTEs. Most commonly the structure of phytochelatin involves 2 to 5 dipeptides (γ-Glu-Cys)_{2–5}-Gly; however, under neutral to slightly alkaline conditions and under PTE stress, an increased number of dipeptides (γ-Glu-Cys) can be found in the structure of phytochelatin [74,77,78]. Increased phytochelatin synthesis from plants was noticed under PTEs stress and increased translocation of phytochelatin to root cells was induced due to PTE accumulation in root tissues [79]. Increased activity of phytochelatin synthase was also induced under increased concentrations of H_{2}O_{2}, Ag,
Pb, Zn, Cu, Hg, Au and Cd. Phytochelatin-complexed metals are transported inside the vacuole through Mg-ATP, ABC and P1B-HMA transporters and inside the vacuole metals form complexes with organic acids or with sulphates contained into the vacuole [6,47,79].

4.3.4. Metallothionein

Metallothioneins can be categorized as low molecular weight proteins of highly variable structure, rich in cysteine and with high affinity in forming complexes with PTEs, while they are mainly found in the cytoplasm. Experimental data indicated that metallothionein effectively reduced the toxic symptoms from Cu, Zn, Cd and As by complexing the metals and mediating their transport to the vacuoles [80]. Contrary to the enzyme mediated synthesis of phytochelatin, metallothionein synthesis occurs after translation of the mRNA coding metallothionein structure [55]. Metallothionein synthesis can be enhanced from PTE increased concentrations, low concentrations of plant nutrients, during osmotic stress and due to exposure to extreme temperatures [6,47,55,76,79].

4.3.5. Nicotinamide

Nicotinamide structure bears carboxylic and nitric groups where elements such as Cu, Ni, Zn and Fe can be complexed. Nicotinamide is found in chloroplasts and in plant vascular systems where there is a lack of biomolecules that have the potential to complex PTEs. However, when high concentrations of nicotinamide are noticed in plant cells, nicotinamide can also be found in the cytoplasm. Furthermore, nicotinamide concentration in plant cells seems to be positively correlated to the concentrations of glutathione, further alleviating stress effects from the elements Cd, Cu and Zn [71,76,81,82].

4.3.6. Phenolics

Plants may overcome various abiotic and biotic stressors by increasing the concentration of phenolic compounds, and their specificity depends on the plant species and the type of stress [83]. Phenolics are vital for plant physiology processes, particularly in response to PTE stress. These secondary metabolites have been demonstrated to increase PTE accumulation in plants without toxic symptoms [84]. Hydroxyl and carboxylic groups found in the structure of phenolic substances can bind metallic ions and phenolic concentration in plant cells increases after exposure to the elements Cu, Cd, Al, Pb and Ni. Moreover, phenolics bind the elements Fe and Cu, leading to the alleviation of the negative effects of reactive oxygen species produced by Fenton reactions. Also, phenolics act as electron donors for $\text{H}_2\text{O}_2$ reduction from the peroxidases [53,85], while excretions of phenolics in the rhizosphere enhance the development of bacteria associated with the degradation of contaminants, making them valuable in phytoremediation strategies [86].

4.3.7. Chaperones

Chaperones are proteins that contribute to the assembly, transport and peptide chain folding of proteins. In plant cells, elements such as Hg, Pb, As, Cr and Cd alter protein structure and result into limited enzyme activity. The majority of chaperone proteins contribute in protein structure stabilization under PTE stress, and also in enhancing the activity of antioxidant mechanisms. Chaperone concentration increases during abiotic stress (high temperature, drought, nutrient deficiency and PTEs). Heat shock proteins are molecular chaperones that are classified as HSP 70, HSP 60 and sHSP and the HSP 70 group of proteins has exhibited a synergistic effect on metallothionein action during PTE stress [9,47,87–89].

4.3.8. Tocopherols

Increased tocopherol concentrations in plant cells can be noticed as a response to increased PTE concentrations, high radiation intensity, low temperature and salinity. Tocopherols, which are composed in plant cells plastids and can be regenerated after glutathione or ascorbate reduction, may control lipid peroxidation leading to the functional and struc-
tural integrity of biological membranes and contribute to the reactive oxygen species scavenging [23,53]. More specifically, tocopherols scavenge singlet oxygen, hydroxyl radicals and prevents further lipid peroxidation, while tocopherol oxidation products (α-tocopherol quinone (TQ) and α-tocopherol quinol (TQH2)) are strong antioxidants [90].

4.3.9. Proline

Proline can act as a stabilizing agent for the structure of macromolecules; it is a structural element of cell walls and contributes to the elimination of reactive oxygen species. Increased proline concentration is noticed when plants are exposed to high salinity, water stress, high levels of ultraviolet radiation, high concentrations of PTEs and oxidative stress. Proline synthesis after plant exposure to stress factors has been attributed to water balance disturbance and the damage inflicted on cell membranes. Furthermore, increased proline concentrations lead to the stabilization of protein structure, reactive oxygen species elimination, increased antioxidant enzyme activity (CAT, POX and SOD), the preservation of redox potential in plant cells, the regulation of the osmotic potential, and pH regulation. The rate of proline synthesis after PTE stress depends on the element that causes the stress (elements that have been found to promote proline synthesis are Hg, Cd, Cu and Pb), element concentration, plant species and the plant tissue exposed to the toxicity [6,54,91].

5. Inorganic Compounds and Their Effect on PTE Stress Alleviation: Hydrogen Sulphide (H₂S)

Hydrogen sulphide (H₂S) is known as a toxic gas, although in small quantities it can play a crucial role in plant functions. In particular, numerous studies have shown that it affects stress tolerance, root growth, stomatal regulation, and photosynthetic efficiency [92–94]. Hydrogen sulphide contributes to the alleviation of stress effects caused by factors such as extreme levels of salinity, drought, high temperature and PTEs concentrations (Cr, Cd and Al) [95]. The priming effect of cellular adaptation to stress factors has been attributed to plant redox signalling and the exogenous application of H₂S has been found to lead to the adaptation to multiple abiotic stresses [96]. According to Nomani et al. [97], H₂S decreased the concentration of copper (Cu) in various plant parts and mitigated the adverse effects of Cu toxicity by increasing the activity of the enzymatic defense system (CAT, POX, SOD). Furthermore, the increased endogenous levels of H₂S enhanced the physiological and biochemical properties and mitigated Cd stress [98].

6. Reactive Oxygen Species in Plant Cells

Reactive oxygen species (ROS) in plant cells are produced from enzymic and non-enzymic reactions when plants are subjected to biotic and abiotic stress. In general, PTEs (e.g., Cu, Ni, Cd, Cr and As) can lead to increased ROS production in plant cells when their concentrations exceed permissible limits [22]. This reactive oxygen species (ROS) production takes place in chloroplasts and peroxisomes, and leads to the activation of ROS scavenging potential in plant cells. More specifically, increased ROS concentrations may result in the inactivation of antioxidant enzymes such as SOD or CAT. Also, PTE toxicity may result in the depletion of low molecular weight antioxidants such as glutathione [9].

However, ROS are continuously produced in unstressed cells as by-products of plant cell metabolic functions (e.g., photorespiration in peroxisomes, redox reactions in cytoplasm, respiration in mitochondria and photosynthesis in chloroplasts) and their levels are controlled by a series of antioxidant enzymes and organic compounds. Exposure to biotic and abiotic stresses increase production of reactive oxygen (ROS) and reactive nitrogen species (RNS) is noticed through enzymic and non-enzymic reactions (Figure 3). When an imbalance between produced and scavenged ROS (in favor of the produced ROS) occurs due to stress factors, increased ROS concentrations can severely affect enzyme functionality, alter protein and membrane structure, and disturb gene expression [7,9,11,69,99,100]. Reactive oxygen species can be generated from oxygen molecules activated from sufficient energy (¹O₂), or through reduction reactions that result in the formation of hydrogen
peroxide (H$_2$O$_2$), superoxide radicals (O$_2^{••}$), and hydroxyl radicals (•OH) [101]. Reactive oxygen species can be categorized as free radicals that bear a non-conjugated electron in their external orbit (superoxide anion (O$_2^{•−}$), hydroperoxyl radical (HO$_2^{•}$), alkoxy radical (RO$^•$) or hydroxyl radical (•OH)) and as non-radical molecules where orbital electrons are in pairs (singlet oxygen (O$_2^1$) and hydrogen peroxide (H$_2$O$_2$)) [9].

Figure 3. Reactive oxygen (ROS) and nitrogen (RNS) species (information obtained from Kapoor et al. [102] and Mansoor et al. [101]).

6.1. Main Reactive Oxygen Species Found in Plant Cells

6.1.1. Singlet Oxygen

Singlet oxygen (Figure 4; O$_2^1$, migration distance: 30 nm, t$_{1/2}$: 1–4 µs) production occurs largely in photosystem II (PSII) during photosynthesis, during plant leaf senescence, and when plant cells are exposed to abiotic stress. Singlet oxygen can oxidize lipids and affect protein structure (amino acids Trp, His, Tyr, Met and Cys were found susceptible to oxidation), while guanine bases in DNA structure can also be affected by guanine (G) reactions with O$_2^1$ [85,100,103]. Singlet oxygen levels can be controlled by low molecular weight lipophilic organic compounds such as β-carotene, tocopherol, and the D-1 protein of PSII. Singlet oxygen seems to have an additive effect with the other reactive oxygen species, leading to alterations in the functions and structure of different biomolecules and in parallel can alter the expression of genes [85,90].

Figure 4. Singlet oxygen and superoxide radical primary reaction stages (information obtained from Mansoor et al. [101] and Dumanović et al. [100]).

6.1.2. Superoxide Anion

Superoxide anion (Figure 4; O$_2^{••}$, migration distance: 30 nm, t$_{1/2}$: 1–4 µs) mostly affects Fe-S proteins [100]. Production takes place mainly in chloroplasts, peroxisomes, mitochondria, cellular membranes and in the apoplast. The production of superoxide anion

\[
\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{OH}^- + \text{OH}^• + \text{Fe}^{3+}
\]

Fenton reactions
can affect a series of biochemical processes inside the cell and its concentration is largely controlled by the activity of the enzyme superoxide dismutase (SOD). This enzyme (SOD) is mainly found in chloroplasts, peroxisomes, the endoplasmatic reticulum and in the plant cell walls catalysing the reaction $O_2^{•−} → H_2O_2$ [69,85,104]. The sites of ROS production and the enzymes involved in this process are summarized in Table 2.

Table 2. Sites of ROS generation and enzymes involved [101].

<table>
<thead>
<tr>
<th>Sites of ROS Production</th>
<th>Enzymes Involved</th>
<th>ROS Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondria</td>
<td>Complex I and III</td>
<td>1–5% (ROS production/O$_2$)</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td>Glycolate oxidation</td>
<td>Main site of intracellular ROS production</td>
</tr>
<tr>
<td>Cell wall, vacuoles, endoplasmic reticulum, nucleus and mitochondria</td>
<td>Oxalate oxidase</td>
<td>NOX transfer $e^−$ from NAD(P)H to O$_2$</td>
</tr>
</tbody>
</table>

6.1.3. Hydrogen Peroxide

Hydrogen peroxide (H$_2$O$_2$, migration distance: more than 1 µm, $t_{1/2}$: more than 1 ms) reacts with proteins (affecting Cys and Met amino acids) and DNA molecules [100]. It is involved in a number of cell functions such as cell death, immunity, regulation of stomatal aperture and is also involved in environmental stress responses. Hydrogen peroxide with a mean lifetime of $10^{−9}$ s can affect biomolecules in its vicinity and subsequently, hydroxyl radicals (OH*) are produced, while its production is reported in chloroplasts, peroxisomes, mitochondrion and in apoplast [105]. It has a major role in the maintenance of plant cell homeostasis and contributes to the regulation of biochemical functions affected in plant cells under stress. More specifically, it affects the expression of several genes involved in plant cell senescence and induction of resistance in biotic and abiotic stress factors (plant pathogens, stress-induced from high radiation levels and from water stress) [69,85,104]. H$_2$O$_2$ can oxidize S-H groups of Cys residues to sulfenic acid that can be further oxidized to sulfonic acid (R-SO$_3^•$H) leading to permanent oxidation damage in protein structure [106].

6.1.4. Hydroxyl Radical

Hydroxyl radical (•OH, migration distance: 1 nm, $t_{1/2}$: 1 ns) production is mediated through Fenton reactions (H$_2$O$_2$ → •OH). It is an extremely reactive oxygen species that mainly affects DNA, RNA, lipids and proteins [100]. Exogenously applied H$_2$O$_2$ may lead to underestimation of the OH* effect that is produced from H$_2$O$_2$ in the plant cell walls or within the cells. Hydroxyl radicals (OH*) production is found to affect reproduction, germination, plant growth and cell death. Contrary to H$_2$O$_2$, for hydroxyl radicals (OH*) there are no enzymes to be involved in OH* detoxification and plant cells rely on a range of antioxidants to limit the possible damage to plant cells [105].

6.2. Mechanisms Affecting ROS Transformations in Plant Cells

Due to PTEs stress, reactive oxygen species (H$_2$O$_2$, O$_2^{•−}$ and OH*) concentrations in plant cells can rise. The effects of the reactive oxygen species on plant cells may be divided in two categories:

1. Although Fe and Cu are not toxic to plant cells in normal concentrations, they contribute to the overall stress inflicted by PTEs, because they are essential for Weiss-Haber and Fenton reactions that lead to the production of hydroxyl radicals (OH*) while used as a substrate of hydrogen peroxide (H$_2$O$_2$).
2. Elements such as Pb, Cd, As, Hg, Ni, Al, Mn and Zn lead to the production of ROS by being attached to the active sites of enzymes (due to their high chemical affinity to SH groups) or by activating the NADPH oxidases [11,76].

ROS production results in high oxidation rates for the residues cysteine (Cys), methionine (Met), lysine, arginine, proline, histidine, threonine, and tyrosine. Especially
the aminoacids cysteine and Met that bear sulfuric groups are highly susceptible to ROS oxidation. Keeping O$_2$•− and H$_2$O$_2$ concentrations at non-toxic levels is of paramount importance for plant cell homeostasis. When O$_2$•−, H$_2$O$_2$ and Fe or Cu ions are present, Fenton reactions occur in plant cells leading to the production of hydroxyl radicals (O$_2$•− → H$_2$O$_2$→ H$_2$O$_2$ + M$^{(n−1)}$ → OH$^−$ + OH• + M$^{(n)}$) that are described as the most toxic reactive oxygen species. Essentially, hydroxyl radicals can react with almost every known biomolecule leading to their oxidation [7,11,85,107]. The ROS production and their effects on cells are presented in Figure 5.

![Figure 5. ROS production and their effects on cells (information obtained from Sharma et al. [22], Mansoor et al. [101], Dumanovic et al. [100], and Aranda-Rivera et al. [106]).]  

6.3. Fenton Reactions

Fenton process reactions can be presented with the following equations, where Fe(II) and Fe(III) react with H$_2$O$_2$ at pH 2.8–3.0, and ROS produced can react with organic molecules. In the following chemical reactions, kinetic data indicate that Fe$^{2+}$ is required for Fenton reactions to proceed effectively:

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{•OH} + \text{OH}^− \quad (k = 40-80 \text{ M}^{-1} \text{ s}^{-1}) \quad \text{(Fenton reaction)}
\]

\[
\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HO}_2^* + \text{H}^+ \quad (k = 0.001 \text{ M}^{-1} \text{ s}^{-1})
\]

Organic compounds + •OH → Oxidation of organic matter (i.e., peroxyl radicals (•OOR), alkoxyl (•OR))

Organic compound radicals may react with Fe ions and alter its redox state. More importantly, Fe$^{2+}$ ions may be regenerated, resulting in further •OH production [106,108,109].

\[
\text{R}^* + \text{Fe}^{3+} \rightarrow \text{R}^+ + \text{Fe}^{2+}
\]

\[
\text{R}^* + \text{Fe}^{2+} \rightarrow \text{R}^- + \text{Fe}^{3+}
\]

\[
2\text{R}^* \rightarrow \text{R}-\text{R}
\]

Hydroxyl and hydroperoxyl radicals can react with Fe$^{3+}$, Fe$^{2+}$ or H$_2$O$_2$ leading to the production of less reactive oxygen species, and Fe redox state alteration [109], as described in the following equations:

\[
\text{Fe}^{3+} + \text{•O}_2\text{H} \rightarrow \text{Fe}^{2+} + \text{O}_2 + \text{H}^+
\]

\[
\text{Fe}^{2+} + \text{•O}_2\text{H} \rightarrow \text{Fe}^{3+} + \text{O}_2\text{H}^−
\]

\[
\text{Fe}^{2+} + \text{•OH} \rightarrow \text{Fe}^{3+} + \text{OH}^−
\]
$\text{H}_2\text{O}_2 + \cdot\text{OH} \rightarrow \cdot\text{O}_2\text{H} + \text{H}_2\text{O}$

except for Fe(II), other metals with multiple oxidation states may be used for Fenton reactions. Cu may be used for non-Fe Fenton reactions with a wide optimum pH range around 7 and in the presence of Fe$^{3+}$, Fe$^{2+}$ ions can be generated upon Cu$^+$ oxidation.

$\text{Cu}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^+ + \cdot\text{O}_2\text{H} + \text{H}^+$

$\text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \cdot\text{OH} + \text{HO}^- \ (\text{Fenton—like reaction})$

Cu$^+$ + Fe$^{3+}$, Cu$^{2+}$ + Fe$^{2+}$ (Fe$^{2+}$ can be used for $\cdot$OH production through Fenton reactions).

Similar to Cu, Mn, Co, Cr or V may be used in parallel with Fe and experimental data indicate that Fe-based heterogenous catalysts result in increased reactivity due to the creation of thermodynamically favorable redox pairs (Fe$^{3+}$ → Fe$^{2+}$ and Mn$^{n+}$ → Mn$^{n+1}$) [109,110]. Ferritins and metallothioneins can store Fe, Cu and Zn, thus limiting ROS production through Fenton reactions. However, Asc or $\cdot$OH can also reduce Fe$^{2+}$ → Fe$^{3+}$ and Cu$^+$ → Cu$^{2+}$, resulting in increased ROS production through Fenton reactions [100,111]. An overview of the effect of redox and non-redox metals is shown in Figure 6.

![Antioxidant mechanisms in plant cells (information obtained from Kapoor et al. [102]).](image)

6.4. Mechanisms That Are Involved in the Alleviation of Reactive Oxygen Species Effects

Under normal conditions when plants are not exposed to any stress, reactive oxygen species are produced due to the metabolic reactions occurring in the plant cells and their levels are controlled by the antioxidant mechanisms of the cell. The enzyme superoxide dismutase has a central role in reactive oxygen species regulation. Three types of superoxide dismutase are found in plant cells: (a) Cu-Zn SOD, found mainly in the cytoplasm (Cu-Zn SOD I); (b) Mn-SOD, found in mitochondria; and (c) Fe-SOD, found in chloroplasts [53,85]. PTEs can affect the function of important enzymes for photosynthesis by forming ligands with sulphate and carboxylic groups and by being complexed to the active site of different enzymes; disturbance of their functionality has been identified for several enzymes including Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phosphoenolpyruvate carboxylase (PEPC). Also, PTEs disturb the function of the mitochondrial respiratory chain (complexes I and III), leading to the imminent increase of reactive oxygen species (ROS) concentration [44,104]. The increased production of reactive oxygen species during PTE plant cell stress leads to the activation of a series of antioxidant mechanisms in order to maintain the homeostasis levels of cells. Superoxide dismutase (SOD), catalase, glutathione and ascorbic acid contribute significantly in reducing oxidative plant cell stress [47,53,104]. More specifically, ROS scavenging mechanisms in plants can be divided into two categories, enzymatic and non-enzymatic. Enzymes that scavenge ROS are SOD, CAT, ascorbate glutathione (AsA-GSH), and glutathione-S-transferases (GSTs).
Non-enzymatic mechanisms that can scavenge ROS are carotenoids, \(\alpha\)-tocopherol, GSH, AsA and proline [101].

Controlling the concentration of hydrogen peroxide (H\(_2\)O\(_2\)) in plant cells is of paramount importance because H\(_2\)O\(_2\) is the substrate of Weiss-Habber and Fenton reactions that lead to hydroxide radical production. For H\(_2\)O\(_2\) to be maintained at levels that do not impede plant cell physiological functions, antioxidant enzymes such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), catalase and the organic compound ascorbic acid must be activated [11,47,76]. Also, the enzymes involved in glutathione metabolism (glutathione transferase (GSTs) and glutathione peroxidase (GPX)) are important in maintaining redox potential in levels that are not inhibiting other biological reactions. Additionally, the enzyme glutathione reductase is essential for glutathione function, when glutathione is found in the reduced form; sulphate group of the amino acid cysteine can provide electrons to reactive oxygen species and glutathione is oxidized leading to the formation of GSSG. Glutathione (GSH) can be regenerated through the enzyme glutathione reductase (GR) and the reducing power required for glutathione regeneration is provided by the oxidation of NADPH. It has been proposed that the level of oxidative stress of plant cells could be safely estimated from the concentration ratio of reduced to oxidized glutathione (GSH/GSSG) [47,104].

7. Conclusions

The availability of PTEs is affected by various factors such as soil physicochemical processes and plant species. Some plant species suitable for taking up these elements and distributing them in aboveground tissues are known as hyperaccumulators. Phytoremediation strategies for PTE-laden soils mainly depend on these plants’ uptake potential. Additionally, plants have developed several mechanisms to mitigate PTE accumulation and toxicity. Reactive oxygen species (ROS), such as singlet oxygen, superoxide anion, hydrogen peroxide and hydroxyl radical, which are formed during PTE stress can lead to oxidative stress that largely depends on the concentration and the physicochemical characteristics of each PTE. The production of ROS induces antioxidant defense systems that involve mainly antioxidant enzymes and non-enzymic compounds. Antioxidant enzymes, include SOD, CAT, AsA-GSH and GSTs, which reduce the oxidized forms. Non-enzymatic mechanisms can act as chelators and promote ROS scavenging such as GSH, ascorbate acids, amino acids and phenolic compounds. These mechanisms increase plant resistance to PTE stress and mitigate their harmful effects. In conclusion, a better understanding of soil chemical processes and plant defense mechanisms will provide a suitable background for developing new approaches for the phytoremediation of contaminated soils. However, further research into PTE absorption, translocation, and accumulation mechanisms is deemed necessary to improve sustainable environmental management and ensure food safety.

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