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Review Article

Phytotoxicity by Lead as Heavy Metal Focus on Oxidative Stress

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In the recent years, search for better quality of life in urban areas has been provoking an increase in urban agriculture. However, this new way of agriculture can bring risks to human health since this land is highly contaminated, due to anthropogenic activities. This way, lead (Pb) phytotoxicity approach must be taken into consideration since it can be prejudicial to human health through food chain. Pb is a common environmental contaminant, which originate numerous disturbances in plant physiological processes due to the bioacummulation of this metal pollutant in plant tissues. This review, focus on the uptake and interaction of lead by plants and how it can be introduced in food chain. Special attention was taken to address the oxidative stress by lead regarding the effects produced in plant physiological and biochemical processes. Furthermore, the antioxidant defence system was taken into consideration. Phytoremediation is applied on site or chronic polluted soils. This emerging technique is useful to bioaccumulate, degrade or decrease risks associated with contaminants in soils, water or air through the use of hyperaccumulaters. In addition, the impact of nanoparticles in plant science was also focused in this article since some improving properties in plants have been increasingly investigated.

1. General Introduction

Metals occur naturally in the environment as constituents of the Earth's crust [1]. They tend to accumulate and persist in the ecosystems due to their stability and mainly because they cannot be degraded or destroyed.

Plants absorb numerous elements from soil. Some of the absorbed elements are referred to as essentials because they are required for plants to complete their life cycle. Certain essential transition elements such as iron, manganese, molybdenum, copper, zinc, and nickel are known as micronutrients because they are required by plants in minute quantity [2]. Other transition metals such as silver, gold and cobalt [3, 4], and nontransition elements like aluminum [5] have proven to have a stimulatory effect on plant growth, but are not considered essential. Moreover, it has been documented elsewhere that plants also absorb elements which have no known biological function and are even known to be toxic at low concentrations. Among these are the heavy metals arsenic, cadmium, chromium, mercury, and Pb. However, even micronutrients become toxic for plants when absorbed above certain threshold values [6].

2. Lead (Pb)

Lead (Pb) is a silvery-white highly malleable metal. Among his physical properties, at normal environmental conditions this metal is presented in the solid state; it is dense, ductiles, and very soft with poor electrical conductivity when compared to most other metals. The chemical symbol for lead, Pb, is an abbreviation of the Latin word *plumbum*, meaning soft metal.

Pb is rarely found in native form in nature but it combines with other elements to form a variety of interesting and beautiful minerals. Galena, which is the dominant Pb ore mineral, is blue-white in color when first uncovered but tarnishes to dull gray when exposed to air [7].

Archeological research indicates that Pb has been used by humans for a variety of purposes for more than 5,000 years. In fact, archeological discoveries found glazes on prehistoric ceramics. The Egyptians used grounded Pb ore as eyeliner with therapeutic proprieties and cosmetic kohl, Pb-based pigments were used as part of yellow, red, and white paint. In ancient Rome-Pb was used to build pipes for water transportation [7–9].

Not so long ago, Pb had a widespread use in all anthropogenic activities, for instance, leaded paints, automobile batteries (as lead oxide), ammunitions, molten Pb as coolant, leaded glass, crystal, and fossil fuels. Until recently, tetraethyllead (TEL) was commonly used in petrol fuels as an inexpensive additive used since 1920. TEL was banned in most industrialized countries in the late 1990s to early 2000s due to environmental and health concerns over air and soil pollution (e.g., the areas around roads) and the accumulative neurotoxicity of Pb. This additive compound, however, is still used today in aviation fuel for piston-engine-powered aircraft. Even today Pb is still used in protective coatings with applications for radiation shielding in medical analysis [10, 11].

According to the U.S. Agency for Toxic Substances and Disease Registry, environmental levels of Pb have increased more than 1,000-fold over the past three centuries as a result of human activity. The greatest increase took place between 1950 and 2000 and reflected the increased use of leaded gasoline worldwide.

Pb commonly occurs in mineral deposits along with other base metals, such as copper and zinc which have been mined on all continents except Antarctica.

Currently, approximately 240 mines in more than 40 countries produce Pb. World mine production was estimated to be 4.1 million metric tons in 2010, and the leading producers were China, Australia, the United States, and Peru, in descending order of output. In recent years, Pb was mined domestically in Alaska, Idaho, Missouri, Montana, and Washington. In addition, secondary (recycled) Pb is a significant portion of the global Pb supply.

World consumption of refined Pb was 9.35 million metric tons in 2010. The leading refined Pb consuming countries were China, the United States, and Germany. Demand for Pb worldwide is expected to grow largely because of increased consumption in China, which is being driven by growth in the automobile and electric bicycle markets [12, 13].

According to Geological Society of America (http://geology.com/usgs/lead/) the worldwide supply and reserves of Pb are present on Table 1.

Table 1: Pb production and reserves. Data from [14].

Country	Production (1000 m^3 ton)	Reserves
USA	400	7000
Australia	620	27 000
Bolivia	90	1600
Canada	65	650
China	1600	13 000
India	95	2600
Ireland	45	600
Mexico	185	5600
Peru	280	6000
Poland	35	1500
Russia	90	9200
South africa	50	300
Sweden	65	1100
Other	330	4000
Total	4100	80 000

3. Pb in Agriculture and Main Causes of Soil Contamination: The Status in the 21st Century

Accordingly to an increased number of studies, food crops accumulate trace metals in their tissues when grown on contaminated soil with Cd, Pb, and Zn from metal smelting activity, irrigation with wastewater, disposal of solid wastes including sewage sludge, vehicular exhaust, and adjacent industrial activity. Long-term use of these wastewaters on agricultural lands often results in the buildup of elevated levels of heavy metals in soils [15, 16].

In addition, in countries with a high demand for food, contaminated arable land is used for crops like rice, cereal grains, and potatoes [6].

Increasing concern on the lack of suitable land for agriculture is prompting urban farmers to use contaminated land, such as waste disposal sites, to produce food crops. This situation is exacerbated by rapid population growth, urbanization and industrialization [17]. Thus, urban agriculture, practiced widely in developing countries, can be at great risk due to the proximity of these contaminant sources [18, 19].

In urban agriculture, wastewater and solid organic wastes are often the main sources of water and fertilizer used to enhance the yields of stable crops and vegetables. This way, municipal or industrial effluents and solid wastes, often rich in trace metals, contribute significantly to metal loadings in irrigated and waste-amended urban soils. However, studies conducted in soils where the atmospheric deposition was the dominant pathway for Pb contamination, revealed that the Pb concentration in those soils decreased with the increasing distance from the road.

Facing the rising population in urban areas, urban agriculture faces problems regarding the balance of the food needs with the potential hazards arising from the use of contaminated urban sites for food production and effluents for irrigation. Previous studies of metal uptake have focused mainly on crop species grown in the developed world and comparatively little information is available concerning vegetables typically grown in periurban environments in developing countries [20].

4. Edible Vegetables Affected by Pb Contamination

Many researchers have shown that some common vegetables are capable of accumulating high levels of metals from the soils [21, 22].

Studies conducted with edible vegetables species revealed the correlations between the Pb content in the soils and environment and its effects in vegetables.

Othman [23] reported a direct positive correlation of Zn and Pb levels between soils and vegetables. In this study, edible portions of five varieties of green vegetables (collected from several areas in Dar Es Salaam, Africa) were analyzed for Pb, Cd, Cr, Zn, Ni, and Cu [16].

Tangahu and colleagues [24] demonstrated Pb accumulation in plant tissues (mg/g dry weight) of the roots, shoots, and leaves from different species. They suggested that several plants could accumulate Pb in their tissues to more than 50 mg/g dry weight of plant. Among those species are *Brassica campestris* L, *Brassica carinata A. Br.*, *Brassica juncea (L.) Czern,* and *Brassica nigra (L.) Koch* that could accumulate more than 100 mg Pb/g dry weight [24]. Also Uwah [22] suggested that certain species of *Brassica* (cabbage) are hyperaccumulators of heavy metals into their edible tissues.

More studies (De la Rosa et al. [25]) suggested that some wild plants (*Prosopis sp.* and *Salsola kali*) edible by humans and/or animals were recently identified as potential hyperaccumulators of Pb and Cd, respectively.

5. Pb Uptake by Plants

As Pb is not an essential element, plants do not have channels for Pb uptake. Instead, this element is bound to carboxylic groups of mucilage uronic acids on root surfaces [26, 27], but it is still unknown how this element goes into the root tissue. Although some plants species tolerate Pb through complexation and inactivation (*Allium cepa, Hordeum vulgare* and *Zea mays*), other species experience toxicity (*Brassica napus* and *Phaseolus vulgaris*) because Pb hampers some metabolic pathways [28]. In a few plant species, the excess of Pb inhibits seed germination, plant growth, and chlorophyll synthesis, among other effects [6].

Pb is considered to have low solubility and availability for plant uptake because it precipitates as phosphates and sulfates, chemicals commonly found in the rhizosphere of plants [29]. Also, Pb is immobilized in soil when it forms complexes with the organic matter [6].

Several studies have shown that most of the absorbed Pb remains accumulated in the roots, making the root the first barrier for the Pb translocation to the above ground plant parts, [29] acting like a natural barrier. Moreover, the increase in accumulation level is directly proportional to the amount of exogenous Pb.

Uptake behavior is known to depend on total soil concentration, soil physico-chemical conditions, and the species and genotypes of the plants involved [30]. Authors have reported the effect of pH variation in Pb uptake, in different plant species: in low pH soils (3.9) an increased mobility of Pb was observed, resulting in higher uptake. Also, in addition to soil factors and plant species, previous studies have shown that trace metal concentrations may differ between cultivars of individual crop species when grown on the same soil, making the risks associated with contaminated soils, with trace metals, difficult to assess [20].

Once inside the root cortex, Pb moves in the apoplastic space, using the transpiration conductive system [28, 31]. It can also bypass the endodermis and gain symplastic access in the young root zone and in sites of lateral root initiation [32]. Pb has been shown to enter and move within the cytoplasm and proteins mediating cross-membrane movement of Pb have been identified [33, 34]. Most of the Pb absorbed by roots is in the form of extracellular precipitate (as phosphate and carbonate) or is bound to ion exchangeable sites in the cell walls [35]. The unbound Pb is moved through Ca channels accumulating near the endodermis [36].

Previous experimental results suggest that at low concentration, the Casparian strip of the endodermis is a partial barrier for Pb movement into the central cylinder tissue [37]. Depending on the plants exposed, different cellular types can be used to store Pb [36]. Varga et al. [38] found that in roots of wheat, Pb is fixed to the cell wall but it can be removed as a complex using citric acid, However, Marmiroli [39] reported that in European walnut (*Juglans regia*), Pb is retained in the lignocellulosic structure of roots. On the other hand, a small portion can also be translocated upwards to stems, leaves, and probably seeds [6].

Results from the Gardea-Torresdey research group have shown (unpublished data) that in hydroponically grown honey mesquite (*Prosopis sp.*) associated with *Glomus deserticola* and treated with high-Pb concentrations (more than 50 mg Pb L^{-1}), Pb concentrates in the phloem tissues, which suggests the Pb movement through the xylem to leaves, returning through the phloem to the plant body. As described by Cobbett [40], Pb, like other toxic elements, is complexed by the cysteine-rich low molecular weight polypeptides widely known as phytochelatins. However, in *Sesbania drummondii* Pb is transported to stems and leaves in structures similar to Pb-acetate, Pb-nitrate, and Pb-sulfide [41]. In addition, López et al. [42, 43] have reported the formation of different Pb complexes in stems and leaves of alfalfa.

6. Pb in Food Chain

According to Ma [44] and Rossato et al. [45] one way of exposure of humans and mammals to Pb is via the food chain. It has long been recognized that the heavy metal accumulation in soil may result in potential health risk to plants, animals, and humans [46].

Published studies illustrating the transport of Pb in the food chain are scarce, and further research is needed to establish the role of the plant Pb compounds in the transference and metabolism of Pb in the food chain. Other researchers have reported that in humans, two binding polypeptides (thymosin and acyl-coA binding protein) are responsible for the Pb binding in kidneys [47]. Pb in blood serum is bound to proteins or complexed with low-molecular-weight compounds such as sulfhydryl groups (e.g., cysteine, homocysteine) and others as citrate, cysteamine, ergothioneine, glutathione, histidine, and oxylate [48].

Lead (II) acetate (also known as sugar of Pb) was used by the Roman Empire as a sweetener for wine, and some consider this to be the cause of dementia that affected many of the Roman Emperors [6].

Zhuang and colleagues [46] performed a study where they evaluated heavy metal transfer along a plant-insect-chicken food chain on metal contaminated soil. They concluded that chicken fed with insect-larva accumulated significantly high Pb in the liver, suggesting that the accumulation of heavy metals in specific animal organ should not be ignored. In their study they also demonstrated decreases of heavy metals along the soil-plant-insect-chicken food chain. Interestingly, cadmium (Cd) steadily declined with increasing trophic level, but concentrations of zinc (Zn) and copper (Cu) slightly increased from plant to insect larva. An important route to avoid bioaccumulation was the elimination of the four elements in feces of insect and chicken. Metal concentrations in liver, muscle, and blood of chickens were highly variable; however, the highest concentration was in liver and the lowest in blood [46].

Many people could be at risk of adverse health effects from consuming common vegetables cultivated in contaminated soil. The condition of the soil is often unknown or undocumented and therefore, exposure to toxic levels can unconsciously occur [49]. Xu and Thornton [50] suggested the existence of health risks from consuming vegetables with elevated heavy metal concentrations. The populations most affected by heavy metal toxicity are pregnant women or very young children [51]. Low birthweight and severe mental retardation of newborn children have been reported in some cases where the pregnant women ingested toxic amounts of heavy metal through direct or indirect consumption of vegetables [52]. Some of the reported effects of heavy metal poisoning are neurological disorders, central nervous system (CNS) destruction, and cancers of various body organs [16, 48].

Taking the health risks encountered in human diet as a result of high levels of heavy metals in vegetables, agricultural good practices should be implemented. This way, educational and official programs should be implemented and broadcasted to educate farmers on the problems associated with the excessive use of fertilizers and other chemicals, as well as the irrigation of crops with waste and all sorts of polluted water, and the need to grow crops with safe levels of heavy metals [16].

7. Pb Phytotoxicity

Pb is known to negatively affect some of the most classical endpoints of plant toxicity like seed germination rate, seedling growth, dry mass of roots and shoots, photosynthesis, plant water status, mineral nutrition, and enzymatic activities [53]. In general, effects are more pronounced at higher concentrations and continuance. In some cases, lower concentrations can stimulate metabolic processes and the enzymes involved in those processes [36].

These negative effects can be expressed as symptoms in the form of chlorotic spots, necrotic lesions in leaf surface, senescence of the leaf, and stunted growth. Germination of seeds is drastically affected at higher concentrations. Development and growth of root and shoot in seedling stage are also affected roots being more sensitive.

Pb negatively influences growth by reducing the uptake and transport of nutrients in plants, such as Ca, Fe, Mg, Mn, P, and Zn, and by blocking the entry or binding of the ions to ion-carriers making them unavailable for uptake and transport from roots to leaves [54]. Thus, Pb interferes with several physiological and biochemical processes; photosynthesis being one of the most affected [36].

Many European countries have adopted a bioavailability based rationale to improve the reliability of assessments of metal uptake [55]. Current legislation in most countries still uses total soil metal concentration as a simple index of hazard in contaminated soils, even though this approach does not take account of soil characteristics which influence the bioavailability of metallic pollutants in contaminated soil [56].

This has major implications for diet-related risk assessments as these often rely on a generic vegetable approach to predict the transfer of trace elements to the human diet (Section 6).

8. Metals and Oxidative Stress

8.1. General Considerations. Reactive oxygen species (ROS) are formed and degraded by all aerobic organisms, leading to either physiological concentrations required for normal cell function or excessive quantities, a state called oxidative stress [57]. Under normal physiological conditions a balance is maintained between the formation of ROS and the cells protective antioxidant mechanism. However, this balance can be disturbed with many environmental stresses including temperature, salinity, drought, flooding, nutritional imbalances and postanoxia stress, a range of gaseous pollutants (ozone, nitrogen oxides, volatile organic compounds, etc.), heavy metals, pathogens attack, and herbicides which have been indicated to increase oxidative stress, leading to overproduction of ROS overcoming the cellular antioxidant capacity [58, 59].

These stresses lead to a series of changes in the plant resulting in deficient plant growth and development by affecting molecular, biochemical, morphological, and physiological, processes [59]. The changes caused by various stressful conditions are frequently due to a secondary stress (usually osmotic or oxidative) that perturbs the structural and functional stability of membrane proteins and disrupts cellular homeostasis [60, 61].

ROS are molecules with an unpaired electron making them highly reactive, by interacting nonspecifically with a variety of cellular components [62]. All aerobic organisms are totally dependent upon redox reactions, and the transfer of single electrons and many life processes (e.g., oxidative respiration, photorespiration, photosynthesis, lipid metabolism, and cell signaling) involve free radical intermediates, molecular oxygen, and activated oxygen species such as the superoxide radical anion ($O_2^{\bullet -}$), the hydroxyl radical ($HO^{\bullet}$), and peroxyl radicals ($ROO^{\bullet}$), as well as nonradical derivatives of molecular oxygen (O_2), such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), singlet oxygen O_2, and peroxynitrite ($ONOO^{-}$) [63–65]. Although H_2O_2 per se does not contain any unpaired electrons, it is ascribed to ROS, as it can be easily converted into more aggressive radical species, for example into $HO^{\bullet}$ via Fentoncatalyzed reduction. Moreover, H_2O_2 is membrane permeable and diffusible, proving it suitable for intracellular signaling. Uncontrolled ROS production may ultimately attack macromolecules such as polyunsaturated fatty acids (PUFAs) of the chloroplast membranes, leading to toxic breakdown products and trigger lipid peroxidation [66, 67]. Peroxidation injury of the cell membrane leads to leakage of cellular contents, failure of cell function, rapid desiccation, and, eventually to a breakdown in structural integrity which can lead to necrosis [68].

Scandalios [69] described some damages induced by ROS on biomolecules:

(i) Oxidative damage to lipids occurs via several mechanisms of ROS reacting with fatty acids in the membrane lipid bilayer, leading to membrane leakage and cell death. In foods, lipid peroxidation causes rancidity and development of undesirable odors and flavors.

(ii) In proteins, oxidative damage is due to site-specific amino acids modifications since specific amino acids differ in their susceptibility to ROS attack. Other effects of protein oxidative damage are: fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electrical charge, increase of susceptibility to proteolysis, oxidation of Fe-S centers by $O_2^{\bullet -}$, destroying enzymatic function, oxidation of specific amino "marks" proteins for degradation by specific proteases and oxidation of specific amino acids (e.g., Try) leading to cross-linking.

(iii) DNA damage by ROS leads to DNA deletions, mutations, translocations, base degradation, single-strand breakage, and cross-linking of DNA to proteins.

In plants, ROS are produced within the cellular compartments like chloroplast, mitochondria, cytosol, plasma membrane, microbodies (peroxisomes and glyoxisomes), and in the cell walls during metabolic pathways as photosynthesis and photorespiration, which is the most obvious oxygenation pathways in the chloroplast [70]. The main types of active O_2 species are superoxide and H_2O_2. In peroxisomes and glyoxisomes, however, just H_2O_2 is produced.

Hydrogen peroxide (H_2O_2) is an interesting form of ROS since it has been considered to be a second messenger for signals generated by ROS due to the capacity to easily diffuse through the membranes and it's relatively long life [70]. Many studies have suggested the existence of a close interaction between intracellular H_2O_2 and cytosolic calcium in response to biotic and abiotic stresses. In fact, environmental stress might trigger a rapid and transient increase in calcium influx, which enhances the generation of H_2O_2. Yang and Poovaiah [71] and other authors have proposed calcium/calmodulin (CAM) a controlling mechanism of H_2O_2 homeostasis in plants. They also verified that increasing cytosolic Ca^{2+} can downregulate H_2O_2 levels by means of Ca^{2+}/CaM-mediated stimulation of catalase activity in tobacco leaves.

The characterization and monitorization of the oxidative stress can be assessed by many parameters: plant membranes integrity evaluation, lipid peroxidation estimation through thiobarbituric acid reactive substances, measurement of redox potential and stress-related metabolites (H_2O_2, ascorbic acid, and glutathione), enzymes like poly (ADP-ribose)-polymerase, screening for heat-shock proteins (HSP), enzymes associated with cell cycle,and evaluation of antioxidant enzymes [72]. According with Wang et al. [73], biological monitoring is a direct test of biological responses to environmental contaminants and has been proposed to complement the information given by chemical analysis. Thus, the use of biochemical or physiological parameters as biomarkers of ecotoxicity is under constant development and has the advantage of delineating effects before observed symptom.

Several techniques can be applied to assess ROS-induced DNA/chromosome injuries such as, flow cytometry (measurement of changes in chromosome number and DNA content), microdensitometry, and fluorescent in situ hybridization (FISH) (look for somatic recombination) or others that detect DNA sequence mutations such as microsatellites, restriction fragment length (RFLP), and amplified fragment length polymorphism (AFLP) [72].

Plants respond in different ways to heavy metal ion stress including exclusion, chelation, compartmentalization, and expression of stress protein genes. This way, being Pb one of the main sources of environmental pollution, previous studies have shown that Pb inhibits metabolic processes such as nitrogen assimilation, photosynthesis, respiration, water uptake, and transcription. In fact, Pb causes two types of unfavorable processes in biological systems. Firstly, Pb inactivates several enzymes by binding with their SH-groups. Secondly, Pb ions can lead to oxidative stress by intensifying the processes of reactive oxygen species (ROS) production. These processes are mutually connected and stimulate each other by destructively affecting cell structure and metabolism, resulting in a possible decreased efficiency of oxidation-reduction enzymes or the electron transport system leading to fast production of ROS in the cell. Pb can exert a negative effect on mitochondria by decreasing the number of mitochondrial cristae, which in turn can lower the capacity of oxidative phosphorylation during photosynthesis and respiration [74].

9. Plant Protection Mechanisms against Oxidative Stress: Antioxidant Defense System

Plants have different defense strategies to cope with the toxicity of heavy metals. The primary defense strategy consists in avoiding the metal entry into the cell by excluding or binding it to a cell wall. The secondary defense system is composed of various antioxidants to combat the increased production of ROS caused by metals [45].

These antioxidants are substances that (either directly or indirectly) protect cells against adverse effects of xenobiotics, drugs, carcinogens, and toxic radical reactions.

In plant cells, the antioxidant defense system is essentially constituted by superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione (GSH), ascorbate (vitamin C), tocopherol (vitamin E), and carotenoids among others. These species are distributed through the cell and are present in vacuoles and chloroplasts in higher amounts.

The following distribution for the main antioxidant components was suggested by Scandalios [69]: 73% in the vacuole (ascorbate, glutathione, and peroxidase); 17% in chloroplasts (carotenoids, α-tocopherol, ascorbate, ascorbate peroxidase, glutathione, glutathione reductase, Cu/Zn-SOD, monodehydroascorbate radical reductase, and dehydroascorbate reductase); 5% in the cytosol (ascorbate peroxidase, CuZn-SOD, catalase, peroxidase, glutathione, ascorbate, glutathione reductase, and monodehydroascorbate radical reductase); 4% in the apoplast (peroxidase and ascorbate); 1% in the mitochondria (catalase, glutathione, glutathione reductase, Mn-SOD, and monodehydroascorbate radical reductase) and peroxisomes (catalase; Cu/Zn-SOD).

Besides the antioxidative system, stress proteins (also called heat-shock proteins, HSPs) are also activated in plant species under adverse conditions [73], and the accumulation of some organic compounds in plants such as polyamines (diamine, putrescine, triamine spermidine, and tetramine spermin) and L-proline play significant roles in plant adaptation to a variety of environmental stresses [75, 76].

Clearly, plant response to Pb contamination is a key research problem, and a special effort is being undertaken in seeking factors affecting the reduction of Pb absorption or toxicity in plants [74].

Selenium (Se) is one of the potential antagonists to Pb. Recent publications indicate that Se addition may also alter the total content of heavy metals in animal tissues by reducing their uptake by plants [77–81]. Magdalena Mroczek-Zdyrska and Wójcik [74] showed that cell viability was enhanced at low concentrations whereas at high concentrations Se was pro-oxidant and increased the lipid peroxidation and cell membrane injury. On the other hand, addition of Se controlled the accumulation of Pb and Cd in lettuce and enhanced absorption of some nutritional elements (Fe, Mn, Cu, Ca, and Mg) [77].

Rossato et al. [45] discussed that Pb stress triggered an efficient defense mechanism against oxidative stress in *Pluchea sagittalis*, but its magnitude was depending on the plant organ and of their physiological status.

10. Phytoremediation

10.1. General Considerations. Heavy metals, with soil residence times of thousands of years, pose numerous health dangers to higher organisms. They are known to affect plant growth, ground cover and to have a negative impact on soil microflora. It is well known that heavy metals cannot be chemically degraded and need to be physically removed or transformed into nontoxic compounds [24].

The generic term “phytoremediation” consists of the Greek prefix phyto (plant), attached to the Latin root remedium (to correct or remove an evil) [82, 83]. Generally, according to Erakhrumen and Agbontalor [82], phytoremediation is defined as an emerging technology using selected plants to clean up the contaminated environment from hazardous contaminants to improve the environment quality [24].

The uptake mechanisms through phytoremediation technology are divided between organic and inorganic contaminants. For organics, it involves phytostabilization, rhizodegradation, rhizofiltration, phytodegradation, and phytovolatilization. For inorganics, mechanisms involved are phytostabilization, rhizofiltration, phytoaccumulation, and phytovolatilization [24].

Plants have developed highly specific and very efficient mechanisms to obtain essential micronutrients from the environment, even when these are present at low ppm levels. Plant roots, are able to solubilize and take up micronutrients from very low levels in the soil, even from nearly insoluble precipitates. Plants have also developed highly specific mechanisms to translocate and store micronutrients. The same mechanisms are also involved in the uptake, translocation, and storage of toxic elements, whose chemical properties simulate those of essential elements. Thus, micronutrient uptake mechanisms are of great interest to phytoremediation [84].

Metal accumulating plant species can concentrate heavy metals like Cd, Zn, Co, Mn, Ni, and Pb up to 100 or 1000 times more than those taken up by nonaccumulator (excluder) plants. In most cases, bacteria and fungi living in the rhizosphere closely associated with plants may contribute to mobilize metal ions, increasing the bioavailable fraction [24].

There are several factors affecting the uptake mechanisms like: plant species characteristic, properties of medium agronomical practices developed to enhance remediation (pH adjustment, addition of chelators, and fertilizers), and addition of chelating agent [24].

11. Phytoremediation Advantages and Limitations

Phytoremediation has several advantages but remains controversial in some aspects. We will describe below some of the main advantages and limitations of this strategy applied to metals (e.g., Pb).

Advantages can be: low cost (is lower than traditional processes); applicablity for a wide range of contaminants; effective in contaminant reduction; environmental friendly method and less disruptive than current techniques of physical and chemical processes (e.g., metal precipitation or otherwise attached to an insoluble form through adsorption or ion exchange [85]; solidification and stabilization are other possibilities [86]); plants can be easily monitored; possibility of recovery and reuse of valuable metals (by companies specializing in “phytomining”); aesthetically pleasing.

Limitations of phytoremediation technology are: surface area and depth occupied by the roots; slow growth and low biomass production require a long-term commitment—it is a time-consuming method; the age of plant; the survival of the plants is affected by the toxicity of the contaminated land and the general soil condition; climatic condition; soil chemistry; the contaminant concentration; bioaccumulation of contaminants, especially metals, into the plants which, then, pass them into the food chain from primary level consumers upwards or requires the safe disposal of the affected plant material; the impacts of contaminated vegetation—with plant-based systems of remediation, it is not possible to completely prevent the leakage of contaminants into the groundwater (without the complete removal of the contaminated ground, which in itself does not resolve the problem of contamination) [24].

Heavy metals uptake, by plants using phytoremediation technology, seems to be a prosperous way to remediate heavy-metals-contaminated environment. In fact it has some advantages compared with other commonly used conventional technologies. However, several factors must be considered in order to accomplish a high performance of remediation result being the most important factor a suitable plant species which can be used to uptake the contaminant. Even if the phytoremediation technique seems to be one of the best alternatives, it also has some limitations. Further research is needed.

12. Nanotechnology Applications in Plant Science

Nanomaterials and nanotechnology have been widely applied all over the world in this last decade [87].

Despite nanotechnology being mainly focused on animal science and medical research (in regard of biological applications), nanotechnology can also be applied to plant science research in order to analyze plant genomics and gene function as well as improvement of crop species [87]. However, in 1996, USEPA (United States Environmental Protection Agency) evidenced several negative effects of nanoparticles (NSPs) on growth and development of plantlets.

More recently, some phytotoxicity studies applied in higher plants, using nanoparticles, have been developed. Some examples are given as follows: improvement of the level of seed germination and root growth; increase of source of iron (or other micronutrients); enhancement of Rubisco carboxilase activity; effect on growth of specific species. The species that have been studied are *Raphanus sativus, Brassica napus, Lolium multiflorum, Lactuca sativa*, *Cucumis sativus, Lolium perenne* (using ZnO nanoparticles), *Zea mays* (magnetic nanoparticles), *Spinacia olerace* (TiO_2 nanoparticles), and *Phaseolus vulgaris* (nano alumin particules), *Triticum aestivum* (Cu nanoparticles) among others [88–91]. In order to understand the possible benefits of applying nanotechnology to agriculture, the first step should be to analyze the level of penetration and transport of nanoparticles in plants. It is established that these particles tagged to agrochemicals or to other substances could reduce the injury to plant tissues and the amount of chemicals released into the environment. Some contact is however inescapable, due to the strong interaction of plants with soil growth substrates [87].

Deposition of atmospheric particulate matter on the leaves leads to remarkable alteration in the transpiration rates, thermal balance, and photosynthesis. Da Silva et al. [92] showed that nanoparticles may enter leaf surface.

Since nanoparticles are introduced into the soil as a result of human activities, among the many fields that nanotechnology takes into consideration, it is also important to recall the interactions between nanoparticles, plants, and soil. There are many gaps in our knowledge on the ecotoxicity of NSPs and there are many unresolved problems and new challenges concerning the biological effects of these NSPs [87].

The elements for acceptable catalytic metal nanoparticles have been restricted to groups VIII and IB of the periodic table, especially palladium, platinum (Pt), silver, and Au [93]. The majority of studies involving Pb nanoparticles were driven to electrochemical materials such as exploration of electrically conductive adhesives (ECAs) for surface mount technology and flip chip applications as Pb-free alternatives [94]. fabrication of pure Pb nanoparticles with nonoxidized surfaces due to Pb particles being readily oxidized even at ambient temperature and in high vacuum [93]; Synthesis of lead dioxide nanoparticles by pulsed current electrochemical method to use as the cathode of lead-acid batteries [95].

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