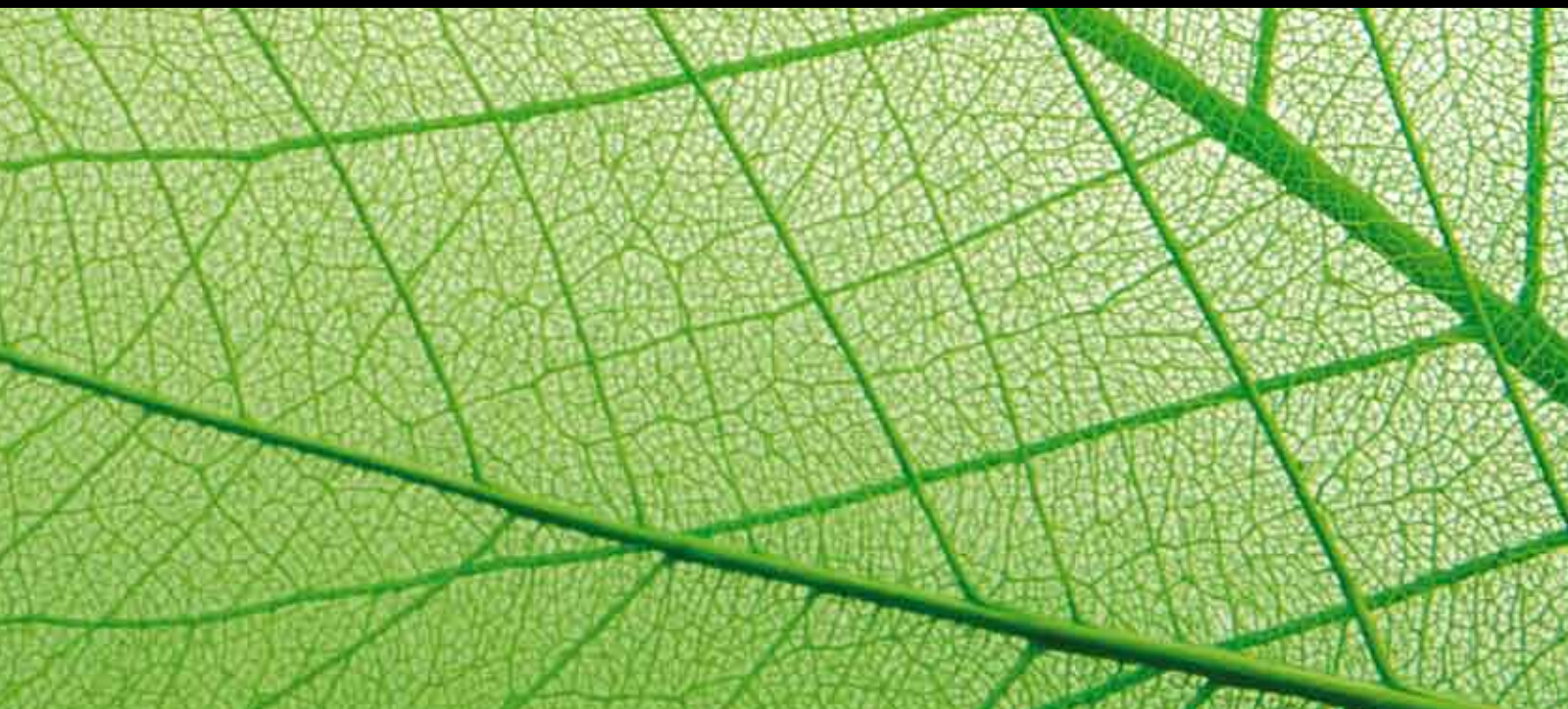


# LEGACY AND EMERGING CONTAMINANTS IN PLANTS: FROM THE GENE TO THE FIELD

GUEST EDITORS: CONCEIÇÃO SANTOS, HELENA OLIVEIRA, JOANNA DECKERT, AND JASON C. WHITE





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# **Legacy and Emerging Contaminants in Plants: From the Gene to the Field**

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From the Gene to the Field**

Guest Editors: Conceição Santos, Helena Oliveira,  
Joanna Deckert, and Jason C. White



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## Editorial

# Legacy and Emerging Contaminants in Plants: From the Gene to the Field

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It has become increasingly clear over the last decades that the interdependence of economic development and environment sustainability is a crucial matter for human health and well-being and as such, far surpasses the restricted forums of scientific academia. In particular, and despite increasing legislation to restrict soil and water contamination in most countries, contamination in agricultural areas remains a growing problem, with clearly recognized negative impacts on plant and animal life. Mining, industry, overuse of pesticides and chemical fertilizers, and improper waste disposal can all contribute to soil contamination. Moreover, some of the most intense effects of soil and water contamination involve putative decreases of crop yield and/or food contamination and ultimately the complete loss of usable land (and habitats).

The papers of this special issue, discuss various facets of soil contamination—plant interactions that are of critical importance in the third millennium. The papers were thoughtfully designed as reviews covering diverse subjects of plant responses to different types of soil contaminants, thereby providing an overview of the recent trends in this field of study.

The arrangement of subjects is as follows: B. Ladeiro discusses and updates some recent aspects related to soil degradation resulting from salt stress and the opportunities of saline agriculture in these areas. The authors also include brief considerations on the valorization of halophyte crops in

order to address the requirements for agriculture improvement and adjustments in the 21st century.

Some other papers in this issue explore the traditional problem of soil contamination by metals and the resulting impacts on plant performance and agriculture. In spite of the fact that metals' adverse effects have been known for decades, exposure to this type of contaminant continues and is even increasing in several countries. In this issue authors review the toxicity of some metals, such as lead, aluminum, mercury, and chromium. Lead is a common environment pollutant, though less studied as a toxicant to plants, than other metals such as cadmium. S. Pinho and B. Ladeiro discuss lead phytotoxicity, reviewing some aspects of toxicity mechanisms in plants, focusing on metabolic pathways, in particular oxidative stress—an issue yet to be fully clarified—and its accumulation in crops and putative perspectives of phytoremediation. S. Silva clearly describes some of the most recent advances in aluminum-induced phytotoxicity, with emphasis on aspects such as root growth inhibition, oxidative stress, alterations on cell wall and plasma membrane, nutrient unbalances, callose accumulation, and disturbance of cytoplasmic  $\text{Ca}^{2+}$  homeostasis.

R. Azevedo and E. Rodriguez deal with the phytotoxicity of mercury in plants, a still poorly understood pollutant. The authors cover different environment aspects of this pollutant and, in particular update most recent data on Hg accumulation and genotoxicity in some crops.

H. Oliveira reviews several aspects of chromium accumulation in soil and its phytotoxicity. Evaluating the chromium toxicity profile is complex due to several valence states, which have different pathways of entrance, and act in different ways inside the cell.

Finally, two reviews deal with two classes of emerging contaminants, reviewing key aspects of nanoparticle and pesticide phytotoxicity. In the first review, C. Remédios and co-authors discuss the main topics of nanoparticle contamination and the potential toxic effects of metal-based nanoparticles in plants, which remain largely unknown. In the latter, M. C. Dias reviews and updates some crucial aspects of fungicides' (extensive) use in agriculture. The author highlights some recent effects of fungicides in plant physiology with particular emphasis on photosynthesis.

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## Review Article

# Saline Agriculture in the 21st Century: Using Salt Contaminated Resources to Cope Food Requirements

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With the continue increase of the world population the requirements for food, freshwater, and fuel are bigger every day. This way an urgent necessity to develop, create, and practice a new type of agriculture, which has to be environmentally sustainable and adequate to the soils, is arising. Among the stresses in plant agriculture worldwide, the increase of soil salinity is considered the major stress. This is particularly emerging in developing countries that present the highest population growth rates, and often the high rates of soil degradation. Therefore, salt-tolerant plants provide a sensible alternative for many developing countries. These plants have the capacity to grow using land and water unsuitable for conventional crops producing food, fuel, fodder, fiber, resin, essential oils, and pharmaceutical products. In addition to their production capabilities they can be used simultaneously for landscape reintegration and soil rehabilitation. This review will cover important subjects concerning saline agriculture and the crop potential of halophytes to use salt-contaminated resources to manage food requirements.

## 1. Human Population Growth and Agriculture Challenges in the 21st Century

It is estimated that in November 2011, the mankind has reached the 7 thousand million people, and the United Nations (2008) predicted a population increase up to 8.01 thousand million people in 2025. This represents a duplication of human population in approximately 50 years. So, agriculture strategies for feeding all people represent one of the most important challenges in the 21st century. Therefore, there is enormous demographic and economic pressure to rise, within the next 40 years, leading to an increase of the crop production by about 50% years in a sustainable manner to fulfil the world food necessities [1, 2]. Some other facts are aggravating this demand, namely, (a) the increase of land occupation for biofuel supply that deviates arable soils from food crops [3], (b) the challenges posed by increasing occasional episodes of extreme environmental conditions and even natural disasters often associated with the generally called “climate change events”, (c) some excesses in soil pressure posed by postgreen revolution practices; (d) the raise of soil degradation and/or the increase of saline soils is growing dramatically, reducing the area of

arable land. The duality faced by mankind concerning food supply was summarized by Rudel [4] stating “*our ability to supply the growing global demand for food, fiber, and fuel, while maintaining a landscape able to provide a full suite of environmental services, hinges on our ability to produce more on less land. This pressure is particularly high in developing countries*”.

Agriculture is the first human activity and represents the major use of land across the world [5]. Agriculture can be defined as an “artificial management to enhance the food value of cultivated land” and represents “the major land use across the globe” [6]. Some emerging strategies to enhance food production involve, for example, increasing yield and cropping intensities, genetic modification [7] which may involve converging strategies of seed and germplasm preservation and improvement [2], and/or developing strategies for use of contaminated or dried soils, meanwhile abandoned as nonproductive.

This increase is particularly emerging in developing countries that present the highest population growth rates, and often the high rates of soil degradation (the Mediterranean Basin [8], Australia, Central Asia, and the Middle East and North of Africa). This pressure is so important that it is

considered in the United Nations Millennium Development Goals [9]. In 2004 the Food and Agriculture Organization of the United Nations (FAO) estimated that there was 852 million food-deprived people worldwide in 2000–2002, with being the most critical places the sub-Saharan Africa and Southern Asia. However, in 2004, FAO also highlighted that despite in most places these hunger issues translate a real lack of food and deficient agricultural techniques. This problem is however the convergence of more complex problems [5]. This is evident by the statistical data pointing to approximately 9 million hungry people in industrialized countries where in theory there is no lack of food [10]. In 2008, FAO expected an increase of food requirements by 20% in developed countries and 60% in developing countries.

It is therefore recognised that food requirements are increasing quicker than crop production, and researchers and politicians increasingly highlight the urgent need to improve alternative agricultural strategies [5, 6, 11, 12]. In other words, to produce more and better it is necessary to invest in better technology within the scopes of the Millennium Development Goals strengthening practices leading to sustainable agriculture without destroying lands and natural resources [5].

Despite all these emerging food problems, in 2011 FAO reported a study highlighting the losses occurring along the entire food chain suggesting that roughly one-third of food produced for human consumption is lost or wasted globally, which amounts to about 1.3 billion tons per year. In this study, FAO revealed that much more food is wasted in the industrialized world than in developing countries, estimating a per capita food waste by consumers in Europe and North-America of 95–115 kg/year, while this figure in Sub-Saharan Africa and South/Southeast Asia is only 6–11 kg/year.

The causes of food losses and waste in low-income countries are mainly connected to financial, managerial, and technical limitations in harvesting techniques, storage, and cooling facilities in difficult climatic conditions, infrastructure, packaging, and marketing systems. While in medium- and high-income countries food is to a significant extent wasted at the consumption stage, meaning that it is discarded even if it is still suitable for human consumption.

This way, FAO, [13], states that one of the first mean to cope with world food requirements is to also promote food loss reduction which alone has a considerable potential to increase the efficiency of the whole food chain. It is important to emphasise that the reducing food losses should not be a forgotten priority.

Agriculture improvement and adjustment to the 21st challenges also needs to take into consideration the type of land and water available [11, 14].

The area of irrigated land increased enormously, between 1960' and 1980's decades. Irrigation schemes cover only 15% of the cultivated land worldwide, though it contributes with two-third of the world's food production [15]. It is also estimated that irrigation limits production in approximately 600 million hectares of "potentially suitable arable land" [11]. Taken together these data, one must be careful on predicting agriculture improvement in quantity and quality. However,

some interesting work has been done using halophytes to complement agriculture needs [16, 17].

It is estimated that the agricultural production increase will need around  $202 \times 10^6$  ha of arable soil in developing countries, but only approximately  $93 \times 10^6$  ha seem available [14, 18]. These data support the need to develop strategies of sustainable agriculture contrarily to what it should be expected if real sustainable agriculture practices dominated. Millions of ha of agricultural land are lost every year, mostly during the last 50–60 years of agricultural development (due to, e.g., unsustainable irrigation practices, excessive use of fertilizers, soil contamination, urban pressure, climate changes). This issue was well addressed by the USA Department of Agriculture [19] which estimated that approximately 10 million ha/year of arable soils are lost in the globe.

Fortunately, it is increasing the number of countries that subscribe the principles inherent of sustainable development and address the Millennium Development Goals, so pressure to use sustainable strategies is rising (e.g., soil and water conservation), despite the increasing pressure for food supply and urbanization.

## 2. Insufficient Freshwater, Salt Contamination, and Soil Degradation

Despite it may be considered as having an ubiquitous distribution in all the continents of the world, most of the arid and semiarid regions are located in developing countries [20]. These problems also are present in regions of the United States of America, Australia, Israel, or the Mediterranean Basin [21], and some of these countries have long tradition in circumventing soil degradation using that land for agriculture. For example, in the USA a large amount of waste land is being used for fields biodiesel plant production. In Australia, on the other hand, a major problem arises with dry land salinity due to rising water tables resulting from clearing the original native vegetation due to changing the type of plant population [15]. On counterpart, other countries, like Israel, possess crop production practices with unconventional water resources irrigation, and the use of brackish water deserves particular attention. Also, in Tunisia strong investigations were addressed to this thematic [22, 23] and one can find agriculture practices based in alternative plant species, most of them are halophytes, which are able to tolerate high temperatures and/or low water availability.

Salinity is one of the most widespread soil degradation processes on the Earth. Soil salinisation affects an estimated 1 to 3 million hectares in Europe, mainly in the Mediterranean countries. It is regarded as a major cause of desertification and therefore is a serious form of soil degradation being salinisation and sodification among the major degradation processes endangering the potential use of European soils. For instance, in Spain 3% of the 3.5 million hectares of irrigated land is severely affected, reducing markedly its agricultural potential while another 15% is under serious risk.

Other examples of salt-affected soil in Europe are the Caspian Basin, the Ukraine, and the Carpathian Basin (Hungary) [24].

The availability of freshwater is a major limiting factor in sustainable agriculture. The decrease of water availability is found in these developing regions of burgeoning population pressure, and limits the area of arable land and crop production for these people. As stated by Galvani [5], when it comes to extreme environments, such as arid and semiarid areas, pressure must be put in major adjustments in alternative agriculture [25].

### 3. Soil and Water Availability and Saline Agriculture

An innovative strategy for enhancing land and water availability is the use of salted soils and salted water, in a strategy designated as saline agriculture. This strategy is not new, as, for example, the use of seawater for crop production in coastal deserts has already been suggested in the last three decades [26–29].

This way, a possible definition for saline agriculture can be as follows.

Profitable and improved agricultural practices using saline land and saline irrigation water with the purpose to achieve better production through a sustainable and integrated use of genetic resources (plants, animals, fish, insects, and microorganisms) avoiding expensive soil recovery measures [30].

The saline water that may be used in halophyte crop irrigation can be, for example, seawater, salt-contaminated phreatic sheets, brackish water (from e.g., Estuaries), drainage water from other plantations irrigation, drainage water from humanized areas, for example, sewage [31, 32], or even water derived from aquaculture waste [33]. It was suggested that around half of the irrigation systems are susceptible to salt contamination or waterlogging, probably due to low quality of used water, leaching, and rising water tables [34]. It is therefore clear that, facing the human population pressure, the technological advances, and the increase of salinized soils and reduction of arable land usable by conventional agriculture, the use of these salinized soils in alternative agriculture may be regarded as a strategy to cope with food demand [17].

### 4. Saline Agriculture: An Opportunity for Saline Soils Use

Soil salinization has been worldwide recognized as being among the most important problems for crop production in arid and semi-arid regions [35]. As reported above, some of the emerging regions in risk of increasing levels of salinization of their soils are, for example, the Mediterranean Basin [36], Australia, Central Asia, the Middle East, and Northern Africa [32, 35].

Soil salinization has numerous origins, namely, natural causes provoked by, for example, the microscopic salt particles carried by the wind to inland from the oceans,

or, as discussed above, some anthropic causes (secondary salinization) [32], among which irrigation water quality is one of the most important.

The use of salinized land through drainage/irrigation without using high-quality water, but instead also some salinized water, may be, therefore, the solution but demands exploration of the potential of halophytes as new emerging crops and changing mankind habits to incorporate this new crops in daily diet [25].

There is a need for more studies on the potential use of halophytes in saline agriculture and their use and incorporation in the consumers' diet. Also, the adequate conditions for increasing to industrial levels the halophyte species production (e.g., physiological studies, organoleptic and nutritional properties, etc.) deserve more attention. As stated by Koyro et al. [37] there are some requisites for the selection of tolerant plants with promising yields and characteristics that make them interesting as crops in saline agriculture: (a) screen of literature for their natural habitats, and so forth; (b) after selecting the species, determining the salinity threshold [15].

For halophytes succeed as irrigated crops, four basic conditions must be gathered:

(1) high yield potential; (2) the irrigation needs must not exceed the conventional crops and be harmless to the soil; (3) the products from halophyte crops must be able to replace the conventional crop products; (4) high-salinity agriculture must be applicable to the existing agricultural infrastructure [38].

### 5. Which Halophyte Crops Can We Use?

The potentiality of using halophytes in saline agriculture has been explored in the last decades. The use of halophytes in commercial cultures/exploitation, though still limited, is already being applied for some species. Also, the project "Greening Eritrea" from the Seawater Foundation [39] represents an example of how to convert a desertified region into a useful soil.

Halophytes can be improved into new, salt-resistant crops, or used as a source of genes to be introduced into conventional crop species that in general have their economical production decreased as soil salt levels increase. We'll discuss here some of the potential of halophytic species to use as emergent or already used crops in arid and semi-arid regions, in a perspective of sustainable development. The sustainable use of halophytes has multiple purposes as stimulating productive ecosystems and regreening degraded areas. About 2,600 halophytic species are known and only few are extensively studied for their potential in agriculture and as biological resources with economical potential as sources of oils, flavours, gums, resins, oils, pharmaceuticals, and fibbers [5], or with environmental potential for protection and conservation of ecosystems (e.g., improvement of soil structure and fertility, habitat for wildlife, source of biomass for the production of biodiesel) [40, 41].

We'll discuss below some of these species.

**5.1. Food Yielding Halophytes.** *Aster tripolium* (also known as Sea Aster or Sea Spinach) belongs to the family of the Asteraceae (Compositae). This Northern European plant, present in salt marches and estuaries, is very productive and can be cut several times with a regrowth of young shoots every 3–4 weeks. This is a familiar plant in The Netherlands since it was known as a famine food during harsh times and has become a delicacy nowadays. According to Brock et al. [42], *Aster tripolium* grows in temperate regions in its natural form, close to the coast mainly in the salt meadows. Also, some interesting research is being conducted concerning the response of *Aster tripolium* and *Puccinellia maritima* to atmospheric carbon dioxide enrichment and their interactions with flooding and salinity [43].

*Salicornia bigelovii* (Chenopodiaceae) is a very well-studied species. As a typical halophyte it is a succulent (having CAM metabolism) plant being cultivated for its oilseed (both for human and animal use) and straw. The residual seed meal is very rich in protein (approximately 33–34% crude protein). This oilseed halophyte has a yield and seed quality similar to the soybean, reaching yields of 2 t/ha of seed containing 28% oil and 31% protein [38]. In fact, field trials with *Salicornia bigelovii* conducted in Puerto Penasco, Mexico (a coastal desert environment), demonstrated a production of 18 t/ha of biomass and 2 t/ha of seed over a 200-day growing cycle [18] in which the seed contained 31% protein and 28% oil rich in polyunsaturated fatty acids (being the linoleic acid 74% of the total). The growth rates of animals fed with *Salicornia bigelovii* are equivalent to those fed with conventional forages on equal amounts such as alfalfa and wheat straw [18].

**5.2. Oilseeds.** Seeds of various halophytes, such as *Suaeda fruticosa*, *Arthrocnemum macrostachyum*, *Salicornia bigelovii*, *S. brachiata*, *Halogeton glomeratus*, *Kochia scoparia*, and *Haloxylon stocksii* possess a sufficient quantity of high quality edible oil with unsaturation ranging from 70–80%. Seeds of *Salvadora oleoides* and *S. persica* contain 40–50% fat and are a good source of lauric acid—a potential substitute for coconut oil.

Also *Diploaxis tenuifolia* is a promising species for saline agriculture, as it lives naturally in saline/dry ecosystems, or with strong influence of sea. This plant has a potential for food (salads) and forages. *Diploaxis tenuifolia* exposed to salt showed halophyte-like behaviour similar to those of known halophyte plants as *Cakile maritima* [44] for example or fodder beet [45]. In a recent study by Guerra [46], *Diploaxis* plants survived, grew, and reproduced in all salinity up to 300 mM NaCl, despite a small growth reduction was found in the higher concentration, with good values of growth at 100 mM and with no loss of nutritional value. This suggests that this species has high potential in large scale production of salt water/soils.

*Diploaxis* L. (DC.) genus is original from the Mediterranean Basin in dry, nutrient-rich, and sandy soils [47, 48], despite nowadays is relatively distributed around the world [49, 50]. It can be grown with other halophytes as *Cakile maritima* in dunes and in road verges usually at a small distance

from the sea [46]. Some studies have been developed with this species, for example in The Netherlands and in Portugal. This last has a coast with unique characteristics, potential use of large-scale production of this and other halophytes, and opening perspectives for an emerging market opportunity.

**5.3. Fuel Wood and Timber.** More than a billion people in developing countries rely on wood for cooking and heating. Quite often fuel wood is obtained from salt-tolerant trees and shrubs, which may include species of *Prosopis*, *Tamarix*, *Salsola*, *Acacia*, *Suaeda*, *Kochia*, *Capparis*, *Casuarina*, *Pithecellobium*, *Parkinsonia*, and *Salvadora*. In addition species like *Dalbergia sissoo*, *Pongamia pinnata*, *Populus euphratica*, and *Tamarix* spp. could provide good-quality wood. In coastal areas the mangroves species of *Rhizophora*, *Ceriops*, *Avicennia* and *Aegiceras* are good fuel woods and also contribute to charcoal production.

On the other hand, the agricultural applications on biofuels to cope with the global energy requirements have been increasing since high oil prices are creating new markets for agricultural commodities that can be used as feedstock for the production of bio-fuels. This way, bio-fuels are being promoted as contributing to a wide range of policy objectives, as providing greater energy security with regard to liquid fuels, increasing rural incomes, lowering greenhouse gas emissions, and providing economic opportunities for developing countries [51].

Fuels such as biodiesel can be produced from biomass ranging from cow manure to wood chips. The advantage of developing biofuel from halophytes as opposed to other types of biomass is that saltwater plants are not dependent on fresh water, which is in increasingly short supply, and can instead be irrigated using plentiful seawater supplies.

Suitable areas around the world for cultivating halophytes include the Sahara desert, Western Australia, South-west USA, parts of the Middle East, and parts of Peru. Scientists claim that an area smaller than the Sahara desert could yield enough biomass to replace the world's fossil fuel requirements. Furthermore, in the case of halophyte production, as these plants are grown in the desert, they will produce a cooler, wetter, land surface, which could lead to rainfall in areas of the world where rainwater is in short supply [52–54].

This way, the innovation and development of biofuels and the controversies around the sustainability of conventional crops as a feedstock for biofuel will likely help halophytes to get timely focus and advantage since the most currently used biofuel sources are conventional crops such as corn, sugar cane, rape oilseed, and palm oil and do not meet for most of the sustainability related issues. Specific examples include bioethanol, made from sugar and starch crops, and biodiesel, made from vegetable oils, animal fats, and other recycled greases.

Aspects such as competition with food, land use, energy efficiency, pressure on other important resources including freshwater, rain forest, and in some cases political instability are issues of ongoing controversy around conventional



biofuel which in turn can lead to an increase on food prices [53, 55, 56].

**5.4. Source of Chemicals.** A kind of soda is obtained in large quantities from *Suaeda*, *Salicornia*, *Salsola*, and *Haloxylon* species, used in soap making and in glass industry. Seeds of *Annona glabra* are a source of insecticide [57].

**5.5. Ornamental.** Many halophytes are useful ornamentals; these include among others *Aster tripolium*, *Limoniastrum monopetalum*, *Batis maritima*, *Tamarix nilotica*, *Tamarix amnicola*, *Cistanche fistulosum*, *Atriplex halimus*, *Sesuvium portulacastrum*, and *Noronia emarginata* [57].

**5.6. Environmental Protection.** Some species of halophytes can give an important contribute to the coastline protection and restoration of coastal ecosystems such as *Spartina alterniflora*, *Spartina maritima*, and *Avicennia marina* [58, 59]. Research is being undertaken to explore the potential of *Avicennia germinans* (black mangrove) to restore coastal and back-barrier salt marshes by taking advantage of the plants woody structure and extensive root system, providing sustainability and habitat [60].

## 6. Environmental and Economic Impact of Saline Agriculture

The economic analysis of saline water irrigation has three main aspects: (1) concerning the reclamation of saline and sodic soils preceding cultivation, (2) the constant use of saline water for irrigation and, (3) the reuse of drainage water for irrigation and the drainage installations [61].

According to a governmental report on “Saline agriculture farmer participatory development project in Pakistan” performed between 2002 and 2008 [30], the impact on the economy and environment was demonstrated.

In regard to the *economic benefits*, tree plantations are sustainable sources of raw materials required for a variety of industries, for example, pulp or paper, match manufacturing, sports goods, panel products, furniture timber, plywood, saw wood, fiberboard, and fuel wood. Vast quantities of dung can be saved by using fuel wood from plantings, enriching agricultural fields.

Concerning the *environmental management* impact, trees can be an important help to recover salt-affected land. Vegetation over saline soils tends to decrease salt concentration in the top soil due to increased infiltration and reduced capillary rise of water. This approach can allow farmers to get instant economic returns by growing field crops and also immediate economic benefits from saline wasteland with the help of trees. Such planting is preferred to various expensive engineering methods since it is cheaper and lasting. The vital impact of trees on the microclimate, soil erosion, and floods is well known. Moreover, large plantations create more favorable conditions to obtain rainfall.

An additional and interesting advantage demonstrated on this report is the ability of the trees to act as scavenging pollutants by removing water condensation nuclear particles

and reducing fog containing gases harmful for life since the branches, leaves, and stems filter and precipitate dust (carried by the wind) [30].

Saline agriculture can also be a potential strategy for reducing CO<sub>2</sub> in the atmosphere in degraded salt-affected areas. Other studies refer the use of halophyte crops to reclaim saline soils [62] since these plants can reduce the salt content of soil over time [63].

The environmental impact assessment (EIA) needs equally to concentrate on means in which positive impacts can be enhanced and negative impacts mitigated [11].

Yamaguchi and Blumwald [12] consider the “identification of key genetic determinants of stress tolerance” a precondition to the knowledge expansion on salt tolerant crops. These same authors consider two different genetic approaches, first the exploitation of natural genetic variations through marker-assisted breeding and second the generation of transgenic plants, a very popular subject that is being currently addressed by researchers. In fact, the use and improvement of conventional and molecular breeding (as well as molecular genetic modification—GM) are subjects of research to adapt our existing food crops to increasing temperatures, decreased water availability in some places and flooding in others, rising salinity [64], and changing pathogen and insect threats [65]. An environmental and important good of such research is to increase the efficiency of crops nitrogen uptake and use, due to nitrogenous compounds in fertilizers being the main contributors to waterway eutrophication and greenhouse gas emissions [64].

## 7. Conclusion and Future Perspectives

The increase in nutrient demand by the prosperous society and the decreasing availability of arable land and freshwater lead to the problem of agriculture sustainable development. With these perspectives, saline agriculture is coming up as an emerging role.

In the future this agricultural area may be of extreme importance in Mediterranean countries due to the increasing soil degradation in some regions and to their geographic and climatic conditions [46].

Without a comprehensive and long-term strategy adaptable to the prevailing economic, climatic, social, as well as edaphic and hydrogeological conditions, it is not considered possible to meet the future challenges of irrigated agriculture using poor-quality water.

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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 bioaccumulation 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 hyperaccumulators. 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.

### 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

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

Country	Production (1000 m <sup>3</sup> 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

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 hyper-accumulators 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<sup>-1</sup>), 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 peroxy 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 Fenton-catalyzed 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 its 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,  $\alpha$ -tocopherol, ascorbate, ascorbate peroxidase, glutathione, glutathione reductase, Cu/Zn-SOD, monodehydroascorbate radical reductase, and dehydroascorbate reductase); 5% in the cytosol (ascorbate peroxidase, Cu/Zn-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); applicability 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 carboxylase 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<sub>2</sub> 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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## Review Article

# Aluminium Toxicity Targets in Plants

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Aluminium (Al) is the third most abundant metallic element in soil but becomes available to plants only when the soil pH drops below 5.5. At those conditions, plants present several signals of Al toxicity. As reported by literature, major consequences of Al exposure are the decrease of plant production and the inhibition of root growth. The root growth inhibition may be directly/indirectly responsible for the loss of plant production. In this paper the most remarkable symptoms of Al toxicity in plants and the latest findings in this area are addressed. Root growth inhibition, ROS production, alterations on root cell wall and plasma membrane, nutrient unbalances, callose accumulation, and disturbance of cytoplasmic  $\text{Ca}^{2+}$  homeostasis, among other signals of Al toxicity are discussed, and, when possible, the behavior of Al-tolerant versus Al-sensitive genotypes under Al is compared.

## 1. Introduction

Aluminium (Al) ranks third in abundance among the Earth's crust elements, after oxygen and silicon, and is the most abundant metallic element. A large amount of Al is incorporated into aluminosilicate soil minerals, and very small quantities appear in the soluble form, capable of influencing biological systems [1].

Al bioavailability, and in consequence, toxicity, is mainly restricted to acid environments. Acid soils (with a pH of 5.5 or lower) are among the most important limitations to agricultural production. The production of staple food crops, in particular grain crops, is negatively influenced by acid soils [2]. Some agricultural practices, as removal of products from the farm, leaching of nitrogen below the plant root zone, inappropriate use of nitrogenous fertilizers, and build-up in organic matter, are causing further acidification of agricultural soils.

When pH drops below 5.5, aluminosilicate clays and aluminium hydroxide minerals begin to dissolve, releasing aluminium-hydroxy cations and  $\text{Al}(\text{H}_2\text{O})_6^{3+}$  ( $\text{Al}^{3+}$ ), that then exchange with other cations. On that conditions,  $\text{Al}^{3+}$  also forms the mononuclear species  $\text{AlOH}^{2+}$ ,  $\text{Al}(\text{OH})_2^+$ ,  $\text{Al}(\text{OH})_3$ , and  $\text{Al}(\text{OH})_4^-$  [3]. The mononuclear  $\text{Al}^{3+}$  species and  $\text{Al}_3$  are considered as the most toxic forms [4, 5].

Although some crops (e.g., pineapple, tea) are considered tolerant to high levels of exchangeable Al, for most crops it is a serious constraint. Species and genotypes within species greatly differ in their tolerance to Al. For most crops, fertilization and attempts of soil correction (e.g., liming) may not be enough per se to reduce Al toxicity (e.g., as the soil reaction remains strongly acid), and in most target countries these strategies may also be jeopardized by economical constraints [6]. Therefore, it is imperative to fully understand the mechanisms that are used by the Al-tolerant species to cope Al toxicity, as well which genotypes, within the most resistant/tolerant cereal species, are more suitable to grow in acidic soils in order to increase world cereal production. Furthermore, the development of new cultivars (or the reinvestment in ancient genotypes from Al rich regions) with increased Al-tolerance is fundamental and economic solution to increase world food production.

## 2. Aluminium Toxicity

**2.1. Root Growth.** A major consequence of Al toxicity is the inhibition of root growth, and this outcome has been reported during the last century (e.g., [7]) for innumerable species [8–15]. Consequently, root growth inhibition has been widely used to assess Al toxicity.

Root growth is the combination of cell division and elongation. Only during the last decade, researchers started to look at the cell cycle (de)regulation induced by Al, with some works focusing unbalances on mitosis phase and very few on other interphase phases (e.g., [15]). Decrease of mitotic activity was reported as a consequence of Al exposure in root tips of several species as wheat [16, 17], maize [18, 19], barley [20], and bean. [19]. Some authors defended that inhibition of cell elongation was the primary mechanism leading to root growth inhibition [21, 22]. The reason for that is that root growth inhibition could occur within a short time period—30 min in Al-sensitive maize [23]—and that cell division is a slow process (cell cycle takes usually several hours to be completed). However, Doncheva et al. [18] reported inhibition of cell division (decrease of S-phase cells) in the proximal meristem after 5 min Al exposure and inhibition of root cell division in the apical meristem within 10 or 30 minutes. Furthermore, Al can accumulate in the nuclei of cells in the meristematic region of the root tip within 30 minutes [15]. Therefore, whereas inhibition of cell elongation or cell division is the primary mechanism leading to root growth inhibition is still unclear. More recently, Yi et al. [24] reported that Al exposure led to abnormal progress through mitosis and induced micronuclei formation in *Vicia faba* roots, which is in agreement with Al-induced chromosome aberrations found in wheat roots [25] and Al-induced chromosome stickiness and breaks in *Oryza sativa* [26]. From the literature review, it is evident that Al leads to cell cycle unbalances, but many questions still remain to clarify. For example it still remains unclear how and where Al exerts its influence throughout the cell cycle, if these changes are species and region dependent (most studies are performed in root apices), how the putative changes are exerted through time, and/or if they may be reversible after Al removal.

The root growth inhibition and increase in root diameter observed in roots exposed to Al [27] suggested that plant cytoskeleton could be a cellular target of Al phytotoxicity [28]. Blancaflor et al. [28] and Horst et al. [29] studied Al-induced effects on microtubules and actin microfilaments and showed that microtubules and microfilaments are altered, in their stability, organization, and polymerization, when exposed to Al. Also, in *Triticum turgidum* Al treatment led to disorganization of actin filaments and formation of actin deposits [30]. Zhang et al. [31] showed that Al inhibited actin and profilin genes. Profilin, as an actin-binding protein, provides cells with the ability to remodel the cytoskeleton [32]. In *Arabidopsis thaliana* a decrease in profilin expression resulted in an elongation defect [33]. Furthermore, Sivaguru et al. [34] and Čiamporová [21] showed that organization of cytoskeleton is most sensitive in the distal transition zone of the root apex, providing evidence that this zone represents a potential target with respect to Al toxicity.

The most sensitive root zone to Al toxicity is under great attention. Earlier, it was hypothesized that root cap played a major role in the mechanism of Al toxicity/protection [35]. However, Ryan et al. [9] demonstrated that the removal of the root cap had no effect on the Al-induced inhibition of root growth in maize. Furthermore, the same authors

also suggested that the meristem is the primary site of Al toxicity. Later, Sivaguru and Horst [36], applying Al to 1 mm root segments, reported that Al accumulation in the distal transition zone (DTZ: 1–2 mm) led to a rapid inhibition of the root elongation and suggested that this root zone is the primary target of Al in an Al-sensitive maize cultivar.

**2.2. Oxidative Stress.** Al-induced oxidative stress and changes in cell wall properties have been suggested as the two major factors leading to Al toxicity [22, 37]. Oxidative stress occurs when any condition disrupts the cellular redox homeostasis. The reactive oxygen species (ROS) have the capacity to oxidize cellular components such as lipids, proteins, enzymes, and nucleic acids, leading to cell death. Metals are known to act as catalysts in ROS production and to induce oxidative damage in plants. Al itself is not a transition metal and cannot catalyze redox reactions; however, Al exposure leads to oxidative stress [37–43]. Because aluminium ions form electrostatic bonds preferentially with oxygen donor ligands (e.g., carboxylate or phosphate groups), cell wall pectin and the outer surface of the plasma membrane seem to be major targets of aluminium [37]. Al binding to biomembranes leads to rigidification [44], which seems to facilitate the radical chain reactions by iron (Fe) ions and enhance the peroxidation of lipids [38].

Al induction lipid peroxidation has been reported for some species, including barley [45], sorghum [46], triticale [42], rice [40], greengram [47], and wheat [48]. Yamamoto et al. [37] found that, for *Pisum sativum* seedlings treated with Al in a simple Ca solution, Al accumulation, lipid peroxidation, and callose production had a similar distribution on the root apex surface and were accompanied by root growth inhibition. However, the loss of membrane integrity was only detected at the periphery of the cracks on the surface of the root apex. Furthermore, Yamamoto et al. [38] concluded that the Al enhancement of lipid peroxidation is an early symptom of Al accumulation and appears to cause partly callose production, but not root growth inhibition. Later, however, in maize, Al treatment did not induce lipid peroxidation, indicating that lipids are not the primary cellular target of oxidative stress in maize [39]. So, it seems that cellular target of oxidative stress depends on plant species.

Plant cells are equipped with a defensive system composed by enzymatic antioxidants such as catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (G-POX), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione-S-transferase (GST), and glutathione reductase (GR) and nonenzymatic antioxidants such as ascorbate (AsA), glutathione (GSH),  $\alpha$ -tocopherol, and carotenoids that help to detoxify the ROS. Some works reported ROS production and alterations in the antioxidant system as a consequence of Al exposure. In pea seedlings, ROS production is detected in root apex after two hours of Al exposure and increased with time exposure [38]. In maize roots, Al treatment also led to increase in ROS production rate in all epidermal cells, only within 10 min of Al exposure and continued to increase during Al exposure [41]. APX and

SOD activity increased in roots of both Al-resistant and Al-sensitive triticale cultivars (with higher magnitude in the sensitive one), but changes were detected first in the sensitive cultivar (6 h) and then in the resistant (12 h) [42]. Boscolo et al. [39] reported for maize root tips an increase of SOD and APX activities. Furthermore, these authors found that SOD and APX activity is inversely proportional to root growth rate and, therefore, suggested that the increase of  $O_2^-$  and  $H_2O_2$  production is related to Al toxicity. An increase in SOD, APX, and GR activities was reported for greengram seedlings, whereas a decrease in CAT activity and glutathione and ascorbate contents was also found at higher Al concentrations [47]. These authors justified the decrease in CAT activity due to the fact that this enzyme is photosensitive and, therefore, needs constant synthesis and suggested that glutathione and ascorbate may be able to detoxify the ROS directly [47]. Devi et al. [49] found an increase in manganese superoxide dismutase (MnSOD) activity in both sensitive and tolerant cell lines of tobacco and in AsA and GSH contents, mostly in the tolerant line. These data indicated that AsA and GSH seem to be in part responsible for the tolerance mechanisms of the tolerant line to Al. Activities of SOD, CAT, and APX also increased in roots of plants and in cultured tea cells exposed to Al [50]. However, plants of this species provide a complex scenario compared with other models, as aluminium may show a stimulatory effect on plant growth. That increase seemed to result in increased membrane integrity, since lipid peroxidation reduced with Al exposure [50].

These findings reporting increase of antioxidants (enzymatic and nonenzymatic) are accompanied with others that prove gene regulation associated with oxidative stress. For example, Ezaki et al. [51] expressed nine genes derived from *Arabidopsis*, tobacco, wheat, and yeast in *Arabidopsis* ecotype Landsberg. An *Arabidopsis* blue-copper-binding protein gene (*AtBCB*), a tobacco glutathione-S-transferase gene (*parB*), a tobacco peroxidase gene (*NtPox*), and a tobacco GDP-dissociation inhibitor gene (*NtGDII*) conferred a degree of resistance to Al: significant differences in relative root growth and decrease in Al content and oxidative damages. They also showed that overexpression of three Al-induced genes in plants conferred oxidative stress resistance. Furthermore, overexpression of the *parB* gene simultaneously conferred resistance to both Al and oxidative stresses. Therefore, Ezaki and coworkers concluded that some of the genes induced during Al exposure and oxidative stresses play protective roles against both stresses. Cançado et al. [52] identified a maize Al-inducible cDNA encoding a glutathione-S-transferase (GST). Expression of that gene (GST27.2) was upregulated in response to various Al concentrations in both Al-tolerant and Al-sensitive maize lines. Recently, using Al-sensitive *Medicago truncatula* cultivar Jemalong genotype A17, 324 genes were upregulated and 267 genes were downregulated after Al exposure [53]. Upregulated genes were enriched in transcripts involved in cell-wall modification and abiotic and biotic stress responses, while downregulated genes were enriched in transcripts involved in primary metabolism, secondary metabolism, protein synthesis and processing, and the cell cycle. Known markers of Al-induced

gene expression including genes associated with oxidative stress and cell wall stiffening were differentially regulated in that study [53]. For maize plants, Al exposure led to alteration in gene expression, mostly in the Al-sensitive genotype. Although Al-sensitive genotype showed changes in the expression of more genes, several Al-regulated genes exhibited higher expression in the tolerant genotype [54]. So, it is clear that expression of some genes confers Al resistance and contributes to reduce oxidative stress.

### 2.3. Cell Wall, Plasma Membrane, and Nutrient Unbalances.

Al accumulation is primarily and predominantly in the root apoplast (30–90% of the total absorbed Al) (e.g., [42, 55]) of peripheral cells and is only very slowly translocated to more central tissues [19, 56, 57]. The primary binding of  $Al^{3+}$  in the apoplast is probably the pectin matrix, with its negatively charged carboxylic groups [57, 58].

Several works reported increases of pectin levels in Al-sensitive genotypes [29, 43, 57–60], and some also detected increase in Al contents in the same sensitive genotypes [29, 57, 60]. These findings indicated that pectin plays a major role in the binding of Al and suggested that some of the additional Al accumulation in sensitive genotypes bound in the newly formed cell wall pectin [43, 57, 58]. Binding of Al to the pectin matrix and other cell wall constituents could alter cell wall characteristics and functions such as extensibility [61], porosity, and enzyme activities thus leading to inhibition of root growth [57]. Another mechanism for Al toxicity targeted to the apoplast invokes a rapid and irreversible displacement of  $Ca^{2+}$  from cell wall components by Al ions [22, 61]. Accumulation of Al occurs predominantly in the root apoplast. Nevertheless, Al accumulates also in the symplast and with a fast rate [19]. Recently, Xia et al. [62] reported a transporter, *Nrat1* (Nramp aluminium transporter 1), specific for  $Al^{3+}$  localized at the plasma membrane of all rice root tips cells, except epidermal cells. Those authors referred that the elimination of the *Nrat1* enhanced Al sensitivity, decreased Al uptake, increased Al binding to cell wall and concluded that this transporter is required for prior step of final Al detoxification through sequestration of Al into vacuoles. Furthermore, given its physicochemical properties, Al can interact strongly with the negatively charged plasma membrane. For instance, Al can displace other cations (e.g.,  $Ca^{2+}$ ) that may form bridges between the phospholipid head groups of the membrane bilayer [63]. Furthermore, Al interaction with plasma membrane could lead to depolarization of the transmembrane potential (e.g., [64]) and/or reduction of  $H^+$ -ATPase (e.g., [65]) which, in turn, can alter the activities of ions near the plasma membrane surface and impede the formation and maintenance of the transmembrane  $H^+$  gradient [2]. Moreover, Al changes in plasma membrane can modify the uptake of several cations (e.g.,  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $K^+$ ,  $NH_4^+$ ) [8, 66–68]. These changes are related to direct  $Al^{3+}$  interactions with plasma membrane ion channels [69] and changes in membrane potential.

Nutritional unbalances induced by Al exposure were reported for several plant species. Eleven families of pteridophytes presented different nutritional unbalances (mostly



in Ca, Mg, P, K) depending on Al accumulation [70], and in maize, Al had negative effects on the uptake of macro- and micronutrients, with Ca and Mg being the macro- and Mn and Zn the micronutrients more affected [68]. Also, the maize Al-tolerant genotypes accumulated higher concentration of Ca, Mg [68], and K [71] than the sensitive genotypes. In wheat, both sensitive and tolerant genotypes presented a decrease in K and Mg contents in roots, whereas Ca, Al, Si contents increased [72]. However, the sensitive wheat genotype showed more nutritional unbalances and Al accumulation than the tolerant one in both roots and shoots [72]. Al exposure led to an increase of Ca accumulation in rye-sensitive genotype, contrarily to the tolerant rye genotype [73]. However, other studies reported different results in Al-induced nutritional imbalances in maize: Lidon et al. [74] referred that all elements in roots, except K, Mn, and Zn, increased in Al-treated roots and that in shoots Ca and Mg had little variation. Reference [67] reported that only the specific absorption rate of B was correlated to the Al-induced root growth inhibition. Al exposure led to decrease in K, Mg, Ca, and P contents and uptake in rice plants, and, as observed in maize, the tolerant cultivar presented less negative effects in nutrient content than the sensitive one [75]. In tomato cultivars, Al exposure decreased the content of Ca, K, Mg, Mn, Fe, and Zn in roots, stems, and leaves [76]. Zobel et al. [27] related changes in fine root diameter with changes in concentration of some nutrients, as N, P, and Al. It seems that the differential tolerance to Al may be due to their differences in uptake, ability to keep adequate concentrations and to use the nutrients efficiently. Differences in nutrient uptake, accumulation, and translocation are evident between plant species and within each species. Furthermore, since each author utilized different Al concentrations, diverse nutritive solutions and time exposures, it is difficult to make a general and accurate model of Al-induced nutritional unbalances.

**2.4. Cytoplasmic  $\text{Ca}^{2+}$ .** Disturbance of cytoplasmic  $\text{Ca}^{2+}$  homeostasis is believed to be the primary target of Al toxicity [77] and may be involved in the inhibition of the cell division or root elongation by causing potential disruptions of  $\text{Ca}^{2+}$ -dependent biochemical and physiological processes [34, 77, 78].

In wheat root apices, [44] found that Al inhibits  $\text{Ca}^{2+}$ -dependent phospholipase C, which acts on the lipid substrate phosphatidylinositol-4,5-bisphosphate. The authors hypothesized that phosphoinositide signaling pathway might be the initial target of Al. In accordance, Zhang et al. [31] found Al-induced inhibition of genes related to phosphoinositide signaling pathway and hypothesized that the gene inhibition could result in disruption of this pathway. Also, it was reported that components of the actin-based cytoskeleton interact directly with phospholipase C in oat [79].

Most works reported an increase in cytoplasmic  $\text{Ca}^{2+}$  when plants were exposed to Al [13, 80, 81]. However, Jones et al. [82] reported a decrease in cytoplasmic  $\text{Ca}^{2+}$  in tobacco cell cultures in the presence of Al. Furthermore, Zhang and Rengel [80] reported an increase in cytoplasmic  $\text{Ca}^{2+}$  in two lines with different tolerance to Al and correlated it with the

inhibition of root growth in both lines. Moreover, Ma et al. [13] correlated cytoplasmic  $\text{Ca}^{2+}$  to root growth response. Moreover, alteration in cytoplasmic  $\text{Ca}^{2+}$  homeostasis can occur within few minutes (20–30 minutes) in root hair tips of *Arabidopsis thaliana* [82].

It is certain that Al exposure influences cytoplasmic  $\text{Ca}^{2+}$  homeostasis, but it is still unclear if it is a primary cause of Al-induced inhibition of root growth or a secondary effect. The source of  $\text{Ca}^{2+}$  for the increase of cytosolic  $\text{Ca}^{2+}$  activity could be extracellular and/or intracellular but is still insufficiently documented, as well the effects on increased cytosolic  $\text{Ca}^{2+}$  (for review see [77]).

**2.5. Callose.** The induction of callose (1,3- $\beta$ -D-glucan) formation in Al-exposed roots has been reported in many plant species (e.g., [20, 41, 67, 83–86]). Al-induced callose formation in root tips is recognized as an excellent indicator of Al sensibility [81, 86–90], and some works negatively correlated root elongation with callose formation during Al exposure (e.g., [86, 91]). Recently, it was reported that Al induced callose accumulation not only in the root meristematic regions but also in mature zones, in both wheat and rye genotypes [72, 73]. In maize roots, Jones et al. [41] found a close spatial and temporal coordination between Al accumulation and callose production in roots. Also, in wheat, callose accumulation in root tissues was progressive with Al-exposure, and, contrarily to the tolerant genotype, the sensitive one presented callose deposition at inner cell layers [72, 73]. Still, Tahara et al. [86] reported that, in some Myrtaceae species, induction of callose formation was not accompanied by root growth inhibition and suggested that callose formation is a more sensitive indicator to Al than root elongation.

Since Al induces a transient rise of cytosolic  $\text{Ca}^{2+}$ , an increase of callose accumulation under Al stress is not unexpected. Cytosolic  $\text{Ca}^{2+}$  is one of the prerequisites for the induction of callose synthesis, but not the only factor modulating increases in callose synthesis and deposition [81]. Callose formation, as response to Al, is described in sensitive and, to a lesser extent, in tolerant roots [85, 87]. In a less extent, callose deposition has been considered as a mechanism to prevent Al from penetrating into the apoplast. Also, this accumulation is reported to inhibit the symplastic transport and cell communication by blocking plasmodesmata, avoiding Al-induced lesions in the symplast [92]. However, callose deposition in sensitive roots has also been shown to lead to uncontrolled rigidity of cell walls [41] leading ultimately to protoplast degradation.

**2.6. Others.** Al-induced effects/damages are first detected in the root system [18, 93]. Changes in the root system may affect nutrient uptake, which can lead to nutritional deficiencies in shoots and leaves [94]. Except for Al-accumulator plants, Al accumulates more in roots than in leaves [95]. In some species, Al-induced alterations in leaves were considered indirect, since Al accumulation was not detected in leaves [94]. Nevertheless, alterations in leaves induced by Al exposure were reported for many species. Several works reported leaves biomass reduction [96], thickness [95], lipid

peroxidation [97], nutritional imbalances [98], changes in the photosynthetic performance [99], and changes in chlorophyll contents [96, 97, 99, 100], among others. Reductions in carbon dioxide (CO<sub>2</sub>) assimilation rate due to Al toxicity are reported for several species [94, 99–101], and some works indicated that Al exposure induced damage of the photosystem II [97, 102]. Very few works focused on the consequence of Al treatment in the carbohydrate metabolism. The effects of Al exposure on Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCo) content and activity are still unclear, and the few reports available were performed in citrus [99, 100] and in wild rice [103].

### 3. Conclusions

Most studies on Al toxicity are performed with different media composition, Al concentration, and period of exposure. Also, there is a large variation between genotypes. This battery of nonharmonized experimental data needs caution during interpretation, mostly concerning generalizations of functional models. So, it would be important to uniform the experimental procedures in order to better comprehend the plant response to Al exposure and the mechanisms of Al tolerance.

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## Review Article

# Environmental Nanoparticles Interactions with Plants: Morphological, Physiological, and Genotoxic Aspects

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Nanoparticles (NPs) are characterized by their small size (less than 100 nm) and large surface area, which confer specific physicochemical properties as strength, electrical, and optical features. NPs can be derived from natural or anthropic sources, such as engineered or unwanted/incidental NPs. The composition, dimension, and morphology of engineered NPs enable their use in a variety of areas, such as electronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalysis, and materials science. As nanotechnology is an innovative and scientific growth area with an exponential production, more information is needed concerning the impacts of these nanomaterials (NMs) in the environment and, particularly, in animals/humans health and in plants performance. So, research on NPs as emerging contaminants is therefore a new field in environmental health. This minireview describes, briefly, the NPs characterization and their occurrence in the environment stating air, water, and soil. Finally, particular emphasis is given to the interaction of NPs with plants at different levels: morphology, physiology, and genotoxicity. By analyzing this compiled information, it is evident that research on NPs phytotoxicity is in the beginning, and more comprehensive studies are needed not only on NPs cytotoxicity and genotoxicity but also on the best and the most reliable methods of assessing NPs toxicity.

## 1. Introduction

**1.1. Historical Overview.** In 1959, Richard Freynman, an American physicist and Nobel laureate, firstly used the concept of nanotechnology, reporting the lecture “There’s plenty room at the bottom” [1]. The Japanese researcher Professor Norio Taniguchi, in 1974, was the first to use the term nanotechnology, reporting “nanotechnology mainly consists of the processing of separation, consolidation, and deformation of materials by one atom or by one molecule.” Later, in 1980, Dr. K. Eric Drexler explored the concept of this emerging field, in particular promoting technological significance of nanoscale phenomena and devices. More recently, in 2000, to coordinate federal nanotechnology research and development, the United States National Nanotechnology Initiative was founded (e.g., see <http://www.nano.gov/>).

Nowadays, nanotechnology is a revolutionary science, with predicted evolution within the next decades that may have equivalence with the ones observed for other industries, as the one of computers during the second half of the

last century or earlier with the automobile industry (please see <http://www.nanotechnologyresearchfoundation.org/nanohistory.html>). This emerging nanoparticles (NPs) industry is expected to contribute to diverse products and services and to serve multiple consumers’ purposes. However, and despite the success of nanotechnology, the release of NPs to the environment remains unknown, mostly due to the lack of scientific knowledge concerning the potential health and environmental risks associated with nanomaterials (NMs). In this minireview, we will describe the potential hazards of NPs in the environment and their interactions with plants. Despite this, NPs are being considered in the restrict group of emerging contaminants by, for example, some European countries (e.g., <http://files.nanobio-raise.org/Downloads/scenihr.pdf>) and the United States Environmental Protection Agency (EPA) (e.g., [http://www.epa.gov/fedfac/pdf/emerging\\_contaminants\\_nanomaterials.pdf](http://www.epa.gov/fedfac/pdf/emerging_contaminants_nanomaterials.pdf)).

**1.2. Nanoparticles Description.** The unit nanometer was derived from the Greek “nano” which means “dwarf,” since

nanometers are referred to particles smaller than 1000 nm or 1  $\mu\text{m}$  [2]. However, NMs have been considered to be substances with a dimension less than 100 nm [2–10].

NPs, considering the building blocks of nanotechnology [6], have at least one dimension at 100 nm or less, which provides a high surface/volume ratio, leading to high reactivity (increasing the potential to cross cell membranes) [10] or intrinsic toxicity of the surface [11, 12]. These factors act together and can change or enhance properties of NPs, such as strength, electrical properties, and optical characteristics [10]. Moreover, their small size and large surface area can determine the harmful potential of NPs [12].

## 2. Nanoparticles Classification

NPs can be classified regarding to their dimensionality, morphology, composition, uniformity, and agglomeration [2], since they can be spherical, tubular, irregularly shaped and can also exist in fused, aggregated or agglomerated forms [4]. Dimensionality refers to their shape or morphology, which can classify them on their number of dimensions, such as one-dimensional (1D), two-dimensional (2D), three-dimensional (3D) NMs.

The characteristics to be taken relatively to NPs morphology are flatness, sphericity, and aspect ratio. NPs can be composed of single material or several materials; composites of various agglomerated materials can be found in nature, although single composites can be easily synthesized today. In respect to uniformity and agglomeration, NPs can exist as dispersed aerosols, suspensions/colloids, or in agglomerate state, due to their different chemistry and electromagnetic properties. Despite some diverse classifications of NPs that can be found, one can in general divide the following classes according to their composition (e.g., [13]):

- (1) carbon-based materials (e.g., C60 fullerene), with particular potential in carbon nanotubes technology,
- (2) metal-based substances (e.g., Ag, Au, and nanometal oxides), among the last ones, for example, those of titanium oxide are of particular interest,
- (3) dendrimers, polymers consisting of branched units,
- (4) bio-inorganic complexes (e.g., titanium with attached DNA strands) [13].

## 3. Nanoparticles Sources

NPs can be derived from natural and anthropogenic sources (engineered and unwanted or incidental NPs) [4, 14–16]. The natural processes that produce NPs can be photochemical reactions, volcanic eruptions, forest fires, simple erosion, and by plants and animals (shedding of skin and hair) [2]. Otherwise, anthropogenic sources refer to the following:

- (1) engineered NMs classified as carbon NMs, metal oxide NPs, zero-valence metal NPs, quantum dots (QDs) and dendrimers [10],
- (2) unwanted or incidental NPs from by-products of simple combustion, food cooking, and chemical

manufacturing; welding or refining and smelting; combustion in vehicle and airplane engines; combustion of treated pulverized sewage sludge; and combustion of coal and fuel oil for power generation.

From their multiple conformation, composition, and nature, NPs present multiple functions and almost infinite applications. Nanotechnology has, therefore, gained a place as an emerging science with a recent rise in interest of NPs, mostly due to our increasing ability to synthesize and manipulate such particles.

Thus, nanoscale materials have a variety of uses in different areas, such as electronic, biomedical, pharmaceutical, cosmetic, energy, environmental, catalytic, and material applications [4], as described below.

## 4. Nanoparticles Applications

NMs have diverse applications, which can be summarized as follows [14, 17]: sunscreens and cosmetics (nanosized titanium dioxide and zinc oxide in sunscreens, nanosized iron oxide in some lipsticks); shampoos and detergents (nanosized alumina); window panes and ceramic tiles (nanostructured titanium oxides); pharmaceutical products; medical implants; food packaging; biosensors and agents for environmental remediation; water treatment technology; composites (mechanical, optical, electrical, and magnetic properties); clays (construction materials); coatings and surfaces; tougher and harder cutting tools; paints; displays (television screens and computer monitors); batteries (growth in portable electronic equipment); fuel additives; carbon nanotube composites; lubricants (nanosized “ball bearings”); magnetic materials; machinable ceramics; and military battle suits.

However, and considering the statement that all technology comes with a price, nanotechnology could lead to environmental hazards and adverse health effects [1].

## 5. Nanotoxicology

Primary geogenic NPs may form as aerosols from, for example, sprays of salts and sulfates mostly from the sea, or as sulfate aerosols from volcanic emissions, or even as forest fires (soot and/or polycyclic aromatic hydrocarbons) [18].

Moreover, NPs may be introduced in the environment by anthropic reasons, being introduced, for example, intentionally to remediate groundwater and contaminated soil [19, 20], or also unintentionally by several sources, as research and clinical settings and solid or liquid waste streams production facilities [21]. The biggest risk of release of this material may occur during transportation to good manufacturers and by intentional releases for environmental application [20, 21].

The toxicity and degradation of these compounds in the environment cannot be accurately assessed yet because it depends on the NPs type, physicochemical properties, and also the environment media in which they partition and the respective conditions. So, this is a field that requires intense research. For example, it is recognized that research needs to

be performed in the following fields: (a) detection of NPs in the environment, (b) measurement of emissions of NPs, (c) life-cycle of NPs in the environment, (d) toxicity of NPs to the environment, and (e) immediate and longer impact on environment (e.g., <http://www.nanocap.eu/>).

The concern of NPs exposure is due to some important issues, as these materials half-lives that may be months to possibly years, the increase on concentration of anthropic substances since NPs are often produced in a large scale, and because this material is expected to be accumulated and transformed by time and local environmental conditions [20, 22].

The current knowledge about NPs and the challenges of nanotoxicity assessment lead to a lack of effective regulation of NPs use. Some governmental entities, as EPA, have been conducting efforts in order to not only comprehend the properties of NPs and their potential risks for human health and the environment but also to regulate the use, storage, and disposal of these materials to allow a safe and sustainable nanotechnology development. Although there are no specific environmental laws, NPs cut across some existing EPA regulations. For example, several NMs are considered chemical substances under the Toxic Substances Control Act, and pesticides containing nanotechnology products are regulated under the Federal Insecticide, Fungicide, and Rodenticide Act (see <http://www.epa.gov/nanoscience/>). Still, a recent report from EPA's Office of Inspector General stated that "... EPA does not currently have sufficient information or processes to effectively manage the human health and environmental risks of nanomaterials..." and that "... EPA has the statutory authority to regulate nanomaterials but currently lacks the environmental and human health exposure and toxicological data to do so effectively" [23]. According to this report, some proposed policies have failed, and others that await approval are facing significant barriers to their effectiveness [23].

## 6. Transport and Interactions

Transport of NPs through environment partition is the most critical parameter to evaluate NPs impact, expecting high mobility of NPs that are designed to be released into the environment [24]. Transport and, therefore, NPs mobility are determined by forces, such as London-van der Waals and double-layer forces that are responsible for attachment. This phenomenon is called Brownian motion (diffusion). Gravity and fluid motion are also factors that influence transport of NPs [7, 8, 14].

NPs are predicted to have high efficiency of transport to collector surfaces due to Brownian diffusion, and their potential mobility may be predicted by knowing the exact surface properties of NPs. The surface properties of engineered NPs are of essential importance for their aggregation behavior, and thus for their mobility in aquatic and terrestrial systems and interactions with algae, plants, and fungi [19, 25, 26].

NPs can interact with toxic compounds, and it can both amplify the toxic effect and have a positive role in

the environment, alleviating the toxicity of the compounds. There are two scenarios of possible NP-pollutant-cell interactions: NPs may adsorb (absorb) the pollutant and decrease the free concentration, resulting in a reduction of pollutant uptake by organisms, and there is also the possibility of NP-pollutant uptake (adsorbed) by the organisms, in which the toxic effect could be caused either by NPs pollutant or the synergistic way by both together [4].

One of the positive applications of NPs in the environment is precisely its use in bioremediation and as anticontaminants in the removal of pollutants from soil and water systems. Their unique surface properties and size have an advantageous role in the direct conversion of the contaminant, in the pollutant-NP surface reaction, and in the aggregation/incorporation of the pollutant into colloids (contaminants are transported with colloids and removed) [4, 19, 27]. Engineered NPs can be synthesized with controlled properties to enhance their selectivity for the pollutant and reactions steps. This can lower the costs of removing pollutants from the environment and can also reduce the concentration of NPs needed for this purpose [14, 27, 28]. For instance, nanoscale zero-valent iron (nZVI—made by reduction of solutions of metal salts) is widely used for groundwater, sediments, and soils remediation. Quinn and collaborators [29] and Elliott and Zhang [30] injected nZVI into the groundwater and obtained successful results in remediation. nZVI removes nitrates by reduction and, being a metallic iron, is effective in degrading a wide variety of common contaminants (chlorinated ethenes, chlorinated methanes, brominated methanes, pesticides, and dyes) [4, 27]. Nevertheless, more *in vitro* tests should be carried out before *in situ* large-scale tests are performed. Despite the control of the production of enhanced NPs (controlled size, composition, and morphology) more precaution is needed, because this nanoscale material is too reactive and their benefic role may turn into a negative one when released into the environment.

## 7. Fate and Behavior of Nanoparticles in the Environment

**7.1. Air.** There is a natural background of NPs (ultrafine particles—smaller than  $0.1\ \mu\text{m}$ ) in the atmosphere and, despite little work has been done on ecological system, it is known that, when compared to anthropogenic sources and potential released manufactured NPs, the natural background present in the air is considerably low [19, 31].

NPs have short residence time. However they attach to accumulation mode particles and remain in the atmosphere as long as the accumulation mode particles participate in the atmospheric chemistry and physics. Diesel NPs are one of the concerning NPs released into the atmosphere. These NPs contribute to global warming since they absorb light and also endanger human health.

When ultrafine particles are directly released from the source, they are called primary particles; volatile NPs (secondary NPs) composed by nucleation mode may be formed in the atmosphere by photochemical processes.



The key species in the atmosphere are sulfuric acid, nitric acid, and organic gases [4, 14]. Some conditions (e.g., temperature, residence time, dilution rate, dilution ratio, and concentration of carbon particles) are necessary for production of these secondary NPs [32–35].

Therefore, this event will result in an increasing of particle number concentration above their natural concentration present in the atmosphere; besides, these nucleation particles may continue to grow by vapor condensation and coagulation, which result in atmospheric optical effects, building soiling, and climate change [4, 14, 36, 37].

**7.2. Water.** Industrial products and wastes tend to end up in waterways, increasing the possibility of NPs contamination, although almost no current data on behavior and fate of NPs in aquatic systems is available [4].

Colloids, particles in the 1 nm to 1  $\mu$ m size range, are macromolecules and molecular assemblies. They include organic (e.g., primarily humic substances (HS) and fibrillar material, usually protein and polysaccharide exudates from microbes) and inorganic matter (e.g., Fe, Mn, Al, and Si oxides, and thus analogous to manufactured metal oxide NPs), and also biological material (e.g., bacteria) [4, 19]. Colloidal fate and behavior are determined by aggregation. Colloids tend to aggregate to particles ( $>1 \mu$ m), and their transport depends on the first one, being denominated sedimentation. Like metals, manufactured NPs tend to aggregate and subsequently settle. This process has positive effects on water bodies' purification, because this behavior results in a transfer of NPs from water column to sediments, resulting in a pollutant loss.

Despite this possible pollutant loss, natural colloids interact with NPs and this will affect the NPs behavior, being more difficult to predict the NPs effect on the aquatic system. Knowing NPs size, surface charges, and chemical reactivity, it is possible to predict some potential biological effects in the aqueous environment, such as generation of ROS, phototoxicity and photocatalytic activity, and interaction with soluble pollutants [25], but due to the aquatic system complexity this physical behavior is hard to predict.

Oceans exhibit changes in physicochemical characteristics with depth (e.g., temperature) that may influence aggregation and colloid chemistry. Analogously to freshwater aggregates of NPs may sink into the ocean floor, as well as accumulate at the interface between cold and warm currents or be recycled by biota. This may threaten pelagic species since they feed at these zones, while deposition in the sediment may present a risk of exposure to benthic species. NPs may also accumulate on the surface microlayers of the oceans, presenting a route of aerosol exposure risk to marine birds and mammals, as well as the organisms living on the surface microlayer [38, 39]. Oberdörster et al. [40] exposed freshwater crustaceans *Daphnia magna*, the marine copepod *Hyalella azteca*, and fish, such as *Pimephales promelas* and *Oryzias latipes*, to C60 (fullerenes), and toxicity was reported. Kashiwada [41] reported an increase in toxicity with the salinity enhancement, along with a greater tendency for the particles to form aggregates, when eggs of Japanese

medaka were exposed to fluorescent NPs (30 mg/L) at different salinities. At salinities similar to the ones found in normal seawater, accumulation decreased, but egg mortality remained high.

Therefore, it is not possible to predict or evaluate the impact of NPs on aquatic systems; however, ecotoxicological studies show that aquatic organisms, both unicellular and animals, are affected by NPs toxicity [4, 42, 43].

**7.3. Soil.** Soil and sediments are the ultimate sinks of NPs and, whether directly or indirectly released (e.g., via sewage treatment plants, aerial deposition, or waste handling), NPs will end up in soil; this system may present the most significant exposure avenue for assessing environmental risk. Being soil one of the main sinks, it raises concern about the entry of NPs into food webs and human access to contaminated agriculture [44].

In terrestrial ecosystems, natural NPs are present (soil colloids), such as organic matter, clays, iron oxides, and other minerals that play an important role in biogeochemical processes. Therefore, the introduction of manufactured NPs in this ecosystem may change the profile of the natural NPs and interfere with soil development (pedogenesis) or behavior (e.g., crusting and dispersion) [19, 45].

The fate of NPs in terrestrial ecosystems is difficult to elucidate, and there is few data that report transport of NPs through soils. NPs are made to have different properties than their similar bulk and macroscopic material, and these differences may change fate and effects of NPs in terrestrial environment [19, 46, 47]. The high surface area, reactivity, size, aggregation, and absorbance are important determinants of their adhesion to soil, and thus their mobility and transport [48].

Bioavailability of NPs also depends on specific properties of each NP, in addition to composition of the soil matrix, porosity, hydraulic conductivity, groundwater gradient and flow velocity, and geochemical properties (e.g., dissolved oxygen, pH, ionic strength, and concentrations of nitrate, nitrite, and sulfate) [4, 19, 43]. Unrine et al. [44] reported absorption, biodistribution through tissues and statistically significant decrease in reproduction of earthworms *Eisenia fetida* when exposed to Au NPs. This study demonstrates the importance of considering food chain accumulation and trophic transfer, but information that NPs end up in higher-level organisms is extremely limited [49].

NPs have been extensively used as antimicrobial agents against pathogenic bacteria, but this abusive use is leading to negative consequences against the populations of soil microbes that play beneficial roles in the environment, such as promoting plant growth, element cycling, and degradation of pollutants. The NPs of Ag, CuO, and ZnO showed both toxicity on pathogenic bacteria (e.g., *Escherichia coli* and *Staphylococcus aureus*) and beneficial effects on microbes, as *Pseudomonas putida*, which has bioremediation potential and is a strong root colonizer [50, 51]. These NPs showed higher toxicity when compared to their equivalent bulk materials [22, 52].

Therefore, being terrestrial systems the larger and most reactive sink for NPs, more research and standardized testing protocols are needed for fate/behavior and food web accumulation of NPs.

## 8. Interaction of Nanoparticles with Plants

As pointed before, the increasing applications and use of NPs are directly related to their release in the environment. The effects of NPs have been described in a wide variety of organisms, such as microorganisms [53, 54], protozoa [55], invertebrates [56, 57], and vertebrates [58].

However, interactions of NPs with plants and other organisms that share similarities with plant cells, such as algae, have been poorly studied, remaining unclear the general consequences of NPs exposure for plant cells [59]. The lack of these data leads as well to a defective understanding of how NMs are transferred and accumulate in the various food chain levels [60]. We will describe below some of the most relevant studies on NPs toxicity in plants.

### 8.1. NPs Phytotoxicity: Morphological and Physiological Data.

The effects of  $\text{Fe}_3\text{O}_4$ ,  $\text{TiO}_2$ , and carbon NPs were assessed on cucumber plants [61]. These NPs were found to negatively affect seed germination rate, root elongation, and germination index. Also, five NPs (multiwalled carbon nanotubes (MWCNTs), Ag, Cu, ZnO, and Si) were tested on *Cucurbita pepo* in suspensions up to 1000 mg/L [62]. The authors found different effects from the NPs and their counterparts, concerning seed germination, root elongation and biomass [62]. No exposure reduced germination rates, but Cu NPs reduced emerging root length. Also, exposure to Ag NPs decreased plant biomass and transpiration. These authors concluded that they demonstrated "... that standard phytotoxicity tests such as germination and root elongation may not be sensitive enough or appropriate when evaluating nanoparticle toxicity to terrestrial plant species" [62].

In another study, Zhang and collaborators [59] compared the phytotoxicity of nanoparticulate  $\text{Yb}_2\text{O}_3$ , bulk  $\text{Yb}_2\text{O}_3$ , and  $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$  to cucumber plants. They studied the toxicity, distribution, and biotransformation of the three materials in plant roots and found that with the nano- $\text{Yb}_2\text{O}_3$  exposure,  $\text{YbPO}_4$  deposits were found in the cytoplasm of root cells. The putative toxicity of these compounds was then discussed by the authors [59].

Other study on root cells of *Allium cepa* reported that ZnO NPs exert cytotoxic and genotoxic effects, including lipid peroxidation, decreasing of the mitotic index, and increasing of the micronuclei and chromosomal aberration indexes [63]. Ma and colleagues [64] evaluated the effects of four rare earth oxide NPs (nano- $\text{CeO}_2$ , nano- $\text{La}_2\text{O}_3$ , nano- $\text{Gd}_2\text{O}_3$ , and nano- $\text{Yb}_2\text{O}_3$ ) on root elongation of seven higher plant species. Different NPs caused distinct effects on root growth, and these effects also varied between plant species, being lettuce the most sensitive one. The surface modification of NPs has been shown to alter the phytotoxicity induced by these materials. A recent study reporting the interaction of  $\text{SiO}_2$  NPs with algae showed that

alumina coated  $\text{SiO}_2$  NPs were less toxic to *Pseudokirchneriella subcapitata* than bare  $\text{SiO}_2$  NPs [65].

On the other hand, several studies demonstrated that NPs can also have no significant or positive effects on plants.  $\text{Al}_2\text{O}_3$  NPs at concentrations up to 4000 mg/L had no significant toxic effects on seed germination, root elongation, and number of leaves of *Arabidopsis thaliana* [66]. One study showed that 10–40 mg/L of carbon nanotubes increased seed germination and growth of tomato plants [67]. The authors suggest that these effects are due to carbon nanotubes ability to penetrate the seed coat and enhance the crucial water uptake, although these specific mechanisms were not reported. Nano- $\text{TiO}_2$  was also found to improve energy utilization and conversion efficiency in D1/D2/Cyt b559 complex of spinach [68], which could explain the enhancement of spinach growth by  $\text{TiO}_2$  reported in other articles.

Larue and collaborators [69] studied the effects of  $\text{TiO}_2$ -NPs on *Triticum aestivum*, *Brassica napus*, and *Arabidopsis thaliana*. They showed that these NPs were absorbed by plants and did not affect their germination and root elongation. The authors also highlighted the need of more studies of NPs toxicity, and in particular on NPs interaction with plants.

Despite the studies reported above, Rico and collaborators [70] recognized that "most of the studies reported the effect of nanomaterials on seed germination or 15-day-old seedlings, and few describe the biotransformation of nanomaterials in food crops, and the possible transmission of the nanomaterials to the next generation of plants exposed to nanomaterials is unknown." So, it is urgent not only to further elucidate the effects of NPs in plants in order to characterize the uptake, phytotoxicity, and accumulation of NPs, but also to understand how nanoscale materials can affect food chains and, ultimately, to human health risk assessment.

Moreover a main question remains, that is, which are the best standard phytotoxicity tests that may be used in assessing NPs toxicity? For example, most of the studies up to moment were based on germination and root elongation, which are not sensitive enough or appropriate when evaluating NP toxicity to terrestrial plant species [71].

### 8.2. NPs and Genotoxicity in Plants.

Few is known on the NPs induced genotoxicity in plants and most of the few available information dates from the two last years. Atha and collaborators [72] reported for the first time that copper oxide NPs damaged DNA in some agricultural and grassland plants (*Raphanus sativus*, *Lolium perenne*, and *Lolium rigidum*). It seems that oxidatively modified compounds accumulated and led to mutagenic DNA lesions, which inhibited plant growth. This isolated study on NPs genotoxicity in plants strongly supports the urgent need to evaluate the putative genotoxicity of the different NPs classes in plants and at which concentrations. Another issue that deserves attention is the analysis of genotoxic endpoints for NPs genotoxicity. For example, Comets, FCM-HPCV, and micronuclei have provided similar information in metal

genotoxicity in plants (e.g., [71]), but any generalization to NPs-induced phytogenotoxicity should be done carefully.

## 9. Conclusion

NPs technology is an emerging field with multiple potentialities and applications. However, and due to the particular characteristics that NPs have when compared to their individual components, NPs may present also different toxicity profiles. Despite NPs have always been present in the environment, the concern on their toxicity has only emerged during the last decade, with the increase of NPs industry and multiple applications. Few studies are available and most are focused on microorganisms and animals/human cells. On the other hand, putative toxicity in plants is by far less studied. It is so important to understand that not all NPs are toxic. From the few available data, some remain controversial, as some NPs seem to be nontoxic and others appear to have beneficial health effects. On the contrary, others seem to be cytotoxic for different organisms, and even genotoxicity was already described for plants.

So, several countries recognized the need to study NPs toxicity and considered them as emerging contaminants. More information is needed regarding the potential impacts that NPs release may have on environmental and animals health.

The uptake, bioaccumulation, biotransformation, and risks of NMs for food crops are still not well understood. Very few NMs and plant species have been studied, mainly at the very early growth stages of the plants. Most of the studies, except one with MWCNTs performed on the model plant *Arabidopsis thaliana* and another with ZnO NPs on *Ryegrass*, reported the effect of NMs on seed germination or 15-day-old seedlings. Very few references describe the biotransformation of NMs in food crops, and the possible transmission of the NMs to the next generation of plants exposed to NMs is unknown. The possible biomagnification of NPs in the food chain is also unknown.

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## Review Article

# Phytotoxicity of Mercury in Plants: A Review

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With the advent of the industrial revolution, heavy metal contamination has become an ever increasing concern. Thus, it is of major importance to understand the extent of the toxicity in plants and animals and the consequences from the ingestion of contaminated food. Hg is easily modified into several oxidation states, and it can be spread in many ecosystems. Due to the recurrence of Hg pollution and due to the lack of knowledge about the effects of this heavy metal in plants, the aim of the present text is to provide a comprehensive review of the literature regarding Hg phytotoxicity.

## 1. Heavy Metal Pollution: Environmental Issue

Agriculture had a major impact in humans, being the major force behind the passage from a hunter-gatherer/forager society to a sedentary one, becoming a crucial tool for human sustainability and the development of economics worldwide. However, the passage to a sedentary and ever developing society was accompanied by a drastic transformation of the environment and the inherent exposure to new risks caused directly or indirectly by those transformations [1].

One of the many new risks that might have affected those early societies was the continuous exposure to pollutants, namely, heavy metals. The first cases of human exposure to heavy metals, beside the naturally occurring phenomena from Earth processes (e.g., volcanoes and rock constituents), were soon reinforced by the inadvertently discharge of these elements to the environment by human activities (e.g., from ore mining and smelting) [2, 3]. Since then, and with the advent of the industrial revolution, heavy metal contamination has become an ever increasing concern [4, 5]. Moreover, due to their stability and as they cannot be degraded, heavy metals tend to accumulate easily spread in a wide variety of ecosystems [6]. For most of the heavy metals, contamination arises from industrial applications, mining, smelters, combustion of fuel, and byproducts. From these sources, contaminants can be present in the ecosystem as airborne

particles, wastewaters, and sludge [7], polluting not only sites near the source but also locations thousands of kilometers apart.

Within the many occurrences of ecosystems' pollution provoked by heavy metals, it easily comes to mind the magnitude and reach of the Minamata disaster (1950), which caught the world unaware of the damage that long exposure to heavy metals can induce in organisms. Mercury (Hg) was the main pollutant, and the subsequent associated diseases caused 2,265 casualties just from direct exposure to Hg [8].

The uncontrolled release of highly polluting substances from mining wastes (e.g., cyanide, arsenic, Hg, and sulphurs) in the region of Oruro (Bolivia) transformed a unique ecosystem in a desert. Nearly 53,000 inhabitants suffer from water pollution, the salinization, and desertification of thousands of acres of land, beside the diseases and death (human and livestock) associated to exposure to these pollutants [9].

In the mid 80's, water used to extinguish a major fire carrying 30 tonnes of a fungicide containing Hg was used in the Upper Rhine. The substantial increase in Hg levels lead to massive fish death in an area over 100 km [10].

In 1998, a nature reserve in Spain was contaminated after the rupture of a dam, releasing sludge and contaminated wastewater. The wastewater entered the Guadiamar River, polluting the river with heavy metals such as cadmium, lead, zinc, and copper. It affected an area of 4.634 hectares,

contaminating 2.703 hectares with sludge and 1.931 with acidic water. Experts estimated that Europe's largest bird sanctuary, as well as Spain's agriculture and fisheries, would suffer permanent damage from the pollution that was accumulated in this ecosystems (DPPR/SEI/BARPI—collapse of the dam of a tailings pond).

Due to the critical risks associated with metal toxicity to human health and environment [11, 12], scientists have been studying the effects of heavy metals with more emphasis on the bioremediation/chelation of the metal ions by organisms [13] and the severe effects in humans [3].

Among the different models available to study heavy metal toxicity, plants present some unique features that make them interesting subjects for this type of assays. Firstly, as primary producers of the food chain, understanding the toxic effect of this metal in the plant status as well as the risks of biomagnifications of these toxicants for consumers is highly important. Also, because plants lack the ability to escape from contaminated sites, these organisms evolved mechanisms to handle exposure to toxicants [14]. For instance, certain species can regulate the amount of pollutants which are taken from the surroundings, resort to sequestration and inactivation in subcellular compartments [15], or tolerate the deleterious effects of heavy metals. Baker 1981 [16] proposed the classification of plants, according to their capability to accumulate toxicants, in three categories: excluders, accumulators, and indicators. The excluders are all of those that can grow in contaminated soils while maintaining the concentration of toxicants at low levels, when compared to the concentrations in the soil. The accumulators are those species that can survive despite concentrating contaminants at high doses in the aerial portion. Plants considered to be indicators can regulate the uptake and transport of the pollutant to the aerial part, being that the internal concentration is often similar to the one observed in the soil's parent soil [17].

Among the heavy metals that have been deemed of highest concern by the European Union, some have been the target of many investigations (e.g., Cd) while, for others, the level of understanding about the mechanism and extent of their phytotoxicity (e.g., Hg) is insufficient.

The toxicity of metals and their compounds largely depends on their bioavailability, that is, the mechanisms of uptake through cell membranes, intracellular distribution, and binding to cellular macromolecules [18]. From the literature available, it is generally agreed that the mechanistic evolving the toxicity of heavy metals is originated by a complex pattern of interactions between cellular macromolecules and the metal ions. The entrance of the metal in the cell can mobilize several metabolic and signal transduction pathways and genetic processes to neutralize the source of toxicity [18]. Although the relative toxicity of different metals to plants can vary with the genotype and experimental conditions, most heavy metals act through one of the following: changes in the permeability of the cell membrane, reactions of sulphhydryl (–SH) groups with cations, affinity for reacting with phosphate groups and active groups of ADP or ATP, replacement of essential ions, and oxidative stress [13, 19].

Considerable attention has therefore been paid to understanding the structural, kinetic, and thermodynamic details

of these interactions in a number of laboratories, as a key requirement for unraveling and discussing the mechanisms of action and physiological roles of metal ions in living systems (e.g., [20, 21]).

In the case of Hg, it is known that this metal is easily modified into several oxidation states and it can also be spread through many ecosystems [22, 23]. These modifications can be resumed in two major cycles, one occurring in a global extent which involves the circulation of Hg as elemental Hg ( $Hg^0$ ) in the atmosphere. The other, with a low range, depends on the intervention of organisms that can methylate the inorganic Hg into organic Hg compounds, being the latter the most toxic for the living organisms [22].

Due to the recurrence of Hg pollution and also because of the lack of knowledge about the effects of this heavy metal in plants, it is urgent to evaluate and understand the extent of Hg-induced phytotoxicity. In the past, management and regulatory responses to the problem of bioaccumulation generally have been constrained by a lack of information on sources, methods of transport, chemical interaction, and biological significance of Hg in the environment. To comprehend the extent of the toxicity and the proprieties that make this heavy metal so interesting to study, the following sections of the introduction will be dedicated to elucidate the reader about the Hg, mainly, the inorganic form ( $Hg^{2+}$ ), which is the predominant form in agricultural soils and thus the most interesting for this paper.

## 2. Chemical Forms of Hg

Among metals, Hg is unique in that it is found in the environment in several physical and chemical forms: for example, elemental Hg ( $Hg^0$ ), inorganic Hg ( $Hg^{2+}$ ), associated with ions (SHg, ClHg<sub>2</sub>), mercurous chloride or calomel ( $Hg_2Cl_2$ ), and organic Hg (e.g.,  $CH_3-Hg$ ) [8, 23, 24].

Hg is a metal with different and singular proprieties than those of the others transition metals, being the only one that, at room conditions, exists in liquid form. It is a good electric conductor, being applied in many technological areas, such as informatics, production of batteries, and light bulbs.

The high solubility in water and easiness with which Hg shifts to the gaseous phase [23] are two of the most important properties of this heavy metal. These proprieties explain the ability and effectiveness of Hg to move in several ecosystems and remain in the atmosphere for long periods, being later on deposited in the soil or water bodies [25].

Hg can form salts with oxygen, sulphur, chlorine, and amalgams (alloys) with most metals, except iron and platinum. The inorganic forms of Hg can include the vapour Hg and the liquid form and mercuric Hg. The liquid is volatile and releases a monatomic gas usually referred to as Hg vapour. This species plays a key role in the global cycling of the heavy metal because it can exist as a cation with an oxidation state of 1+ (mercurous) or 2+ (mercuric) [22, 23].

The first oxidation state ( $Hg^+$ ) is the mercurous Hg form, and it is encountered in the form of calomel and mercurous chloride ( $Hg_2Cl_2$ ). Like shown in Figure 1, mercuric mercury ( $Hg^{2+}$ ) forms the divalent state of the Hg cycle, and it is

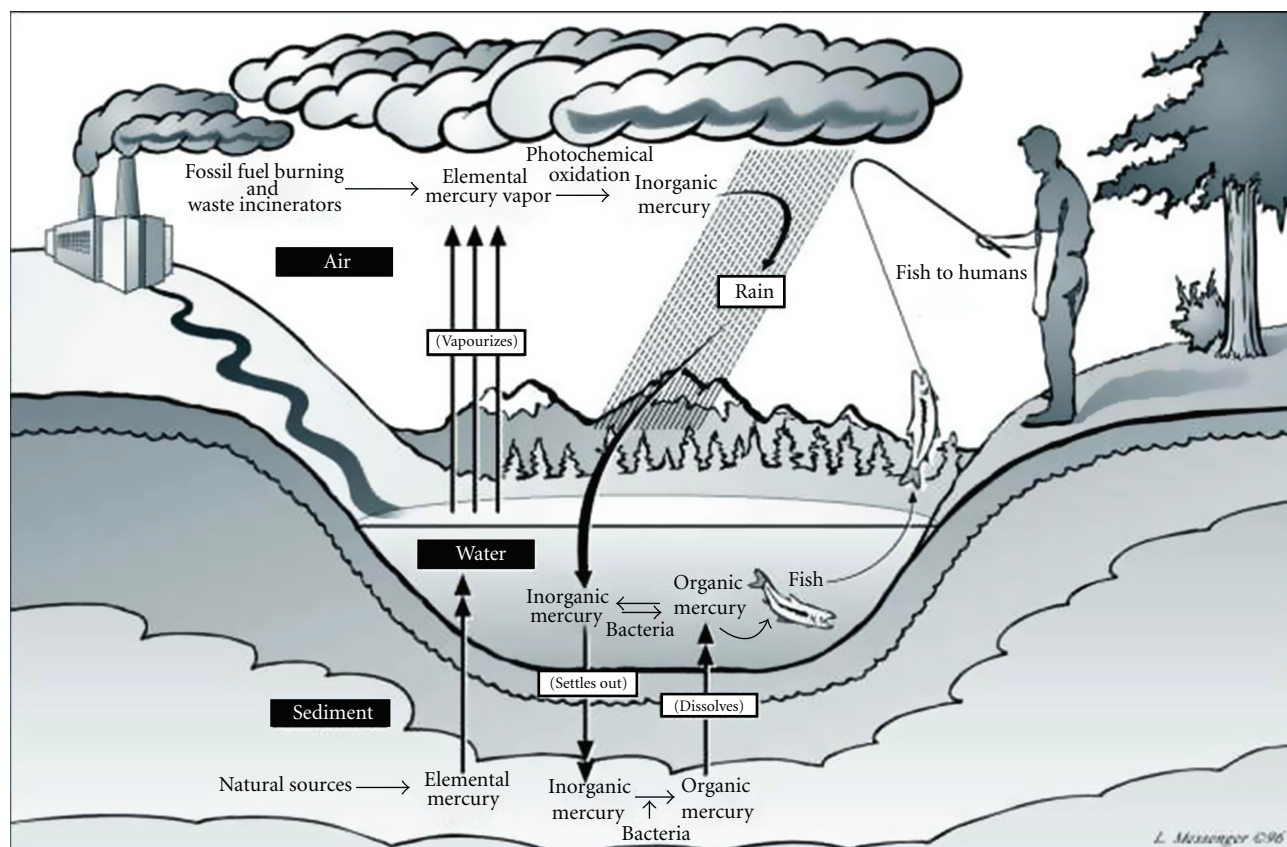


FIGURE 1: The Biogeochemical cycle of Hg. Briefly, it starts with the evaporation of Hg from natural and anthropogenic sources, which is then oxidized to inorganic Hg. This element is spread by the rain. Once in the soil, Hg can be transformed into organic compounds by bacteria (taken from [http://www.mercury.utah.gov/atmospheric\\_transport.htm](http://www.mercury.utah.gov/atmospheric_transport.htm)).

responsible for the formation of practically all the inorganic and organic form encountered in the environment and organisms.  $\text{Hg}^{2+}$  is a product of the metabolism of vapour Hg as well as, from the organic compounds of Hg. Due to these proprieties, this form plays a key role in the cycle of Hg and in the toxicology of this heavy metal in living organisms [23]. High levels of this form have strong phytotoxic effects; when present in toxic concentrations can induce visible injuries and physiological disorders in plant cells triggering the production of ROS (reactive oxygen species) leading to cellular disruption. Mercuric Hg has high affinity for thiol groups, special for the anions  $\text{R-S}^-$ . The easiness of the movement between thiol groups and in entering the cells is due to the high speed with which the reversible reactions between Hg's ionic forms occur. When mercuric Hg is in the form of water-soluble salts, as Hg chloride, it can be a highly potent poison. As a matter of fact, legends tell that the Chinese emperor Qin Shi Huang, the first to unify China, died after ingesting Hg pills concocted by his physicians in an attempt to obtain immortality.

Studies regarding Hg toxicity are mostly from animals and humans, being known that it is linked to autoimmune diseases [26]. The toxicity of inorganic Hg forms (e.g.,  $\text{HgCl}_2$ ) is at least in part explained by the element's great affinity for biomolecules containing sulfhydryl (SH) groups

[27] and by a lower affinity for carboxyl, amide, and amine groups [27]. Inorganic Hg arrives to the ecosystems as the result of the Hg cycling, and in the presence of the right conditions  $\text{Hg}^{2+}$  can be transformed in organic Hg forms.

Some organisms, like bacteria or fungus, can naturally modify the Hg available in the environment ( $\text{Hg}^{2+}$ ) by methylation of this ion, making the final product more dangerous and toxic than its precursor [5, 22, 23]. The more common organic forms are methyl-Hg ( $\text{CH}_3\text{-Hg}^+$ ) and ethyl-Hg, which despite the chemical differences have similar proprieties. Methyl-Hg is the most toxic of the organo-compounds [23, 28]. It is produced by biomethylation and it can be incorporated into trophic chains, mostly in the aquatic ones. The intact organomercurial cation is believed to be the toxic agent responsible for the damage provoked in cells; for instance, in humans, Hg can be modified to methyl-Hg that is capable of causing damage to the nervous system, liver, and ultimately cause death by multiorgan failure [29].

### 3. Ancient and Modern Applications of Hg

Hg and its compounds have been used by mankind since ancient recorded civilizations. Hg has been extracted from mines for centuries; Almaden (originated from the Arabic "the metal") is one of the biggest mines in the world, and



it has been used to extract Hg since the Roman Empire age, to produce explosives [23]. Egyptians used this heavy metal as a medicinal compound and in tombs, as a preservative. In medicine, mercurials use has been as vast as treatments for syphilis and various skin disorders, used as antiseptic or to treat diuretic and chemotherapeutic problems. Hg components were also employed to produce high-quality hats: Hg nitrate was applied to treat the fur used to make hats.

Since the industrialization era, Hg has been used as a compound of electric equipment, batteries, and explosives. It has also been used in medicine, cosmetics, and for agricultural purposes [19]. Activities like the smelting of copper and lead and the extraction of precious metals (e.g., gold and silver) contribute with a high percentage of the Hg pollution of aquatic systems [30]. The burning of fossil fuels, the chloralkali industries, production of electric equipment, and paint are the largest consumers of Hg [19, 31].

All of these anthropogenic activities promote an accumulation of this heavy metal in terrestrial and aquatic ecosystems, which can persist in these locations and in the living organism for over 100 years after the source of pollution has closed [23, 31].

It is understandable that Hg and its compounds present a big dilemma to those interested in making use of its many proprieties. If, on the one hand, Hg has great and useful advantages, on the other, it has great toxicity and is easily spread throughout diverse ecosystems.

#### 4. Uptake of Hg by Plants

Contamination of soils by Hg is often due to the addition of this heavy metal as part of fertilizers, lime, sludges, and manures. The dynamics between the amount of Hg that exist in the soil and its uptake by plants is not linear and depends on several variables (e.g., cation-exchange capacity, soil pH, soil aeration, and plant species). The uptake can be reduced when the soil's pH is high and/or there is an abundance of lime and salts [13, 19].

Another factor affecting the level of accumulation of Hg is the species and the variety [32] as a matter of fact; at least 45 plant families include metal-accumulating species [33]. Most of the plants that uptake Hg tend to accumulate it on the roots [31], and some are even able to accumulate moderate amounts in the shoots [34, 35] either due to translocation or direct absorption of the vapour form. The work done by Suszcynsky and Shann [36] showed that plants exposed to  $Hg^0$  can uptake and accumulate it in shoots, but there is no translocation to the roots.

Toxic metal ions are thought to enter plant cells by the same uptake process as micronutrients, competing with these elements for absorption. Hg, which is a class B metal [14], preferentially binds with sulphur and nitrogen ligands and is thought to enter the cell through ionic channels competing with other heavy metals like cadmium or essential metals like zinc, copper and iron [37]. However, this information is mostly based on experiments in animal cells and the authors believed that the uptake of Hg can occur via other processes which still remain unclear.

### 5. Hg-Induced Phytotoxicity

**5.1. General Effects.** The interaction between Hg and plant systems is of particular importance due to the highly employment in seed disinfectants, fertilizers, and herbicides [38]. Ross and Stewart 1962 [39] have shown that some Hg compounds used on tree foliage as fungicides can be translocated and redistributed in plants.

At the cellular level, the possible mechanisms which heavy metals can damage comprise the blocking of important molecules (e.g., enzymes and polynucleotides), the transport of essential ions, displacement or substitution of metal ions from molecules (such as Mg from chlorophyll), denaturing or inactivation of proteins, and disruption of cell membranes or organelles [13]. Relative to Hg, the possible mechanisms of its phytotoxicity can be through the change of the permeability of cells membrane, high affinity to react with the sulphhydryl (SH) groups, affinity for reacting with phosphate groups, and the replacement of essential ions and its ability to disrupt functions involving critical or nonprotected proteins [13, 19].

Hg is known to affect the antioxidant defence system, by interfering with the modulation of the nonenzymatic antioxidants glutathione (GSH) and nonprotein thiols (NPSH) and the enzymatic antioxidants superoxide dismutase (SOD), ascorbate peroxidase (APX), and glutathione reductase (GR) [2, 40, 41].

Many forms of Hg have been related to seed injuries and reducing seed viability. When Hg interacts with the SH groups to form the S–Hg–S bridge, disrupting the stability of the group, it can affect seed's germination and embryo's growth (tissues rich in SH ligands). Hg chloride has been shown to reduce elongation of *Zea mays*' primary roots as well as an inhibition of the gravimetric response of the seedlings [13]. The same author also discussed that, with increasing concentration of this heavy metal, the respiration rates of *Vigna radiata* seedlings declined, as did the total nitrogen and sugars content and the DNA and RNA content.

Exposure to Hg can also reduce photosynthesis, transpiration rate, and water uptake and chlorophyll synthesis. Both organic and inorganic Hg have been showed to cause loss of potassium, magnesium, and manganese and accumulation of iron [22]. These decreases explain the changes in the permeability of cell membrane by compromising its integrity.  $Hg^{2+}$  is one of the forms of Hg that can affect the plasma membrane and might explain the toxicity provoked in the aerial part of plants; however some authors believe that it is the damage in the roots that explain the toxicity observed in the shoots.

**5.2. Genotoxicity.** Studies considering Hg's genotoxicity are scarce, and the cellular and molecular mechanistic involving the toxicity of this metal are practically unknown. However, it has been demonstrated that this heavy metal can introduce deleterious errors in the genetic materials of crop plants' species.

Inside the cells, Hg ions tend to form covalent bonds, because of their easily deformable outer electron shells. A number of potentially reactive sites for Hg bonding are

present in DNA, depending on external conditions such as ionic strength, presence of different competing ions, and base composition [13].

The effects of Hg's forms depend of the concentration and time of exposure to plants, with very marked effects in S-phase, when it can induce damage leading to severe clastogenicity [13].

The binding of Hg to DNA results in potential toxic effects: chronic intake of methyl-Hg<sup>2+</sup> at subtoxic levels results in chromosomal damage in humans, presumably due to its direct interaction with DNA [8]. In plants, it has been demonstrated that low doses of Hg can provoke c-mitosis, sister chromatid exchanges, chromosomal aberrations, and spindle alterations [13].

Despite that the interaction of metal ions with the sulfur atoms of nucleosides and amino acids bearing the thiol group has provided the dominant mechanism for explaining Hg's toxicity, certain effects (e.g., mutagenic effects) cannot be totally explained by this process. Ribose/ribosephosphate groups and purine/pyrimidine bases present several N and O atoms, which might be potential binding sites for Hg. The ligation of Hg to nucleobases can lead to nucleobase impairing; this phenomenon has been suggested as being relevant to the mutagenic potential of Hg. By this way, Hg can induce alteration on the amino acid sequences of proteins to be synthesized [29].

Despite of all these, there still plenty of unknown aspects regarding Hg's genotoxicity, namely, the mechanistic, target, and extent of its effects in plants.

## 6. Concluding Remarks

As it has been explored throughout this paper, Hg is a critical pollutant which can be easily spread through many ecosystems causing several toxic effects in many biological processes. Unfortunately, very little is known about Hg-induced phytotoxicity, even though plants assume a fundamental role as the base of many trophy chains and in particular of mankind subsistence and economy. It is therefore necessary to increase the level of knowledge about the mechanism by which Hg is uptake by plants and which processes are targeted by this pollutant.

What little is known about Hg toxicity is presented in this paper, which the authors expect, could be a valuable source for other researchers working with Hg phytotoxicity and, possibly, to entice further investigation in this area of research.

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## Review Article

# Chromium as an Environmental Pollutant: Insights on Induced Plant Toxicity

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In the past decades the increased use of chromium (Cr) in several anthropogenic activities and consequent contamination of soil and water have become an increasing concern. Cr exists in several oxidation states but the most stable and common forms are Cr(0), Cr(III) and Cr(VI) species. Cr toxicity in plants depends on its valence state. Cr(VI) as being highly mobile is toxic, while Cr(III) as less mobile is less toxic. Cr is taken up by plants through carriers of essential ions such as sulphate. Cr uptake, translocation, and accumulation depend on its speciation, which also conditions its toxicity to plants. Symptoms of Cr toxicity in plants are diverse and include decrease of seed germination, reduction of growth, decrease of yield, inhibition of enzymatic activities, impairment of photosynthesis, nutrient and oxidative imbalances, and mutagenesis.

## 1. Introduction

Chromium (Cr) is the 17th most abundant element in the Earth's mantle [1]. It occurs naturally as chromite ( $\text{FeCr}_2\text{O}_4$ ) in ultramafic and serpentine rocks or complexed with other metals like crocoite ( $\text{PbCrO}_4$ ), bentorite  $\text{Ca}_6(\text{Cr,Al})_2(\text{SO}_4)_3$  and tarapacaite ( $\text{K}_2\text{CrO}_4$ ), vauquelinite ( $\text{CuPb}_2\text{CrO}_4\text{PO}_4\text{OH}$ ), among others [2]. Cr is widely used in industry as plating, alloying, tanning of animal hides, inhibition of water corrosion, textile dyes and mordants, pigments, ceramic glazes, refractory bricks, and pressure-treated lumber [1]. Due to this wide anthropogenic use of Cr, the consequent environmental contamination increased and has become an increasing concern in the last years [3].

Chromium exists in several oxidation states, but the most stable and common forms are Cr(0), the trivalent Cr(III), and the hexavalent Cr(VI) species. Cr(0) is the metallic form, produced in industry and is a solid with high fusion point usually used for the manufacturing of steel and other alloys. Cr(VI) in the forms of chromate ( $\text{CrO}_4^{2-}$ ), dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ), and  $\text{CrO}_3$  is considered the most toxic forms of chromium, as it presents high oxidizing potential, high solubility, and mobility across the membranes in living organisms and in the environment. Cr(III) in the forms of oxides, hydroxides, and sulphates is less toxic as it is relatively

insoluble in water, presents lower mobility, and is mainly bound to organic matter in soil and aquatic environments. Moreover, Cr(III) forms tend to form hydroxide precipitates with Fe at typical ground water pH values. At high concentrations of oxygen or Mn oxides, Cr(III) can be oxidized to Cr(VI) [4, 5].

As Cr(VI) and Cr(III) present different chemical, toxicological, and epidemiological characteristics, they are differently regulated by EPA, which constitutes a unique characteristic of Cr among the toxic metals [6]. Cr(VI) is a powerful epithelial irritant and also considered a human carcinogen [7]. Cr(VI) is also toxic to many plants [8] aquatic animals [9], and microorganisms [10]. Contrarily to Cr(VI), Cr(III) is considered a micronutrient in humans, being necessary for sugar and lipid metabolism [11] and is generally not harmful. In plants, particularly crops, Cr at low concentrations ( $0.05\text{--}1\text{ mg L}^{-1}$ ) was found to promote growth and increase yield, but it is not considered essential to plants [5, 12]. In this context, accumulation of chromium in edible plants may represent a potential hazard to animals and humans.

## 2. Chromium in the Environment

**2.1. Chromium in Water.** Chromium may enter the natural waters by weathering of Cr-containing rocks, direct discharge



from industrial operations, leaching of soils, among others. In the aquatic environment Cr may suffer reduction, oxidation, sorption, desorption, dissolution, and precipitation [6].

The aqueous solubility of Cr(III) is a function of the pH of the water. Under neutral to basic pH, Cr(III) will precipitate and conversely under acidic pH it will tend to solubilize. The forms of Cr(VI) chromate and dichromate are extremely soluble under all pH conditions, but they can precipitate with divalent cations [6]. The recommended limits for Cr concentration in water are  $8\text{ }\mu\text{g L}^{-1}$  for Cr(III) and  $1\text{ }\mu\text{g L}^{-1}$  for Cr(VI). In the effluents in the vicinity of Cr industries the levels of Cr range from 2 to  $5\text{ g L}^{-1}$  [13].

**2.2. Chromium in Soil.** The concentration of Cr in the soils may vary considerably according to the natural composition of rocks and sediments that compose them [6]. The levels of chromium in the soil may increase mainly through anthropogenic deposition, as for example atmospheric deposition [14], also dumping of chromium-bearing liquids and solid wastes as chromium byproducts, ferrochromium slag, or chromium plating baths [6]. Generally, Cr in soil represents a combination of both Cr(III) and (VI). As in aquatic environment, once in the soil or sediment, Cr undergoes a variety of transformations, such as oxidation, reduction, sorption, precipitation, and dissolution [6]. The oxidants present in the soil (e.g., dissolved oxygen and  $\text{MnO}_2$ ) can oxidize Cr(III) to Cr(VI) [15]; however, it seems that oxidation of Cr(III) by dissolved  $\text{O}_2$  is residual when compared with  $\text{MnO}_2$ . The forms of Cr(VI) are on the other hand reduced by iron, vanadium, sulphides, and organic materials [16]. However, when the reducing capacity of the soil is overcome, Cr(VI) may persist in the soil or sediment for years, especially if the soils are sandy or present low levels of organic matter.

López-Luna et al. [17] compared the toxicity of Cr(VI), Cr(III), and Cr tannery sludge respecting to Cr mobility in the soil and toxicity in wheat, oat, and sorghum plants and found that Cr(VI) was more mobile in soil and caused higher toxicity on those plant seedlings, while tannery sludge was the least toxic [17].

### 3. Chromium in Plants

**3.1. Chromium Uptake.** The pathway of Cr uptake in plants is not yet clearly elucidated. However, being a nonessential element, Cr does not have any specific mechanism for its uptake and is also dependent on Cr speciation. Plant uptake of Cr(III) is a passive process, that is, no energy expenditure is required by the plant [3, 18]. The uptake of Cr(VI) is thought to be an active mechanism performed by carriers for the uptake of essential elements such as sulphate [19, 20]. Cr also competes with Fe, S, and P for carrier binding [8].

Cr(VI) has higher solubility and thus bioavailability is more toxic at lower concentrations than Cr(III), which tends to form stable complexes in the soil [17]. There are conflicting results concerning the uptake and translocation of Cr(VI). While some authors defend that Cr(VI) is reduced to Cr(III) on the root surface [21, 22], others suggest that dissolved Cr(VI) is taken up by plants without reduction [23].

Thus, Cr toxicity is dependent on metal speciation, which is determinant for its uptake, translocation, accumulation. Cr is toxic for agronomic plants at about  $0.5$  to  $5.0\text{ mgm L}^{-1}$  in nutrient solution and  $5$  to  $100\text{ mg g}^{-1}$  in soil [24]. Under normal conditions, concentration of Cr in plants is less than  $1\text{ }\mu\text{g g}^{-1}$  [25].

**3.2. Chromium Accumulation and Translocation.** Cr accumulates mainly in roots and shoots; however roots accumulate the major part, being usually only a small part translocated to the shoots [12, 26]. In pea plants exposed to Cr there was an increase in concentration of Cr in different parts of the plant with the increase of Cr supply. Accumulation of Cr in the different parts of the plant was in the following order roots > stem > leaves > seed [27]. Corroborating these results are the findings of several works and for instance, Huffman and Allaway [28] found that bean seeds accumulated about 0.1% Cr, while roots accumulated 98%. Furthermore, Liu and coworkers [29] studied hydroponically grown *A. viridis* L. under different concentrations of Cr(VI) and found that Cr was accumulated primarily in roots [29]. Another study performed by Vernay et al. [30] in *Lolium perenne* grown in the presence of  $500\text{ }\mu\text{M}$  of Cr(VI) showed that roots accumulated 10 times more Cr than leaves. Spinach (*Spinacia oleracea* L. cv. “Banarasi”) grown in the presence of Cr(VI) showed more accumulation of Cr in the roots than in leaves and stem showed the least accumulation [31]. Also, in celery seedlings grown in the presence of Cr(III) most Cr was accumulated in roots [32].

López-Luna and coworkers [17] found that roots of wheat, oat, and sorghum accumulated more Cr than shoots; however in spite of that, wheat, oat, and sorghum showed Cr translocation from roots to shoots. Furthermore, Zayed et al. [33] tested Cr(III) and Cr(VI) translocation in several crops and found that translocation of both Cr forms from roots to shoots was very low and accumulation of Cr by roots was 100-fold higher than in shoots, despite of the Cr species. However, Skeffington and coworkers [18] found that more  $^{51}\text{Cr}$  was transported from root to shoot when Cr(VI), rather than Cr(III), was supplied to the plant. At high Cr doses ( $1\text{ mM CrCl}_3$ ) roots accumulated very high levels of Cr and translocation was mainly to cotyledonary leaves and only small amounts in hypocotyls. Chatterjee and Chatterjee [34] also found low levels of translocation of Cr from roots to the shoots in cauliflower (*Brassica oleracea*) grown on sand with  $0.5\text{ mM Cr(III)}$ .

These results may conclude that Cr is mainly accumulated in roots, followed by stems and leaves; however only small amounts of Cr are translocated to leaves. This pattern seems independent of the form of Cr tested.

**3.3. Plants with Potential of Phytoremediation of Chromium Contamination.** In phytoremediation, hyperaccumulator plants are used to extract and transform toxic metals, as Cr, into nontoxic and immobile compounds [35]. Cr hyperaccumulator plants can accumulate  $>1,000\text{ mg Cr kg}^{-1}$  (DW), in plant leaves. These plants can tolerate metals through

chelation with appropriate high-affinity ligands, biotransformation with reductants, and compartmentalization in the cytoplasm or in the vacuole. Thus, Cr immobilization in vacuoles in plant root cells may represent an important mechanism of Cr detoxification by the plant [8, 36].

The bioconcentration factor (BCF) and translocation factor (TF) are usually used to evaluate plant ability to tolerate and accumulate heavy metals. The BCF is the ratio of metal concentration in the plant tissue to the soil and TF is the ratio of metal concentration in plant shoots to the roots. Plants exhibiting a shoot BCF > 1 are suitable for phytoextraction, and plants with a root BCF > 1 and TF < 1 have the potential for phytostabilization [37].

Rafati and coworkers [37] evaluated the ability to uptake Cr from the soil by different organs of *Populus alba* and *Morus alba*. Leaves accumulated higher levels of Cr than stems or roots. However, neither *P. alba* nor *M. alba* showed potential of Cr phytostabilization, since presented TF > 1 and root BCF < 1; also these plants are not suitable for phytoextraction as they presented a BCF < 1. In another study, Gafoori and coworkers [38] evaluated the potential accumulation of heavy metals, including Cr in *Dyera costulata*. This specie presented high potential to retain high amounts of Cr in leaves, suggesting that this specie has high phytoremediation potential, as presented high translocation factor and low BCF factor. *Pluchea indica* also shown a good potential of phytoremediation, as it presented high levels of Cr accumulation and translocation to the leaves [39]. Mellem and coworkers [40] found that *Amaranthus dubius* tolerate high Cr(VI) concentrations as indicated by the BCF value > 2, showing good potential for phytoremediation. Furthermore, Gardea-Torresdey and coworkers [41] found that *Convolvulus arvensis* L. exposed to 20 mg L<sup>-1</sup> of Cr(VI) demonstrated capability to accumulate more than 3800 mg of Cr kg<sup>-1</sup> dw tissue, showing that this specie can be used in phytoremediation of Cr(VI) contaminated soils. Also, the concentration of Cr in leaf tissue (2100 mg kg<sup>-1</sup> dw) indicates that this plant species could be considered as a potential Cr-hyperaccumulator.

*Ipomoea aquatica* is a chromium hyperaccumulator that shows no toxicity symptoms when exposed to high levels of Cr(VI). Up to 28 mg L<sup>-1</sup> Cr(VI), *I. aquatica* exhibits uniform absorption characteristics showing over 75% removal of added Cr(VI). Over 90% Cr(VI) is accumulated in stems and leaves, that is, aerial regions [42]. Furthermore, Mant and coworkers [43] found that *Pennisetum purpureum* and *Brachiaria decumbens* exposed to 20 mg L<sup>-1</sup> of Cr(III) showed a metal removal efficiency of 78% and 66%. Also, Barbosa and coworkers [44] found that *Genipa americana* has potential for Cr(III) phytoremediation in contaminated watersheds, since its seedlings uptake elevated amounts of Cr(III) from the solution and it presented high capacity of immobilizing and storing the metal on their roots.

### 3.4. Growth and Development

**3.4.1. Germination.** The presence of Cr in the medium may compromise several processes in plants, as for instance plant germination. Thus, the ability to germinate in the presence

of Cr may indicate the degree of tolerance to Cr [45]. Oat seed germination was severely diminished (84%, resp. to the control) in tannery sludge soil with 4000 mg Cr kg<sup>-1</sup>, while in tannery sludge soil containing 8000 mg Cr kg<sup>-1</sup> both oat and sorghum seed germination was suppressed [17]. When comparing the sensitivity of sorghum, wheat, and oat germination to Cr, López-Luna and coworkers found that germination of sorghum and wheat were markedly affected at 500–1000 mg Cr(III) kg<sup>-1</sup> soil respectively, while oat germination was not affected in levels of Cr(III) below 4000 mg kg<sup>-1</sup> soil [17]. With respect to Cr(VI) it affected wheat and sorghum germination at the maximum concentration of 500 mg kg<sup>-1</sup> soil [17]. Germination of *T. aestivum* seeds was also affected by exposure to 100 mg L<sup>-1</sup> of Cr(VI) [46]. *Echinochloa colona* (L.) seeds showed lower rates of germination when exposed to contaminated medium from chromite minewaste dumps [47]. The effect of Cr contamination on the germination medium was also tested in mungbean (*Vigna radiata* L.) tolerant/sensitive cultivars and results showed that in sensitive plants, germination rate decreased in plants exposed to 96 or 192 µM Cr(VI), while in tolerant plants germination was not affected [48]. Maize seeds exposed to Cr(VI) also presented decreased rates of germination when exposed to concentrations of 100–300 mg L<sup>-1</sup> of Cr(VI) [49]. Zeid [50] found that germination of beans (*Phaseolus vulgaris*) was reduced in the presence of 5 × 10<sup>-2</sup> M Cr(III). Similar results were found by Peralta et al. [45] in alfalfa seeds exposed to Cr(VI). In another study Scoccianti and coworkers [32] found that Cr(III) at concentrations of 0.01 to 10 mM inhibited germination of celery seeds; indeed at 10 mM a total inhibition was detected.

In spite of the findings above, Corradi et al. [51] suggested that Cr(VI) treatment may not affect seed germination, but instead inhibit radicles growth when they emerge and contact Cr solution. Nevertheless, decrease in germination is a common response upon exposure to heavy metals, such as Cd, Pb, and Hg [52–54]. This response of low levels of germination upon Cr exposure can be related with decrease in α and β amylase activities under Cr stress [50]. Amylase hydrolysis of starch is essential for sugar supply to developing embryos. Decrease in amylase activity under Cr treatment decreases sugar availability to developing embryo which may contribute to inhibition of seed germination [46].

**3.4.2. Root Growth.** Besides germination, also root growth is frequently affected by heavy metals. Peralta and coworkers [45] showed that 5 mg L<sup>-1</sup> of Cr(VI) increased root growth comparatively to the control, and at higher doses (20 and 40 mg L<sup>-1</sup>) there was a dose-inhibition effect. Cr(VI) in concentrations up to 200 mg L<sup>-1</sup> decreased growth of paddy (*Oriza sativa* L.) [26]. Sensitive mungbean cultivars also showed decreased root growth when exposed to Cr(VI) [55]. Samantary [48] found that there was no root elongation in mungbean exposed to Cr(VI) concentrations between 96 and 1928 µM, but in lower concentrations, sensitive cultivars showed root elongation similar to the control. Also, development of lateral roots and root number was also affected by Cr exposure [48]. Moreover, roots of *Zea mays* L. treated with Cr(VI) were shorter and brownish and presented less

number of roots hairs [56]. López-Luna et al. [17] found that root growth of oat and sorghum was decreased by Cr concentrations in the soil of 100 mg Cr(VI) kg<sup>-1</sup> soil [17]. Decrease in root growth in presence of Cr(VI) can be explained by inhibition of root cell division and/or elongation, which might have occurred as a result of tissue collapse and consequent incapacity of the roots to absorb water and nutrients from the medium [57] combined with extension of cell cycle [26]. Reduced root surface in Cr(VI) stressed plants may contribute to decreased capacity of plants to search for water in the soil contributing to water stress. Despite of these results, stimulation of growth under low concentrations of chromium was also described (e.g., [58]). For example, Peralta et al. [45] found that roots of alfalfa plants exposed to 5 mg L<sup>-1</sup> of Cr(VI) grew 166% more than the controls.

**3.4.3. Stem Growth.** Stem growth is another parameter usually affected by Cr exposure. Mallick and coworkers [56] found that shoot length of *Zea mays* L. decreased significantly at 9 µg mL<sup>-1</sup> Cr(VI) after 7 days. Also, Rout and coworkers [47] found reduction of plant height and shoot growth due to Cr exposure in sensitive mungbean plants. In *T. aestivum* L. seedlings exposed to 100 mg L<sup>-1</sup> of Cr(VI) for 7 days, Dey and coworkers [46] found decrease in root length by 63% and in shoot length by 44%, comparatively to the control. Concentrations of Cr(VI) in soil of 500 mg kg<sup>-1</sup> also affected shoot growth of wheat and oat [17]. This decrease in plant height could be due to the reduced root growth and consequent decreased nutrients and water transport to the higher parts of the plant. Moreover, Cr transport to the aerial part of the plant can directly impact cellular metabolism of shoots contributing to the reduction in plant height.

**3.4.4. Leaf Growth.** Cauliflower grown on sand with 0.5 mM Cr(III) showed suppression of growth and leaves were smaller, chlorotic, and wilted comparatively to the control [34]. Leaf area is also usually decreased in response to increase of Cr concentration [53, 59]. Reduction of leaf area can be a consequence of reduction of the number of cells in the leaves stunted by salinization or reduction in cell size [60]. Watermelon plants growing in the presence of Cr(VI) showed reduced number and size of leaves and turned yellow, wilted, and due to loss of turgor hung down from petioles [61]. With continued Cr supply the lamina of affected old leaves became necrotic, permanently wilted, dry, and shed [61].

**3.4.5. Yield.** Plant yield is dependent on leaf growth, leaf area, and number. As Cr affects most of the biochemical and physiological process in plants, productivity and yield are also affected. Cr(VI) in irrigation water decreased significantly grain weight and yield (kg ha<sup>-1</sup>) of paddy (*Oriza sativa*) up to 80% under 200 mg L<sup>-1</sup> of Cr [26].

### 3.5. Physiological Processes

**3.5.1. Photosynthesis.** As other heavy metals, Cr may affect plant photosynthesis leading to decrease in productivity and ultimately to death. In a recent work, Rodriguez and

coworkers [62] showed that exposure to Cr(VI) induced a reduction of both chloroplast autofluorescence and volume in pea plants. Moreover, both Cr(III) and Cr(VI) can cause ultrastructural changes in the chloroplasts leading to inhibition of photosynthesis [63].

Respecting to pigments, Samantary [48] found chlorophyll degradation in mungbean sensitive cultivars exposed to Cr(VI) and decrease in chlorophyll a and chlorophyll b contents. Furthermore, pea plants grown in sand under different concentrations of Cr(VI) presented reduced chlorophyll contents in leaves [27]. Dey and coworkers [46] also found that total chlorophyll content decreased in shoots of *T. aestivum* L. with increasing Cr(VI) concentration. Concerning Cr(III), Chatterjee and Chatterjee found a decrease in chlorophyll contents in cauliflower grown on sand with 0.5 mM of Cr(III) [34]. Also, in celery seedlings, Cr(III) reduced chlorophyll contents mostly at concentrations of 1 mM [64].

When comparing the effects of Cr(III) and Cr(VI) on photosynthesis parameters of water hyacinth, Paiva and coworkers [12] found that Cr(III) was much less toxic than Cr(VI), and might eventually increase photosynthesis and chlorophyll content. In another study, Zeid [50] found that low and moderate concentrations of Cr(III) (10<sup>-6</sup> and 10<sup>-4</sup> M) in irrigation solution increased pigment content in leaves, but higher Cr(III) concentrations (10<sup>-2</sup> M) reduced the contents of chlorophyll a, chlorophyll b, and carotenoids.

This general profile of decrease in chlorophyll content at high Cr concentrations suggests that chlorophyll synthesis and/or chlorophyllase activity is being affected. Vajpayee and coworkers [65] showed that Cr affects pigment biosynthesis by, for instance, degrading δ-aminolaevulinic acid dehydrates, an essential enzyme in chlorophyll biosynthesis. Vernay and coworkers [30] also presented evidence that Cr competes with Mg and Fe for assimilation and transport to leaves, affecting therefore pigment biosynthesis. As the levels of reactive oxygen species (ROS) usually increase as a result of Cr exposure (e.g., [63, 66]), Juarez and coworkers [67] showed that ROS damages pigment-protein complexes located in thylakoid membranes followed by pheophytinization of chlorophylls (substitution of Mg<sup>2+</sup> by H<sup>+</sup> ions) and destruction of thylakoid membranes.

Considering the effects of Cr on plant fluorescence parameters, Liu and coworkers [29] found that *A. viridis* L. exposure to Cr(VI) resulted in decreased net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO<sub>2</sub> concentration. Also, chlorophyll fluorescence parameters  $F_v/F_m$ ,  $F_v'/F_m'$ , ΦPSII, and  $q_p$ , decreased in Cr(VI)-treated, but  $q_N$  and NPQ showed an increase in Cr(VI)-treated plants [29], indicating that the photochemical apparatus might have been compromised. In another study, Vernay and coworkers [30] found that Cr(VI) affected *L. perenne* fluorescence parameters associated with PSII. In another study, these authors compared the effects of Cr(VI) and Cr(III) on *Datura innoxia* and found that Cr(VI) had a more toxic effect on those plants than Cr(III) [68]. In plants stressed with Cr(VI), a decrease in the quantum yield of PSII electron transport (ΦPSII),  $F_v'/F_m'$  and  $q_p$  was observed [68]. ΦPSII represents the number of electrons transported across a PSII reaction center per mole of quantum absorbed



by PSII,  $F_v'/F_m'$  represents the excitation capture efficiency of open PSII reaction centers, while ( $q_p$ ) reflects the number of open reaction centers and it is an indicator of the capacity of photochemical processes [69].

**3.5.2. Mineral Nutrition.** Cr, being structurally similar to other essential elements, may affect plant mineral nutrition. Mallick et al. [56] found that Cr exposure decreased Cu absorption in *Zea mays* roots, while leaves were not affected. Uptake of both macronutrients (e.g., N, P, K) and micronutrients decreased with increase of Cr(VI) in irrigation of paddy [26]. Also, decreased uptake of the micronutrients Mn, Fe, Cu, and Zn was detected by Liu et al. [29] in *A. viridis* L. exposed to Cr(VI). High content of Cr may displace the nutrients from physiological binding sites and consequently decrease uptake and translocation of essential elements. In watermelon plants grown in the presence of Cr, an increase in concentrations of P and Mn and decrease in Fe, Cu, Zn, and S contents in leaves was observed [61]. In *L. perenne*, Vernay and coworkers [30] found that Cr(VI) exposure affected mineral contents mostly Fe, Ca, and Mg. Cr(VI) also decreased Fe concentration in spinach [31] and sunflower [70]. The decrease in Fe concentration in leaf tissue in response to Cr toxicity is suggestive of Cr(VI) interference in the availability of Fe, leading to impairment of Fe metabolism [71].

**3.5.3. Enzymes and Other Compounds.** The activity of antioxidant enzymes, namely, peroxidase, catalase (CAT), glucose-6-phosphate dehydrogenase and superoxide dismutase (SOD) increased in case of Cr-sensitive of mungbean exposed to different Cr concentrations. However, the level of antioxidant enzymes decreased in Cr-tolerant cultivars [48]. SOD and CAT activities decreased in *T. aestivum* L. grown in the presence of  $K_2Cr_2O_7$  in roots and shoots [46]. CAT activity also decreased in *A. viridis* L. exposed to Cr(VI) but an increase in SOD and guaiacol peroxidase (POX) activity was observed with increase of Cr(VI) concentration [29]. POX decreased in roots and increased in shoots of *T. aestivum* exposed to Cr(VI) [46]. Prado et al. [72] evaluated the metabolic responses to Cr(VI) exposure in floating and submerged leaves of *Salvinia minima* plants and found that Cr affected sucrose contents which were higher in Cr-treated leaves, while glucose contents showed an inverse pattern. Invertase activity also was also affected and suffered a decrease in floating leaves [72]. Zaimoglu and coworkers [73] studied the antioxidant responses of *Brassica juncea* and *Brassica oleracea* to soils enriched with Cr(VI) and found that total enzymatic activity was higher in *B. oleracea* than in *B. juncea*. Cr(VI) and also a decrease in CAT activity in both species [73]. Cellular antioxidants play an important role in protecting *Brassica* sp. to Cr-induced oxidative stress. This high activity of antioxidant enzymes and consequent detoxification of ROS contributes to relative tolerance of these species to Cr(VI). Furthermore, Guédard and coworkers [74] found that leaf fatty acid composition of *Lactuca serriola* was affected by the presence of Cr in metallurgic landfill soil.

**3.6. Genotoxicity.** Zou and coworkers [58] evaluated the effects of Cr(VI) on root cell growth and division of root tips of *A. viridis* L. and found that the mitotic index decreased with increased concentration of Cr(VI). Furthermore, Cr(VI) also affected chromosome morphology with increase in the frequency of c-mitosis, chromosome bridges, anaphase bridges, and chromosome stickiness [58].

Pea plants grown in the presence of Cr(VI) showed significant variations on cell cycle dynamics and ploidy level in leaves; however roots presented a cell cycle arrest at G2/M phase of the cell cycle; also polyploidization at both 2C and 4C levels was detected [75]. Moreover, in leaves and roots, an increase in DNA damage, assessed both by comet assay, and an increase in full peak coefficient of variation (FPCV) of G0/G1 were also detected [75]. Labra et al. [76] found hypermethylation of DNA and increase in DNA polymorphism in *Brassica napus* in response to Cr(VI) exposure. Cr(VI) also induced genotoxicity detected by AFLP analysis in *Arabidopsis thaliana* (L.) [77]. Furthermore, Knasmüller and coworkers [78] compared Cr(VI) and Cr(III) with respect to their ability to induce micronucleus in *Tradescantia* and found that only in Cr(VI)-exposed plants there was an increase in micronucleus frequencies. Moreover, Wang [79] in a survey to assess the genotoxic effects of Cr in water extracted from contaminated soil found that it was able to induce micronuclei in *Vicia faba* roots. Furthermore, Vannini and coworkers [80] evaluated the molecular changes induced by Cr(III) and Cr(VI) on germination kiwifruit pollen and concluded that neither Cr species induced a genotoxic effects. Both Cr species induced a strong reduction of proteins involved in mitochondrial oxidative phosphorylation and a decline in ATP levels [80].

## 4. Concluding Remarks

This paper includes an overview of the literature about Cr toxicity in the environment, especially in water and soil and provides new insights about Cr toxicity in plants. Cr exists mainly in three oxidative states Cr(0), Cr(III), and Cr(VI), which are the most stable forms of Cr. As Cr(0) is the metallic form, the forms of Cr(III) and Cr(VI) are the most preponderant in soils and water. Once in water/soil, Cr suffers a variety of transformations such as oxidation, reduction, sorption, desorption, precipitation, and dissolution. While Cr(III) solubility is dependent on pH, Cr(VI) is extremely soluble under all pH conditions. Cr as being a nonessential element for plants does not have any specific mechanism for its uptake. Cr(III) uptake is a passive process, whereas Cr(VI) uptake is performed by carriers of essential elements such as sulphate. Cr accumulates mainly on plant roots, being translocated to shoots in small levels, independently of Cr specie. Despite known toxicity of Cr to plants, there are several plants that hyperaccumulate this metal contributing to its removal from soil/water, showing good potential for application in Cr phytoremediation strategies. Cr affects several processes in plants, namely, seed germination, growth, yield and also physiological processes as photosynthesis impairment and nutrient and oxidative



imbalances. Also, it has been shown that Cr is able to induce genotoxicity in several plant species.

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## Review Article

# Phytotoxicity: An Overview of the Physiological Responses of Plants Exposed to Fungicides

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In the last decades, the use of fungicides in agriculture for fungi diseases control has become crucial. Fungicide research has produced a diverse range of products with novel modes of action. However, the extensive use of these compounds in the agriculture system raises public concern because of the harmful potential of such substances in the environment and human health. Moreover, the phytotoxic effects of some fungicides are already recognized but little is known about the impact of these compounds on the photosynthetic apparatus. This paper presents a comprehensive overview of the literature considering different classes of fungicides and their effects on plant physiology, with particular emphasis on photosynthesis.

## 1. Introduction

Fungicides are chemical compounds or biological organisms that destroy or inhibit the growth of fungi or fungal spores [1]. The use of fungicides for an effective control of plant diseases has become crucial in the last decades in the agriculture system since it is estimated that fungal infections cause yield reductions of almost 20% of crops worldwide [1]. Due to their relatively low cost, ease of use, and effectiveness, fungicides became the primary means of fungi control [2]. However, the extensive use of these compounds to control fungal disease in plants raises the appearance of new strains of pathogens that have become resistant to the available commercial products [3].

Fungicide toxicity is not always restricted to the target pest organism, having also been demonstrated in mammals [4] including humans [5]. The extensive use of fungicides in plant protection against fungal disease generates long-term residues in food and in the environment [6, 7]. In the annual EU report, EFSA (European Food Safety Authority), where vegetables and fruits of 27 countries were surveyed for pesticides contamination, the results highlighted that dithiocarbamates are among the most common residual contaminants. Thus, the abusive use of such compounds in agriculture has mobilized public concern because of the

harmful potential of such substances in the environment and in the food chain representing a risk for human health [6].

Most of the work dealing with the impact of fungicides in agriculture is focused on their efficiency against fungal pathogens or their residues in crops [6, 8]. Several reports appoint that some fungicides may enhance plant defences through phytoalexin synthesis and cell wall lignification or stimulate enzymes involved in the synthesis of phenolic compounds [8–10]. Others describe the putative protective role of fungicides for crops against various types of stress. Wu and Von Tiedemann [11] reported that triazoles protect *Hordeum vulgare* and *Arachis hypogaea* against ozone exposure or salt stress by stimulating antioxidative enzymes. Moreover, azoxystrobin and epoxiconazole fungicides induced a delay of senescence of *Triticum aestivum* mainly due to an enhancement of the antioxidative potential protecting the plants from harmful active oxygen species [11]. Muthukumarasamy and Panneerselvam [12] described an induction of the synthesis of photosynthetic pigments and proteins in fungicide-exposed plants. However, few studies have addressed the question of whether these products alter or inhibit physiological and metabolic activities in the plant [3], and the negative effects of some fungicides on photosynthesis, pigment content, growth, and alterations in the reproductive organs were poorly explored [7, 8]. The available data report



modifications on the CO<sub>2</sub> assimilation and photosynthetic efficiency [7, 8, 13–15]. Photosynthesis reduction strongly conditions biomass production and growth rates, which are strictly related with crop productivity and yield. Information on fungicides effects on plant physiology (photosynthesis) is crucial for the understanding of the underlying regulatory mechanisms as a precondition to judge the phytotoxicity of a compound. In this paper I present a comprehensive overview of the literature considering different classes of fungicides and their effects on plant physiology, with particular emphasis on photosynthesis. Finally, some general conclusions are drawn.

## 2. Types of Fungicides

Fungicides that are used to control plant fungi can be applied before infection to protect the plant from fungi invasion. This type of fungicides have a protective action. Others can be used to eliminate or eradicate an established infection. Fungicides can be classified in two main categories: contact (nonsystemic) and systemic fungicides.

**2.1. Contact Fungicides.** Contact fungicides have preventive action by killing or inhibiting fungi or fungal spores before the mycelia can grow and develop within the plant tissues [16]. However, once the infection is established, this fungicide may not have any function. Thus, this kind of fungicides can be used only as protectants.

Inorganic copper compounds such as Bordeaux mixture and copper carbonate and inorganic sulfur in the form of elemental sulfur and lime sulfur are some examples of the main contact fungicides available for plant protection [3]. Within the organic contact fungicides, for example, dialkyldithiocarbamates, which include the fungicides thiram, ferbam, and ziram, are a group of fungicides with a high role in the worldwide control of plant diseases since they are generally more effective and less toxic than the inorganic compounds (e.g., sulfur and copper fungicides) [4, 17]. These multisite inhibitors have several kinds of toxic action in fungal cells such as metal chelation, mixed disulfide formation, and transport of heavy metals across membranes. Dialkyldithiocarbamates inhibit a wide range of fungal enzymes, but the pyruvic dehydrogenase system is particularly sensitive to these fungicides [4]. Another group of organic contact fungicide widely used is the ethylenebis-dithiocarbamates, which include zineb, maneb, metiram, and mancozeb. The mode of action of this type of fungicide differs from that of the dialkyldithiocarbamates: they undergo transformation to ethylenediisothiocyanate, which inactivates thiol groups of enzymes and metabolites in fungal cells.

Contact fungicides are inexpensive and fungal resistance rarely occurs. Therefore, they are still widely used for plant disease control even though many newer, more potent systemic fungicides have been developed

**2.2. Systemic Fungicides.** The other category of fungicides, systemic fungicides, are absorbed by the plant and carried by

translocation to the site of infection. These kind of fungicides can kill the fungus after the mycelia has penetrated the parenchyma of the plant tissue, stopping the dispersal or infection within the plant [16]. Systemic fungicides can be used as protectants, eradicants, or both and are the most recently developed and the most promising type of fungicide for the future [3]. However, since systemic fungicides usually have a very specific site of action in the target fungus, fungi may readily develop resistance to them if they are not managed appropriately.

Systemic fungicides comprise a wide group of compounds with several modes of action. For example, the largest and most important group of systemic fungicides used to control plant fungal diseases is the dicarboximide. The mode of action of this fungicide seems to be related to the inhibition of triglyceride biosynthesis in the fungi [18]. The dicarboximide fungicides, iprodione, procymidone, vinclozolin, chlozolinate, and metomeclan are particularly valuable for the control of plant diseases caused by species of *Botrytis*, *Sclerotinia*, *Monilinia*, *Alternaria*, *Sclerotium*, and *Phoma*.

Benzimidazoles are a group of organic fungicides with systemic action that are also extensively used in agriculture. These types of compounds control a broad range of fungi at relatively low application rates [19]. For example, benomyl is one of the most effective and extensively used benzimidazoles in crop protection [3]. The benzimidazoles benomyl, carbendazim, and thiabendazole and the phenylcarbamate diet-hofencarb specifically interfere with the formation of microtubules, which function in a variety of cellular processes, including mitosis and maintenance of cell shape. These fungicides bind specifically to protein subunits called tubulin and prevent their assembly to form microtubules [20].

Since their introduction in the 1960s, systemic fungicides have gradually replaced the older nonsystemic products, establishing higher levels of disease control and developing new fungicide markets [1]. Compared with the nonsystemic, systemic fungicides are approximately twice as valuable in terms of sales [21].

## 3. Plant Physiological Responses to Fungicide Exposure

The widely accepted assumption that fungicide has low phytotoxicity has started to be outdated with the publication of more detailed analysis at the cell level that demonstrated several damages at the photosynthetic apparatus (e.g., [7, 8, 15]). Some reports appointed that application of fungicides has consequences on plant physiology, such as growth reduction, perturbation of reproductive organ development, alteration of nitrogen, and/or carbon metabolism [8]. This former physiological trait is fundamental for plant culture and is reflected by both photosynthetic rate and mobilization of carbohydrate reserves [7]. Physiological studies after fungicide application on several species reported modifications of both photosynthetic activity and chlorophyll *a* fluorescence [2, 8, 15, 22, 23]. Decreased CO<sub>2</sub> assimilation in fungicide-treated plants has been attributed to both stomatal (due to stomatal closure) [2] and nonstomatal effects due to

a disruption in the capacity of RuBisCO carboxylation, decrease of RuBisCO content, and/or reduction of the ribulose 1.5 biphosphate regeneration [7, 24].

Net CO<sub>2</sub> assimilation reductions accompanied by changes in stomatal conductance and intercellular CO<sub>2</sub> concentration were reported in *Malus domestica* and *Cucumis sativus* after fungicide application [2, 15]. The application of a nonsystemic fungicide, fludioxonil, in *Vitis vinifera* induced a decrease in net CO<sub>2</sub> assimilation and in the intercellular CO<sub>2</sub> concentration but stomatal conductance was not affected [7]. In another study reported by Saladin et al. [8], the application of the same fungicide, fludioxonil, and a systemic fungicide, pyrimethanil, in *in vitro* plants and fruiting cuttings of *Vitis vinifera* promoted different physiological responses: in *in vitro* plants, both fungicides decreased net CO<sub>2</sub> assimilation, transpiration rate, stomatal conductance, and intercellular CO<sub>2</sub> concentration; in the fruiting cuttings, the fungicides did not affect CO<sub>2</sub> exchange neither transpiration rates.

Some reports suggested that the systemic fungicide strobilurin may improve the water status and stress management of plants under conditions of drought stress [25, 26]. Nason et al. [24] showed that the application of beta-methoxyacrylate, a strobilurin fungicide, improve the water use efficiency only in well-watered *Triticum aestivum* and *Hordeum vulgare* plants. However, when these plants are under drought stress, strobilurin strongly reduces net CO<sub>2</sub> assimilation, intercellular CO<sub>2</sub> concentration, transpiration rate, and rate of stomatal conductance to water. In this study, net CO<sub>2</sub> assimilation reduction seems to be related to stomatal conductance decrease. It is possible that stomata respond to strobilurin-induced changes in mesophyll photosynthesis either by sensing changes in the intercellular CO<sub>2</sub> concentration or by responding to the pool size of an unidentified C-fixing substrate. It is also possible that the effects of strobilurin fungicides are mediated via ABA-based chemical signaling [24].

The analysis of several chlorophyll *a* fluorescence parameters of plants treated with fungicides [2, 15, 22, 23, 27] demonstrated that light reactions of photosynthesis are also sensible to fungicide exposure. Bader and Abdel-Basset [27] showed, for the first time, that fungicides of the triforine type (a systemic and contact fungicide) strongly inhibit electron-transport reactions of chloroplasts. Moreover, the application of systemic fungicides, benzimidazoles and triazole, and a dithiocarbamate contact fungicide affected the effective quantum yield of PSII ( $\Phi_{PSII}$ ) as well as the maximal quantum efficiency of PSII ( $F_v/F_m$ ). This reduction was attributed to the decrease in photochemical quenching (qP) [2, 15, 23]. In *Glycine max*, strobilurin fungicides application reduced the ratio of  $F_v/F_m$  [24]. Strobilurin fungicides seem to block the transport of electrons between PSII and PSI by binding to the Q<sub>i</sub> site of the chloroplast cytochrome *b<sub>f</sub>* complex [24].

Since plants depend on photosynthesis to assimilate carbon for further growth and overall vigor, photosynthesis impairment has negative consequences in plant biomass production and yield. Several reports support a decrease in biomass production in fungicides-treated plants: benomyl,

a systemic fungicide, reduced the growth of *Gossypium hirsutum*, *Helianthus annuus*, *Cucumis sativus*, *Lactuca sativa*, and *Pinus taeda* [3, 13]. Moreover, the application of carbendazim (systemic benzimidazole fungicide) in *Nicotiana tabacum* affected negatively plant biomass [3].

Pigment biosynthesis is appointed by Ahmed et al. [13] to be inhibited by the systemic fungicide, benomyl. This fungicide induces a considerable reduction on the chlorophyll *a*, chlorophyll *b*, carotenoids, and the total pigments content of *Helianthus annuus* plants [13]. Similarly, the treatment of *Vitis vinifera* with fludioxonil and *Nicotiana tabacum* with carbendazim also decreases the chlorophyll and carotenoid content [3, 8]. Mihuta-Grimm et al. [28] and Van Iersel and Bugbee [23] reported leaf chlorosis after benomyl application on *Impatiens walleriana*, *Cucumis sativus*, *Celosia plumose*, *Petunia hybrid*, and *Lycopersicon esculentum*.

Modifications of dark respiration were reported after mancozeb (contact fungicide) and flusilazol (systemic fungicide) application in *Malus domestica* [15]. The increase in dark respiration can be explained by additional energy requirement, metabolic breakdown of the compound, and/or activation of the alternative, cyanide-insensitive, respiration. Curiously, the treatment with strobilurin fungicides induced different responses: while in *Triticum aestivum* and in *Spinacia oleracea* plants respiration was inhibited [25, 29], in *Triticum aestivum* dark respiration was reduced [24].

## 4. Conclusion

The available studies in the literature have demonstrated that fungicide application impairs photosynthesis. However, the reports available are in general based on few physiological parameters using a large variety of plant species and different types of fungicide leading in some cases to controversial results that jeopardize a comprehensive knowledge of the main photosynthetic targets of fungicides. Thus, future investigation on the subject should be considered in order to produce more reliable data to identify fungicide photosynthetic targets and build a comprehensive model of the physiological response of plant exposed to fungicides.

It is expected that fungicides remain an essential tool for plant disease management and will continue to play a crucial role in optimizing yields from the world crops. Therefore, the development of new compounds with lower negative impact in plant physiology is a future challenge. This will provide benefits not only for plants yield but also for the environment and human health.

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