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Treball Final de Grau

Metabolomic approach to assess arsenic stress in plants and algae
Metabolòmica per a l'avaluació de l'estrès per arsènic en plantes i algues

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Hi ha una força motriu més poderosa que el vapor, l'electricitat i l'energia atòmica: la voluntat.

Albert Einstein

Vull expressar el meu agraïment al Dr. José Fermín López Sánchez per haver accedit a tutoritzar aquest TFG i pel seu temps en llegir i corregir el treball. També vull agrair als meus pares i amics, i al Gorka, per la comprensió i ànims que m'han donat durant aquests mesos.

REPORT

IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (SDG)

This TFG work is a review of the recent literature about plant and algae metabolisms under the stress of arsenic exposure and the metabolomic approaches used to study them. Therefore, no new results have been generated on this topic since it is mainly a bibliographic work. However, it can constitute a useful introductory summary of information and be of interest to anybody involved in this type of studies.

Contamination by arsenic is an environmental and health concern, and, consequently, research carried out on this field can be aligned within the great areas of People and Planet, which belong to the ONU's 5 Ps classification. In particular, this type of investigation is directly related to several topics of the Sustainable Development Goals (SDG) of the United Nations, like:

3 – Good Health and Well-Being, since contamination by arsenic and other metalloids can have significant effects, no only on plant crops or marine algae but also on animals and fish that feed on them, and ultimately on humans.

6 – Clean Water and Sanitation, because contamination of groundwater by arsenic is one of the main ways to spread this metalloid among plants and algae.

14 – Life Below Water, because as stated above, algae growing on water streams and reservoirs might be affected by arsenic contamination, as well as other organisms living in such environments.

15 - Life on Land, since arsenic contamination of soils can affect plants and eventually reach animals and humans.

Maybe more indirectly, this research topic could also be related to the SDG of 2- Zero Hunger, since contamination by arsenic can also affect plant growth and, therefore, the crops of food stock plants, like rice and wheat, among others.

Altogether, the studies on the different ways arsenic is uptaken by plants and algae and on the effects at different levels that it has on their metabolism (ie. generation of ROS, alteration of metabolism under arsenic-induced stress,…), mediated by the use of metabolomic methodologies such as those mentioned and cited in this TFG, are important and relevant for the above mentioned aspects, and they fit well within the SDG's identified as fundamental for the chemical community by the American Chemical Society.

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1. SUMMARY

Arsenic, a highly toxic metalloid, is widely distributed in soil and groundwater, especially in areas with mining or geothermal activity, raising environmental and health concerns. This contamination poses a threat to food security by affecting crop productivity and quality. The presence of arsenic in drinking water and crop irrigation compromises human and animal health, linking to chronic diseases and cancer, thus becoming a global threat to all living beings.

Plant exposure to arsenic induces morphophysiological disorders, affecting their growth and development and resulting in decreased productivity. Physiologically, arsenic induces changes in various cellular structures, such as chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum, the cell wall and the plasma membrane. It also causes the overproduction of ROS, which damage the cell by breaking down lipids, proteins, and DNA. Biochemically, arsenic interferes with crucial metabolic processes such as photosynthesis, respiration, amino acid synthesis, cellular signaling and gene regulation.

Plant tolerance to arsenic-induced oxidative stress mediated by ROS is crucial for their survival. Those with enhanced antioxidant defense systems demonstrate greater resistance by neutralizing excess ROS and protecting cells from oxidative damage.

The diversity of plants, their tolerance or susceptibility to arsenic, and their ability to accumulate or respond to its toxicity influence the mechanisms of accumulation, absorption, or response to the metalloid. In the field of phytoremediation, hyperaccumulating plants such as rice (*Oryza sativa*) can extract arsenic from the soil, reducing its availability to other organisms. However, this approach presents challenges such as low biomass and waste management.

Another strategy involves the use of tolerant plants through conventional genetic improvement or genetic engineering, such as tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*), which maintain acceptable performance under arsenic stress conditions.

The identification of regulators of the arsenic response is essential for the development of tolerant plants, contributing to viable phytoremediation strategies. This bibliographic work focuses on the growing interest in arsenic toxicity in crops, providing a detailed explanation of the mechanisms activated in plants when exposed to arsenic and the strategies they have developed to tolerate and mitigate the damage caused by this metalloid. The latest studies and data on the influence of arsenic on the metabolism, physiology, and ecology of terrestrial and

aquatic plants are reviewed to offer an updated and comprehensive bibliographic summary on this topic.

Keywords: Arsenic, toxicity, oxidative stress, plants, algae, metabolomics, physiological responses, biochemical responses.

2. RESUM

L'arsènic, un metal·loide altament tòxic, es troba àmpliament distribuït al sòl i a l'aigua subterrània, especialment en zones amb activitat minera o geotèrmica, generant preocupacions ambientals i de salut. Aquesta contaminació amenaça la seguretat alimentària a l'afectar a la productivitat i a la qualitat dels cultius. La presència d'arsènic a l'aigua de consum i a la irrigació de cultius compromet la salut humana i animal, vinculant-se a malalties cròniques i càncer, i convertint-se, per tant, en una amenaça global per a tots els éssers vius.

L'exposició de les plantes a l'arsènic provoca trastorns morfofisiològics que afecten el seu creixement i desenvolupament, resultant en una disminució de la productivitat. Fisiològicament, l'arsènic indueix canvis en diverses estructures cel·lulars, com el cloroplast, la mitocòndria, el peroxisoma, el reticle endoplasmàtic, la paret cel·lular i la membrana plasmàtica. També causa la sobreproducció de ROS, les quals danyen la cèl·lula en desintegrar lípids, proteïnes i l'ADN. A nivell bioquímic, l'arsènic interfereix en processos metabòlics crucials com la fotosíntesi, la respiració, la síntesi d'aminoàcids, la senyalització cel·lular i la regulació gènica.

La tolerància de les plantes a l'estrès oxidatiu per ROS és crucial per a la seva supervivència davant la toxicitat de l'arsènic. Aquelles amb sistemes de defensa antioxidant millorats demostren una major resistència al neutralitzar l'excés de ROS i protegir les cèl·lules del dany oxidatiu.

La diversitat de les plantes, la seva tolerància o susceptibilitat a l'arsènic, i la seva capacitat d'acumulació o resposta a la seva toxicitat, influeixen en els mecanismes d'acumulació, absorció o resposta al metal·loide. En l'àmbit de la fitoremediació, plantes hiperacumuladores com, per exemple l'arròs (Oryza sativa), poden extreure l'arsènic del sòl, reduint-ne la disponibilitat per a altres organismes. Tanmateix, aquest enfocament presenta reptes com la baixa biomassa i la gestió de residus.

Una altra estratègia implica l'ús de plantes tolerants mitjançant millora genètica convencional o enginyeria genètica, com el tabac (Nicotiana tabacum) i el tomàquet (Solanum lycopersicum), que mantenen un rendiment acceptable sota condicions d'estrès per arsènic.

La identificació de reguladors de la resposta a l'arsènic és essencial per al desenvolupament de plantes tolerants, contribuint així a estratègies viables de fitoremediació.

Aquest treball bibliogràfic se centra en el creixent interès respecte a la toxicitat de l'arsènic en els cultius. La recerca bibliogràfica abasteix l'explicació detallada de tots els mecanismes que es posen en marxa en les plantes quan s'exposen a l'arsènic, així com les estratègies que aquestes han desenvolupat per tolerar i mitigar el dany causat per aquest metal·loide. Es revisen els estudis i dades més recents sobre la influència de l'arsènic en el metabolisme, la fisiologia i l'ecologia de les plantes terrestres i aquàtiques, amb l'objectiu d'oferir un resum bibliogràfic actualitzat i complet sobre aquest tema.

Paraules clau: Arsènic, toxicitat, estrès oxidatiu, plantes, algues, estudi metabòlic, respostes fisiològiques, respostes bioquímiques.

3. INTRODUCTION

3.1 ARSENIC AS AN ENVIRONMENTAL CONTAMINANT

Arsenic is a naturally occurring non-essential metalloid element with both metallic and non-metallic properties. It can exist in four oxidation states: -3, 0, 3, and 5. Arsenic can form a variety of compounds with other elements, but most arsenic occurs in mineral forms such as orphiment (As_2S_3), enargite (Cu_3AsS_4), realga (As_4S_4) and arsenopyrite (FeAsS) [1]. Some of these compounds are more toxic than others, depending on their chemical form and solubility.

Arsenic has a high potential to cause serious health problems in humans and plants since it is a pollutant present in the air, water and soil, and, as a consequence, it can affect the food chain. It can be released into the environment through natural processes such as volcanic eruptions, rock erosion and geothermal activity, or through human activities such as mining, smelting, agriculture and industrial processes [2]. A major source of human exposure is drinking water containing high concentrations of arsenic, particularly in some parts of the world such as Bangladesh, China, India and Mexico [3]. Other sources of exposure include food and tobacco. It also affects plant growth and development, reducing yield and quality and increasing susceptibility to pests and diseases.

However, the use of arsenic is restricted and regulated in many countries due to its harmful effects. The World Health Organization (WHO) sets the arsenic content of drinking water at 10 micrograms per liter (µg/L) [4] and recommends the use of alternative water sources or treatment methods to reduce arsenic levels. The Food and Agriculture Organization (FAO) and the Codex Alimentarius Commission have also set maximum levels of arsenic in foods such as rice, cereals, fish and seafood. In addition, the International Agency for Research on Cancer (IARC) has classified arsenic and its compounds as human carcinogens.

3.2 ASSESSING THE SIGNIFICANCE OF ARSENIC TOXICITY

Recently, research on the effects of stress on plants has increased using omics methods. Among the omics sciences, metabolomics is the study of metabolites that can provide valuable information about the biochemical and physiological responses of plants to various stresses such as drought, salinity, temperature, and heavy metals, including arsenic.

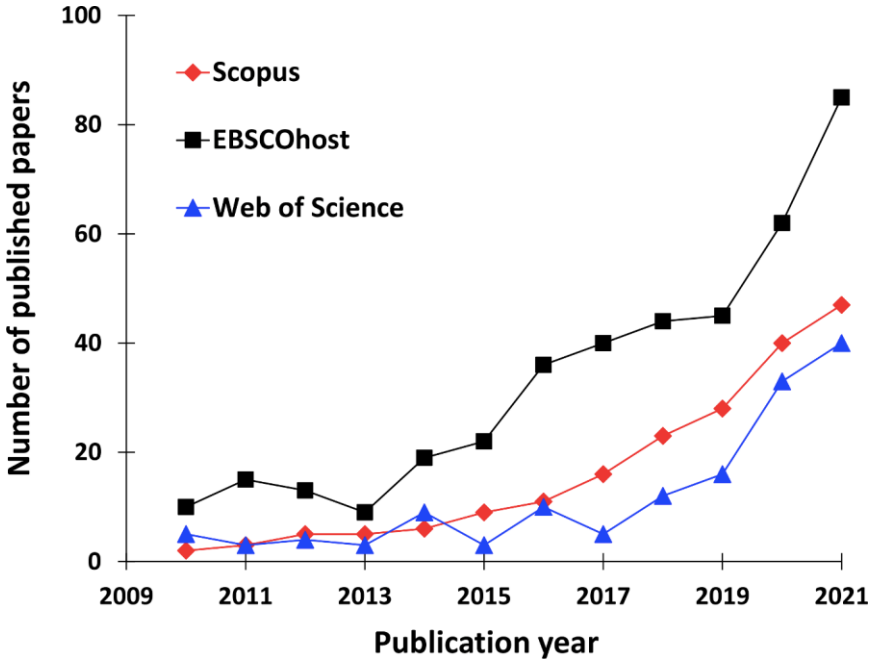


Figure 1. Analysis of trends in metabolomics research over the last decade reveals a focus on plant stress research. This graphic was made with data from Scopus, EBSCOhost and Web of Science.

(image taken from J.I. Martínez-Castillo et al. "Arsenic stress in plants: A metabolomic perspective" 2022)

Figure 1 shows that the number of plant stress studies using metabolomics has increased significantly over the last decade. This indicates that this omics science will be revealing in the coming years. The great interest in finding remedies for arsenic toxicity comes from its connection with the food chain through plants. Plants are the most important producers of organic matter and the most important source of food for humans and animals. Therefore, understanding how plants cope with stress and how stress affects plant quality and safety is essential for food security and human health. Metabolomics can help to identify key metabolites and response pathways to arsenic stress and to discover new biomarkers and targets to improve arsenic tolerance and phytoremediation potential of plants.

To carry out these studies, several analytical techniques are used to facilitate the separation, detection and characterization of metabolites. Metabolomic analysis can be conducted through the utilization of nuclear magnetic resonance (NMR) spectroscopy or by employing gas or liquid chromatography or capillary electrophoresis in conjunction with mass

spectrometry (GC-MS, HPLC-MS, and CE-MS, respectively) to achieve high resolution, specificity and accuracy in metabolic profiling.

To obtain a more comprehensive understanding of the metabolome, NMR and MS can also be used simultaneously. NMR is particularly adept at non-destructively identifying and quantifying a wide range of metabolites, while MS excels at providing detailed structural information. By comparing the results of both techniques, researchers can validate the identification and quantification of metabolites, discover interactions between metabolites, and identify new biomarkers or metabolic pathways. In addition, innovative research uses the direct coupling of NMR and MS in a single instrument, such as NMR-MS or HPLC-NMR-MS. This integration allows sequential or simultaneous analysis of a sample, minimizing sample loss and variability, and improving data correlation and integration.

Some metabolomic studies on the response of plants that have been exposed to arsenic are summarized in Table 1.

Analytical techniques	Study	References
^{1}H NMR spectroscopy and (HRMAS*) NMR spectroscopy	Investigates the effects of arsenic exposure on the metabolic profile of a green microalga, *Chlorella vulgaris*	[5]
GC-MS	Investigates how different rice cultivars cope with arsenite in their growth environment	[6]
HPLC-MS	Investigates how arsenic exposure affects the biochemical composition of rice plants	[7]

*HRMAS, High-Resolution Magic Angle Spinning

Table 1. Examples of metabolomic studies with the corresponding analytical techniques used.

While NMR and MS play a fundamental role, complementarity with other techniques further enhances the depth and breadth of metabolomic analyses. Among these complementary techniques, the use of infrared spectroscopy (IR), ultraviolet spectroscopy (UV) and

fluorescence spectroscopy stands out. These techniques offer advantages such as high sensitivity, selectivity and simplicity, providing valuable information on the functional groups, chemical bonds and chemical environments of the metabolites, as well as their concentration and distribution. The combination of all these techniques allows a more complete understanding of the metabolome, solving the deficiencies of the individual methods.

3.3 EFFECTS ON MORPHOLOGY AND PLANT GROWTH

Arsenic has the ability to cause a series of morphological and physiological deterioration in plants. Its harmful effects are manifested as it infiltrates plant tissues through the roots, causing significant damage to the processes of growth and development of shoots. The adverse consequences of arsenic exposure extend beyond mere toxicity, affecting complex plant morphological mechanisms and physiological functions, thus posing significant challenges to overall plant well-being. Table 2 shows all the visible effects that exposure to arsenic produces in plants [2], [8].

Morphological effects of arsenic on plants	
Leaf senescence	Loss in fertility
Chlorotic leaf appearance	Stunted growth, arresting expansion
Necrosis and leaf defoliation	Reduction in biomass
Reduced leaves number	Inhibit root extension & proliferation
Leaf wilting and leaf curling	Low yield and food production

Table 2. Arsenic toxicity in plants: morphological symptoms.

4. OBJECTIVES

The toxicity of arsenic and its effects on the metabolism of living organisms have been widely studied in the scientific literature. Arsenic is a metalloid that can disrupt several metabolic

pathways and cause oxidative stress, DNA damage and cell death. However, some algae and plant species have developed various strategies to cope with arsenic stress, including reducing uptake, storing it in vacuoles, or converting it to less toxic forms.

Metabolomics is a powerful tool for studying the metabolic response of organisms to environmental stressors such as arsenic. Metabolomics can detect changes in the levels and composition of metabolites, which are the end products of cellular processes. Using advanced analytical techniques such as mass spectrometry and nuclear magnetic resonance, metabolomics can provide a comprehensive picture of arsenic-induced biochemical changes in algae and plants.

The aim of this study is to provide a review of recent literature about metabolomic studies on arsenic stress in algae and plants. The particular topics covered in this review are:

- Arsenic sources, forms and toxicity, as well as effects on algae and plant metabolism.
- Mechanisms of arsenic uptake by plants and factors affecting the bioavailability and transport of arsenic in plant tissues.
- Tolerance and defense mechanisms of plants against arsenic stress and the metabolic pathways involved in the detoxification and adaptation processes.
- Summary of the results of two illustrative metabolomic studies on the effects of arsenic in plants.

5. METHODS

For this project, a literature review was conducted during the months from September 2023 to January 2024. The primary emphasis was on prominent biochemical databases and sources, including Crai UB, Pubmed, ScienceDirect, SpringerLink, and Web of Science (WOS). The most relevant works and articles that have been published in recent years (from 2000 to present) were chosen. All of them are closely related to the topic being reviewed. Particular attention was paid to articles that examined the metabolic reaction caused by the interaction of arsenic with plants.

The search was predominantly carried out in English, given its status as the vehicular language in the biochemical field. Arsenic, toxicity, oxidative stress, plants, algae, metabolomics, physiological responses, and biochemical reactions were among the keywords selected for this study.

The keywords were carefully combined using boolean operators, "AND," "OR," and "NOT," to find publications that supported the study objectives. Notably, the database search became less confusing by minimizing the use of the "NOT" connector. The almost synonymous terms "physiological responses" and "biochemical responses" were put together between parenthesis, and the "OR" connector was used. Furthermore, all keywords were used with the "AND" connection to improve the search's sensitivity and specificity.

To determine the inclusion and exclusion criteria, articles that met the following characteristics were taken into account:

- Open access articles published after 2000 and in the format of systematic reviews, meta-analyses or randomized biochemical trials.
- Articles that explain the methods by which contact with arsenic affects terrestrial and/or aquatic plants, causing them a stress situation.

On the other hand, articles were disqualified if they:

- Were released before 2000.
- Investigated how arsenic interacts with living organisms other than plants.

6. RESULTS AND DISCUSSION

6.1 ARSENIC SPECIES UPTAKE IN PLANTS

The absorption of arsenic by plants is a complex process influenced by its chemical form, soil availability and transformation of arsenic in the soil. Arsenic can enter plants through roots, the main absorption path, or through leaves [9].

In soil, arsenic exists as arsenate (As^{V}), arsenite (As^{III}), and methylated forms (MMA and DMA) (figure 2). Plants selectively absorb different arsenic species through specific

mechanisms and transporters. Understanding these absorption mechanisms is crucial for mitigating arsenic contamination's adverse effects on the environment and human health.

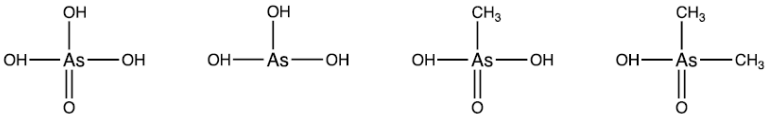


Figure 2. Structures of Arsenic compounds found in soils and terrestrial plants

In aerobic conditions, As^{V} is the predominant form of arsenic, mainly derived from mineral decomposition. As^{V} mimics inorganic phosphate (iP), as their oxyanions are structurally analogous. Phosphate is vital for plant nutrition, contributing to processes like ATP formation and plant growth promotion. Physiological studies show that, due to structural similarities, As^{V} competes with phosphate for its uptake by plant roots in various plants, favoring phosphate absorption due to a higher affinity by transporters [10].

While numerous phosphate transporters are identified in higher plants, the PHT1 (plant high affinity Phosphate Transporter 1) family, primarily found in roots, is crucial in transporting As^{V} from the external environment [11] (Appendix 1). Studies suggest that PHT1 proteins play a role in As^{V} uptake in plants that hyperaccumulate arsenic, such as *Pteris Vittata* or *Arabidopsis Thaliana* [12]. Besides inhibiting phosphate uptake, As^{V} also disrupts gene expression related to phosphate deficiency response, affecting the phosphate sensor and signaling pathway.

On the other hand, in anaerobic environments, As^{III} is the predominant and potentially toxic form of arsenic as under low oxygen conditions arsenic tends to exist in its lowest oxidation state. Unlike As^{V}, As^{III} remains mostly neutral (H_3AsO_3) at pH levels below 8, facilitating its absorption by plant roots through various channels [13]. To cope with As^{III}, plants use aquaporins, especially nodulin 26 (NIPs) (figure 3), crucial for As^{III} transport into root cells [14], but also for other uncharged molecules. Studies show NIPs from different plant species, including *Arabidopsis thaliana*, play vital roles in As^{III} absorption.

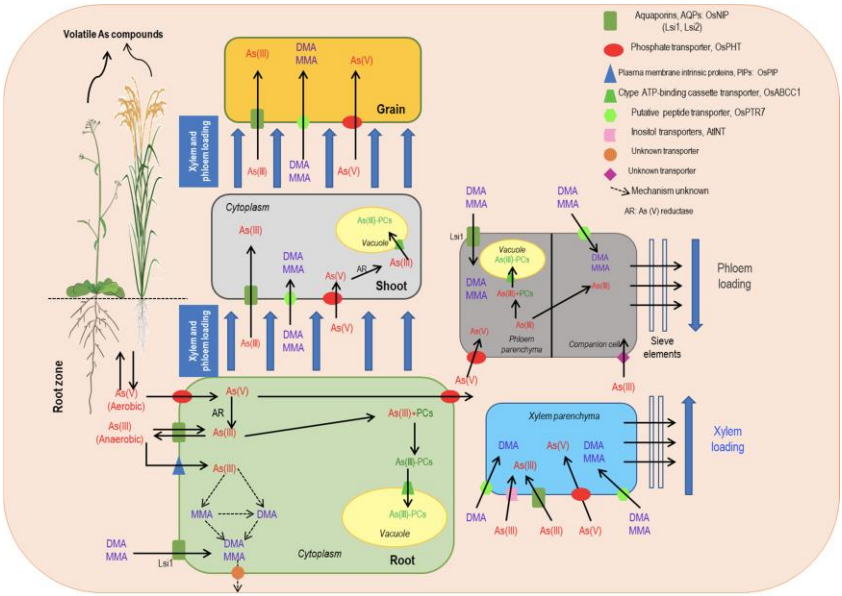


Figure 3. Schematic illustration of As uptake, translocation, accumulation and detoxification in plants. The dotted arrows indicate that the pathways/mechanisms are not known properly. *Image taken from Nahar et al,* [15]

Organic forms of arsenic, like MMA and DMA, are present in soil, possibly originating from past arsenic pesticide use or synthesized by microorganisms and algae through methylation [16]. Despite being less toxic and mobile than inorganic forms, plants can still absorb them, especially rice, which accumulates a concentration of DMA twice as high as that of inorganic arsenic [17].

OsLsi1 (OsNIP2;1) is crucial for the entry of MMA and DMA into rice roots, confirming its role in absorption, as the mutant that lacks this protein has a much lower absorption capacity than the wild type [18]. However, OsLsi1 doesn't seem to be involved in organic arsenic efflux. Factors affecting MMA and DMA uptake include membrane permeability, pH, and substrate properties. Hydroxyl groups also affect entry through plasma membranes, since MMA and DMA can form hydrogen bonds with the aquaporin protein, facilitating their entry through the plasma membrane [19]. While slower than inorganic arsenic, the absorption of MMA and DMA by rice roots exhibits greater mobility within the plant, possibly due to their lower toxicity and higher solubility. The mechanism behind this process remains unknown.

6.2 OXIDATIVE STRESS IN PLANTS

One of the major effects of arsenic exposure is the induction of oxidative stress, which is the imbalance between the production and scavenging of reactive oxygen species (ROS).

ROS can damage cellular components such as lipids, proteins and DNA, leading to cell death or dysfunction. They are normally produced as by-products of various metabolic processes, especially in respiratory and photosynthetic electron transport chains and, more generally, of many reactions occurring in cellular compartments with strong electron flow like mitochondria or chloroplasts [20]. Most of them are free radicals, which are inherently unstable because of the presence of one or more unpaired electrons. As a result, they are highly reactive.

ROS production is not only a consequence of normal cellular metabolism but also a response to various stress factors, such as temperature, light, nutrient availability and various kinds of contaminants, including arsenic. They are regulated by antioxidant systems, which can neutralize them by donating electrons or hydrogen atoms or by catalyzing their conversion to less harmful molecules. However, when plants are exposed to arsenic, the antioxidant systems are overwhelmed by the excessive generation of ROS, either directly by arsenic or indirectly by its interference with cellular metabolism. For example, arsenic can replace phosphate in ATP, disrupting the energy flow and oxidative phosphorylation [21]. Arsenic can also bind to thiol groups of enzymes and proteins, inhibiting their functions and altering their structures. It can also affect the chloroplast, mitochondria, peroxisome, endoplasmic reticulum, cell wall, and plasma membrane, altering their integrity and permeability [2].

Apart from the previously mentioned defense system, plants are capable of triggering other mechanisms, such as regulating the expression of antioxidant genes, chelating arsenic with phytochelatins (PCs), sequestering arsenic in vacuoles or cell walls, or excluding arsenic from the roots by efflux transporters. The effectiveness of these mechanisms varies with plant species, arsenic concentration, valence state and exposure duration. Some plants, such as *Pteris vittata*, are able to hyperaccumulate arsenic without showing toxicity symptoms, while others, such as *Oryza sativa*, are highly sensitive to As and show reduced growth and yield [21].

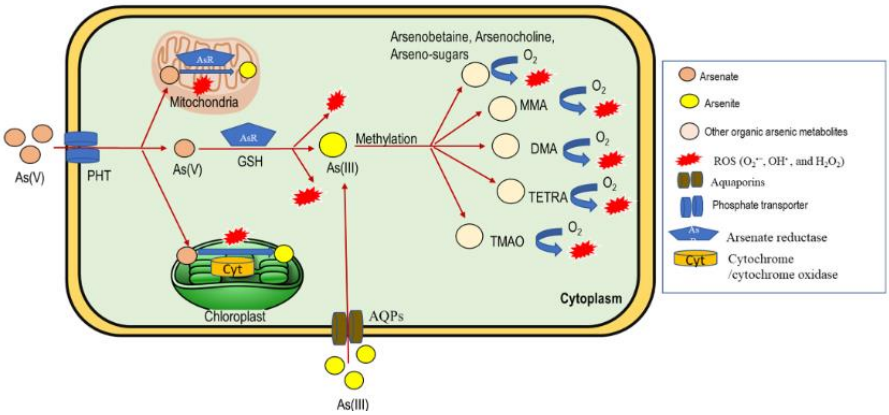


Figure 4. Schematic overview of ROS generation in plant cell under As-induced stress. Phosphate transporters (PHT) and aquaporins (AQPs) facilitate entry of As^{V} and As^{III}, respectively, into the cell. Initial ROS burst occurs due to arsenate reductase (AsR) mediated and non-enzymatic transformation of As^{V} into AsI^{II} in cytoplasm, chloroplast and mitochondria. Subsequently, a second sequential ROS burst occurs due to the methylation of As^{III} into other organic arsenic metabolite like MMA, DMA, tetraethylarsonium ion oxide (TETRA), trimethylarsonium oxide (TMAO), arsenobetaine, arsenochlorine and arsenosugars. *Image taken from Nahar et al,* [15].

As observed in figure 4, different forms of arsenic generate ROS through distinct mechanisms, as indicated by recent research. Although both forms exhibit cytotoxic effects, their actions within the cell differ.

6.2.1 Arsenate toxicity

A crucial mechanism of As^{V} toxicity could involve the substitution of inorganic phosphate in biochemical reactions, disrupting various cellular processes. Cellular metabolism, biosynthesis, information storage and retrieval, and cellular signaling are sensitive to As^{V}, with reactions involving iP or iP-ester substrates susceptible to disruption [22].

As^{V} particularly impacts glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a key enzyme in glycolysis, hindering cellular respiration and reducing energy production. As mentioned, As^{V} competes with iP in cellular reactions, inhibiting GAPDH activity and disrupting oxidative phosphorylation, leading to decreased ATP synthesis and increased ROS production, causing oxidative stress and damage to cellular components. Despite the structural similarities

between iP and As^{V}, recent studies indicate that arsenic does not substitute phosphorus in DNA or RNA [23]. This implies the existence of mechanisms that prevent the binding of arsenic to nucleic acids, potentially involving the recognition of arsenic by specific enzymes or the exclusion of arsenic from the nucleus. However, As^{V} still affects gene expression in stress response, detoxification, and metabolism.

When plants receive As^{V}, over 90% of the absorbed arsenic in both roots and shoots is converted to As^{III}, indicating efficient reduction [24]. This transformation can take place through enzymatic or non-enzymatic means [22], with glutathione (GSH) and GSH reductase playing a role in the non-enzymatic way and arsenate reductase (AR) being crucial in the enzymatic reduction. This reduction can be considered a detoxification mechanism, as AsIII is more mobile and easier to sequester or excrete. However, it comes with adverse effects, including electron leakage and enzyme inhibition, leading to the generation of ROS [25], [26].

Following As^{V} reduction, arsenic methylation occurs within plant cells, involving the transfer of methyl groups from S-adenosyl methionine (SAM). This process is also recognized as a detoxification mechanism since it creates less toxic and more easily excreted methylated arsenic metabolites like MMA^{III} and DMA^{III} [27]. However, methylation also contributes to the generation of ROS, due to the heightened reactivity of the methylated arsenic metabolites with O_2, and it releases redox-active iron (Fe) species from ferritin [28], catalyzing ROS formation through Haber-Weiss reactions (figure 5). Cytochrome oxidase can further produce the highly reactive superoxide radical ($O2^{\bullet-}$) during the conversion of As^{V} to As^{III}, using O_2 as the final electron acceptor.

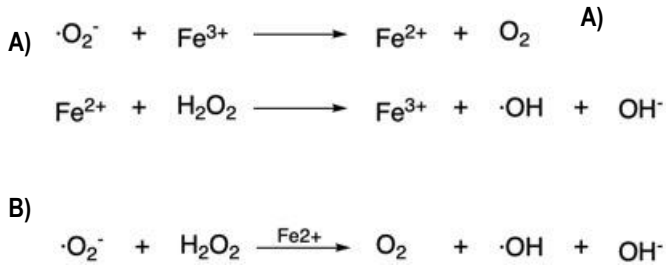


Figure 5. **(A)** Fenton and **(B)** Haber-Weiss reactions resulting in the generation of hydroxyl radicals in the presence of iron.

Numerous studies across plant species and arsenic concentrations confirm ROS accumulation during arsenic transformation, highlighting its impact on plants like *Triticum aestivum*, *Oryza sativa*, *Spinacia oleracea*, *Pisum sativum*, *Vicia faba, Anadenanthera peregrina* and *Myracrodruon urundeuva* [29]–[34].

6.2.2 Arsenite toxicity

As^{III} operates distinctly from As^{V}, primarily causing harm by binding to the thiol groups (-SH) present in proteins (due to Cys) and cofactors rich in sulfur (like CoA), leading to inhibition of their functions and cellular death [12]. This interaction alters molecular structure and function, leading to a deterioration of enzymatic activity and causing protein degradation and oxidative stress. For example, As^{III} affects the synthesis and function of GSH, a vital antioxidant in plants. As^{III} forms complexes with numerous free GSH molecules, promoting detoxification but depleting the GSH reserve. This depletion, as highlighted by several studies [27], not only affects the plant's ability to counteract oxidative stress but also impairs the functionality of several enzymes that depend on glutathione as a substrate, like glutaredoxins, which serve as electron carriers and play a role in salicylic acid signaling, and adenyl sulfate reductase, an enzyme in the sulfur assimilation pathway.

This interaction extends to pyruvate dehydrogenase [35], a component of the mitochondrial respiration complex. The consequence of such binding is the obstruction of the active sites of this enzyme, hindering the synthesis of crucial energetic compounds and antioxidant molecules, including NAD+, within the respiratory chain. Consequently, this disruption leads to the generation of $O2^{\bullet -}$, further contributing to the overall oxidative stress induced by arsenite exposure. Another direct mechanism involves the activation of NADPH oxidase, which facilitates electron transfer to O_2, leading to the production of superoxide radicals and hydrogen peroxide [36].

Additionally, arsenite disrupts the structure of ferritin, causing the release of iron, which catalyzes the formation of hydroxyl radicals through the Fenton reaction and contributes to the overall oxidative stress induced by arsenite exposure [37].

Indirectly, As^{III} impacts protein folding within the endoplasmic reticulum (ER). Arsenite binds to sulfhydryl groups in proteins, inhibiting disulfide bond formation and resulting in the

emergence of misfolded or unfolded proteins, triggering specific ER stress, which, in turn, instigates the production of ROS and inflicts damage on DNA [38].

Moreover, proteins with zinc in their structure, critical for transcription and DNA repair, show heightened sensitivity to As^{III}. In this context, As^{III} displaces zinc from its binding site, resulting in the inactivation of the enzyme [39]. Molecules substituted by arsenite are particularly susceptible to oxidative processes initiated by ROS. During this oxidative reaction, As^{III} is released, allowing its reintroduction into the cycle of protein damage.

Arsenite's final toxicity mechanism discussed in this review involves disrupting various metabolic pathways, particularly affecting oxidative carbon metabolism, as well as nitrogen and sulfur assimilation. In oxidative carbon metabolism, vital for plant growth, arsenite exerts inhibitory effects on key enzymes that participate in the Calvin cycle, such as Rubisco and GAPDH, impairing carbon fixation and sugar production [40]. This inhibition reduces photosynthetic efficiency, hindering the supply of energy to plant cells. In addition, As^{III} impacts chlorophyll biosynthesis and functionality, which are essential for light absorption. Arsenite-induced iron insufficiency or enzyme leads to a decreased chlorophyll content and altered leaf colour [2]. This damage disrupts the balance between heat dissipation and photochemical processes, affecting energy management, gas exchange and inducing oxidative stress [41].

The impact varies among plant types, varieties and environmental conditions. Some plants have developed strategies to tolerate or accumulate high levels of arsenite. These strategies include converting arsenite into volatile compounds, storing it in vacuoles, or forming complexes with PCs.

6.3 PHYTOCHELATINS AND VACUOLAR SEQUESTRATION

Plants employ strategies to counter arsenic-induced oxidative stress, with chelation and vacuolar sequestration being key mechanisms for detoxification and regulation within the plant system.

Chelation involves binding arsenic to organic molecules like PCs and GSH, which can reduce its toxicity or facilitate its excretion. PCs, derived from GSH and triggered by the presence of metalloids and heavy metals, form stable complexes with different arsenic species, protecting cells from oxidative damage. Non-hyperaccumulator plants can tolerate arsenic by elevating the concentrations of GSH and PCs after its exposure, as observed in different plant

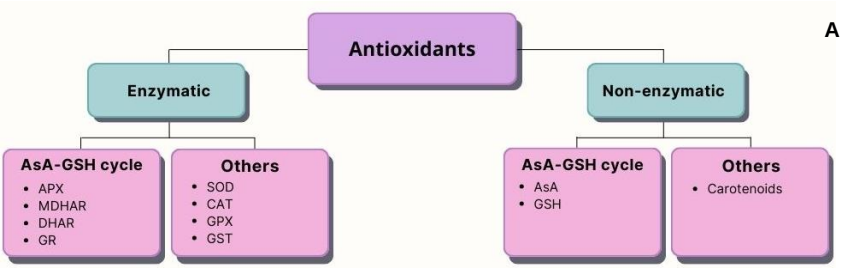
species, such as *Rauvolfia serpentina*, *Holcus lanatus*, *Helianthus annuus*, *Brassica juncea* and in *Pteris cretica* [42]–[45]. After chelation, arsenic complexes are stored in vacuoles, reducing harm to plant metabolism and limiting availability for uptake by other organisms [46]. Sequestration may be facilitated by ABC transporters that can pump arsenic complexes into vacuoles (Figure 3) (Appendix 1), as demonstrated by some studies made in *Arabidopsis thaliana* [47] and in *Oryza Sativa* [48].

Non-hyperaccumulator plants, reduce arsenic toxicity by rapidly forming As-PC complexes and isolating them in the vacuoles of root cells. On the other hand, hyperaccumulator species store As^{III} in the vacuoles of root cells [49], with unclear transport mechanisms to shoots.

The combination of chelation and sequestration enhances plant tolerance to arsenic toxicity by reducing its bioavailability and toxicity. However, these mechanisms can also affect arsenic accumulation and distribution in plant tissues and in the environment.

6.4 ANTIOXIDATIVE SYSTEM

Apart from the previous defense mechanisms explained, there have been documented instances of the activation of enzymatic and non-enzymatic antioxidant molecules within plants in order to counteract the deleterious impacts of oxidative stress induced by arsenic exposure.



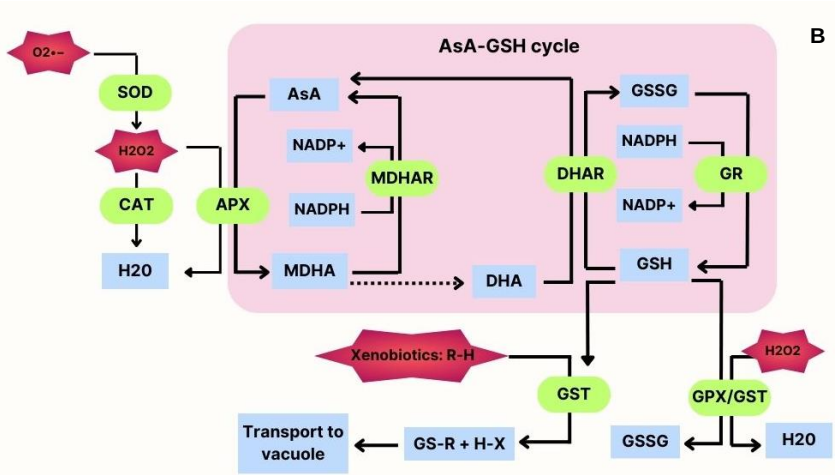


Figure 6. Overview of the plant antioxidant defense system: **(A)** categorization of antioxidants; **(B)** possible mechanisms of the antioxidant defense system in plants responding to As-induced oxidative stress.

6.4.1 Enzymatic Antioxidative System

Table 3 provides a summary of antioxidant enzymes involved in the plant defense system, along with their catalyzed reactions and cellular locations.

Antioxidants	Reactions catalyzed	Catalytic reaction sites
SOD	$O_2^{\bullet-} + 2\,H^+ \rightarrow O_2 + H_2O_2$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
CAT	$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$	Peroxisomes, glyoxisomes, cytosol, mitochondria and root nodules
APX	$H_2O_2 + AsA \rightarrow 2\,H_2O + MDHA$	Chloroplast, peroxisomes, cytosol, mitochondria
GR	$GSSG + NADPH + H^+ \rightarrow GSH + NAD^+$	Chloroplast, cytosol, mitochondria
GPX	$H_2O_2 + GSH \rightarrow H_2O + GSSG$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
GST	R-X + GSH → GS-R + H-X	Cytosol, chloroplast, mitochondria

Table 3. Reaction mechanisms of major ROS scavenging enzymatic antioxidants.

6.5.1.1 Superoxide Dismutase

Superoxide anion $O_2^{\bullet -}$ is a highly reactive oxygen species that can cause oxidative damage to cellular components, such as lipids, proteins and DNA. To counteract its effects, cells employ a group of metal-containing enzymes called superoxide dismutases (SODs), categorize into copper/zinc (Cu/Zn), manganese (Mn), or iron (Fe) SODs. In plants, Cu/Zn SODs are distributed across the cytosol, peroxisome, plastid and root nodules, whereas Mn SODs are exclusively found in the mitochondrial matrix. Meanwhile, Fe SODs are primarily situated in the plastid stroma [27].

Arsenic disrupts SOD function and expression in plants. Low or moderate arsenic concentrations enhance SOD activity in various plant species [50], [51], acting as a compensatory response to arsenic-induced oxidative stress. Conversely, exposure to high arsenic concentrations hinders SOD transcripts and inhibits its enzymatic activity [26], potentially due to SOD inactivation by H_2O_2, a by-product of SOD-mediated $O_2^{\bullet -}$ dismutation. This vulnerability to oxidative harm suggests that ROS-detoxifying enzymes, including catalase and those in the AsA–GSH cycle, are activated during abiotic stress.

The harmonious interaction among SODs, AsA–GSH enzymes and metal ion chelation is essential for balancing oxygen and hydrogen peroxide, preventing additional ROS generation through metal-catalyzed reactions [52].

6.5.1.2 Catalase

Catalase (CAT), responsible for neutralizing H_2O_2 produced during cellular metabolism, is a protein composed of four subunits and a heme prosthetic group. The connection between CAT and the heme prosthetic group is confirmed by its irreversible inhibition by substances such as cyanide, azide and hydroxylamine, all of which act as inhibitors for heme-containing proteins [53].

CAT is widely distributed in various cellular compartments, including peroxisomes, glyoxisomes, cytosol, mitochondria and root nodules [54], [55]. It does not require any cellular reducing equivalents to perform its function, thus saving energy and protecting the cell from oxidative stress [26].

CAT activity is influenced by various factors, such as arsenic exposure. Studies suggest higher CAT activity in As-tolerant plants like Pteris Vittata compared to As-suscpetible plants

like *Pteris ensiformis* and *Nephrolepis exaltata* [50] indicating a role in arsenic detoxification. However, some plants, such as *Vigna* radiata and *Taxithelium nepalense* [56], show reduced CAT activity under arsenic exposure, possibly due to interference with its heme group or its thiol groups essential for its catalytic activity. Thiol-inhibiting substances like aminotriazole and mercaptoethanol confirm the presence of a thiol group near the active site of catalase [2].

6.5.1.3 Ascorbate Peroxidase

Ascorbate peroxidase (APX) is as a crucial enzyme in the AsA-GSH pathway, providing an alternative means of detoxifying H_2O_2 in higher plants. Given the absence of CAT in the chloroplast, APX plays a key role in converting H_2O_2 to water using ascorbate (AsA) as a reducing agent [26], preventing the inhibition of Calvin cycle enzymes caused by hydrogen peroxide. In the presence of APX and two AsA molecules, hydrogen peroxide is reduced to water, generating two molecules of monohydroascorbate (MDHA) [2].

Acting as an active ROS scavenger, APX isoforms are present in various cellular compartments. They have been isolated and characterized from diverse plant species, including, for example, *Pisum sativum* [57] and *Oryza sativa* [58]. APX activity, influenced by factors like light, temperature, and metal toxicity, increases in response to arsenic exposure [56], [59], providing protection against oxidative stress in plants.

6.5.1.4 Glutathione Reductase

Glutathione reductase (GR) regulates GSH levels and is crucial for various cellular functions including antioxidant defense, redox signaling and detoxification[60]. Predominantly found in chloroplasts, with smaller amounts in mitochondria and cytosol [61], GR converts GSSG to reduced GSH using NADPH as a reducing equivalent [26]. This process maintains cellular redox levels by during oxidative stress. Additionally, GSH plays a role in regenerating AsA from MDHA, a by-product of the action of APX on hydrogen peroxide [62].

Influenced by various biotic and abiotic stress factors, including pathogens, drought, salinity and metalloids, arsenic exposure impacts GR in higher plants, stimulating its activity.

While increased GR activity has been detected in the roots of *Pteris vittata*, *Pteris ensiformis* and *Nephrolepis exaltata* [26], variations exist among species, highlighting distinc roles in arsenic tolerance and accumulation across different plant organs.

6.5.1.5 Glutathione Peroxidases

Glutathione peroxidase (GPX) belongs to an extensive peroxidase family with diverse substrate specificity, reducing various peroxides, using GSH as a reducing substrate [63]. It catalyzes the transformation of H_2O_2 and forms GSSG, which is subsequently reduced back to GSH by GR [26].

Distributed across plant cell compartments, including the cytosol, chloroplasts, mitochondria, peroxisomes, and apoplast, GPX isoforms vary based on sequence and subcellular location. In *Arabidopsis* T*haliana*, for example, there are identified GPX isoforms, denoted as AtGPX1 to AtGPX7 [64]. Among these, AtGPX1 and AtGPX7 are situated in chloroplasts and provide antioxidant protection and coordinate immune responses.

GPX activity is influenced by environmental factors, including As-induced oxidative stress, with studies reporting increased activity in various plant species [50]. However, higher arsenic concentrations may lead to decreased GPX activity, potentially impairing its function under excessive arsenic exposure [65].

6.5.1.6 Glutathione S-transferase

Glutathione S-transferases (GST) constitute a group of enzymes present in the plant cytosol and various cellular compartments. These enzymes utilize GSH as a co-substrate or coenzyme [50], [60] to catalyze the conjugation of GSH to different electrophilic centers of cytotoxic products [26] (Figure 6). This reaction makes the molecules more polar and easier to transport to the vacuole or the apoplast, where they can be further degraded or excreted [2].

GSTs are induced by various environmental factors that cause oxidative stress in plants, such as toxic metals, drought, salinity and pathogens [55]. They are involved in the defense mechanism against As toxicity, as they can conjugate GSH to As and facilitate its sequestration or removal.

Numerous investigations have revealed an increase in both the activity and expression of glutathione S-transferase in plants following exposure to As, with the results depending on the plant species and the chemical form of the arsenic found. For example, in the context of rice plants, a remarkable upregulation of at least 10 GST genes is observed in response to As^{V}, whereas the downregulation affects no more than 2 GST genes in the case of As^{III} [48]. This underscores the idea that different chemical forms of arsenic exert different effects on GST regulation. Similarly, under arsenic-induced stress, mesquite and maize plants show an

elevation of their GST activity. In contrast, *Pteris vittata* shows a reduction in GST activity, especially when exposed to high concentrations of arsenic [66].

6.5.2 Non-Enzymatic Antioxidative System

Table 4 summarizes the antioxidant molecules participating in the plant defense system, including their catalyzed reactions and cellular locations.

	Antioxidants	Reactions catalyzed	Catalytic reaction sites
Non-enzymatic	Ascorbic acid (AsA)	Scavenges $O_2^{\bullet -}$, H_2O_2, $^{\bullet}OH$, and $^1O_2$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
	Glutathione (GSH)	Scavenges H_2O_2, $^{\bullet}OH$, and $^1O_2$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
	Carotenoids	Scavenges mainly $^1O_2$	Chloroplast

Table 4. Reaction mechanisms of major ROS scavenging non-enzymatic antioxidants.

6.5.2.1 Ascorbate (Ascorbic Acid)

Ascorbate, a vital antioxidant in plants, is found in multiple cellular compartments and is synthesized chemically from glucose throught enzymatic reactions.

AsA plays a key role in protecting the photosynthetic machinery from ROS by either directly scavenging them or by serving as a co-factor for the APX enzyme [67]. AsA also contributes to the regeneration of carotenoids and α-tocopherol, both essential for avoiding lipid peroxidation, through its involvement in the AsA-GSH cycle. This cyclic process involves the conversion of H_2O_2 into water by APX, generating the oxidized form MDHA. MDHA can be restored to AsA or undergo disproportionation into AsA and DHA, enhancing the antioxidant defense system in various cellular organelles [68]. DHA is able to be back-reduced to AsA, which occurs through the enzymatic activity of DHA reductase. This particular biochemical transformation needs the presence of GSH and relies on NADPH as the essential reducing equivalent [26], as mentioned before. The AsA-GSH cycle functions within various cellular organelles and increases the antioxidant defense system.

It was observed that within the fronds of the arsenic As-hyperaccumulator *Pteris vittata*, there was a notable and significant increase in the concentration of reduced AsA and the ratio AsA/DHA one day following exposure to arsenic [67]. This was contrasted with the results of As-

sensitive *Pteris ensiformis*, where such changes were not as pronounced. It suggests that AsA may be involved in the detoxification of As and the protection of photosynthesis in *P. vittata*. Conversely, in cucumber plants subjected to arsenic treatment, a reduction in AsA concentration was noted in the roots, but the hypocotyls exhibited an increase under the same arsenic exposure conditions [69]. This indicates that AsA may have different roles in different tissues and organs of plants under arsenic stress. Further research is needed to elucidate the mechanisms and functions of AsA in plant adaptation to arsenic toxicity.

6.4.2.2 Glutathione

Glutathione is a trimeric thiol peptide that includes the amino acids cysteine, glycine and glutamate. Distinguished by a peptide bond linking the carboxyl group of glutamate and the amino group of cysteine. GSH emerges as a major antioxidant within cells, as it plays a fundamental role in preserving cellular redox balance, protecting against ROS and binding with metals and metalloids, including As, facilitating its detoxification from the body [70], [71].

GSH is synthesized in two ATP-dependent steps. The first step is the limiting step in GSH synthesis and involves the enzyme γ-glutamylcysteine synthetase (γ-GCS or glutamate-cysteine ligase), which catalyzes the formation of γ-glutamylcysteine (γ-GGC) from L-glutamate and cysteine. The second step involves the enzyme glutathione synthetase (GS), which attaches glycine to the terminal C of γ-GGC to form GSH. γ-GCS plays a major role in regulating GSH synthesis and is induced by oxidative stress and metal toxicity [72].

GSH assumes multiple roles in the defense against metals and metalloids. It serves as an electron donor for arsenate reductase [73], which reduces arsenate to arsenite, which can be sequestered in vacuoles as thiol-peptide complexes. In addition, GSH acts as a precursor to the synthesis of phytochelatins, improving the sequestration of metals. It also binds directly with ROS, detoxifying them through a GST-catalyzed reaction [26]. In addition, GSH modulates the expression and activity of genes and enzymes involved in metal and metalloid homeostasis and metabolism, including metallothioneins, phytochelatin synthases, metal transporters, nitrate reductase, sulfite reductase and selenocysteine lyase [74].

Several studies have reported changes in GSH and PCs levels in plants exposed to As. These studies suggest that GSH has a vital role in plant adaptation to arsenic stress. For example, it was found that protection from oxidative damage by a greater level of AsA-GSH is associated with arsenate tolerance in the As-hyperaccumulator *Pteris vittate* [67]. Also, some

authors reported that rapid arsenate influx resulted in GSH depletion and PC production in *Holcus lanatus* [75]. Others have demonstrated a notable increase in GSH and PCs in tolerant plants like *Hydrilla verticillata* upon arsenic exposure [76]. In addition, it has been shown that exogenous GSH and cysteine supplementation alleviate oxidative stress and restore the growth of rice seedlings exposed to arsenic [59].

In essence, GSH emerges as a versatile molecule capable of protecting plants from the harmful impacts of metals and metalloids. Despite the recognized influence of these elements on GSH levels and plant redox status, it still seems to be difficult to find a comprehensive understanding. Studies indicating alterations in GSH and PC levels in plants exposed to these elements underline the vital role of GSH in plant adaptation to metal and metalloid stress. However, further research is essential to explain the complex mechanisms and functions of GSH in various plant tissues and organs subject to metal and metalloid toxicity.

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6.5.2.3 Carotenoids

Carotenoids constitute a category of lipid-soluble pigments located within the thylakoid membrane of chloroplasts, playing a vital role in capturing light and safeguarding the membrane against oxidative damage. In their capacity as antioxidants, carotenoids play a crucial role in shielding the photosynthetic machinery [26]. They achieve this by counteracting the effects of a triplet sensitizer (Chl_3), singlet oxygen and various harmful free radicals that arise as natural byproducts during the process of photosynthesis [77].

An observation has been made regarding a decline in carotenoid levels in oat and red clover plants cultivated in soil contaminated with arsenic [51], [78]. This reduction is hypothesized to be attributed to arsenic-induced disruptions leading to thylakoid membrane swelling and starch accumulation in the chloroplast under arsenate stress. In contrast, it was noted in the hyperaccumulator *Pteris vittata* that carotenoid levels increased, while in *Pteris ensiformis*, there was a decrease observed after exposure to arsenic for a duration of 10 days [67]

6.6 TWO ILLUSTRATIVE METABOLOMIC STUDIES

By exploring the complex network of molecular responses to specific situations, metabolic studies play a fundamental role in identifying metabolites affected by various reactions. The results of two metabolomic studies are presented below, which demonstrate the previously explained effects.

A search in literature databases crossing the terms “arsenic contamination” AND “metabolomics”, followed by filtering of the results with the terms “plants” OR “algae” did not provide many results (some of them are indicated in Table 1), which reflects that this is an area relatively unstudied. In this section, two representative papers about research on this field are commented.

Arora et al, [5] studied how a green microalga, *Chlorella vulgaris*, responds to different forms and concentrations of arsenic. They exposed *C. vulgaris* to various levels of As^{III} and As^{V} and measured the changes in its metabolites. As stated in section 3.2, this metabolomic study has been carried out using NMR analytical techniques, which have allowed the quantification, identification and the analysis of the changes produced in metabolites that are involved in biological processes of interest.

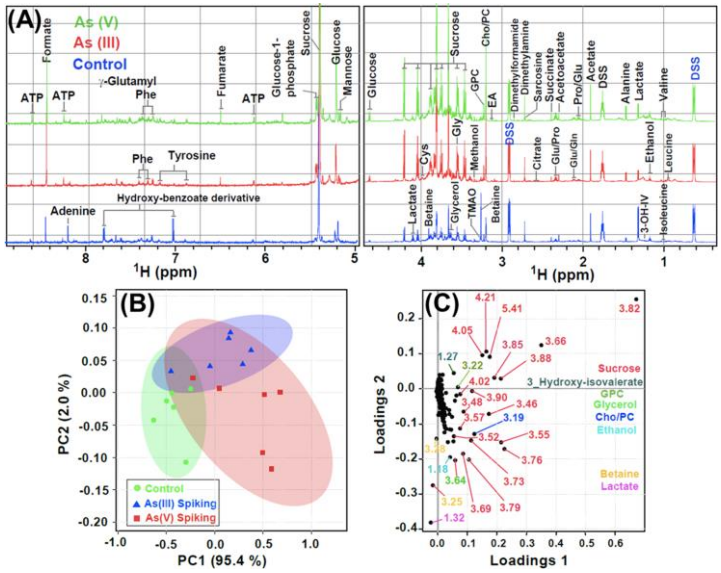


Figure 7. ^{1}H-NMR spectrum resulting from the metabolic study. (A) Comparison between the control sample (blue) and the samples that have been contaminated with As^{III} (red) and As^{V} (green). (B) The principal component analysis (PCA) and (C) PCA loading plot help to reveal the metabolites responsible for the pattern of discrimination. *Image taken from Arora et al,* [5]

Analyzing NMR data for metabolite assignment revealed significant differences in peak patterns for carbohydrates (sucrose, glucose, and mannose), amino acids (leucine, alanine, valine, serine, and cysteine), ATP, organic acids (fumarate, succinate, citrate, and acetate), and nucleotides between control algae extracts and those treated with As^{III} and As^{V}. Variations were also observed between the two arsenic species (Figure 7a). To validate these differences between treatments, a PCA was carried out, which demonstrates a statistically significant clustering of six biological replicates and different variations between control and arsenic-treated samples, as well as between As^{III} and As^{V} (Figure 7b). The PCA loading plot highlights relevant metabolites that follow a discrimination pattern (Figure 7c). The farther the metabolite is from the origin (0,0), the more it contributes to the discrimination group.

The study by Arora et al. on the microalga *Chlorella Vulgaris* reveals that the alga is able to cope with arsenic stress by modulating its metabolic pathways, including glycolysis, the pentose phosphate pathway, amino acid metabolism and biosynthesis of fatty acids, as observed in figure 8. For example, it increased the production of antioxidants such as GSH in order to combat ROS generated by arsenic exposure. It also synthesized osmolytes such as proline, choline and betaine to maintain cellular water balance and membrane stability and adjusted its lipid composition and fatty acid profile to cope with membrane damage and oxidative stress caused by arsenic. In addition, the results show that the responses of *C. vulgaris* vary according to the form and concentration of arsenic, with As^{III} being more toxic than As^{V}, with different tolerance thresholds for these two forms of arsenic.

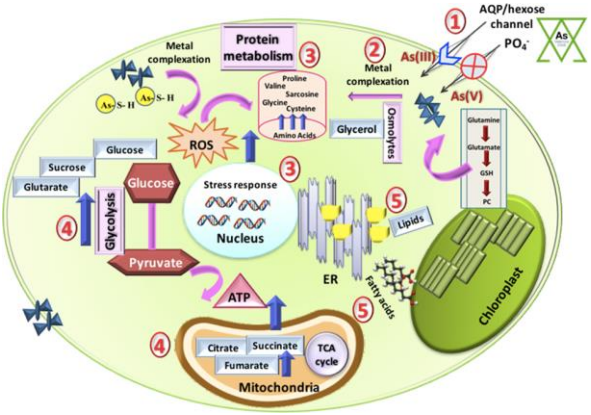
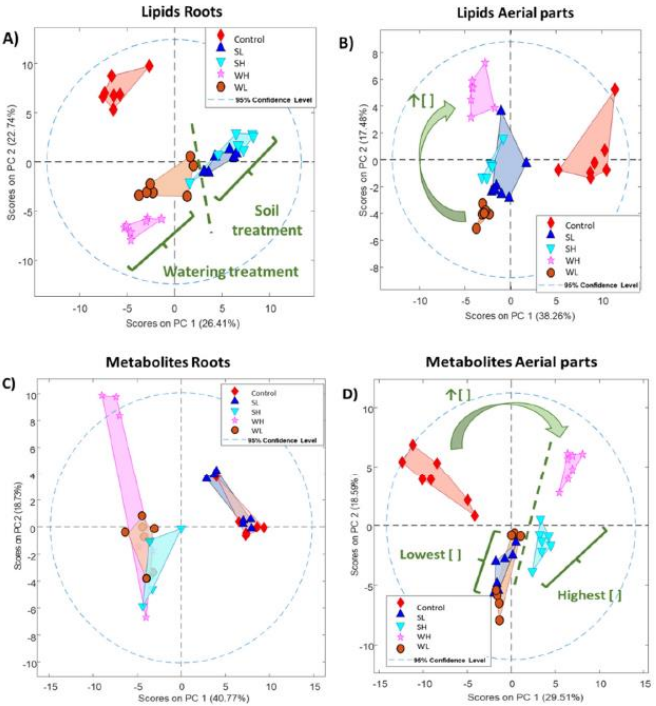


Figure 8. Representative scheme of altered metabolism due to exposure to As^{V} and As^{III}, taken from *Arora et al,* [5]

A second study performed by Miriam Pérez-Cova, Romà Tauler and Joaquim Jaumot [7], constituted a comprehensive exploration of the impact of arsenite on rice cultivars using non-targeted metabolomics and lipidomics, based on liquid chromatography coupled to mass spectrometry (HPLC-MS). The main objective of this work was to explore the mechanisms of absorption and translocation of arsenic in rice, as well as its possible consequences on the plant's metabolome and lipidome.

Arsenic was supplied through two main routes: irrigation with contaminated water or soil containing arsenic. Samples were analyzed by using a UPLC-MS system, and the results were treated using PCA as a multivariate data analysis method to determine the differences between rice samples exposed to different arsenic treatments (figure 9). The PCA analysis allowed them to reduce the dimensionality of the data and visualize the main sources of variation in the metabolomic and lipidomic profiles of the rice tissues. They also used other multivariate methods such as PLS-DA (Partial Least Squares Discriminant Analysis) and ROIMCR (Regions of Interest Multivariate Curve Solving) chemometrics strategy to complement the PCA results and improve the identification and quantification of metabolites and lipids altered.



S, soil; L, low; W, water; H, high

Figure 9. PCA score plots obtained in the analysis of both tissues, roots, and aerial parts. (A,B) Represent the lipodomic analysis of roots and aerials tissues, respectively. (C,D) Represent the metabolomic analysis of roots and aerials tissues, respectively. *Image taken from Pérez-Cova et al,* [7]

Figure 9 shows that both the lipidomic and metabolomic samples extracted from the roots have a more pronounced impact due to the treatment applied, overcoming the influence exerted by variations in the level of arsenic concentration. Interestingly, this was in contrast with the results obtained from samples located in aerial tissues, where the opposite pattern emerged. Specifically, concentration levels played a more crucial role in influencing the lipidomic and metabolomic profiles of aerial tissues compared to the effects induced by the treatment itself.

The results obtained in the study reveal significant insights into the impact of arsenic exposure on metabolomic profiles in different tissues. Annotated metabolites were primarily found in three groups: WH (water-high As concentration), SH (soil-high As concentration), and WL (water-low As concentration), with key altered-level metabolites such as palmitic acid, allantoin, norvaline, succinic acid, tryptophan and isoleucine prominently present.

An exhaustive analysis of the affected metabolic pathways by arsenic exposure in all tissues was conducted using MetaboAnalyst [79]. The six principal metabolic pathways influenced by arsenic included: aminoacyl-tRNA biosynthesis (A); alanine, aspartate, and glutamate metabolism (B); (C) glycine, serine, and threonine metabolism; (G) arginine biosynthesis; (H) phenylalanine, tyrosine, and tryptophan metabolism; (I) arginine and proline metabolism. The results obtained are visually presented in figure 10a, where the key pathways influenced by arsenic exposure are highlighted using letters.

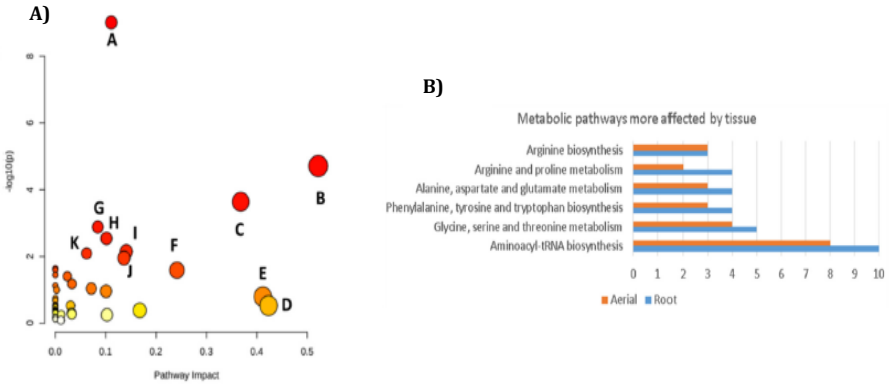


Figure 10. (A) Visualization of MetaboAanalyst results indicating the most affected metabolic pathways regarding arsenic. (B) Comparison of the number of significant metabolites related to the main metabolic pathways affected. *Image taken from Pérez-Cova et al,* [7]

Furthermore, figure 10b provides a comparative illustration of pathways based on the analyzed tissues. While there were overlapping altered pathways in both roots and aerial tissues, it is important to note that the specific metabolites associated with these pathways did not necessarily coincide. In other words, common pathways were affected, but the individual metabolites within those pathways differed between the two types of tissues.

7. CONCLUSIONS

All the data in the literature indicates that exposure to arsenic generates harmful effects on plants, affecting both biochemical and molecular levels. It can interfere with various metabolic processes and pathways, either directly as a competitive inhibitor of phosphate or indirectly by modifying the activity of key enzymes, thereby affecting crucial physiological responses such as seed germination, root and shoot growth, and other developmental processes during the early stages of the plant.

The main mechanism by which arsenic causes damage is the induction of oxidative stress, which occurs when there is an imbalance between the production and removal of ROS. Arsenic can increase the production of ROS or decrease their elimination, leading to the oxidation and deterioration of lipids, proteins, and nucleic acids. The specificity of this As-induced oxidative stress is due to two factors: the substitution of phosphate by As^{V} and the high affinity of As^{III} for sulfhydryl groups, resulting in the overproduction of ROS and the subsequent oxidative stress. However, plants have antioxidant defense mechanisms that are activated in response to oxidative stress. These mechanisms include the synthesis of antioxidant metabolites and the action of antioxidant enzymes, which have a role in protecting plants against oxidative damage.

The modification of various metabolic pathways, such as GSH synthesis, in response to oxidative stress increases the tolerance of plants to arsenic. The significant variability among species in sensitivity to arsenic suggests the possibility of discovering new metabolic pathways related to arsenic tolerance in the near future. Further research is needed to fully understand the molecular mechanisms involved in the plant's response to arsenic and identify biochemical markers that indicate the level of stress and damage caused.

Metabolomic studies emerge as valuable tools to evaluate how arsenic alters plant metabolism, affecting the quality and bioactive properties of crops. They could contribute to the development of solutions to reduce or eliminate arsenic from plants and to take advantage of the metabolites of interest for pharmaceutical, nutritional or industrial purposes. High Performance Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS) and NMR

stand out as crucial techniques to identify metabolites in plants affected by arsenic, thanks to their high sensitivity and specificity.

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12. ACRONYMS

26-like Nucleoglyceroporins Intrinsic Protein: **NIP**

γ-Glutamylcysteine: **γ- GGC**

γ-Glutamylcysteine Synthetase: **γ-GCS**

Arsenate Reductase: **AR**

Ascorbate: **AsA**

Ascorbate peroxidase: **APX**

Catalase: **CAT**

Dimethylarsinic Acid: **DMA**

Dehydroascorbate: **DHA**

Endoplasmic Reticulum: **ER**

Food and Agriculture Organization: **FAO**

Glutathione: **GSH**

Glutathione Disulfide: **GSSG**

Glutathione Peroxidase: **GPX**

Glutathione Reductase: **GR**

Glutathione Synthetase: **GS**

Glutathione S-transferase: **GST**

Glyceraldehyde-3-Phosphate Dehydrogenase: **GAPDH**

Monohydroascorbate: **MDHA**

Inorganic Phosphate: **iP**

International Agency for Research on Cancer: **IARC**

Monomethylarsinic Acid: **MMA**

Phytochelatins: **PCs**

Plant High Affinity Phosphate Transporter 1: **PHT1**

Reactive Oxygen Species: **ROS**

Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase: **Rubisco**

S-adenosyl Methionine: **SAM**

Superoxide Dismutase: **SOD**

Web of Science: **WOS**

World Health Organization: **WHO**

APPENDICES

APPENDIX 1: ARSENIC TRANSPORTERS IN PLANTS

Transporter group	Plant species	Transporter name	As species	Location	Processes	References
Phosphate transporter	*Arabidopsis thaliana*	AtPHT1;1	As^{V}	PM	Uptake	[80], [81]
		AtPHT1;4	As^{V}	PM	Uptake	[80], [81]
		AtPHT1;5	As^{V}	PM	Uptake	[82]
		AtPHT1;7	As^{V}	PM	Uptake	[81]
		AtPHT1;8	As^{V}	PM	Uptake	[83]
		AtPHT1;9	As^{V}	PM	Uptake	[83]
	Oryza sativa	OsPHT1;1	As^{V}	PM	Uptake	[84]
		OsPHT1;8	As^{V}	PM	Uptake; root to shoot translocation	[85]
NIP aquaporin	*Arabidopsis thaliana*	AtNIP1;1	As^{III}	PM	Uptake	[86]
		AtNIP1;2	As^{III}	PM	Uptake	[86]
		AtNIP3;1	As^{III}	PM	Uptake; root to shoot translocation	[87]
		AtNIP5;1	As^{III}	PM	Transport	[86], [88]
		AtNIP6;1	As^{III}	PM	Transport	[88]
		AtNIP7;1	As^{III}	PM	Uptake	[14]
	Oryza sativa	OsNIP1;1	As^{III}	PM	Uptake	[89]
		OsNIP3;1	As^{III}	PM	Uptake	[89]
		OsNIP3;2	As^{III}	PM	Transport	[88]
		OsNIP3;3	As^{III}	PM	Transport	[90]

		OsNIP2;1 (OsLsi1)	As^{III}, MMA, DMA	PM	Influx	[88], [89]
		OsNIP2;2 (OsLsi6)	As^{III}	PM	Uptake	[89]
	Lotus japonicus	LjNIP5;1	As^{III}	PM	Transport	[88]
		LjNIP6;1	As^{III}	PM	Transport	[88]
ABC transporter	*Arabidopsis thaliana*	AtABCC1	As^{III}-PCs	Tp	Transport into vacuoles	[47]
		AtABCC2	As^{III}-PCs	Tp	Transport into vacuoles	[47]
	Oryza sativa	OsABCC1	As^{III}-PCs	Tp	Transport into vacuoles	[91]

PM, plasma membrane; Tp, tonoplast

APPENDIX 2: ARSENIC-INDUCED PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN PLANTS

Plant species	Growth medium	As concentration	Observations	References
Trifolium pratense L	Soil	As^{V} (5;10;50 mg kg^{-1})	Decrease in GSH content at 5 and 50 mg kg-1 but it increased at 10 mg kg^{-1} by 8%	[51]
Zea mays L.	Soil	As^{V} (0,40; 80; 120 mg kg^{-1})	Elevation in shoot concentrations of As and iP accompanied by a decline in the concentrations of chlorophyll pigments.	[8]
Anadenanthera peregrine, Myracrodruon urundeuva	Soil	As^{V} (0; 10; 50; 100 mg L^{-1})	Elevation in hydrogen peroxide and lipid peroxidation.	[34]