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# HALOPHYTES AND PROLINE: A PROMISING POSSIBILITY FOR HEAVY METAL REMEDIATION AND AGRICULTURAL RESTORATION

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The continuous rise in soil salinity and the expansion of saline areas present a worldwide threat to agriculture by reducing the amount of arable land available for crop cultivation. Halophytes, naturally inhabitants of saline environments, are the sole group of plants able to populate and thrive in saline soils. With highly efficient adaptive strategies, these plants tolerate extreme saline conditions indefinitely. Some of these species are metal hyperaccumulators, particularly those growing under saline conditions frequently rich in heavy metals that are often polluted. One mechanism enabling them to tolerate these extreme conditions is the accumulation of osmoprotectant metabolites. Even though these protective compounds are generally produced by plants to help them tolerate extreme environmental conditions, usually halophytes accumulate more osmolytes than glycophytes. Although many publications discuss the potential of halophytes in the remediation of areas polluted by heavy metals, relatively little is documented about the role of compatible solutes in alleviating the toxic effects of heavy metals. This review focuses on the role of the most common osmoprotectant, that is, proline, produced by halophytes to mediate cellular damage caused by the hyperaccumulation of metal ions and addresses the biosynthetic pathways of this compound. Reclaiming land polluted by heavy metals by populating it with halophytes and enhancing osmoprotective solute production through genetic engineering in halophytes presents viable solutions to restore pollution-ridden areas for potential agricultural use worldwide.

**Keywords:** halophytes, proline, heavy metals, toxicity, mitigation

## 1. Introduction

Natural terrestrial habitats where enhanced levels of soil salinity and heavy metals (HMs) greatly diminish crop yield and quality are considerably widespread throughout the world, restricting the productive use of arable land [1]-[3]. Owing to the progressively decreasing amount of precipitation, irrigation with saline water and inadequate agricultural practices, the amount of land contaminated by saline soils is continuously increasing. It has been estimated that about 20% of arable land (45 million ha) has already been negatively affected by salinity. By 2050, 50% of agricultural land will become saline and inappropriate for common crop cultivation [1]. On the other hand, human industrial activities and mining have also had detrimental effects on fertile soils in the environment, leading to HM pollution and retention in soils [2],[4]. These activities have contaminated soils with potentially toxic elements and HMs, including arsenic (As), silver (Ag), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb) and zinc (Zn) [5]-[6].

Salinity generates osmotic stress and a water deficit in the soil, reducing its water potential and altering the water balance in plants. Plants have evolved a diverse range of morphological, physiological and biochemical adaptive mechanisms allowing salinity tolerance [7]-[11]. High $Na^+$ and $Cl^-$ concentrations in chloroplasts decrease photosynthetic activity, affecting carbon metabolism and photophosphorylation, while photosynthetic electron transport is less sensitive to saline conditions [12]. High levels of salinity also alter the composition of plasma membrane protein and lipids as well as cause an ionic imbalance across membranes, ultimately leading to hyperosmotic stress [13]. Salt stress triggers osmotic stress and ion toxicity. $Na^+$ is toxic when its concentration exceeds 10 mM in the cytosol, but 100-200 mM of $K^+$ is needed in the cytosol for

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physiological functions. Many enzymes are sensitive to $Na^+/K^+$ ratios and excessively high cytosolic $Na^+$ concentrations could impair their activities. To achieve optimal metabolic conditions, the cytosol should have a low $Na^+$ concentration and low $Na^+/K^+$ ratio [14].

Halophytes, accounting for only approximately 1% of terrestrial plants, can survive and thrive in naturally saline soils containing at least 200 mM of NaCl [15]-[16]. Saline soils are frequently polluted with HMs, increasing the likelihood of salt and HM stress. To alleviate the damaging effects of factors causing environmental stress (drought, salinity, extreme temperatures, heavy metals) and as a HM detoxification mechanism, halophytes accumulate osmoprotective solutes [17]-[19]. Osmoprotectants, or compatible solutes, are small, highly soluble molecules with low molecular weights and a neutral charge that do not exhibit toxic effects at molar concentrations [20]. Osmolytes mitigate cellular injury by stabilizing proteins and membranes as well as maintaining the cellular osmotic potential during periods of stress. While not all plant species respond to stress by producing osmolytes, a range of stress-tolerant species upregulate the biosynthesis of these highly effective protective molecules [18]-[19]. Furthermore, osmoprotectants are also key molecules for maintaining turgor pressure and protecting cells from oxidative damage by scavenging detrimental reactive oxygen species (ROS). Aside from the direct quenching activities of ROS, they may also exert their antioxidant defense potential indirectly, triggering the enhanced synthesis of antioxidants and the upregulation of antioxidant enzymatic activities [7, 9, 21]. Osmoprotectants are in the form of amino acids (proline, alanine, arginine), ammonium compounds (glycine betaine and polyamines) and soluble carbohydrates (sugars and polyols) as compatible solutes to modulate cellular osmotic potential. Here the essential role of proline (Pro) in alleviating damage as a result of heavy metals in halophytes is described.

## 2. Proline biosynthesis in plants

The synthesis of compatible solutes such as Pro is one of the strategies that plants use to cope with HM stress [22]-[23]. Pro is synthesized both under adverse and non-stressed physiological conditions. Although Pro biosynthesis occurs via two pathways, namely the glutamate and ornithine pathways, the glutamate pathway accounts for stress-induced Pro accumulation [24]. Glutamate-derived Pro is synthesized in the cytoplasm in a reaction catalyzed by 1-pyrroline-5-carboxylate synthetase (P5CS), which generates pyrroline-5-carboxylate (P5C) from glutamate in a two-step reaction. The intermediate product is glutamate-γ-semialdehyde (GSA), synthesized in an ATP and NADPH-dependent reaction. Next, P5C is further reduced to Pro. Pro is reduced in a NADPH-dependent reaction catalyzed by P5C reductase (P5CR) [25]. Although the activity of the enzyme P5CS represents the rate-limiting step of Pro biosynthesis, it should be mentioned that P5CS consists of two isoforms, P5CS1 and P5CS2. Székely et al. demonstrated [7] that the two isoforms appear with non-redundant functions, with chloroplast-localized P5CS1 having an impact on stress-induced Pro synthesis, while the activity of cytosolic P5CS2 may cause embryo abortion during the later stages of seed development in Arabidopsis.

On the other hand, ornithine-derived Pro is synthesized in the mitochondria in a reaction catalyzed by ornithine-δ aminotransferase (OAT), which converts ornithine into glutamate-semialdehyde (GSA) and P5C, that is finally transformed into Pro. The catabolic pathway is mitochondrial; Pro is oxidized into glutamate over two steps. In the first rate-limiting step, Pro catabolism is catalyzed by flavin-dependent proline dehydrogenase (ProDH), which reduces Pro to P5C. Next, P5C is oxidized to glutamate by NAD+-dependent P5C dehydrogenase (P5CDH) [24]. In plants, two isoforms of ProDH have been detected with different localizations. Similarly to isoforms of P5CS, ProDH1 and ProDH2 appear with non-redundant functions; ProDH1 is expressed at the whole plant level, while ProDH2 shows a vasculature-specific expression [26]. The reciprocal regulation of P5CS and ProDH genes is critical in order to control Pro levels during and after periods of stress [27]. Intracellular Pro levels are determined by its biosynthesis, catabolism and transport between organelles and cells. Proline biosynthetic enzymes (P5CS1, P5CS2 and P5CR) are thought to be localized in the cytosol, whereas the enzymes of Pro catabolism (e.g. ProDH1, ProDH2, P5CDH and OAT) are considered to be located in mitochondria [17].

## 3. Multifaceted roles of proline with regard to the adaptation of halophytes to environmental stresses

Halophytes accumulate Pro in response to salinity stress, and their capacity to accumulate Pro reflects their salinity tolerance [28]. Free Pro accumulation can be as high as 10-20% of the shoot dry weight in various species of halophytes with Pro representing the most abundant component of the amino acid pool in salt-tolerant plants [17],[28]. A later work demonstrated that aside from salinity other environmental stress factors (e.g. water deficit, extreme temperatures or heavy metal contamination) may also activate Pro synthesis. Pro accumulation proved to be one of the most important defense responses of plant cells encountering the aforementioned stress factors [17],[29]. Many halophytes such as *Sesuvium portulacastrum*, *Thellungiella salsuginea*, *Mesembryanthemum crystallinum* and *Lepidium crassifolium* accumulate a significant amount of Pro to benefit from its protective effects [30]-[32]. The major effects of proline in mitigating the negative impacts of adverse environmental stress factors in plant cells are illustrated in *Figure 1*.

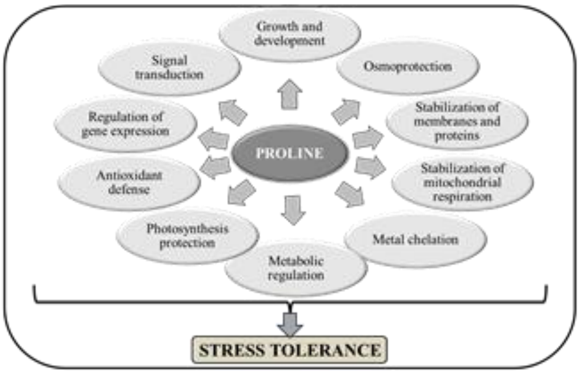

*Figure 1:* Roles of proline in plant adaptation to environmental stress conditions

*Table 1:* Selected reports describing the accumulation of proline in halophytes in response to heavy metal exposure

| Osmo-protectant | Halophyte species | Heavy metals | Ref. |
|---|---|---|---|
| Proline | *Aeluropus littoralis* | Cd, Co, Pb, Ag | [35] |
| | *Mesembryanthemum crystallinum* | Cd | [41] |
| | *Zygophyllum fabago* | Cd, Zn | [42] |
| | *Atriplex halimus* | Cd | [39] |
| | *Atriplex maritima* | Zn | [43] |
| | *Cakile maritima* | Cd | [44] |

A clear correlation between HM accumulation and an increase in Pro level has been documented in many species of halophytes [22, 33–34]. While few HM-tolerant species such as *Armeria maritima*, *Deschampsia cespitosa* and *Silene vulgaris* contain high Pro concentrations under environmental stress conditions unaffected by metal ions, increased levels of Pro accumulation have generally been associated with HM tolerance [33]. As an osmolyte, Pro alleviates damage induced by HMs, can stabilize cell membranes by interacting with phospholipids, protects protein structures [35] and acts as a chelator to bind metals [33]. Pro also plays a role in stress protection as an antioxidant, neutralizing ROS by quenching singlet oxygen, superoxide, hydrogen peroxide and hydroxyl radicals [7, 9, 32, 36]. Pro also enhances the photochemical activities of Photosystem II [37] and maintains a low NADPH to $NADP^+$ ratio in Photosystem I, lowering the likelihood of singlet oxygen formation. Furthermore, Pro alleviates the harmful effects of singlet oxygen and hydroxyl radicals on Photosystem II, delays plant senescence [38] and suppresses apoptosis under stress conditions [17],[32]. Furthermore, Pro stabilizes mitochondrial respiration, protecting Complex II of the mitochondrial electron transport chain. Similarly, increased Pro levels under cadmium (Cd) stress mitigate the adverse effects of stress-induced ROS in the halophyte *Atriplex halimus* [39]. Nevertheless, it is documented that Pro protects key enzymes such as glucose-6-phosphate dehydrogenase and nitrate reductase against the toxic effects of Zn and, to a lesser extent, Cd through the reduction of free metal ions due to the formation of metal-proline complexes. This data suggests that osmoregulation and enzymatic protection rather than metal sequestration may represent the primary roles of metal-induced Pro accumulation [40].

To further underscore the importance of proline, some species of halophytes that accumulate Pro as a defense strategy to withstand different types of HM pollution are presented in *Table 1*. The amino acid proline is essential for halophytes to endure HMs. According to the studies mentioned in *Table 1*, the accumulation of Pro is a common response in halophytes like *Aeluropus littoralis*, *Mesembryanthemum crystallinum* and *Atriplex halimus* when exposed to HMs such as Cd, Co, Pb and Ag. These results highlight the widespread efficiency of Pro in different plant species and HM-polluted environments. Moreover, the accumulation of other amino acids such as leucine, isoleucine and valine enhance HM tolerance. *Suaeda salsa*, for example, accumulates several amino acids when exposed to Cd and Zn, suggesting a well-defined and diverse strategy for osmoprotection. The variety of amino acids involved demonstrates how complex the interaction between metabolic adaptations is to alleviate HM stress.

Other species of halophytes such as *Walsura monophylla* respond by increasing their level of glutamine when exposed to enhanced levels of Ni [1], while *Deschampsia cespitosa* accumulates asparagine when experiencing Zn toxicity [46]. Similarly, the presence of Cd triggers an increase in the levels of amino acids like arginine, leucine, isoleucine, glutamate, glutamine, phenylalanine, valine, tryptophan and tyrosine in *Suaeda salsa*. However, it is unclear whether such accumulation can be directly attributed to metal tolerance or is a consequence of protein degradation [47].

## 4. Concluding remarks

The ability of halophytes to remediate HM stress-induced damage reflects a complex interplay between physiological and biochemical processes. Generally, Pro accumulation is associated with enhanced tolerance to stress. However, because of the extremely versatile strategies to tolerate stress, the exact mechanisms by which enhanced levels of Pro accumulation help plants cope with HM stress still remain unknown. Further research into the roles of Pro in mitigating HM toxicity may be useful in different areas: elucidating gene expression patterns and signaling pathways could help to determine molecular stress responses; studying the synergistic effect of Pro with other stress-protective molecules could identify interactions more effectively with regard to stress tolerance compared to Pro alone; developing transgenic plants with a high level of Pro biosynthesis could provide a new strategy to improve metal tolerance in crops; and exploring plant species that accumulate Pro in phytoremediation strategies to remediate HM contaminated soils might provide an ecological solution for environmental management. By concentrating on these future goals, researchers can further exploit the potential of proline to enhance the resistance of plants to HM stress, contributing towards sustainable agriculture and environmental protection.

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