

Metal-biomolecule complexes in plants: Occurrence, functions, and applications

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The occurrence and role of metal complexes in plants is discussed. Different mechanisms, often involving the biosynthesis of a metal complexing ligand, are overviewed. The potential role of hyperaccumulating plants in phytoremediation is emphasized.

Plants growing in metal contaminated environments would inevitably accumulate trace and abundant metal ions. Certain trace metals are important for metabolism of plants while a number of them are not essential imposing stress on the metabolic functions (Fig. 1). Since plants cannot move unlike animals and organisms having powerful locomotory organs, they efficiently compartmentalize these toxic metals into various parts (mostly in roots and/or older leaves) and organelles of cell where damage due to their accumulation is least [1,2]. Thus, organisms exposed to excess of toxic metals would eventually evolve mechanisms of metal resistance (Tabs. I and II). The published data indicates that metal compartmentation at the whole plant level can be divided into 3 categories (Figs. 2a-c).

Mechanisms of resistance to metal toxicity in plants

Primarily two types of mechanisms may explain the resistance to the toxicity of metal ions in plants. They are (i), avoidance, involving various ways of preventing toxic ions to reach their target sites, and (ii), tolerance to metal ions that entered the symplasm [1,2]. Various types of heavy metal adaptation strategies in plants are depicted in figure 3. The prominent metal complexation processes are the synthesis of phytochelatins and of other metal chelating peptides [1-6]. Phytochelatins were found to be induced by Ag, Bi, Cd, Cu, Hg, Ni, Sn, W, Zn whereas no induction was noticed in the case of Na, Mg, Al, Ca, V, Cr, Sb, Te, Mn, Fe, Co and Cs (Tabs. III and IV).

Glutathione

Glutathione is a precursor of phytochelatin synthesis. Metal induced phytochelatin production decreases cellular levels of glutathione. Glutathione and its homologues *viz.*, homoglutathione and hydroxymethyl-glutathione are the abundant

Table I. Mechanisms of metal detoxification by biomolecules.

Mechanism	Biomolecule
Induction of proteins	Metallothionein Heat-shock protein Phytochelatin Ferritin Transferrin
Induction and activation of antioxidant enzymes	Superoxide dismutase Catalase Glutathione peroxidase
High turnover of organic acids	phytate, malate, citrate, oxalate, succinate, aconitate, -ketoglutarate

Table II. Functions of metal-biomolecule complexes.

Protein	Metal	Function
Transferrin	Cu, Fe, Mn, V	Transport
Ferritin	Fe	Storage
Ceruloplasmin	Cu	Transport
Albumin	Cu, Ni, Zn	Transport
-Macroglobin	Zn	Transport
Metallothionein	Cd, Cu, Hg, Zn	Storage

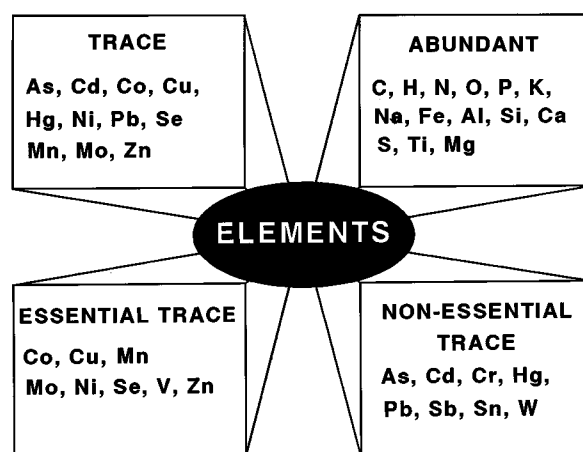


Figure 1. Metabolic and nutritional importance of elements and their classification.

Table III. Metal complexation processes in plants. Recent reviews (in alphabetical order according to author).

Broad subject	Reference
Metal tolerance in plants	Ernst et al. (1992) [7]
Biochemical, physiological and structural effects of excess copper in plants	Fernandes and Henriques (1991) [8]
Trace metals	Prasad (1997) [1]
Phytochelatin	Rausser (1990, 1996) [3, 4]
Heavy metal binding proteins/peptides: occurrence structure synthesis and functions. A review	Reddy and Prasad (1990) [5]
Mechanisms of plant resistance to aluminium and heavy metals	Rengel (1997) [2]
The heavy metal-binding peptides of plants	Steffens (1990) [6]
Metallothioneins	Stillman (1995) [10]

Table IV. Metal ion interaction with vascular plants, algae and pteridophytes reported to synthesize the metal chelating peptides. This is not an exhaustive list.

Vascular plants	Pteridophytes	Algae
<i>Agrostis sp.</i>	<i>Athyrium yokescence</i>	<i>Bumilleriopsis filiformis</i>
<i>A.gigantea</i>	<i>Polygonum</i>	<i>Chlamydomonas reinhardtii</i>
<i>Avena sativa</i>	<i>Equisetum</i>	<i>Chlorella ellipsoida</i>
<i>Brassica sp.</i>		<i>C. fusca</i>
<i>Daucus</i>		<i>C. pyrroidosa</i>
<i>Datura innoxia</i>		<i>Dunaliella bioculata</i>
<i>Deschampsia cespitosa</i>		<i>Euglena gracilis</i>
<i>Eichhornia</i>		<i>Fragillaria crotonensis</i>
<i>Glycine max</i>		<i>Monoraphidium minutum</i>
<i>Lycopersicon</i>		<i>Navicula pelliculosa</i>
<i>Mimulus</i>		<i>Phaeodactylum tricornutum</i>
<i>Nicotiana tabacum</i>		<i>Porphyridium cruentum</i>
<i>Oryza sativa</i>		<i>Sargassum muticum</i>
<i>Pisum sativum</i>		<i>Scenedesmus acutiformis</i>
<i>Rauwolfia serpentina</i>		<i>S. bijugatus</i>
<i>Silene cucubalus</i>		<i>S. quadricauda</i>
<i>S. vulgaris</i>		<i>Stegioclonium tenue</i>
<i>Spinaceae</i>		<i>Stichococcus</i>
<i>Zea mays</i>		<i>S. bacillaris</i>
		<i>Synechococcus</i>

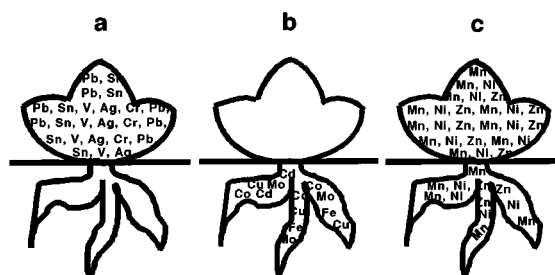


Figure 2. Metals which accumulate a) rather in shoot (stems and leaves) than in roots and rhizomes. e.g. Ag, Cr, Pb, Sn; b) rather in roots and rhizomes than in shoot, e.g. Cd, Co, Cu, Fe, Mo; c) uniformly in root and shoot, e.g. Mn, Ni, Zn.

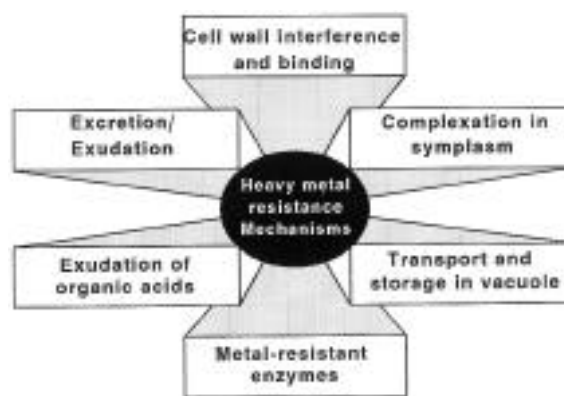


Figure 3. Heavy metal adaptation strategies being depicted in plants.

low molecular weight thiols in plants. Glutathione was implicated to play a major role in plants exposed to metal stress [1]. Glutathione synthesis is an ATP-dependent process. Sulphur (1 mM) reduced the toxicity of cadmium to the chloroplast pigments in leaves of young sugar beet plants probably by influencing the glutathione [11].

Alteration of thiol pools in maize exposed to Cd could be regulated by intermediates and effectors of glutathione synthesis. Direct evidence of glutathione involvement in phytochelatin synthesis was demonstrated in tomato cells. Glutathione depleted due to Cu-induced phytochelatin causing oxidative stress in *Silene cucubalus* (Ref. [1] and references therein). Oxidative damage in plants is also caused either due to excess or deficiency of heavy metals, viz., Zn, Fe, Cu, or of other elements such as B, Mg and K (Ref. [2] and references therein).

Buthionine-S-sulfoximine (BSO) is an inhibitor of γ -glutamylcysteine synthetase (EC 6.3.2.2). Thus, its inhibition prevents the formation of metal chelating peptides, i.e. phytochelatin. However, *Chlamydomonas reinhardtii* treated with BSO bound 3-fold more Cd than untreated cells, suggesting that some component other than phytochelatin may bind Cd. Further, the Cd binding component induced by BSO appears to be localized in the cell membrane or in the cell wall and may not be a phytochelatin [12].

Exudation of organic acids

An increased organic acid exudation as a metal resistance mechanism was shown in a number of plant species [2]. Aluminum stress in buckwheat (*Fagopyrum esculentum Moench.*) caused secretion of oxalic acid into rhizosphere and detoxified Al-oxalate complex was taken up by roots and translocated into leaves [11]. Al is excluded from the symplasm by complexation with organic anions into the rhizosphere [2].

Enzymes

Chromium (IV) altered the activities of oxido-reductases in *Chlorella pyrenoidosa* (superoxide dismutase, catalase and peroxidases). Lead treatment to maize seedlings reduced the activity of nitrate reductase, piling up nitrate amount. Also, lead treatment increased aspartate amino transferase activity, while alanine aminotransferase activity decreased [11]. Rauser be referred for additional information on enzymology of phytochelatins and related peptides [4].

Pigment biosynthesis

Heavy metals are reported to inhibit Chl biosynthesis particularly by inhibiting δ -aminolevulinic acid dehydrogenase and protochlorophyllide reductase [13]. Sulphydryl group interaction has been proposed as the mechanism for this inhibition. Cd inhibited the synthesis of 5-aminolevulinic acid and formation of photoreactive protochlorophyllide reductase complex with its substrates. Cadmium affects the free photoconvertible protochlorophyllide reductase complex due to its interference with sulphydryl site on the reductase protein.

Heavy metals usually decreased total Chl and Chl *a/b* ratio in higher plants. In general, carotenoids were less affected by heavy metals, resulting in a lower Chl/carotenoid ratio in higher plants. Cadmium reduced the Chl and accessory pigments before photosynthetic function (Ref. [1] and references therein). Recently, Krupa et al. investigated the relative changes in the content of anthocyanins, Chl *a+b* and total carotenoids in the first leaves of rye seedlings treated with Cd, Pb, Ni and Zn, and concluded that the determination of Chl and total carotenoids seem to be the reliable markers of heavy metal toxicity in higher plants [14].

Heavy metal substitution in chlorophyll

In submerged aquatic plants growing in heavy metal contaminated environment, the central ion of isolated Chl was substituted by heavy metal ions (*in vitro* and *in vivo*). It was discovered that the substitution of the central atom of Chl magnesium by heavy metals (Hg, Cd, Cu, Ni, Zn, and Pb) *in vivo* is an important damage mechanism in metal stressed plants. This substitution prevented photosynthetic light-harvesting in the affected Chl molecules, resulting in the breakdown of photosynthesis, however, the extent of damage

varies with light intensity. In low light irradiance all the central atoms of the Chl are accessible to heavy metals, with heavy metal-Chl being formed, some of which are much more stable towards irradiance than Mg-Chl. Consequently, plants remain green even when they are dead. In high light irradiance, almost all Chl decays, showing that under such conditions most of the Chl are inaccessible to heavy metal ions [15].

Co-stress manifestations

Plants often exhibit multiple stress resistance mechanisms. Seedlings of different plants when pretreated with heavy metals acquired tolerance to lethal and sub-lethal temperature. Conversely, when exposed to heat, the heat-shock proteins synthesized would help to prevent cellular damage against heavy metal toxicity [1]. Thermoprotection by heavy metals by heat-shock cognates, role of heat-shock proteins in protecting membranes from toxic heavy metals in plants is a manifestation of co-stress which evoke coping mechanisms for *vice versa* stress resulting in adaptation at cellular and molecular level [1,9].

Application of metal ion complexation processes in phytoremediation

In the area of environmental management hyper accumulators of metals have tremendous scope (Fig. 4) [16,17].

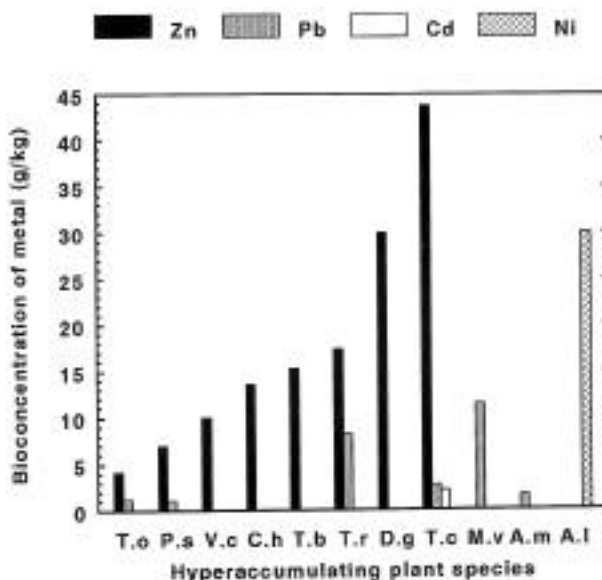


Figure 4. Hyperaccumulators of Zn, Cd, Pb and Ni. A.m - *Armeria maritima* (Plumbaginaceae), C.h - *Cardaminopsis halleri* (Brassicaceae), D.g - *Dichapetalum gelonioides* (Dichapetalaceae), M.v - *Minuartia verna* (Caryophyllaceae), P.s - *Polycarpea synandra* (Caryophyllaceae), T.b - *Thlaspi brachyepetalum* (Brassicaceae), T.c - *Thlaspi caerulescens* (Brassicaceae), T.o - *Thlaspi ochroleucum* (Brassicaceae), T.r - *Thlaspi rotundifolium* (Brassicaceae), V.c - *Viola calaminaria* (Violaceae), A.l - *Alyssum lesbiacum*. (Data from Ref. [17]).

Phytodecontamination, phytomining, phytoextraction and phytostabilization involve the usage of hyperaccumulators. Thus, phytoremediation having reached the brink of commercialization, the knowledge of metal ion complexation in plants seems to be the promising area not only for mineral resource but also for cleanup of the metal contaminated environment.

Acknowledgements

Trace metal research in the author's laboratory is currently being funded by CSIR (38/901/95 EMR- II, Dt 30.10.95), New Delhi; Ministry of Environment and Forests (19/33/95 Dt 28.2.97), Govt. of India, New Delhi.

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