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Review

Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance

Manomita Patra [a,*], Niladri Bhowmik [b], Bulbul Bandopadhyay [c], Archana Sharma [d]

[a] *Department of Biochemistry, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India*
[b] *Department of Genetics and Plant Breeding, Uttarbanga Krishi Biswavidyalaya, Coochbehar, India*
[c] *Department of Botany, Bijoygarh College, Calcutta, India*
[d] *Department of Botany, Centre for Advanced Study, University of Calcutta, 35 Ballygunge Circular Road, Calcutta 700 019, India*

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Abstract

Metals can, when present in excess, or under wrong conditions, and in the wrong places, produce errors in the genetic information system. The present review is limited to three examples of heavy metal genotoxicants, namely arsenic (As), lead (Pb) and mercury (Hg) on plant systems. Exposure to lead is mainly through atmospheric pollutants, to mercury through soil and to arsenic through drinking water.

Toxic metal ions enter cells by means of the same uptake processes as essential micronutrient metal ions. The amounts of metal absorbed by a plant depend on the concentrations and speciation of the metal in the soil solution, its movement successively from the bulk soils to the root surface, then into the root and finally into the shoot. Excessive concentrations of metals result in phytotoxicity through: (i) changes in the permeability of the cell membrane; (ii) reactions of sulphydryl (–SH) groups with cations; (iii) affinity for reacting with phosphate groups and active groups of ADP or ATP; and (iv) replacement of essential ions.

Mercuric cations have a high affinity for sulphydryl groups and consequently can disturb almost any function where critical or non-protected proteins are involved. A mercury ion may bind to two sites of a protein molecule without deforming the chain, or it may bind two neighbouring chains together or a sufficiently high concentration of mercury may lead to protein precipitation. With organomercurials, the mercury atom still retains a free valency electron so that salts of such compounds form a monovalent ion.

The effect of lead depends on the concentration, type of salts and plant species involved. Though effects are more pronounced at higher concentrations and durations, in some cases, lower concentrations might stimulate metabolic processes. The major processes affected are seed germination, seedling growth, photosynthesis, plant water status, mineral nutrition, and enzymatic activities.

The phytotoxicity of arsenic is affected considerably by the chemical form in which it occurs in the soil and concentration of the metalloid. Due to its chemical similarity to phosphorus, arsenic participates in many cell reactions. Specific organo-arsenical compounds have been found in some organisms and arsenic has been reported to replace phosphorus in the phosphate groups of DNA. In view of the variety of reactions in plants that involve sulphydryl groups and phosphorus, arsenites and arsenates may interfere with physiological and biochemical processes which constitute growth in a number of ways.

Mercury, lead and arsenic are effective mitotic poisons (turbagens) at particular concentrations, due to their known affinity for thiol groups and induce various types of spindle disturbances. The clastogenic effects are S-dependent. The availability of

* Corresponding author. Tel.: +91-33-2497-0396.
E-mail address: manomita@vsnl.net (M. Patra).

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cations affect the number of aberrations produced quantitatively. Effects of metallic salts are related directly to the dosage and duration of exposure. Plants, following lower exposure, regain normalcy on being allowed to recover.

Studies on genotoxicity of metals discussed in this review showed that genotoxic effects could be in part responsible for metal phytotoxicity, deserving further examination to elucidate the underlying mechanisms. The most noticeable and consistent effect of mercurials was the induction of c-mitosis resulting in the formation polyploid and aneuploid cells, and c-tumours. Inorganic salts of lead induced numerous c-mitoses together with strong inhibition of root growth and lowering of mitotic activity. As(III) is a weak mutagen but potent comutagen. Genotoxic evaluation of chemical mixtures from soil containing arsenic as component by *Tradescantia* micronucleus assay showed clastogenic effects, but not related specifically to arsenic.

Plants growing on metal-contaminated sites need to develop some degree of tolerance to metal toxicity in order to survive. Since all plants contain at least some metal in their tissues, they clearly are incapable of completely excluding potentially toxic elements, but simply of restricting their uptake and/or translocation. The mechanisms for metal tolerance proposed are: (a) metal sequestration by specially produced organic compounds; (b) compartmentalization in certain cell compartments; (c) metal ion efflux; (d) organic ligand exudation. Inside cells, proteins such as ferritins and metallothioneins, and phytochelatins, participate in excess metal storage and detoxification. When these systems are overloaded, oxidative stress defence mechanisms are activated.

Bacterial plasmids encode resistance systems for toxic metal ions including mercury, lead and arsenic. Chromosomal determinants of toxic metal resistance are also known. For mercury and arsenic, the plasmid and chromosomal determinants are basically the same. The largest group of metal resistance systems functions by energy-dependent efflux of toxic ions.

Mercury-resistant bacteria have genes for the enzymes mercuric ion reductase and organomercurial lyase, which are often plasmid-encoded, and more rarely by transposons and bacterial chromosome. All mercury resistance genes are clustered into an operon. The expression of the operon is regulated and is inducible by Hg(II).

Lead tolerance in *Festuca ovina* is an inherited characteristic, evolved by the production of compounds within the plants, specifically for protection against the toxic effects of heavy metals. A small number of genes are probably producing the major effects, and modifiers for dominance are present, which are probably affected by the genome as a whole.

Arsenic tolerance appears to be genetically controlled in a fairly simple Mendelian manner but the specific mechanisms may be one or several, acting in cohesion. The *ars* operon provides resistance to arsenicals and as well antimonials. Arsenic-resistant bacterial and yeast strains may prove an important tool for identifying the genes for arsenic transporters in higher plants.

Keywords: Arsenic; Lead; Mercury; Genotoxicity; Tolerance

Contents

The problem of metal genotoxicity has acquired new dimensions with the advent of the industrial era. Millions of tonnes of new (not used earlier) trace elements continue to be produced every year from the mines in response to new uses and demands for newer materials. Such metals are released in the biosphere through air, water and soil and ultimately affect the plant, animal and human systems. The annual toxicity of all metals mobilised exceeds the combined total toxicity of all radioactive and organic wastes generated every year from all other sources (Nriagu and Pacyna, 1988).

Metal ions are essential in the maintenance and evolution of all life systems, and also mediate all stages of dissemination of genetic information carried in the genetic code. At the same time, the same metals can, when present in excess, or under wrong conditions, and in the wrong places, produce errors in the genetic information system. These effects can be manifested as alterations in chromosome structure, chromosome number and spindle disturbances, and monitored accordingly. The extensive work carried out by our group has screened the cytotoxic effects of a large number of metallic pollutants (see Sharma and Talukder, 1987).

Although the relative toxicity of different metals to plants can vary with plant genotype and experimental conditions, the metals which, when present in excessive amounts, are the most toxic to higher plants and microorganisms are Hg, Cu, Ni, Pb, Co, Cd and possibly also Ag, Be and Sn (Kabata-Pendias and Pendias, 1984). Metals like mercury, lead and arsenic may be increasingly taken up by plants, especially crops, and transferred further to the food chain (Beijer and Jernelöv, 1986; Devkota and Schmidt, 2000; Jamil and Hussain, 1992; Jung and Thornton, 1997; Kiss et al., 1992). Food plants, which tolerate a relatively high concentrations of these potentially hazardous metals, are likely to create a greater health risk than those which are more sensitive and show definite symptoms of toxicity (Alloway, 1990; Carbonell-Barrachina et al., 1999a,b; Wierzbicka and Antosiewicz, 1993).

The present review is limited to three examples of heavy metal genotoxicants, namely arsenic (As), lead (Pb) and mercury (Hg) on plant systems. These three elements were selected because of the increasing levels of these metals in a variety of crop plants and exposure of populations to high levels following rapid industrialization in India (The Times of India, 1999), presenting three aspects. Exposure to lead is mainly through atmospheric pollutants, to mercury through soil and to arsenic through water, which has been observed to be a major calamity (Chakraborty et al., 1998; Chatterjee and Mukherjee, 1999; Mandal et al., 1996).

Majority of the studies for evaluation of metal genotoxicity was carried out in animal and microbial systems. Studies on putative genotoxic effects of the three metals in higher plants are relatively few, though arsenic, lead and mercury are now known to induce both clastogenic and mutagenic effects in higher plants. It has been shown that the metals induce chromosomal abnormalities and also decreases the rate of cell division. The genotoxic effects depend on the oxidation state of the metal, its concentration and duration of its exposure. In general, effects are more pronounced at higher concentrations and at longer duration of exposures (Bandyopadhyay, 1997; Bhowmik, 2000; Lerda, 1992; Patra, 1999). Plant species respond differently to exposure to the same metal depending on the number of diploid chromosomes, total length of the diploid complement and the number of metacentric chromosomes (Ma et al., 1995). The response was also found to vary with the method of treatment and the plant parts used for exposure (Bhowmik, 2000).

An attempt has been here made to review, in detail, the various studies conducted to determine the impact of the three metals, viz. arsenic, lead and mercury on genetic systems in plants. The study primarily deals

Table 1
General properties of Hg, Pb, and As

Metal	Group in Periodic table	Atomic number and atomic mass	Oxidation state	Common forms	Mode of action with biological ligands
Lead (Pb)	IVa	82 207.19	+2 and +4	Oxides, sulphides, acetates, chlorates and chlorides	(γ Glu–Cys)$_2$Gly, (γ Glu–Cys)$_3$Gly and (γ Glu–Cys), cysteine, acid-soluble thiol, glutathione Lead binds strongly to a large number of molecules like amino acids, several enzymes, DNA and RNA; thus it disrupts many metabolic pathways
Mercury (Hg)	IIB	80 200.6	+1 and +2	Organometallic compounds, inorganic salts	Thiol groups of proteins, $CONH_2$, NH_2 of amino acids and proteins, COOH of amino acids, phosphate group in DNA, cysteine, glutathione High affinity for sulphydryl (–SH), able to disturb functions involving critical or non-protected proteins. A mercury ion may bind to two sites of a protein molecule without deforming the chain, or it may bind two neighbouring chains together. High concentrations induce protein precipitation. With organomercurials, the mercury atom still remains a free valency electron so that salts of such compounds form a monovalent ion
Arsenic (As)	VA	33 74.9216	+3 and +5	Arsenious and arsenic acid, tetra alkylarsonium compounds, cacodyl derivatives, esters of arsenious acids, trimethyl arsine oxide, arsenobetaine and arsenocholine	Phosphate group of DNA, sulphydryl groups of proteins and thiol groups, cysteine, acid-soluble thiol, glutathione, participate in many cell reactions, either by replacing phosphorus in the phosphate groups of DNA or by reacting with enzymes

with the clastogenic and mutagenic potency of the three metals, and explores the mechanism of genetic effects, the factors affecting the intensity of genotoxicity, variable effect in different plants and finally the induction of tolerance against the toxic effects.

The mode of uptake and assimilation of the three metals (Table 1) has been briefly described in the beginning because better knowledge of these processes in various plant parts is crucial in understanding the genetic effects of the metals on plant systems and in studying the tolerance of plants to these metals.

1. Uptake and assimilation in plants

Toxic metal ions enter cells by means of the same uptake processes that move essential micronutrient metal ions. Class A metals (e.g. K, Ca, Mg) preferentially bind with oxygen-rich ligand (e.g. carboxylic groups), class B metals (e.g. Hg, Pb, Pt, Au) preferentially with sulphur- and nitrogen-rich ligands (e.g. amino acids), and borderline metals (e.g. Cd, Cu, Zn) show intermediate preferences, with the heavier metals tending towards class B characteristics (Nieboer and Richardson, 1980).

Plants may receive trace metals from either their aboveground surfaces, their roots or some combination of the two. The amounts of metal absorbed by a plant depend on: (i) the concentrations and speciation of the metal in the soil solution; (ii) its movement from the bulk soils to the root surface; (iii) transport from the root surface into the root; and (iv) its translocation from the root to the shoot (Wild, 1988). The potential mobilization of metals in soil depends primarily on metal content, dissolved organic matter, soil pH and soil characteristics like clay, oxides and cation exchange capacity (Kalbitz and Wenrich, 1998). Plant uptake of mobile ions present in the soil solution is largely determined by the total quantity of this ion in the soil but, in the case of strongly adsorbed ions, absorption depends more upon the amount of root produced. Excessive transfer of metal ions from contaminated soil to the food chain is controlled by a 'soil–plant barrier', which sometimes fails to work for certain metals like arsenic, lead or mercury, leading to contribution of more than half of the human lead intake through food of plant origin (Dudka and Miller, 1999).

1.1. Mercury

Since mercury is not very phytotoxic in normally occurring concentrations, information is meagre about its uptake and metabolism in plants. The absorption of organic and inorganic mercury from soil by plants is low (Lodenius, 1990; Rauter, 1976) and there is probably a barrier to mercury translocation from plant roots to tops. However, mercury containing pesticide/fungicide spray residues are, in some cases, taken up by plants (e.g. rice) and translocated to edible portions. Mercury salts in soil may be reduced by biological and chemical reactions to metallic or methylated compounds, which may volatilize and be taken up through the leaves in plants grown in enclosed spaces. Factors affecting plant uptake include external mercury concentration and exposure time, soil or sediment organic content, carbon exchange capacity, oxide and carbonate content, and redox potential (Cho and Park, 1999; Crowder, 1991). Differences in mercury content between tissues of different types and ages constitute a major source of within-site variation between trees of the same species (Rasmussen et al., 1991).

A part of mercury emitted from the source into the atmosphere is absorbed by plant leaves, and migrates to humus through fallen leaves. Airborne mercury thus seems to contribute significantly to the mercury content in crops (Mosbaek et al., 1988). Higher mercury content was recorded in fruits of plants grown close to highly industrialized areas (Wojciechowska-Mazurek et al., 1995).

Accumulation, toxicity response, and distribution of mercury differed between the exposure to elemental Hg^0 through shoots or ionic Hg^{2+} through roots. In plants exposed to Hg^0, mercury accumulated in the shoots with no movement to roots. Root-exposed plants showed accumulation of mercury with movement to the shoots by tenth day. Inhibition of root and shoot growth occurred at 1.0 μg/ml and above, with very limited tissue damage at higher treatment levels (Suszcynsky and Shann, 1995).

1.2. Lead

Plant absorbs lead and accumulation has been reported in roots, stems, leaves, root nodules and seeds, etc. which increases with the increase in the exogenous lead level. Majority of the lead absorbed by plants reside in the root with only a small fraction was translocated upward to the shoots. Histochemical observations in barley and maize seedlings showed that, in roots, lead was distributed in outer root cap and slime covering root and cap surface, in cell walls of rhizodermal and cortical cells. Almost no lead penetrated endodermis and entered the stele. Thus, endodermis acts as a barrier to lead uptake to shoots (Sobotik et al., 1998). Soil solution lead is the immediate source of plant roots; concentration in roots being linearly related to the total soil concentration. Low soil pH (3.9) caused increased mobility of lead and resulted in higher uptake (Ernst et al., 2000). Increasing soil pH (3.9–6.7) caused a reduction of lead uptake by Italian ryegrass in pot experiments (Gorlach et al., 1990). In addition to soil factors, uptake and accumulation of lead differ with plant species.

1.3. Arsenic

Arsenic in inorganic and organic forms used previously as pesticides, plant defoliants, and herbicides may accumulate in agricultural soils and in plants. The phytoavailability of arsenic is primarily determined by arsenic species and concentration in the medium (Burlo et al., 1999; Carbonell-Barrachina

et al., 1999a). Plants readily take up arsenite and arsenate, the major forms of arsenic, which is greatly influenced by soil texture and competing phosphates. Low levels of phosphates displace arsenic from soil particles to increase uptake and phytotoxicity, while larger amounts of phosphates compete with arsenic at root surfaces to decrease uptake and phytotoxicity (Peterson et al., 1981). Plant accumulation of arsenic can be affected by other factors, like plant species, type of compound and method of application. It is rare that arsenic accumulation in plants may reach levels that are harmful to animals and man (Bandyopadhyay, 1997; Dudka and Miller, 1999).

Pickering et al. (2000) studied the biochemical fate of arsenic in Indian mustard (*Brassica juncea*). After arsenate uptake by the roots, possibly via the phosphate transport mechanism, a small fraction is exported to the shoot via the xylem as the oxyanions (arsenate and arsenite). Once in the shoot, the arsenic is stored as an As(III)–tris thiolate complex. The majority of the arsenic remains in the root as an As(III)–tris thiolate complex, which is indistinguishable from that found in the shoot and from As(III)–tris glutathione.

The rate of arsenic uptake by plants increases with the rate of plant growth. However, growth per unit of arsenic uptake was higher for plants in untreated soils than plants in arsenic treated soils (Onken and Hossner, 1995). Arsenite uptake was active, and was taken up at approximately the same rate as arsenate (Abedin et al., 2002).

2. Metal toxicity

Studies on micronutrient requirements and toxicity effects of trace metals on both soil organisms and native plants in field conditions are limited. Usually, the effects of metals on key test organisms and native plants are examined in sterile and much simplified laboratory conditions, which may differ from field conditions in different degrees (Ross, 1994). Much research has been directed at the effects of metals on food plant production and, until recently, rather less on trace metal cycling in natural ecosystems.

To understand the effects of toxic metals on soil–plant systems, studies on a number of aspects is required. These include characteristics of the toxic metal, like metal chemical forms; bioavailability; residence time(s) in the organism(s) under study; distribution of dose over time; route of exposure (leaf, shoot and root); mechanisms and effects of toxic metal action; interactions with other metals; cumulative effects; acute and chronic effects; developmental stage of the entity affected; targets of action, like reproductive functions, respiratory functions, photosynthetic processes, genetic material; and increase/decrease in populations.

The main problems in such studies are: (i) phytotoxicity thresholds differ with plant species; (ii) soil properties influence the rates at which metals transfer to plants; (iii) roots may sequester metals and prevent or reduce translocation to the leaves; (iv) no chemical or toxicant interactions are taken into account; and (v) changes in foliar chemistry may be influenced by other environmental factors such as water availability, pH, redox or salinity.

Excessive concentrations of metals result in phytotoxicity through: (i) changes in the permeability of the cell membrane; (ii) reactions of sulphydryl (–SH) groups with cations; (iii) affinity for reacting with phosphate groups and active groups of ADP or ATP; and (iv) replacement of essential ions (mainly major cations).

2.1. Mercury

Mercuric cations have a high affinity for sulphydryl (–SH) and consequently can disturb almost any function where critical or non-protected proteins are involved (Clarkson, 1972). A mercury ion may bind to two sites of a protein molecule without deforming the chain, or it may bind two neighbouring chains together or a sufficiently high concentration of mercury may lead to protein precipitation. With organomercurials, the mercury atom still retains a free electron so that salts of such compounds form a monovalent ion. The toxic action of mercurials may also be related to a non-specific inhibition of a variety of intracellular enzymes and several specific thiol-containing respiratory enzymes in vitro.

Maximum work has been carried out on seed germination and seedling growth of different plant species in field exposed to mercurials (Bonifacio and Montano, 1998; Fargasova, 1994; Setia and Bala, 1994; Varshney, 1991). Mercury increased the levels of photosynthetic pigments viz. chlorophylls and caretenoids

at a shorter exposure time but decreased the same at prolonged duration of exposure. It affects both light and dark reactions of photosynthesis. It strongly inhibits photosynthetic electron transport chain, Photosystem II (PSII) being the most sensitive target. Hg^{++} ions interact with the intermediates Zn^{++} situated in the D_1 and D_2 proteins and with the manganese cluster in the oxygen evolving complex which are located on the donor side of PSII, and also with the chlorophyll a dimer in the core of PSI (P700). Donor side of PSII is affected by preventing chloride binding and/or function and P700 is oxidized in the dark by mercuric chloride. Hg^{++} ions form organometallic complexes with amino acids of chloroplast proteins and also caused depletion of a polypeptide (33 kDa) of PSII submembrane (Bernier and Carpentier, 1995; Bernier et al., 1993; Sersen et al., 1998; You et al., 1999). The extent of toxicity and the mechanism influencing the photosynthetic apparatus depend largely on the system in vitro or in vivo, as well as on the age of the plants used (Krupa and Baszynski, 1995; Shaw and Rout, 1998). Mercury was found to inhibit the water channels in the membrane of higher plant cells. At concentrations >1 mg/l, mercury hastens membrane lipid peroxidation, disrupts membrane structural integrity and increased the membrane permeability in rape seedlings (Ma, 1998). Mercuric chloride was found to reduce the hydraulic conductivity of wheat root cells and rapidly depolarized the membrane potential of the root cells (Zhang and Tyerman, 1999). In leaf discs of *Quercus robur*, $HgCl_2$ (1–20 μM) caused a concentration-dependent decline in both non-protein thiol and glutathione levels and induction of a dose- and time-dependent glutathione *S*-transferase (Gullner et al., 1998). Phenylmercury compounds have been shown to hasten plant senescence. Other aspects include laboratory experiments to test the relative efficacy of mercury compounds against fungal cultures; effects against plant growth (shoot and root) and cell cultures (Thangavel et al., 1999).

2.2. Lead

The effect of lead depends on the concentration, type of salts, soil properties and plant species involved. In general, effects are more pronounced at higher concentrations and durations. In some cases, lower concentrations stimulate metabolic processes and enzymes involved. The major processes affected are seed germination, seedling growth (shoot and root growth), photosynthesis, plant water status, mineral nutrition, and enzymatic activities.

Visible symptoms include chlorotic spots, necrotic lesions etc. in leaf surface, senescence of leaf (due to reduced chlorophyll, DNA, RNA, protein, and dry weight, ratio of acid to alkaline pyrophosphatase activity, activities of protease and RNase), and stunted growth.

Germination of seeds is drastically affected at higher concentrations. Development and growth of root and shoot in seedling stage are also affected, roots being more sensitive. Initiation of lateral roots is most sensitive (Fargasova, 1994; Malone et al., 1978; Mesmar and Jaber, 1991).

Photosynthesis is one of the most sensitive processes to lead. The effects are multifacial affecting both in vivo and in vitro photosynthetic CO_2 fixation. Long term exposure results in reduced leaf growth, decreased photosynthetic pigments, changed chloroplast structure, and decreased enzyme activities for CO_2 assimilation (Parys et al., 1998; Shearan and Singh, 1993). The total chlorophyll content and relative content proportion of Chlorophyll a and b were reduced, through inhibition of chlorophyll biosynthesis (Ernst et al., 2000; Sinha et al., 1993; Van Assche and Clijsters, 1990). The substitution of the central atom of chlorophyll, magnesium, by lead in vivo prevents photosynthetic light-harvesting in the affected chlorophyll molecules, resulting in a breakdown of photosynthesis (Kupper et al., 1996).

Higher concentrations of lead significantly affected plant water status causing water deficit. Transpiration intensity, osmotic pressure of cell sap, water potential of xylem, and relative water content were significantly reduced (Parys et al., 1998). Lead also reduces the size of stomata but increases their number and diffusion resistance. Lead reduced the uptake and transport of nutrients in plants, such as Ca, Fe, Mg, Mn, P and Zn, by blocking the entry or binding of the ions to ion-carriers making them unavailable for uptake and transport from roots to leaves (Xiong, 1997).

2.3. Arsenic

The phytotoxicity of arsenic is affected considerably by the chemical form in which it occurs in the soil and concentration of the metalloid; water-soluble

form being more phytotoxic than other firmly bound forms (Tang and Miller, 1991). Arsenite, As(III) is more phytotoxic than arsenate, As(V) and both are much more phytotoxic than monosodium methane arsenic acid (MSMA) (Sachs and Michael, 1971). In rice, while application of arsenate did not affect plant growth, both As(III) and monomethyl arsenic acid (MMAA) were phytotoxic (Marin et al., 1992). In contrast, Carbonell-Barrachina et al. (1999b) found that organic arsenicals (MMAA and dimethyl arsenic acid (DMAA)) were translocated upward in greater amount in turnip and thus more phytotoxic than their inorganic counterparts (arsenite and arsenate).

Inorganic arsenic inhibits enzyme activity and trivalent inorganic arsenic reacts with the sulphydryl groups of proteins affecting many enzymes containing such groups (Thompson, 1948; Webb, 1966). The marked inhibitory effects of As(III) on mitochondrial respiration mediated by NAD-linked substrates, appear to play a critical role in toxicity.

Due to its chemical similarity to phosphorus, arsenic participates in many cell reactions. Specific organo-arsenical compounds have been found in some organisms and arsenic has been reported to replace phosphorus in the phosphate groups of DNA (Dixon and Webb, 1958). In view of the variety of reactions in plants that involve sulphydryl groups and phosphorus, arsenites and arsenates may interfere with physiological and biochemical processes which constitute growth in a number of ways. It competes with P-uptake of plants and caused P-deficiency resulting in appearance of dark red leaves (Otte and Ernst, 1994). Organo-arsenicals can apparently be metabolized. The carbon–arsenic bond is apparently stable in plants but is rapidly broken down in soils (Von Endt et al., 1968).

Inhibition of various physiological and biochemical processes by the metals result in consequent reduction in morphological characters and economic yield of agricultural and horticultural crops. Major characters affected are tillers (in cereals), plant height, leaf number and area, pod number and length (in legumes), and dry matter production (Bhowmik and Sharma, 1999).

3. Genotoxic effects

Observations on putative metal genotoxicity are scarce and majority of the cellular and molecular aspects of metal toxicity in plants are unknown, even though deleterious effects on crop production have long been recognized. Most metallic salts are effective mitotic poisons (turbagens) at particular concentrations, due to their known affinity for thiol groups and induce various types of spindle disturbances. The clastogenic effects are S-dependent. Most metals, when administered to higher plants in vivo are clastogenic at certain concentrations and durations of exposure.

In plant systems in vivo, solubility of the salt in water is of primary importance. The degree of dissociation and the availability of cations affect the number of aberrations produced quantitatively. The viscosity of the plasma may be changed through changes in the ionic environment, and/or formation of chelated complexes, leading to spindle dysfunction.

Effects of metallic salts are related directly to the dosage and duration of exposure. Plants, following lower exposure, regain normalcy on being allowed to recover. With respect to effects on cell division, metals may be classified in descending order as:

- very marked effects: Cd, Cu, Hg, Cr, Co, Ni, Be;
- marked effects: Zn, Al, Mn, Fe, Se, Sr, Sb, Ca, Ti;
- relatively less active: Mg, V, As, Mo, Ba, Pb.

Salts of groups IV and VII were significantly mitostatic as compared to control, but those of II and VIII less so. Chromosomal abnormalities induced by metallic salts of groups IV and VII were significantly higher than those of group III.

3.1. Mercury

For more than 60 years or more, numerous experiments have been carried out to study the genetic effects of mercury compounds in experimental test systems using a variety of endpoints (See De Flora et al., 1994 for review).

In the earliest work, Sass (1937) recorded multinucleate cells in root tips of corn seedlings exposed to solutions of New Improved Ceresan (a fungicide containing ethyl mercury phosphate). The most noticeable and consistent effect of mercurials was the induction of c-mitosis through disturbance of the spindle activity, resulting in the formation polyploid and aneuploid cells, and c-tumours (Kostoff, 1939, 1940). C-mitosis was induced at similar dosages of all compounds tested, butyl mercury bromide being the most active

Table 2
Genotoxic effects of mercury screened in laboratory tests

Genetic endpoint	Target species	Target organ	Compounds tested	Dose or dose range	Reported effects	Reference
Chromosomal damage	*Zea mays*	Seedlings	New improved ceresan	0.1%	+	Sass, 1937.
	Secale cereale, Triticum durum, T. persicum, T. polonicum, T. aegilopoides, Pisum sativum, Linum usitatissimum, Crepis capillaris	Germinating grains	Granosan	0.1–5%	+	Kostoff, 1939, 1940.
	Allium cepa	Meristematic roots	PMH and PMN	0.01 mg/kg	+	MacFarlane, 1956
			Panogen 5	1.9–2.5 M	+	Ramel, 1969
			Panogen 8	3.2–8.0 M	+	
			MMH	2.0–8.0 M	+	
			PMH	4.0–8.0 M	+	
			MOEMC	16–31 M	+	
			Betoxin	0.2–3%		Fiskesjö, 1969.
			MMC	5–20 M		
			EMC	2–5 M		
			BMC	1–2 M		
			MOEMC	1–10 M		
Mitosis; chromosomal aberration	*Allium cepa*	Meristematic roots	$HgCl_2$	0.5, 1.0 and 5.0 mg (for 12, 24 and 48 h)	+	Agar and Uysal, 1997.
Chromosomal aberration; polyploidy	*Hordeum vulgare*	Seeds	PMA	10^{-1} to 10^{-7} M	+	Gautam et al., 1994.
Mitosis; chromosomal aberration	*Allium cepa, A. sativum*	Meristematic roots	$HgCl_2$ and Hg_2Cl_2	0.001–1 mg/kg	+	Patra, 1999.
	Hordeum vulgare	Meristematic roots	$HgCl_2$	0.001–1 mg/kg	+	Patra, 1999, PhD thesis
	Hordeum vulgare	Seeds	MMC	10^{-5} to 10^{-3} M	+	Subhadra and Panda, 1994.
	Vicia faba	Meristematic roots	MMH	0.1–6.4 M	+	Ramel, 1972.
	Tradescantia virginiana	Meristematic roots	Panogen 15	0.001–0.005 mg/kg	+	Ahmed and Grant, 1972.
	Allium cepa	Exposure of meristematic roots	MOEMC	0.01–10 mg/l	+	Fiskesjö, 1988.
	Hydrilla verticillata	Meristematic roots	$HgCl_2$ and Hg_2Cl_2	Various concentrations	+	Pal and Nandi, 1989, 1990
	Allium cepa	Meristematic roots	Hg^{+2} water	Various concentrations	+	Pal and Nandi, 1989, 1990
	Lathyrus sativus	Exposure of meristematic roots	$HgCl_2$	10^{-1} to 10^{-5} M	+	Gupta and Ghosh, 1992.
	Nigella sativa	Exposure of meristematic roots	Hg	NR	+	Chaudhuri et al., 1993.

Table 2 (*Continued*)

Genetic endpoint	Target species	Target organ	Compounds tested	Dose or dose range	Reported effects	Reference
Micronuclei	*Allium cepa*	Exposure of meristematic roots	$HgCl_2$, MMC, PMA, MOEMC (Emisan-6)	0.0001–0.01 mg/kg	+	Dash et al., 1988.
	Allium cepa	Exposure of meristematic roots	Hg contaminated water	5×10^{-6} to 10^{-5}	+	Panda et al., 1988, 1989.
			MMC	>9.6 mg Hg/kg solid waste	+	
	Eichhornia crassipes	Exposure of meristematic roots	MMC	0.001–0.005 mg/kg	+	Panda et al., 1988.
Mitosis; micronucleus	*Hordeum vulgare*	Exposure of seeds	MMC	10^{-4} M	+	Patra et al., 1995.
	Hordeum vulgare, *Allium cepa*	Shoot cells, root meristematic cells	MMC	–	+	Patra et al., 2000.
	Allium cepa	Exposure of meristematic roots	MMC	1.26×10^{-6} M	+	Panda et al., 1995.
	Vicia faba	Exposure of meristematic roots	Hg^{2+}	0.005 mg/kg	+	Duang and Wan, 1995.
Mitosis; nucleoli	*Allium cepa*	Exposure of meristematic roots	$HgCl_2$	10^{-7} mg/kg	+	Liu et al., 1995a
Meiosis	*Hordeum vulgare*	Pollen mother cells, embryonic shoots	Hg	0.1–5 mg/kg	+	Panda et al., 1992.

+:Toxic effects; NR: not reported. Chemically defined compounds were referred to by the following abbreviations—mercury: Hg; phenyl mercury hydroxide: PMH; phenyl mercury nitrate: PMN; methylmercury hydroxide: MMH; phenylmercury hydroxide: PMH; methoxyethylmercury chloride: MOEMC; methylmercury chloride: MMC; ethylmercury chloride: EMC; butylmercury bromide: BMC; methoxyethylmercury chloride: MOEMC; mercury chloride: $HgCl_2$; phenylmercury acetate: PMA; methylmercury hydroxide:MMH.

Table 3
Genotoxic effects of lead observed in laboratory experiments

Genetic endpoints	Target species	Target organ	Compounds tested	Dose or dose range	Reported result	Reference
Mitosis; mutation	*Allium cepa*	Root meristem cells	$Pb(NO_3)_2$	$3.0\,mg\,dm^{-3}$	Lowering of mitotic activity; numerous c-mitoses; lead chloride more clastogenic than lead nitrate; low lead doses potentially mutagennic	Mukherji and Maitra, 1976; Wierzbicka, 1988
			$PbCl_2$	$2.5\,mg\,dm^{-3}$		
Mitosis	*Allium cepa*	Root meristem cells	$PbCl_2$ or $Pb(NO_3)_2$	$1–3\,mg\,dm^{-3}$	Inhibition of cytokinesis after c-mitosis leading to polyploidy or binucleate cells	Wierzbicka, 1989
			$PbCl_2$		Prolonged (approximately three-fold) cell cycle of binucleate cells	
			$PbCl_2$	3.2.5 μg/ml	Root growth and mitotic activity gradually inhibited, c-mitosis about 40%	Wierzbicka, 1994
			$Pb(NO_3)_2$	10^{-7} to 10^{-2} M	C-mitosis, anaphase bridges and chromosome stickiness; micronuclei in interphase cells; irregularly shaped nuclei and nuclei with decomposed material	Liu et al., 1994, 1995b
			$Pb(NO_3)_2$	Up to 5 mg/l	Reduction in mitotic activity	Ochoa et al., 1992
			Pb salt	0.0001, 0.001, 0.01, 0.05, 0.1, 0.2 mg/kg	Reduction in frequency of mitotic cells	Lerda, 1992
			$Pb(NO_3)_2$	0.001, 0.01, 0.1, 1 mg/kg (for 6, 12, 18, 24, 48, 72, 96, 120 h)	Mitostatic effects; mostly spindle disturbances leading to c-mitosis and polyploidy; period required for recovery directly proportional to concentration and duration	Bhowmik, 2000
	Allium cepa, *Hordeum vulgare*, *Trigonella foenum-graecum*, *Coriandrum sativum*		$Pb(NO_3)_2$	0.001, 0.01, 0.1, 1 mg/kg	Gradual reduction in mitotic index and increase in frequencies of chromosomal aberration, noted with increase in concentration. Relative sensitivity depends on the genotype involved and to the mode of exposure (seed or bulb). *A. cepa* bulbs were found to be most sensitive followed successively by seeds of *H. vulgare*, *C. sativum* and *T. foenum-graecum*	Bhowmik, 2000
	Allium ascalonicum		$Pb(OAc)_2$	10^{-3}, 10^{-4}, 10^{-5} M/ml	Bridges, stickiness, achromatic mass, diplochromosomes, centromeric splits, fragmentation, dissolution of chromosomes	Savic et al., 1989
	Vicia faba		Pb^{2+}	NR	Mitotic stage shortened and interphase prolonged, thus prolonging the cell cycle; mitotic index and frequency of micronuclei increased with increase in the concentration below 1 ppm but decreased with higher concentrations; chromosomal aberrations increased with increase in concentrations below 5 ppm	Qun and Xiao, 1995

Table 3 (*Continued*)

Genetic endpoints	Target species	Target organ	Compounds tested	Dose or dose range	Reported result	Reference
	Zea mays	Seeds	$(Me)_3PbCl_2$, $(Et)_3PbCl_2$- and $(Pr)_3PbCl_2$, $PbCl_2$, $(Me)_4Pb$, $(Et)_4Pb$, $(Pr)_4Pb$	0, 10, 20, 40 μg/g	Mitotic activity decreased with increasing length of alkyl radical chain and increasing lead concentration	Radecki et al., 1989
	Helainthus annuus, *Picea abies*		Pb salt	NR	Toxic effects induced both at low and high concentrations	Chakravarty and Srivastava, 1992; Baycon et al., 1994
Micronucleus	*Allium cepa*, *Tradescantia virginiana*, *Vicia faba*	Root meristem cells in *Allium* and *Vicia*; pollen mother cells in *Tradescantia*	Pb^{2+}	1–1000 mM	Dose-dependent increase in micronucleus (MCN) in all three systems; induction of MCN in *Tradescantia* pmc within a range between 1 and 10 mM; significant induction of MCN in root tip cells only at higher concentrations (10–1000 mM)	Steinkellner et al., 1998
	Tradescantia virginiana	Pollen mother cells	$Pb(OAc)_4$	44×10^{-3} M	Induction of micronucleus	Sandhu et al., 1989
DNA synthesis	*Pisum sativum*	Root meristem cells	Pb^{2+}	10^{-4} M	Diminished DNA synthesis	Gabara et al., 1992
RNA content (Cytophotom-etric analysis)	*Pisum sativum*	Root meristem cells	Pb^{2+}	NR	Reduction of RNA content of nucleolus but enhanced RNA level in nucleus and cytoplasm, resulting in the increase in total amount of RNA in root cells	Lbik-Nowak and Gabara, 1997
Mutation	*Arabidopsis thaliana*	Seeds	$Pb(NO_3)_2$	NR	Mutagenic effects	Dineva et al., 1993
Cell growth (suspension culture)	*Glycine max*	Cells	$(Et)_3Pb$	50–200 μM	Inhibition of growth leading to lethality	Stournaras et al., 1984
	Funaria hygrometrica	Gametophytes	Pb^{2+}	NR	Significant increase in G+C rich repetitive DNA sequences in nucleus; formation of well-defined agglomerates, generally located adjacent to nucleolar region, which increased in both size and number	Bassi et al., 1995
Mitotic cell division in vitro	*Dianthus caryophyllus*, *Musa paradisiaca*	Callus (*Dianthus*); rooted plantlets (Musa)	$Pb(NO_3)_2$	0, 1, 10, 15, 20, 40 ppm (*Dianthus*); 0.001, 0.01, 0.05, 0.1 ppm (Musa)	Reduction in mitotic index was proportional to the increase in concentrations and durations of exposure. Reduction in mitotic index was more in the callus tissue of *Dianthus* compared to root tip cells of Musa	Bhowmik, 2000

NR: Not reported. Chemically defined compounds were referred to by the following abbreviations—lead nitrate: $Pb(NO_3)_2$; lead chloride: $PbCl_2$; lead acetate: $Pb(OAc)_2$; trimethyl lead chloride: $(Me)_3PbCl_2$; triethyl lead chloride: $(Et)_3PbCl_2$; tripropyl lead chloride $(Pr)_3PbCl_2$; tetramethyl lead: $(Me)_4Pb$; tetraethyl lead: $(Et)_4Pb$; tetrapropyl lead: $(Pr)_4Pb$; lead tetraacetate: $Pb(OAc)_4$.

(Fiskesjö, 1969). Organomercurials had been reported to be 200 times more potent than inorganic mercury (bromide) in inducing c-mitosis (Ramel, 1969).

Exposure to inorganic salts of mercury in *Allium cepa* and *Allium sativum* reduced mitotic index in the root tip cells and increased the frequency of chromosomal aberrations in degrees directly proportional to the concentrations used and to the duration of exposure (Patra, 2000). The period needed to regain normalcy after removal of mercury was inversely related to the concentration of the chemical and duration of exposure. The lowest effective concentration tested (LECT) was 10 ppm. Cytotoxic effects of mercuric chloride were greater than mercurous chloride. *A. sativum* was more resistant than *A. cepa* (Patra and Sharma, 2002), possibly due to the presence of greater amount of heterochromatin in the former and to the lower amount of sulphur compounds with affinity for mercury in the latter. *Hordeum vulgare* seeds were less effected than *A. cepa* when exposed to mercuric chloride for short period (Patra and Sharma, 1999). Genotoxic effects of mercury have been summarised in Table 2.

3.2. Lead

Inorganic salts of lead (chlorides and nitrates) induced numerous c-mitoses together with strong inhibition of root growth and lowering of mitotic activity.

Table 4
Genotoxic effects of arsenic observed in laboratory experiments

Genetic effects	Plants used	Exposure to arsenic		Effects	References
		Form	Concentration		
Mitosis	*Allium cepa*	As_2O_3	NR	Decline in mitotic index and induction of chromosomal aberrations. 0.001–0.005 M range of concentrations was found to be most cytotoxic	Sinha et al., 1996.
	Allium cepa, *Hordeum vulgare*	Na_3AsO_3, $Na_2HAsO_4{\cdot}7H_2O$	0.001–10 mg/kg for 6–120 h	Decrease in mitotic index more prominent with As(III) than As(V). Mitotic index reduced by higher doses. Prolonged exposure showed both mitostatic and clastogenic effects. Plants, exposed to lower concentration, regained normalcy on being transferred to arsenic free media	Bandyopadhyay and Maity, 1995; Bandyopadhyay, 1997.
	Hordeum vulgare	As-contaminated ground water	NR	Increase in bridges and fragments in anaphase	Constantin et al., 1980.
Mitosis; micronucleus	*Tradescantia virginiana*	Arsenicals	NR	Micronucleus formation, clastogenic effects	Gill and Sandhu, 1992; Ma et al., 1992.
Micronucleus	*Allium cepa*, *Vicia faba*, *Tradescantia virginiana*	As^{3+}	1–1000 mM	Dose-dependent increase in micronucleus (MCN) in all three systems. Induction of MCN in *Tradescantia* pmc at lower concentration compared to root tip cells	Steinkellner et al., 1998.
Sister chromatid exchange	*Vicia faba*	Arsenicals	0.26–1.07 mg/l for 3 h	Significant increase of sister chromatid exchange	Gómez-Arroyo et al., 1997.

Chemically defined compounds were referred to by the following abbreviations—arsenic trioxide: As_2O_3; sodium arsenite: Na_3AsO_3; sodium arsenate: $Na_2HAsO_4{\cdot}7H_2O$.

However, effects in this range do not constitute a genetic threat in the natural environment since such high doses of lead do not normally occur. The lower dose resulted in slight but statistically significant clastogenic effects without disturbing mitotic activity. Lead chloride was more clastogenic than lead nitrate. Low doses appeared to be potentially mutagenic (Mukherji and Maitra, 1976; Wierzbicka, 1998).

The chemical form of lead only affects lead transport from the medium into the plants and all forms had similar effects on mitosis. Lead nitrate proved to be a weak mutagen but owing to its high toxicity had a synergistic effect in combination with ionizing radiation in some populations (Dineva et al., 1993). The iodides had a greater mutagenic effect than the nitrates, perhaps because the latter dissolved completely in the solution and were supplied as ions, rather than molecules as in the cases of the iodides (Radecki et al., 1989; Reutova, 1993). The genotoxicity of lead in different test systems has been summarised in Table 3.

In species of *Allium* bulbs treated with lead compounds, aberrations of the non-specific type prevailed, like anaphase bridges, stickiness, achromatic mass, diplochromosomes, centromeric splits, fragmentation and dissolution of chromosome (Bhowmik, 2000; Savic et al., 1989; Wierzbicka, 1989).

3.3. Arsenic

Jacobson-Kram and Montalbano (1985) and Leonard (1984) suggested that arsenicals are unable to induce gene mutations in microorganisms or eukaryotes. However, arsenicals can cause damage to DNA as indicated by positive results obtained in Rec-assays on *Bacillus subtilis*. Inhibition of enzymes involved in DNA repair by arsenic may be responsible for the DNA damage (Schaumloffel and Gebel, 1998). As(III) is a weak mutagen but potent comutagen (Hartwig and Beyersmann, 1989; Rossman et al., 11988; see Table 4).

Genotoxic evaluation of chemical mixtures from soil containing arsenic as component by *Tradescantia* micronucleus assay showed clastogenic effects, but not related specifically to arsenic (Gill and Sandhu, 1992; Ma et al., 1992). In *H. vulgare* seeds, Bandyopadhyay and Maity (1995) observed that the percentage of germination was inversely proportional to the concentration of the salt, except for very low doses. Effects on growth rate of roots were variable. The highest concentration was mitostatic. Frequency of chromosomal abnormalities was directly proportional to the concentration of salt and inversely proportional to the period of recovery. Plants, exposed to the lower concentrations, recovered within 96–120 h. The most frequent abnormalities involved disturbance of the spindles and indicated that the principal effect of the chemical is due to its known affinity for thiol groups. In *A. cepa*, mitotic index was observed to be reduced and chromosomal aberrations increased both by sodium arsenate and sodium arsenite, being directly related to the concentration of the chemicals and duration of exposure (Bandyopadhyay, 1997).

4. Tolerance

Plants growing on metal-contaminated sites need to develop some degree of tolerance to metal toxicity in order to survive. Tolerance to metals can either be achieved by avoiding the metal stress, by tolerating it or both (Levitt, 1980). Avoidance by exclusion is the most common mechanism of plant adaptation to metal toxicity. It depends on various kinds of reduced metal uptake: (i) by deposition in cell wall components; and (ii) by chelate secretion (Meharg, 1993).

Tolerance to metal stress relies on plant capacity to detoxify metals having entered cells. The mechanisms for metal tolerance proposed are: (a) metal sequestration by specially produced organic compounds; (b) compartmentalization in certain cell compartments; (c) metal ion efflux; (d) organic ligand exudation.

Plant protection against metal toxicity involves, with others, the control of root metal uptake and of long distance metal transport. Inside cells, proteins such as ferritins, metallothioneins and phytochelatins and related peptides, participate in excess metal storage and detoxification, together with low molecular weight organic molecules, mainly organic acids and amino acids and their derivatives. When these systems are overloaded, oxidative stress defense mechanisms are activated. The naturally tolerant plants which hyperaccumulate metals form the basis for investigations on the improvement of metal resistance (Briat and Lebrun, 1999). The largest group of metal resistance systems function by energy-dependent efflux of toxic ions (Silver, 1996; Silver and Phung, 1996).

The ferritins are a class of ubiquitous multimeric iron-storage proteins able to sequester several thousand iron atoms per molecule (Harrison and Arosio, 1996). Although not documented in plants, it is important to note that animal ferritins are also able to store metals like lead (Price and Joshi, 1982).

Metallothioneins are small proteins that sequester excess amounts of certain metal ions. Their synthesis is transcriptionally activated by metal ions. Plant metallothioneins have received little attention until it was reported that plants indeed contain functional metallothionein homologs. Two *Arabidopsis thaliana* cDNAs, named MT1 and MT2, share all the structural characteristics of yeast metallothioneins (Zhou and Goldsbrough, 1994). Since then, three protein bands, corresponding to six MT genes, have been isolated from *Arabidopsis*, and the amino acids sequenced for nine fragments (Rauser, 1999).

The term phytochelatin (PC) have been given to a unique family of thiol containing metal-binding polypeptides derived from glutathione (GSH) (see Rauser, 1990 for review). Maitani et al. (1996) used root cultures of *Rubia tinctorum* and confirmed that arsenic, lead and mercury induced PCs. The analysis of a PC-deficient mutant of *Arabidopsis* showed a detoxifying role for PCs against mercury (Howden and Cobbett, 1992). Exposure to excess of arsenate and arsenite induced the biosynthesis of phytochelatins in vivo and in vitro. The rapid induction of the metal-binding PCs has been reported in cell suspension cultures of *Rauvolfia serpentina*, in seedlings of *Arabidopsis*, and in enzyme preparations of *Silene vulgaris*. Gel filtration studies and inhibition studies have demonstrated the complexation and detoxification of arsenic by the induced PCs (Grill et al., 1987; Schmöger et al., 2000). Furthermore, activities of PC-deficient mutants of *Arabidopsis* and *Schizosaccharomyces pombe* showed an increased sensitivity towards arsenate (Ha et al., 1999). Conversely, the overexpression of a plant PC synthase in *S. pombe* resulted in increased resistance to arsenite and arsenate (Vatamaniuk et al., 1999).

Mercury-stressed (1–10 mg/l) plant cells showed increased activities of antioxidants like superoxide dismutase and catalase in varying degrees and presented a positive endogenous protection effect. However, the protection effect disappeared at higher levels (50 mg/l) of mercury (Ma, 1998).

Plants exhibit considerable constitutional tolerance to lead and, in some cases, it reaches levels of inducible tolerance (Wierzbicka, 1999). High constitutional tolerance to lead in tomatoes was associated with the highest tissue level of calcium during administration of lead, and with the highest tolerance to calcium deficit. The lead concentration in the media and its absolute amounts in the roots and shoots was not proportional to the degree of lead tolerance (Antosiewicz, 1993). Constitutional tolerance to lead, taking onion roots as a model, shows that after an initial phase in which lead is toxic to cells, defense processes appear. Lead in the root symplast is detoxified in vacuoles, cell walls and dictyosomal vesicles. Initial cells of the meristem (quiescent centre) which plays a basic role in root regeneration processes are protected against lead penetration. This is in agreement with the absence of any symptoms of lead poisoning in plants growing in natural conditions, and suggests that there is a defense mechanism specific only to plant cells (Wierzbicka, 1995). Yang et al. (2000) observed in rice that oxalate compounds secreted from the root may reduced the bioavailability of lead and concluded that this may constitute an important lead tolerance mechanism in rice. In the polluted zone around the lead smelting plant, the gradual disappearance of wood and the appearance of mainly grassland plants confirmed the close connection between the appearance and resistance of plants and between their chromosome and genetic constitutions (Druskovic, 1985).

Using radiolabeled recombinant calmodulin as a probe to screen a tobacco cDNA library, a tobacco protein designated NtCBP4 (*Nicotiana tabacum* calmodulin-binding protein) was identified that modulate plant tolerance to heavy metals and was proposed to be involved in metal uptake across the plant plasma membrane. Transgenic tobacco expressing NtCBP4 exhibit hypersensitivity to lead(II) which is associated with an enhanced accumulation of the metal (Arazi et al., 1999, 2000).

The main soil factor determining the high degree of lead tolerance was the high Pb:Ca ratio. Populations from soils with a low Pb:Ca ratio had a very low degree of tolerance, indicating the presence of a genuine intracellular tolerance mechanism for the accumulation of lead in aerial organs (Brown and Brinkmann, 1992).

Of three poplar clones of *Populus canadensis*, growing in sand culture, the most tolerant clone

accumulated almost twice as much lead (primarily in the roots) as the most susceptible clone. The highest concentrations of lead were found in the roots, with considerably less in 1 year-old shoots, rooted cuttings and leaves. This distribution indicates that lead is not very mobile. Since the tolerant clone took up more lead than the intermediate and susceptible clones, it appears that tolerance depends more on detoxification than on selective absorption. Migration of lead was also less in the tolerant clone than in the susceptible one (Rachwal et al., 1993).

The mechanism of tolerance of higher plants to arsenic may involve one or more of the several methods suggested for metal tolerance like binding of metal to cell wall material (Cumming and Taylor, 1990; Turner and Marshall, 1972); complex-formation with organic acids and then removal to the vacuole (Godbold et al., 1984); and binding to specific thiol-rich proteins or phytochelatins (Grill et al., 1987; Lolkema et al., 1984; Rauser, 1984).

Arsenic triggers tissue and developmental stage specific defense responses of antioxidants (superoxide dismutase and catalase) and detoxification related genes (glutathione *S*-transferase) in maize (Mylona et al., 1998). Cat1 transcript increased in developing and germinating embryos and in young leaves while Cat2 and Cat3 increased at low concentrations of arsenic only in germinating embryos and developing embryos respectively. Sod3 transcript increased in developing, germinating and in leaves. The cytosolic Sod4 and Sod4A increased in germinating embryos, while only Sod4 increased in leaves. Expression of Gst1 was similar to that of Cat1.

In several cases, plant survival has been related to tolerance to arsenic (Porter and Peterson, 1975; Rocovich and West, 1975). All *Andropogon scoparius* plants from a mine site in the USA possessed tolerance to arsenate and in the UK, tolerance in arsenic-toxic mine spoil *Agrostis* plants was specific to water-soluble arsenate (Porter and Peterson, 1977). Only those plants found on soils with over 15,000 μg arsenic/g tolerated 25 μg arsenic/ml. Arsenic is one of the metals which have been successfully identified by suitable tolerant "indicator" plant species, used in identifying ore deposits (Shacklette, 1965). Porter and Peterson (1975) reported the presence of 6640 ppm arsenic levels in the herb Jasione montana; 4130 ppm in heather *Calluna vulgaris* and 3470 ppm in the grass *Agrostis tenuis* in old arsenic mine sites in Cornwall and Devon in England.

5. Genetics of tolerance

Plants growing on media contaminated with trace metals have been observed to exhibit strategies of avoidance or tolerance of metal toxicity, which are selected during evolution. Physiological and genetic factors determine which species can and which cannot evolve tolerance (Baker and Proctor, 1990). The evolution of heavy metal tolerance in vascular plants was first reported by Bradshaw (1952) for *Agrostis* tenuis and later by Wilkins (1957) for *Festuca ovina*.

Bacterial plasmids encode resistance systems for toxic metal ions including mercury, lead and arsenic. Chromosomal determinants of toxic metal resistance are also known, and the distinction between plasmid resistance and those from chromosomal genes has blurred, because for some metals like mercury and arsenic, the plasmid and chromosomal determinants are basically the same. The largest group of metal resistance systems functions by energy-dependent efflux of toxic ions. Some of the efflux systems are ATPases and others are chemiosmotic ion/proton exchangers. The first bacterial metallothionein, binding metal cations by means of cysteine thiolates, has been characterized in cyanobacteria (Silver, 1996, 1998; Silver and Phung, 1996).

5.1. Mercury

Environmental and clinical isolates of mercury-resistant bacteria have genes for the enzymes mercuric ion reductase and organomercurial lyase. These genes are often plasmid-encoded, and more rarely by transposons and bacterial chromosome. All mercury resistance genes are organized on the *mer* operon. Such systems have a world-wide geographical distribution (Osborn et al., 1997). Hg(II) is transported into the cell by the products of one to three genes encoded on the resistance determinants. The expression of the operon is regulated and is inducible by Hg(II). In some systems, the operon is inducible by both Hg(II) and some organomercurials. In gram-negative bacteria, two regulatory genes (*merR* and *merD*) were identified. The *merR* regulatory gene is transcribed

divergently from the other genes and its product represses operon expression in the absence and activates transcription in the presence of the inducers. The product of *merD* coregulates (modulates) the expression of the operon. Both *merR* and *merD* gene products bind to the same operator DNA. In gram positive bacteria, *merR* also positively regulates expression of the *mer* operon in the presence of Hg(II) (Misra, 1992). The *merR* regulatory protein of transpososn Tn501 controls the expression of the mercury resistance (*mer*) genes in response to mercuric ions. MerR is unique among prokaryotic regulatory proteins in that it acts as a repressor [−Hg(II)] and an activator [+Hg(II)] of transcription of the *mer* genes, but binds to a single site on the DNA in both cases. This transcriptional activation process has been postulated to involve a protein-induced conformational change in the DNA that allows RNA polymerase more readily to form an open complex at the promoter. The *mer* genes are inducible, with regulatory control being exerted at the transcriptional level (Foster, 1987). The molecular mechanism of bacterial resistance to organomercurials involves the novel enzyme activity organomercuryl lyase (Walsh, 1994), which cleaves the C–Hg bond and releases Hg(II).

Plasmid pPB confers broad-spectrum mercury resistance to a *Pseudomonas stutzeri* strain. Two pPB regions, separated by 25–30 kb and sharing homology with Tn501 *mer* genes, were cloned separately and shown to carry a cluster of functional and independently regulated *mer* genes. In the broad-spectrum resistant *mer* operon, the 5504 base pairs sequence includes six open reading frames (ORFs), five of which were identified as *merR*, *merT*, *merP*, *merA* and *merB*. The *merB* encoding organomercurial lyase showed lower similarity than the other *mer* genes with those from other broad-spectrum resistance operons. The remaining ORF named *merG*, located between *merA* and *merB*, seemed to be a new gene, which is involved in expression of phenylmercury resistance. Analyses of the mer-polypeptides revealed that pMRA17 *mer* operon expressed mercurial-inducible phenotype and *merB* and *merG* as well as the merA were under the control of *merR* which could be activated both by mercuric ion and organomercurials (Kiyono and Pan-Hou, 1999; Kiyono et al., 1997). Plants expressing the targeted *MerB* proteins and cytoplasmic *MerA* are highly resistant to organic mercury and degrade organic mercury at 10–70 times higher specific activity than plants with cytoplasmically distributed wild-type *MerB* enzyme (Bizily et al., 2002).

5.2. Lead

Lead tolerance in *F. ovina* is an inherited characteristic (Wilkins, 1960), evolved by the production of compounds within the plants, specifically for protection against the toxic effects of the heavy metal (Bradshaw et al., 1965). The tolerance was dominant in some of the crosses and diallel analyses demonstrated strong directional dominance. However, the degree of dominance could vary, and in some instances it was absent. Ashida (1965) suggested that adaptation to metal toxicants might be the result of intensification of some normal physiological activity, and individuals possess this heightened activity would be selected. More than one, but probably a small number of genes, are producing the major effects, and modifiers for dominance are present. This latter were probably affected, in turn, by the genome as a whole (Urquhart, 1971).

The *cadCA* operon of *Staphylococcus aureus* plasmid pI258 confers resistance to lead. The *cadA* gene encodes a P-type ATPase that has been shown to transport Cd(II), Zn(II) and Pb(II) (Rensing et al., 1998). The *cadC* gene encodes a 122-residue transcriptional regulator (CadC) that is a member of the ArsR family of metalloregulatory proteins (Xu and Rosen, 1999). When expressed in *S. aureus*, CadC responds to metals (Corbisier et al., 1993; Tauriainen et al., 1998). CadC protein contains five cysteines at residues 7, 11, 52, 58 and 60. Recently, it has been reported that Cys7, Cys58 and Cys60 are involved in sensing metals and suggest that they could be ligands to Pb(II), Zn(II) and Cd(II) (Sun et al., 2001).

ZntA, a close homolog of CadA, is another P-type divalent metal-translocating ATPase found in *Escherichia coli* which confers resistance to lead among others. Putative ZntA homologs are widespread in prokaryotes and also identified in *A. thaliana* suggesting that higher plants may employ similar resistant mechanisms (Rensing et al., 1998; Sharma et al., 2000). *zntA* gene expression is mediated by ZntR, belonging to the MerR transcriptional regulator family (Binet and Poole, 2000).

5.3. Arsenic

Arsenic tolerance appears to be genetically controlled in a fairly simple Mendelian manner but the specific mechanisms may be one or several, acting in cohesion (Schultz and Hutchinson, 1991). Arsenic tolerance was recorded in *Agrostis capillaris* as a dominant genetic character and controlled by a small number (one or two) of major genes. The genetic architecture of this character allows for heritable variation in degree of tolerance among tolerant plants (MacNair, 1993; Smith and MacNair, 1998; Watkins and MacNair, 1991). Arsenate tolerant genotypes of *Holcus lanatus* have an altered phosphate and arsenate uptake system so that the influx of this compound is appreciably reduced. The close relationship between altered phosphate uptake and arsenate tolerance indicates that these may be due to the pleiotropic effects of the same gene or the genetic linkage may be very tight (Meharg and MacNair, 1991, 1992). Through polycrosses between three tolerant plants from an abandoned mine, three non-tolerant and one less tolerant plants of *H. lanatus*, it was suggested that the tolerance gene may show variable penetrance, depending on the genetic background (MacNair et al., 1992). As(III) tolerant populations of *A. castellana* and *A. delicatula* have a significantly higher maximum root growth (MRG) than sensitive ones (De Koe and Jaques, 1993).

Multiple co-tolerance to arsenic with other metals has also been observed in some plants. Populations of the grass *Deschampsia caespitosa* from Sudbury, Canada were simultaneously tolerant to Cu, Ni, Al, As and Ag, all of which are elevated in the soil (Cox and Hutchinson, 1979).

Bioaccumulation of arsenic has been reported in some higher fungi and appears to be under genetic control (see Vetter, 1993, 1994). Continuous pollution of the soil by arsenic promoted the predominance of As(III)-tolerant fungi (Hiroki, 1993a). The effect on microbial populations was more drastic in paddy fields. Arsenic tolerant *Bacillus circulans* was also isolated from polluted soil (Hiroki, 1993b).

One of the best understood metal resistance systems is the product of the ars operon, which provides resistance to arsenic and antimony (Hedges and Baumberg, 1973; Silver et al., 1981; Wu and Rosen, 1993). As a reflection of the ubiquity of arsenic in the environment, ars operons are found in all species of bacteria, carried in chromosomes, plasmids and transposons. It is regulated at the transcriptional and allosteric levels, through cysteine thiol interaction with arsenite (Cai et al., 1998; Xu et al., 1998).

Bacterial plasmids conferring arsenic resistance encode specific efflux pumps able to extrude arsenic from the cell cytoplasm, thus lowering the intracellular concentration of the toxic ions. The ars operon of the *E. coli* (Gram-negative) plasmids R773 and R46 consists of five genes (*arsR*, *arsD*, *arsA*, *arsB* and *arsC*). The ArsR is a trans-acting repressor protein. The arsD protein is a secondary regulator of transcription of the *ars* operon, with little effect on the level of resistance. The *arsA* gene encodes an arsenite-stimulated ATPase (Ars protein). ArsA contains two nucleotide-binding sites and a binding site for arsenic or antimony. The ArsB protein is an inner membrane protein channel which participates in the pumping of arsenite across the inner membrane. Arsenate resistance is conferred by enzymatic reduction of less toxic arsenate to the more toxic arsenite by the small cytoplasmic ArsC polypeptide (Ji and Silver, 1992; Zhou et al., 2000). In Staphylococcal plasmids (Gram-positive) and *E. coli* chromosomes, comparable *arsR*, *arsB* and *arsC* genes (and proteins) are found, but *arsD* and *arsA* is missing (Cai and DuBow, 1996; Sato and Kobayashi, 1998). The *E. coli* chromosomal ars operon is transcribed as a single mRNA molecule of 2100 nucleotides in length and processed into two smaller mRNA products in a way similar to that found in plasmid-borne ars operon (Cai and DuBow, 1996). In addition to the widespread plasmid arsenic resistance determinant, a few bacteria confer resistance to arsenite with a separate determinant for enzymatic oxidation of more toxic arsenite to less toxic arsenate. In contrast to the detailed information on the mechanisms of arsenic resistance in bacteria very little work has been reported on this subject in algae and fungi (Carlin et al., 1995; Cervantes et al., 1994).

Saccharomyces cerevisiae serve as a model system for the study of arsenic resistance in eukaryotes. A cluster of three ACR (arsenic compounds resistance) genes named *ACR1*, *ACR2* and *ACR3* was revealed from a 4.2 kb region from *S. cerevisiae* chromosome XVI which confers resistance to arsenite and arsenate (Bobrowicz et al., 1997). Acr2p, the first identified eukaryotic arsenate reductase, reduces ar-

senate to arsenite, which is then extruded from cells (Mukhoppadhyay et al., 2000). *S. cerevisiae* has two independent pathways for the removal of arsenite from the cytosol. The first step is the extrusion of arsenite into the medium. Acr3p is a plasma membrane efflux transporter that confers resistance to arsenite by arsenite extrusion from cells. Deletion of *ACR3* was directly correlated with increased arsenite accumulation, reflecting loss of efflux activity. The second pathway is sequestration of As(III) into the vacuole as the glutathione conjugate in a reaction catalyzed by the product of the *YCF1* gene (Ycf1p).

Arsenite detoxification mechanisms are proposed to be nearly universal in nature and the pathways will have similar steps even if they are the products of independent evolution (Ghosh et al., 1999). The first step is the reduction of arsenate to arsenite catalysed by members of three evolutionary diverse families of arsenate reductases (Bobrowicz et al., 1997; Gladysheva et al., 1994; Ji et al., 1994; Mukhopadhyay and Rosen, 1998). The next step is the arsenic removal from the cytosol. Though no homologs of ArsB, Acr3p or Ycf1p was identified in higher plants till to date, yet, the arsenic-resistant bacterial and yeast strains may prove an important tool for identifying the genes for arsenic transporters in higher plants.

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