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Review Paper

**A Review of Formation, Toxicity Of Reactive
Oxygen Species By Heavy Metals And Tolerance In
Plants**

Abstract – In plants, reactive oxygen species are the primary factor of cell mechanism deterioration. The stability of the reduction and regeneration is disturbed under stress environment. Reactive Oxygen Species (ROS) continuously damages the main organelles of cell in plants as well as inactivate several enzymes. Break down of lipids, nucleic acid, proteins, pigments, damages in a membrane which may lead to cell death. ROS is diffusible messages in pathways of signal transduction in numerous developmental pathways in plants. ROS acts as a messenger. Plants protect the cells from misbalancing and also damages ROS production. ROS produced in excess amount rather than required for numerous metabolic reaction. In aerobic respiration, ROS is produced. This whole review tells about the origin of ROS, Cell death, the role of ROS, defence system in numerous developmental pathways.

Keywords: Reactive metabolites, membrane damage, active mechanism, cell death.

INTRODUCTION

Heavy metals are generally defined as metals with relatively high densities, atomic weights, or atomic numbers. Heavy metals are the most significant source of contamination in plants and other living and non-living things on the planet earth. Heavy metals such as Cadmium, Copper, Zinc, Nickel, Cobalt, Chromium, Lead and Arsenic etc. are great source of contamination of agricultural soils. Because of the excessive use of phosphate fertilisers which are rich in amounts of phosphorous that is why these metals are also known as polluting agents (Cobbett et al., 2002). There are many other factors such as sewage sludge application, dust from surroundings, and industrial waste and poor irrigation systems in agricultural land responsible for the pollution of the environment. The exposure of heavy metals in plants results in the generation of reactive oxygen species which contaminates plants, and this becomes harmful to their growth. Heavy metals in plants produce ROS through Haber-Wesis reactions (Generation of $\text{OH}^\cdot$, H_2O_2 , $\text{O}_2^\cdot$). The overproduction of ROS

in plants or oxidative stress can be an excellent source of heavy metal toxicity in plants. Oxidative stress can be indicated by Malondialdehyde (MDA), which is the decomposed product of polyunsaturated fatty acids (Cobbett and Meagher, 2002).

MECHANISM OF ROS PRODUCTION IN PLANTS

The production mechanism of ROS includes factor such as the disruption of the electron transport chain in plants, their reaction with an antioxidant system or the disturbance of metabolism of essential elements. The production of lipid peroxidation (Degradation of lipids and steal the electrons from lipid on cell membrane) in plants is the most disastrous effect on plants caused by heavy metals due to which biomembrane deterioration can occur. Plants have an excellent potential mechanism to face the hazardous heavy metal toxicity present in the environment. In general, plants can produce low molecular weight thiols which have an affinity for toxic metals. Glutathione (GSH) and cysteine are the best examples of low molecular weight thiols (sulphates takes the place of oxygen in hydroxyl group) which react against the toxic metals. GSH is a thiol which contains sulphur. It is a tri-peptide thiol which includes the formula of γ -glutamate-cysteine-glycine (Dar and Roychodury, 2014)

SYNTHESIS OF GSH

GSH is synthesised by the catalization of two ATP dependent enzymes γ -glutamate-cysteine-glycine (GSH1) and Glutathione (GSH2). GSH is a substrate of phytochelatin synthesis. Phytochelatin (PCs) are a small binding material of heavy metals. They are cysteine-rich polypeptides. $(\gamma\text{-Glu-Cys})_n\text{Gly}$ ($n=2-11$) is its general structure. They are found in many plants as well as in fungi and in many other living things. The enzyme known as phytochelatin synthase is catalysed during its synthesis; therefore heavy metals such as cadmium and nickel are detoxified by it. As they form complexes with toxic metal ions in the cytosol and then transport them into vacuoles so they act as best defendants of plants from heavy metals (Clemens, et al.,1999)

HEAVY METALS AND PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS)

Heavy metals are the primary source of production of ROS in plants. The accumulation of ROS in plants depends upon the balance between ROS production and ROS scavenging. The factors affecting the accumulation of ROS in plants depend upon the growth situations such as temperature, light intensity and presence of heavy metals (Assunção et al.,2003). In case heavy metals are present in excessive amount in plants they cause limitation of fixation of CO_2 in the chloroplasts, it results in over reduction of the photosynthetic electron transport and this is the major cause of the production of ROS in plants. Over-reduction of electron transport chain in the mitochondria also generates ROS. Almost 1-5% of O_2 consumed by

mitochondria is converted into ROS. Peroxisomes produce ROS i.e hydrogen peroxide (H_2O_2) when glycolate is oxidised into glyoxylic acid during photorespiration (Gill, 2014). Therefore due to spin inversion, ROS such as singlet oxygen, superoxide anion, H_2O_2 , and hydroxyl radicals are produced in these organelles. The redox heavy metals such as Cu, Cd, Fe, and Zn induce ROS formation directly by participating in Haber-Weiss and Fenton reactions (Kehr,2000).

The properties of ROS are such that they are unstable, they are highly reactive, and they generate free radicals with reaction with other macromolecules. ROS as extremely reactive in nature interact with macromolecules like DNA, pigments, proteins, lipids and many other essential molecules which depends upon the properties like chemical reactivity, redox potential, half-life and mobility leading the cellular system (Campbell, et al.,2003). These all factors leads to a destructive process called "Oxidative stress". $OH^\cdot$ is the most reactive and highly damaging species among ROS, it is short-lived (1 ns) and can oxidise macromolecules within a diffusion distance. Due to this factor ROS produce reversible and irreversible modifications in lipids, proteins, and nucleic acids. As compared to all most of these ROS are damaging and irreversible. ROS act as a signalling molecule which performs the regulation of many physiological reactions such as root hair growth, stomatal movement, cell growth and cell differentiation (when finally regulated by an antioxidative defence system) (Alschner, 1989). ROS produced by NADPH (Nicotinamide adenine dinucleotide phosphate) oxidases during stress are channelled through the plant as it serves as a stress signal for the activation and defence mechanism, which in turn counteract oxidative stress. The fate of ROS in the cellular system depends upon the output of many complex processes that involve the antioxidant system, Signaling cascades, redox alterations etc. Oxidative stress occurs when ROS exceeds that of the scavenging potential of antioxidants (Stobier, et al.,2015).

MECHANISM TO KEEP ROUTINELY PRODUCED ROS AT PHYSIOLOGICAL STATE

Plants have a specific mechanism to keep routinely produced ROS at the physiological limit, as they prevent them from exceeding toxic threshold levels. This mechanism is known as Plant antioxidant defence system. It helps in regulation of ROS levels in the cellular system. The antioxidant system comprises of two components i.e enzymatic and non-Enzymatic. Enzymatic antioxidants are those which are superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase are the key enzymatic antioxidants of the defence system by which free radicals during metabolic reactions are released. Ascorbate and glutathione both functions as cofactors of enzymatic antioxidants (

Ahmad, et al.,2010).These directly quench ROS and regulate the gene expression associated with biotic and abiotic stress responses. The non-enzymatic antioxidants include water-soluble compounds such as ascorbate, glutathione, proline and α -tocopherol. The importance of antioxidants depends upon the increased, or decreased levels are related to an enhanced or declined stress tolerance of stressed plants. Since the evolution of O₂, antioxidants play an essential role in sustaining ROS concentration at an appropriate level that can enhance plant development (Bratteler et al., 2006).

UPTAKE OF HEAVY METALS BY THE PLANTS

The hyperaccumulators can absorb heavy metals from the soil under varying concentration of heavy metals. Heavy metals are taken by hyperaccumulators but their uptake is affected by many factors such as pH, water content and organic substances. The uptake of heavy metals requires a suitable transporting system to enter the plant. The proton secretion by roots further acidifies rhizosphere and enhances metal dissolution. It affects the growth of metal accumulating plant species. The organic substances released from the roots affect growth in hyperaccumulating plants. The organic acids influence Cd solubility by the formation of Cd complexes. The study has revealed that increased Zn uptake is due to overexpression of genes belonging to ZIP (zinc-regulated transporter iron-regulated transporter protein) family encoding plasma membrane located transporter (Baker and Brooks .,1989).

TOXIC EFFECTS OF VARIOUS HEAVY METALS IN PLANTS

The contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence. The regulatory limit of Cd in agricultural soil is 100 mg/kg. Plants which are exposed to high levels of Cd causes a reduction in photosynthesis, because of water uptake and nutrients uptakes, they enter into the bodies of plant. Plants grow in soil which contains high levels of Cd show visible symptoms of injury infected in terms of chlorosis growth inhibition, browning of root tips leading to death (Bernard, et al.,2004).

ROLE OF ZINC (Zn) IN PRODUCTION OF ROS

Zinc and Cadmium, which have now a day's become an integral part of making the soil poisonous and are very harmful to the plants as they get most of their necessary nutrients from the soil. Thus heavy metals like these damage the growth of plants in a variety of ways. Excess amount of these heavy metals in soil is due to some reasons as semi-solid materials produced by various by-products, fertilizers that are given to the plants for their rapid growth, from air, garbage, by combustion of organic substances, by waste products from mining and

industries and many other human activities (Deniau and Pieper, 2006). Zinc (Zn) is also used as an important substance for living organisms for health and body growth. If excess zinc is found in poisonous soil will cause the toxic effect to plant growth. The concentration of zinc which is to be estimated as useful for living being is 150-300mg/kg and if the concentration of soil increase above this point, it causes severe damage to plant. Due to a high concentration of zinc, the plants tend to lose their actual color and become yellow. This damage to plants is more prominent in small leaves as compared to large ones (Blaudez, et al., 2003). Presence of these metals is not only harmful to plants but also induces the presence of some other heavy metals that induce severe damages to plants. It reduces the process of photosynthesis and the leaves become yellow. In some cases, these metals cause a deficiency of needed metals that resist the transfer of these metals from roots to the shoot or other parts of the plant. Another toxic effect is that it causes deficiency of phosphorus which results in the purplish-red color of leaves (Bereczky, et al.,2003).

ROLE OF COPPER (Cu) IN THE PRODUCTION OF ROS

Copper is count to be a very important component for production and growth of different plant parts and play a key role in adsorption and the process of taking in carbon dioxide (CO₂) and production of adenosine triphosphate by some chemical reactions. Cu is also a very important component of protein in respiratory electron transport chain but due to industrial and mining activities, copper is inducing in the soil much more than its need. It is also being added in soil due to some human sources such as extraction process and the ores that contain copper. Many of the minors working in mining throw their waste products and residue in the environment (Bernal, et al., M., 2007). When the soil has an excess amount of copper, it will then be taken up by plants as the plants take their minerals from the soil and it will not only cause damage in plants but is also harmful and toxic for living beings. Such metals slow down the process of plant growth and also lead to yellowish of young leaves. When plants are exposed to large amount of copper, it will generate different type of oxygen species such as superoxides etc and the oxygen due to Cu in soil when coming in contact either plants will cause damage in all the reactions taking place and all the large molecules present in them will get damaged (Yruela, 2005).

ROLE OF MERCURY (Hg) IN PRODUCTION OF ROS

Like other heavy metals, mercury also causes damage to plants. As we know that we get our food from plants and plants get their minerals from soil so if mercury will induce in agricultural land, it will disturb our food chain. Mercury is found in nature in different forms such as HgS, Hg²⁺ etc. and this is the reason that mercury is considered to be different from

other heavy metals. However, in the agricultural environment, the predominant form of mercury is its ionic form. Mercury released in soil is mainly found in solid form. It is well known that plants in water take in mercury in ionic form in large quantity compared to that of others. Hg in large quantity is harmful mainly to the coloration of leaves. For instance, Hg in its ionic form binds with water channel protein and help in the process of opening and closing of stomata and also control the flow of water from roots to shoots and then to all parts of plant body. High level of mercury interferes with the activity of mitochondria and the reactive oxygen species are induced in them as mercury is a good component to start the generation of reactive oxygen species. Hence the process of biomembrane lipids and reaction taking place in cells of plants are disrupted (Patra and Sharma 2000).

ROLE OF CHROMIUM (Cr) IN THE PRODUCTION OF ROS

Due to industrial growth, pollution into the environment has increased in a dramatic manner. Chromium is considered to cause the addition of poisonous or polluting substances in soil, dregs and underground water. The industries in which soft and perishable protein of animals hides or skin are converted into durable and flexible leather is one of the main sources that consume water, use it in their processes and release the contaminated water in the environment that contain a very large amount of chromium from 1.07 to 7.8 mg/ litres. Pollutants from living sources and discharge of chromium in fresh water is about 3550mt. Chromium is toxic as it causes very harmful effects on the epithelial layer in plants and acts as an irritant on the epithelium. Many agencies have proven on the experimental basis that several of human carcinogens are also induced by chromium (Shadreck, Mugadza, 2013). An excess amount of chromium in soil causes reduces growth in plants, discoloration of young leaves and interrupted balance of nutrients. The top of plants and the roots get damaged when plants are supplied with water contaminated by chromium. The plants in terrestrial areas are exposed to discoloration of leaves due to chromium. For example: if 100 μ M of chromium is present then it causes about 40% of resistance in the growth of plants. The process of germination, growth of stem, leaves and roots as light, water and mineral nutrition are also interrupted. Excess of chromium also generates reactive- oxygen species when it has its direct effect on enzymes (Shankar, et al.,2005).

EFFECT OF ROS DUE TO LEAD (Pb)

The Earth's soil contains many heavy metals and is abundant in lead (Pb). The toxicity in soil, due to lead, is the reason of dumping of toxic municipal sewage sludge, paints papers, pulp, gasoline and explosives rich in Pb, extraction of minerals and metals by heating and melting. It, therefore, produces the harmful effects on shape and structure, growth and essential

processes of plants like photosynthesis and respiration. Increased level of Pb also stops or inhibits the original enzymatic activity at the cellular level by combining with the hydrogen and sulphur groups. Increased level of Pb also gives rise to the oxidative stress by increasing the ROS production in plants. The increased level also disturbs the balance of required normal water contents, alters the permeability across the membranes and also on other hands, disturbs the mineral nutrition (Bernal, et al.,2007)

EFFECT OF ROS DUE TO ARSENIC (As)

Arsenate is competitive to phosphate as it fights and for the same carriers for uptake phenomenon in the plasmalemma of plants present in the roots. The tolerance of arsenate has been produced in several plants, like, in grass, the suppression of a high-affinity phosphate or arsenate results in arsenate tolerance. The resulting suppression causes the level of toxicity to be decreased so that it can easily be recovered by the respective constitutive mechanism methods (Tu, et al., 2003). The single gene encoding for the reduced transport of arsenate gives rise to arsenate tolerance. Although this clearly makes us understand the controlling process in As uptake, still, grass being tolerant towards As, gathers the As, however at much less rate as compared to that of the non-tolerant grass. However, the grass growing on the contaminated soil can result in a high concentration of As, e.g. 3470 mg/g As in *Agrostis Tenuis* and 500 mg/g As in *Holcus Lanatus*. The As is also transformed to several less phytotoxic As species. For example, As undergoes transformation and results in the conversion to arsenite, dimethyl arsinic acid (DMA) and monomethyl arsenic acid (MMA) in phytoplankton and macroalgae. Then some As species of methyl is then, after metabolism, converted to organic phospholipids and arsenosugars. Lately, plants relating to terrestrial environment have been studied under the presence relating to arsenate and arsenite. However, a new study has also reported reduced methylated as concentrations in plants, on terrestrial environments, such as MMA and DMA (Dimethylamine) (Finnegan and Chen, 2012).

EFFECT OF ROS DUE TO COBALT (Co)

Earth's crust normally contains cobalt in the form of cobaltite (CoAsS), erythrite ($\text{Co}_3(\text{AsO}_4)_2$) and smatter (CoAs_2). The earth's soil may get increased levels of toxic cobalt due to the deposition from the burning of fossil fuels, wearing of cobaltite alloys and spreading of sewage wet mud and animal wastes (Yruela, 2005). However, cobalt producing risks in the environment are met and managed through the establishment of checking the environment quality and meeting the standards of one's needs. Plants can, themselves, gather a small amount of cobalt from the soil. The Co varies from species to species in plants and its uptake and distribution are controlled by many different mechanisms. However, we know

little regarding the phytotoxic effect of excess Co. But however, the harmful effects are being studied under phytotoxicity of Co in barley, tomato and oilseed rape, which shows producing an effect on the growth mechanism of shoots and biomass. Along with the effect on biomass, the increased rate of Co lemmatize (group infected together) the normally required concentration of other various metals such as iron and other pigments like chlorophyll and substances such as proteins and also in leaves of cauliflower, catalase activity. On other elements like phosphorus, sulphur, manganese, zinc and copper, the increased level of Co effects the translocation from tips to roots of the cauliflower. While comparing to that of copper or chromium, Co tragically reduces the significant water potential and the rate of transpiration. While resistance and hindrance towards diffusion and osmosis increased on exposure to the high content of Co (Baker, et al., 2000).

EFFECT OF ROS DUE TO NICKEL (Ni)

Nickel is one of the transition metals which are to be found in chemical-free soils in a very less quantity to be measured except for those soils which are rich in the availability of minerals and metals and bases. However, many anthropogenic activities are leading to an increased level of nickel in the environmental contents. For example the extraction of different minerals from mines, removal of dumped substances, many combustion processes along with burning of oil, waste products, using of fertilizers and pesticides which are rich in phosphates and phosphate containing compounds. The polluted soil due to previously mentioned reasons may contain an increased level of nickel concentration which is more than the original required levels in the natural environment. The increased levels then cause many harmful effects and changes in the various functions and abilities of plants required in normal mechanisms (Cempel and Nikel, 2006). Like in many plants, it causes decolouration which affects the process of photosynthesis and also causes the death of many plants cells due to an increased rate of toxicity in the plants' cells, for example, rice. Normal functions are disturbed in various plant species which causes the plants in a reduction in normal nutrients uptake and disturbs the physiology of cell membranes in the plants. A plant *Oryzasativa*'s shoot was studied under the increased level of toxicity produced by the nickel and it showed the adverse effects on the lipids produced within. Moreover, when wheat was exposed to a higher level of toxicity produced by nickel, it enhanced the MDA concentration. Such changes in normal metabolic processes of a living being cause the disturbances in the functions of the membranes, like the maintenance of ions, such as potassium ions, across the membranes, or also in the cytoplasm. Other adverse effects on the plant metabolism were related to the normal water uptake and hydro imbalance. As in, it produced a fall in level of the uptake of

water, as it increased the uptake of nickel ions, especially the monocot and dicot plants species. The reduced level of water intake by plants is taken as a symbol for the researchers that respective plant has increased in toxicity due to relative reasons being studied on that plant (Baker, et al., 2000).

MECHANISM OF PLANTS TO COPE WITH HEAVY METALS TOXICITY

We have been studying so far, the toxicity due to several heavy metals. The plants have been coped with these heavy metals by fitting in such mechanisms which gives a defensive shield to a plant towards such heavy metals. Reduced forms of oxygen are produced through several processes happening within a plant, and some species which are related to biochemical damage when an electron is accepted by a molecular oxygen from other molecules and many reactions and processes which are being taken place inside a cell reduces the molecular oxygen species, which took an electron, to other species like superoxide and hydrogen peroxide. Which are comparatively less reactive molecules and can form a hydroxyl radical which causes most of the damage in oxidative processes, mainly photosynthesis and transpiration in the biological system. The reduction taking place with the one electron in the formation of molecular oxygen to the superoxide radical is not favoured regarding the stability while considering the heat changes; however, this can take place when a molecular oxygen species is made to interact with the unpaired electron from another species or the paramagnetic Centre. For example, many transition metals, like iron and copper, are electron efficient and thus can prove to be a very good catalyst towards the processes of oxygen reduction (Clemens,2006).

In the Haber-Weiss reaction, when iron is taken into consideration as a transition metal to catalyze the reaction it is named as Fenton reaction. The Haber-Weiss reaction producing the hydroxyl radicals oxidize many biological molecules, which, as a result, leads to necrosis, i.e. leading to several damages in and out of cell which then causes cell death. Hydroxyl radicals can also be produced near the DNA molecule by either oxidation or reduction, as referred through hydrogen, towards DNA bases or the DNA backbone, depending on the loss and gain, respectively. The changes occurring Hydroxyl-mediated DNA involves the iron to be in a complex structure with a phosphate residue or sometimes it may be indirectly related in coordination with the ringed nitrogens and, or several times being a free species. The process of Haber-Weiss reaction, metals ions also plays an important role in the oxidative modifications of free amino acids and proteins. Commonly referred sited relating to oxidation in proteins and major products are to be identified as cysteine, methionine, proline, lysine, arginine and histidine residues (Bratteler et al., 2006). It has been

studied and observed that only one amino acid is modified by oxidation in a given respective protein. But, it should be related to its specificity towards its target to be modified for example some of the amino acids residues are specifically related to metal binding sites. The breakdown by proteases is being a major consequence of radicals damage which is free of oxygen. Another important feature of oxidation of proteins is also taken into consideration which is the release of iron from a huge group of similar components, such as aconites. At last, the conveyance of oxygen and transition metals to lipid peroxidation is completed, and the membranes related to our natural environment which are abundant in polyunsaturated fatty acids are capable of accepting these reactions. While in the aqueous phase, the ascorbic acid then dampens down the peroxy and alkoxy radicals. Although, the ascorbate, is functioning as a pro-oxidant, can reduce the iron and copper which then allows the change of one form to another of metal catalyzed lipid hydroperoxides (Coupe, et al.,1995).

GLUTATHIONE BIOSYNTHESIS AND ITS REGULATION

Glutathione is an antioxidant tripeptide present in all the organisms which are bearing mitochondria including all eukaryotes including plants. It is the most abundant and has the least molecular weight among all the thiols. The plant's fluid of transportation involves the mechanism regarding the GSH. In many cellular processes which include the mechanism of defence against the ROS, forcibly taking in of heavy metals and in the removal of toxic substances such as xenobiotics (Ahmad et al .,2010). The developmental processes within an organism also require the presence of GSH, such as regulation of cell division which is also referred to as cell mitosis and also included in the phenomenon of flowering. Along with all these GSH has also proved to be beneficial in the major transport system referring to that of sulphur and also helps in keeping the storage form of reduced form of sulphur. The synthesis of GSH requires the involvement of two processes which are being carried out responsibly in the presence of ATP, where γ -glutamyl cysteine synthetase acts as a catalyst in the formation process between the carbonyl group of glutamate and the amino group of cysteine, resulting in the formation of peptide bond and results in the yield of γ -glutamyl cysteine. Under the reviewed considerations of the processes relating to GSH, the first and the initial step is referred to as a control point when the GSH is under a demand of high concentrations. The next step includes the tying up of glutathione synthetase with a glycine residue takes place with γ -glutamyl cysteine to form GSH. GSH1 is restricted to be present in plastids, whereas, GSH2 on the other hand, is not restricted in its presence and can be found in both, cytosol and plastids (16).

Two forms of GSH are to be found in existence; which are i) reduced glutathione (GSH), and ii) oxidized form (GSSG). The potential of glutathione to donate an electron must be determined by the intracellular ratio, i.e. GSH/GSSG. Many factors lead to the changes and variations in the respective ratio of glutathione, which are the following; pH of the environment in which the GSH related processes takes place, total GSH concentration which is to be required in the whole phenomenon, GSH may produce complex molecules within the cellular environment and breakdown of molecules of GSH. When the study was completed it was evident from the research that only a single gene was solely responsible for the encoding of GSH1 and GSH2 (Hossian, et al., 2012).

While the analysis and research done regarding the transcript of GSH2 disclosed that the major part that is more than 90 percent in count, are shortened down during the processes. Truncation resulted in the loss of many metabolic mobile peptides. Hence, study shows that GSH2 is only restricted to the cytosol only and a very less part is being transported outside into the plastids. This makes the plastids automatic that is it can control its compartments itself for the GSH biosynthesis independently while others are dependent organelles. Either on γ -EC or GSH which is being transported outside plastids. Further studies on several suspensions revealed that γ -EC is the major transport out of the plastids. This has also been supported by several other researchers in their research work on GSH distribution in fast-growing leaves (Blaudez, et al., 2003).

Biosynthesis takes the GSH representation in a plant cell. The first enzyme of glutathione metabolic synthesis of GSH1 is vastly positioned in plastids. While, on the other hand, the second enzyme GSH2 is localised in both, cytosol and plastids, being a significant portion located in the cytosol. Both are the genes which are encoded by nuclear genes, having mobile transit, however, on the other hand, the mobile peptide containing a major portion of GSH2 is lost due to vast transcript processing and remained positioned in the cytosol. Models in researchers have shown that γ -EC is vastly metabolised in the chloroplast and a transported mediated efflux of γ -EC, was assumed and predicted to be from plastids to the cytosol. Since both the biological catalysts are present in the chloroplast, therefore, GSH is also metabolised in this compartment. It is unknown the exact amount of GSH to be metabolised out of the cytosol or inside the cytosol. However, it is to be predicted that the transport processes may be regulated in the response to developmental and stress-related cues (Bereczky, et al., 2003).

MECHANISM OF GLUTATHIONE-MEDIATED HEAVY METALS STRESS TOLERANCE IN PLANTS

Glutathione stimulating hormone is considered to be the main part of the cell and found in

almost all parts of the cell. Proteins are exposed to the cells in plants are induced due to this glutathione stimulating hormone and is major source of protein in cells. A group found in GSH is thiole group which is responsible to make it favorable in serving a large range of biological and chemical reactions in all types of living organisms. Positive charge loving nature of that group found in glutathione stimulating hormone (GSH) plays a key role in forming a bond called mercaptide bond with the other metals to make a suitable reaction with the desired electron loving molecules. The reactivity of electrophilic and nucleophilic substances with respect to their stable nature and the property of solublizing water in high range to make it a good biological and chemical component to prevent the plant body from different type of stress due to which the plants get damaged. That stresses include oxidative stress that damage the plant with the help of oxygen components, by the use of heavy metals and with the help of many other organic compounds (Baker and Walker, 1990).

Reactive oxygen species are being introduced in plants when plants are exposed to heavy metals in a direct or indirect way and disturb the reactions taking place within the cells of plants. H_2O_2 present in cell is dangerous to the health of plants as well as animals either its level is low or high. So the glutathione stimulating hormone used to maintain the level of H_2O_2 in plants. The reduction of GSH if make any alteration to oxidize it into another form which is GSSG. H_2O_2 in its broken form is very important in those pathways that signal the process of oxidation-reduction reaction. This ratio of GSH and GSSG is considered to be a signal to maintain the balance of oxidation and reduction of cell thus play a key role in production of oxygen specie in the cell. GSH in its reduced form can also be used as an agent which reduce the oxidation and useful in some ways that it can reduce the value of oxygen species that is harmful to health without any need were generated in stressful conditions. Moreover, GSH plays an important role in the process of removing toxic substances from the cell of those substances that are foreign to the body and many other heavy metals [30]. The first job of GSH is to activate itself and to make a bond in reduction and oxidation process to convert itself into oxidative form. An enzyme is used in GSH which is responsible for making their links with other which is glutathione-S-transferase. The heavy metals which get into plants through soil are not directly attacking the parts of plant body but they are accumulating in the vacuole and this is the reason that plant survives more than that of animals. The heavy metals are accumulating in the cells and are not attacking the cells directly. But its first and foremost use is to detoxify heavy metals and to decrease the content of cytosolic GSH. Thus has a direct impact on GSH/GSSG potential of oxidation and reduction and this gives a signal to the cell exposed to stress. As a result, any increase in high

level of GSH which has its base in the process of breaking will help in maintain the oxidation-reduction of cell. But under some of such conditions, if we are going to maintain the concentration of cell might be harmful to a plant to survive anymore (Bennett, et al., 2003).

Reactive oxygen species have an electron in them which is unpaired and come in contact with anything. In some cases it is harmful while in some cases it may be useful. Under that condition in which the plants are growing, the plants are in the control of the system in which they survive on dead beings such as GSH. ROS are in plants are harmful so it should be removed from there and if ROS does not remove from the plants at time, it will lead to a stressful condition known as oxidative stress. An excess amount of ROS found in the cell can stimulate the addition of oxygen and can introduce oxygen also there where it is not being needed. Moreover, it can modify the processes of amino acids in the cell, and all the other components. This causes disruption in plants and seriously affects the process taking place in plants. This all damages the growth in plants. ROS and GSH start to do their work at the stage when the plant is just exposed to both of these. The insertion of transfer deoxyribonucleic acid in GSH and enzyme is found in them which encode a gene called gama-glutamyl cysteine synthetase. If this enzyme is not present according to its need then it will lead to severe unsuitable diseases. Addition to this, GSH also plays a role in glutaredoxin reactions to control the oxidation-reduction reaction of proteins in many cells. Many kinds of different types of hormones are kept in hydrogenated form with the help of NADPH, glutathione reductase and GSH. Grx also play an important role in the redox- mediated development processes, as it is seen in flowering (Cobbett and Goldsbrough, 2002).

PLANTS ANTIOXIDANT DEFENCE SYSTEM

Antioxidant is a term used to define the class of that useful compounds that are used in protecting the plants from severe damage that are caused when the plants are exposed to some heavy metals that induced reactive oxygen species in them. The network that is formed when the plants are exposed to heavy metals is responsible to remove the agents which are necessary for neutralization. SOD is a useful enzyme that is involved in changing the superoxide radicals produced due to introducing oxygen to molecular oxygen into H_2O_2 and O_2 in the entire compartment found in a cell. H_2O_2 which is produced by the processing of the enzyme SOD is little bit dangerous because it can very easily pass through the membrane of the cell and embed into the other compartments where there is no need of this causing damages in that compartments and as a result many of the other hormones or enzymes are being helpful to locate H_2O_2 . Three main SODs are known to be located in plants on the basis

that they contain the chloroplast Cu-Zn SOD, mitochondrial Mn SOD and the Chloroplastic Fe SOD (David-Assael, et al .,2006)

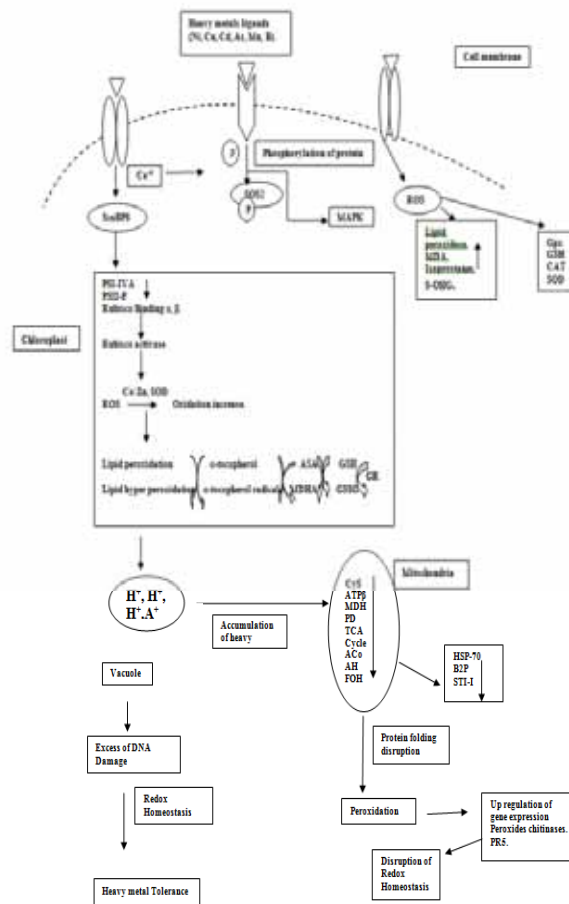
A protein which is commonly used in chloroplast and cytosol is mainly considered as APX which is mainly used in the process of breakdown of H_2O_2 . The substance which is found to activate the process of enzyme that increases the speed of reaction then is ascorbate and the material gain at the end of the reaction which is in the form of radical is mainly reduced to hydrogenated ascorbate with the help of the enzyme MDHAR when a donor of electron is present which is NADPH. CAT is another very necessary enzyme that is used in breaking of large molecules into smaller ones and hence is used in the breaking of H_2O_2 molecule into two smaller molecules such as H_2O and O_2 it is mostly considered to be present in plants and in plants, large entity of this enzyme is found to be peroxisomes. CAT used in the detoxification process of peroxides (reactive oxygen species) that are formed during the process of photosynthesis and respiration (process of formation of oxygen and supply it to environment and process of taking in carbon dioxide from the environment). APX and CAT both are enzymes used to speed up the reaction as well as they are used in the detoxification process, different kinds of these enzymes use to signal for the modification of H_2O_2 when the condition is stressful (Chiang, et al.,2006).

In the above paragraph, all the enzyme about which the details have been given are the components that play a very important role in making the heavy metals less severe during stressful conditions. It has been studied and on continuous experimental basis, it has come to know that if we do any treatment to reduce the action of heavy metals on plants it will eventually generate the class of reactive oxygen species, as due to increasing in oxidizing agent, an increase in APX, CAT and SOD is also observed (Singh, et al.,2015). It has been studied that when plants are exposed to Pb, there is a visible rise in the concentration of SOD, oxygen species, APX and CAT activities are observed. These activities will rise in the concentration of Pb from 50-100 μ M (Srini, et al.,2014). Similar to this, another scientist claimed that the concentration of SOD and CAT can also be raised when plant is exposed to Pb either in 5-50 μ M (Surjendu, et al., 2007) and from all these things it has been resulted that there should be co-operation in the action of oxygen reducing components to detoxify the action of metals under stressful conditions.

Fig- 1. The receptor present on cell membrane binds with incoming ligand that are heavy metals like Ni, Cd, Pb etc which starts the signalling of the production of ROS in response to ROS production occurs indicate high level of MDA in plants matrix. The rise in ROS production causes in certain antioxidants (Isoprostanes, 8OHG, Glutathione, SOD, Cat).

473 Extreme ROS production disrupts the protein in mitochondria matrix i.e 8H ESP 17, SIIB2D
 474 (Heat shocked proteins) causing up regulation of gene expression peroxidase five (PR5)
 475 Chitinase and other lipid peroxidation production. This disruption redox homeostasis
 476 (coordination) cause excess DNA damage in the vacuole indicated by high level of 8OHG.
 477 However, the plant body is designed in nature in such a way that it accumulates all the heavy
 478 metals residues it is vacuole compartment preventing further oxidative damage to the plant
 479 body. This is how the plant has developed self-tolerance property of oxidative damage.

Fig. 1. Mechanism of Heavy metals induced Oxidative stress tolerance in Plants.



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CONCLUSION

484 The exposure of plant body to heavy metals like Cd, As, Pb etc. produce reactive oxygen
485 species which directly hit mitochondria matrix. The origin of ROS causing cell death and
486 disturbing self-defence system of the plant body which is tolerable due to the accumulation of
487 heavy metals produced in the vacuole which is self-phagocytes by the plant body.

488

REFERENCES

- 489 Alscher, R.G.,(1989). Biosynthesis and antioxidant function of glutathione in plants,
490 *Physiol .Plant.*,vol.77,pp.457–464.
- 491 Assunção, A.G.L., Schat, H., and Aarts M.G.M., (2003). *Thlaspi caerulescens*, an attractive
492 model species to study heavy metal hyperaccumulation in plants, *New Phytol .*,vol.159,
493 pp.351–360.
- 494 Ahmad, P., Jallel, C.A, Salem, M.A., Nabi, G., Sharma, S., (2010). Role of enzymatic and
495 non –enzymatic antioxidants in plants during abiotic stress, *Crit Rev Biotechnal.*, vol.30,
496 pp.161-75.
- 497 Arrick, B.A., Nathan, C.F., Griffith O.W., and Cohn Z .A.,(1982). Glutathione depletion
498 sensitizes tumor cells to oxidative cytolysis, *J. Bio. Chem.*,vol.257, pp.1231–1237
- 499 Baker, A.J.M., and Walker, P.L.,(1990). Ecophysiology of metal uptake by tolerant plants,
500 heavy metal tolerance in plants, In: Shaw AJ (ed) *Evolutionary aspects*. CRC, Boca Raton,
501 pp .155–177.
- 502 Baker, A.J.M., and Brooks, R.R., (1989). Terrestrial higher plants which hyper-accumulate
503 metallic elements—a review of their distribution, ecology and phytochemistry,
504 *Biorecovery.*, ,vol.1,pp181–126.
- 505 Baker, A.J.M., McGrath S.P., Reeves R.D., and Smith J.A.C.,(2000). Metal
506 hyperaccumulator plants: a review of the ecology and physiology of a biochemical resource
507 for phytoremediation of metal-polluted soils, In: Terry N, Bañuelos G, Vangronsveld J
508 (eds) *Phytoremediation of contaminated soil and water*. Lewis, Boca Raton, USA, pp 85–
509 107.
- 510 Bernard, C., Roosens, N., Czernic, P., Lebrun, M., and Verbruggen, N., (2004). A novel
511 CPxATPase from the cadmium hyperaccumulator *Thlaspi caerulescens*, *FEBS. Lett.*,
512 vol.569, pp.140–148.

513 Berczky, Z., Wang, H, Y., Schubert, V., Ganai, M., and Bauer, P., (2003). Differential
 514 regulation of Nramp and IRT metal transporter genes in wild type and iron uptake mutants
 515 of tomato, J. Bio. Chem., vol. 278, pp.24697–24704.

516 Bernal, M., Testillano P, S., Alfonso, M., Del Carmen Risueno, M., Picorel, R., Yruela, I.,
 517 (2007). Identification and subcellular localization of the soybean copper P1B-ATPase
 518 GmHMA8 transporter, J. Struct. Biol., vol.158, pp.146–158.

519 Bennett, L.,E, Burkhead, J.L., Hale, K.,L., Terry, N., Pilon, M., and Pilon-Smits, E .A.H.,
 520 (2003). Analysis of transgenic Indian mustard plants for phytoremediation of metal
 521 contaminated mine tailings, J. Environ. Qual., vol.32, pp.432–440.

522 Bernal, M., Testillano P, S., Alfonso, M., Del Carmen Risueno, M., Picorel, R., Yruela, I.,
 523 (2007). Identification and subcellular localization of the soybean copper P1B-ATPase
 524 GmHMA8 transporter, J. Struct. Biol., vol.158, pp.146–158.

525 Blaudez, D., Kohler, A., Martin, F., Sanders, D., and Chalot, M.,(2003). Poplar metal
 526 tolerance protein 1 confers zinc tolerance and is an oligomeric vacuolar zinc transporter
 527 with an essential leucine zipper motif, Plant Cell., vol.15, pp.2911–2928.

528 Bratteler, M., Lexer, C., and Widmer, A.,(2006). Genetic architecture of traits associated
 529 with serpentine adaptation of *Silene vulgaris*, J. Evol.,vol 19, pp.1149–1156.

530 Campbell, E.J., Schenk, P.M., Kazan, K., Penninckx, I.A.M.A., Anderson, J.P., Maclean,
 531 D.J., Cammue, B.P.A., Ebert, P.R., and Manners, J.M., (2003). Pathogen-responsive
 532 expression of a putative ATP-binding cassette transporter gene conferring resistance to the
 533 diterpenoid Sclareol is regulated by multiple defence signalling pathways in *Arabidopsis*,
 534 Plant. Physiol.,vol.133, pp.1272–1284.

535 Chiang, H.C., Lo, J.C., Yeh, K.C.,(2006). Genes associated with heavy metal tolerance and
 536 accumulation in Zn/Cd hyper-accumulator *Arabidopsis halleri*: a genomic survey with
 537 cDNA microarray, Environ. Sci. Technol., vol.40, pp.6792–6798.

538 Clemens, S., (2006). Toxic metal accumulation, responses to exposure and mechanisms of
 539 tolerance in plants, Biochimie.,vol.88, pp.1707–1719.

540 Cobbett, C.S., (2000). Phytochelatin biosynthesis and function in heavy-metal
 541 detoxification, Curr. Opin. Plant. Biol, vol.3, pp.211–216.

542 Clemens, S., Kim, E.J., Neumann, D., and Schroeder, J.I., (1999). Tolerance to toxic
 543 metals by a gene family of phytochelatin synthase from plants and yeast, EMBO. J., vol.18,
 544 pp.3325–3333.

545 Cobbett, S., and Meagher R.B., (2002). *Arabidopsis* and the genetic potential for the
 546 phytoremediation of toxic elemental and organic pollutants, *The Arabidopsis book*.
 547 American Society of Plant Biologists., ISSN.1543–8120, pp.1–22.

548 Cobbett, C., and Goldsbrough, P., (2002). Phytochelatin and metallothioneins, roles in
 549 heavy metal detoxification and homeostasis, *Annu. Rev. Plant. Biol.*, vol.53, pp.159–182.

550 Cempel, M., Nikel, G., (2006). Nickel: A Review of its sources and Environmental
 551 Toxicology, *Polish J. Of Environ. Stud.*, vol. 15, pp.375-382.

552 Coupe, S.A., Taylor, J.E., and Roberts, J.A., (1995). Characterization of an m-RNA
 553 encoding a metallothionein-like protein that accumulates during ethylene-promoted
 554 abscission of *Sambucus nigra* L, *Planta.*, vol.197, pp.442–447.

555 Dar, K., and Roychoudury, A., (2014). Reactive oxygen species (ROS) and response of
 556 antioxidants as ROS – scavengers during environmental stress in plants, *Front*
 557 *Environ,sci.*, 2.

558 David-Assael, O., Berezin, I., Shoshani-Knaani, N., Saul, H., Mizrahy-Dagri, T., Chen, J.,
 559 Brook, E., and Shaul, O., (2006). AtMHX is an auxin and ABA-regulated transporter whose
 560 expression pattern suggests a role in metal homeostasis in tissues with photosynthetic
 561 potential, *Funct, Plant, Biol.*, vol.33, pp.661–672.

562 Deniau, A. X., and Pieper, B., (2006). WMT-B, QTL analysis of cadmium and zinc
 563 accumulation in the heavy metal hyperaccumulator *Thlaspi caerulescens*, *Theor. Appl.*
 564 *Genet.*, , vol.113, pp.907.

565 Gill, M., (2014). Heavy metals stress in plants, *International Journal of Advanced Research.*,
 566 vol. 2, pp. 1043-1055.

567 Finnegan, M.P., and Chen, N., (2012). Arsenic Toxicity : The Effects on Plant Metabolism ,
 568 *Front Physiol.*, vol. 3 , pp.182.

569 Hossian, M.A., Piyatid, P., A, J., Sliva, T.D and Fujita, M., (2012). Molecular Mechanism
 570 of Heavy metal Toxicity and Tolerance in plant: Central Role of Glutathione in
 571 Detoxification of Reactive Oxygen Species and Methylglyoxyl and in heavy metal chelation.
 572 vol.2012. pp.37.

573 Kehrer , P.J., (2000). The Haber Weiss reaction and mechanism of Toxicity , *Toxicology .*,
 574 2000, vol. 149, pp. 43-50.

575 Patra, M., and Sharma, A., (2000). Mercury Toxicity in plants, *The botanical Review.*, vol.
 576 66, pp. 379-422.

577 Stobier, W., Obermayer, A., Peter Stinbacher, P., and Wolf Kraugartner, W.F .,(2015). The
578 Role of Reactive oxygen species in the formation of Extracellular Traps (ETS) in Humans,
579 Biomolecules ., vol.5, pp. 702-723..

580 Shadreck, M., and Mugadza,T.,(2013).Chromium an essential nutrient and pollutant,
581 African Journal of Pure and Applied Chemistry., vol 7, pp 310-317.

582 Shankar, A.K., Loza-Tavera, C.C.H., Yagam, S.A.,(2005). ChromiumToxicity in Plants,
583 Environmental international.,vol. 31, pp.739-753.

584 Singh, S., Parihar, P., Singh,R., Vijay, P., Singh and Prasad,S.M.,(2015). Heavy metal
585 Tolerance in plants, Role of Transcriptomic, Proteomics , Metabolomics and Ionomics.,
586 Front. Plant Sci; vol.6, pp.1143.

587 Srini, V.M ., Vikram, S.S., JC, P., Favas and Perumal, V., (2014).Lead metal toxicity
588 induced changes on growth and antioxidative enzyme level in water hyacinths [Echoronia
589 Crassipes (Mart)], Botanical Studies An International Studies.,vol.55, pp.54.

590 Surjendu K.D., D, Jayashree., Patra, S., and Pothal, D., (2007).Changes in antioxidative
591 enzyme activities and lipid peroxidation in wheat seedings exposed to cadmium and lead
592 stress., Braz. J. Plant Physiol,. vol.19, pp. 53-60.

593 Tu, C., Ma, L.Q., (2003).Effects of Arsenate and Phosphate on their accumulation by an
594 arsenic hyper accumulator. Pteris Vittata L, Plant and soil., vol. 249, pp.373-382.

595 Yruela, I., (2005). Copper in Plants, Toxic metals in Plants, Braz J.Plant., vol. 17, pp. 145-
596 156.