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EFFECT OF HEAVY METALS COPPER AND CADMIUM EXPOSURE ON THE ANTIOXIDANT PROPERTIES OF THE PLANT CLEOME GYNANDRA

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ABSTRACT : Toxic metal contamination of soil, aqueous waste streams and groundwater causes major environmental and human health problems. The most commonly used methods for dealing with heavy metal pollution are still extremely costly. Phytoremediation is the use of plants to extract, sequester and/or detoxify pollutants and is a new and powerful technique for environmental clean-up. Plants are ideal agents for soil and water remediation because of their unique genetic, biochemical and physiological properties. During the exposure of plants to contaminated soils, the antioxidant defence system helps the plant to protect itself from the damage. Antioxidants are substances that protect itself from damage caused by oxidation. In the present work the plant Cleome Gynandra was exposed to the sublethal and half of sublethal concentration of cadmium and copper metal contaminated soils and the antioxidant factors were investigated and the results are discussed.

Key word: Heavy metal cadmium and copper, Phytoremediation, Anti-oxidant and Cleome gynandra plant.

INTRODUCTION

Pollution is the introduction of pollutant (chemical substances, noise, heat, light, energy and others) into the environment which result in deleterious effects of such a nature as to endanger human health, harm living resources and ecosystem. Soil pollution by metals differs from air or water pollution, because heavy metals persist in soil much longer than in other compartments of the biosphere (Lasat, 2002). Over recent decades, the annual worldwide release of heavy metals reached 22,000 t (metric ton) for cadmium, 939,000 t for copper, 783,000 t for lead and 1,350,000 t for zinc (Singh et al.,2003). Sources of heavy metal contaminants in soils include metalliferous mining and smelting, metallurgical industries, sewage sludge treatment, warfare and military training, waste disposal sites, agricultural fertilizers and electronic industries (Alloway,1995).

Heavy metals can be emitted into the environment by both natural and anthropogenic causes. The major causes of emission are the anthropogenic sources specifically mining operations (Hutton and Symon, 1986; Battarbee et al., 1988; Nriagu,1989).The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. Although individual metals exhibit specific signs of their toxicity, the following have been reported as general signs associated with cadmium, lead, arsenic, mercury, zinc, copper and aluminium poisoning:gastrointestinal (GI) disorders, diarrhoea, stomatitis, tremor, hemoglobinuria causing a rust–red colour to stool, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia when volatile vapours and fumes are inhaled (McCluggage, 1991). The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic.

The regulatory limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil (Salt et al., 1995). But this threshold is continuously exceeding because of several human activities. Plants exposed to high levels of Cd causes reduction in photosynthesis, water uptake, and nutrient uptake.

Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips, and finally death (Wójcik and Tukiendorf, 2004; Mohanpuria et al., 2007). Copper is an essential element for growth and development of every living organism, including plants, since it acts as a co-factor in numerous proteins, particularly in those which are involved in the photosynthetic and the respiratory electron transport chains (Linder and Goode, 1991; Burkhead et al., 2009). Excess of Cu in soil plays a cytotoxic role, induces stress and causes injury to plants. This leads to plant growth retardation and leaf chlorosis (Lewis et al., 2001). Exposure of plants to excess Cu generates oxidative stress and ROS (Stadtman and Oliver, 1991). Oxidative stress causes disturbance of metabolic pathways and damage to macromolecules (Hegedus et al., 2001).

Traditional technologies for the remediation of metal-contaminated soils such as excavation and subsequent ex situ solidification, vitrification and soil washing or in the situ encapsulation, attenuation, volatilization and electrokinetics (Pierzynski, 1997) are time-consuming and expensive. In addition, these technologies can generate hazardous waste (Wenzel et al.,1999). Furthermore, unlike organic pollutants, heavy metals cannot be degraded through any known biological processes (Muhammad et al.,2009). Thus there is a need to develop suitable cost-effective biological soil remediation techniques to remove contaminants without affecting soil fertility. As a cost effective and environmentally-friendly alternative, the use of plants to remedy soils contaminated with inorganic and organic xenobiotics has gained increasing attention in recent years, giving rise to the phytoremediation concept (Doty, 2008;Macek et al., 2008; Eapen et al., 2007; Eapen and D'Souza, 2005; Cherian and Oliveira, 2005; Macek et al., 2004). Phytoremediation defines the use of plants to extract, sequester, and/or detoxify various kinds of environmental pollutant (Salt et al., 1998). This field has generated great excitement because it may offer a reasonable cost effective means to restore the hundreds of thousands of square miles of land and water that have been polluted by human activities (Salt et al., 1995; Cunnigham et al., 1996; Salt et al., 1998). The conventional methods of remediation may cost from $10 to 1000 per cubic meter. Phytoextraction costs are estimated to be as low as $ 0.05 per cubic meter (Cunningham et al.,1997). This technology can be applied to both organic and inorganic pollutants present in soil (solid substrate), water (liquid substrate) or the air (Salt et al.,1998;Raskin et al.,1994). The physico-chemical techniques for soil remediation render the land useless for plant growth as they remove all biological activities, including useful microbes such as nitrogen fixing bacteria, mycorrhiza, fungi, as well as fauna in the process of decontamination (Burns et al.,1996).

In many ways, living plants can be compared to solar driven pumps which can extract and concentrate several elements from their environment. From soil and water, all plants have the ability to accumulate heavy metals which are essential for their growth and development. These metals include Mg, Fe, Mn, Zn, Cu, Mo and Ni (Langille and MacLean, 1976). Certain plants also have the ability to accumulate heavy metals which have no known biological function. These include Cd, Cr, Pb, Co, Ag, Se and Hg (Hanna and Grant, 1962; Baker and Brooks, 1989). However, excessive accumulation of these heavy metals can be toxic to most plants. The ability to both tolerate elevated levels of heavy metals and accumulate them in very high concentrations has evolved both independently and together in a number of different plant species (Ernst et al., 1992). The primary response of plants is the generation of reactive oxygen species (ROS) upon exposure to high levels of heavy metals. Reactive oxygen species (ROS) are mainly produced in the mitochondrial respiratory chain and are associated with a variety of diseases, including cancer, neurodegenerative diseases and aging (Forsberg et al .,2001;Ma,2010;Nishikawa,2008;Taupin,2010). Various metals either generate ROS directly through Haber-Weiss reactions or overproduction of ROS and occurrence of oxidative stress in plants could be the indirect consequence of heavy metal toxicity (Wojtaszek, 1997; Mithofer et al., 2004). The indirect mechanisms include their interaction with the antioxidant system (Srivastava et al., 2004), disrupting the electron transport chain (Ogawa et al., 2004) or disturbing the metabolism of essential elements (Dong et al., 2006).

During accumulation of heavy metal, plant exhibit variety of defence mechanism through antioxidant. Antioxidants are important species which possess the ability of protecting organisms from damage caused by free radical-induced oxidative stress (Canadanovic-Brunet et al.,2005). An anti-oxidant is a substance capable of slowing or preventing the oxidation of other molecules.

Oxidation is a chemical reaction that transfer electrons from a substance to an oxidising agent. oxidation reaction can produce free radicals, which start chain reaction that damage cells. .Anti-oxidants terminate these chain reaction by removing free radicals intermediates, and inhibit other oxidation reaction by being oxidised themselves. As a result, anti-oxidants are often reducing agent . Although oxidation reaction are crucial for life, they can also be damaging; hence plants maintain complex system of multiples types of anti-oxidant, such as glutathione, vitamin c ,and vitamin E, as well as catalase , SOD and various peroxidases. Plants have developed an array of defense strategies (antioxidant system) to cope up with oxidative stress. The antioxidative system includes both enzymatic and non-enzymatic systems. The non enzymatic system includes ascorbic acid (vitamin C); ά-tocopherol, cartenes etc. And enzymic system include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and polyphenol oxidase (PPO) etc. The function of this antioxidant system is to scavenge the toxic radicals produced during oxidative stress and thus help the plants to survive through such conditions. An increase or decline in the activity of anti-oxidants is a direct indication of the adaptive response of plants to avoid the metal toxicity.

The plant cleome gynandra belongs to the family of capparaceae. The common names are Africancabbage, spiderwisp(Eng), morotho(northen sotho) and muruthu(venda). The plant prefers light(sandy) and medium(loamy) soils and requires well- drained soil. The plant prefers acid, neutral and basic soils. It cannot grow in the shade. They are, however, also widely used as leafy herbs and vegetables and for various medicinal purposes, it posses antioxidant properties. Hence in the present study it is aimed to investigate the effect of heavy metal copper and cadmium on the anti-oxidant activity of the hyperaccumulator plant Cleome Gynandra .

MATERIALS AND METHODS

Top soil upto 15 cms depth, was collected from the fertile agricultural lands of Kaniyambadi village near Vellore Town, Tamil Nadu, India**.** The samples were air dried, crushed to powder and sieved in 0.5 mm mesh. The sieved soil samples were stored in polythene bags. Physico-chemical characteristics of soil such as pH ,Electrical Conductivity, porosity, specific gravity, organic matter, total nitrogen, phosphorus, potassium, sodium, calcium, salinity and heavy metal such as copper, cadmium were analyzed by the standard method.

Sublethal concentration (300mg/1000kg) and half of sublethal concentration (150mg/1000kg) of copper chloride and Sublethal concentration (200mg/1000kg) and half of sublethal concentration (100mg/1000kg) of cadmium chloride were selected for the toxicity studies of copper and cadmium on the antioxidant properties of the plant cleome gynandra. The plants were exposed to the above metal contaminated soils for four weeks and the antioxidant factors were tested.

Preparation of plant extract

Cold aqueous extracts of the plant were prepared by homogenizing 10 g of each fresh plant materials in 100 mL of water for 5 minutes. Boiled extracts were prepared by boiling 10 g of each macerated plant in 500 mL of distilled water separately for 1 hour, while the methanolic extracts came from 10 g of the fresh material homogenized in 100 mL of methanol for 10 minutes. All the boiled, cold, and macerated mixtures were separately filtered through Whatman No. 1 paper (Whatman International, Maidstone, UK) and dried in an oven at 50°C. The collected dried extracts were stored separately in the refrigerator at 4°C. Various parameter were estimated by the following standard methods.

Total Phenolics was estimated by Folin-Ciocalteau reagent, DPPH free radical scavenging activity by Blois method (1958) ,Proline estimated by ninhydrin method , estimation of SOD by the method of Misra and Fridovich (1972), estimation of glutathione by Ellman ,(1959) and estimation of catalase by Beers and Seizer method (1952).

RESULTS AND DISCUSSION

Table – I shows the physico - chemical factors of the fertile soil used in the present study.

Table-1 PHYSICO-CHEMICAL CHARACTERISTICS OF GARDEN SOIL

Physico-Chemical Factors	Garden soil
Ph	7.5
Electrical conductivity (mmho/cm^2)	0.348
Porosity	2.08
Specific gravity	2.2
Organic matter (%)	5.6
Total Nitrogen (mg/kg)	6527
Phosphorus (mg/kg)	418.8
Potassium (mg/kg)	120
Sodium (mg/kg)	420
Calcium (mg/kg)	380
Salinity (mg/kg)	21.6
Cu (mg/kg)	0.02
Cd (mg/kg)	ND

The physio-chemical characteristics of soil **(Table – I)** shows that the garden soil is free from heavy metals and also the fertility of the soil was so good. Therefore when the garden soil was spiked with heavy metal cadmium and copper solutions any toxicity shown on plant growth and on the anti-oxidant activity of the plant Cleome gynandra was only due to exposure to sublethal and half of sublethal concentration of cadmium and copper metal only. **Table - 2** shows the effect of heavy metal exposure to copper and cadmium on the antioxidant properties of the the plant Cleome gynandra.

Table – 2 ANTIOXIDANT OF THE PLANT CLEOME GYNANDRA

Factors	Garden Soil	Sub lethal copper	Half of Sub lethal copper	Sub lethal cadmium	Half ofSub lethal cadmium
Total Phenolics mg/100g	910	640	850	641	751
AOA %	95	60	86	60	72
Proline g/100g protein	4.1	6.0	5.3	6.0	5.0
SOD µgm / minute / mg protein	622	807	724	806	676
Catalase µmol/min/g	7.1	12.2	6.2	12.3	8.0
GSH mg/g	16.1	19.2	15.5	19.1	17.2

Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind particularly iron and copper . The roots of many plants exposed to heavy metals exude high levels of phenolics (Winkel-shirley,2002). From the above table, the decreased value of phenolics with increased concentration of heavy metal copper and cadmium shows that phenol form chelation with metal and there by reduce the toxicity of the plant during accumulation of the metal.

The antioxidant activity was found to be the maximum (95%) in the plant exposed to control soil. The same has been reduced much in the plant exposed to sublethal copper and cadmium contaminated soil (60% and 60%) whereas it was 86% and 72% in the half of sub lethal copper and cadmium exposed soil. This shows that the free radical scavenging activity is reduced much in the plant exposed to heavy metal contaminated soils.

The proline value were considerably increased with increased concentration of copper and cadmium. This may be because proline is a non essential amino acid and synthesized in the living organism whenever it is subjected to stress such as high or low temperatures, high salinity, sodicity, high heavy metal exposures etc. Thus the increase of proline value shows that plant undergoes oxidative stress due to accumulation of heavy metals and it was partly overcome.

The antioxidant enzymes Superoxide dismutase, catalase and glutathione were increased significantly in the plant sample exposed to heavy metal contaminated soils. The increase in SOD activity in response to stresses has been attributed to synthesis of the enzyme (Slooten et al.,1995). SOD catalyze the dismutation of superoxide into oxygen and hydrogenperoxide. catalases are ubiquitous heme enzymes that are found in aerobic organisms, ranging from bacteria to higher plants and animals. Catalase is a tetramer of four polypeptide chains, each over 500 aminoacids long. It contains four porphyrin heme group that allow the enzyme to react with the hydrogen peroxide. Functionally, catalases are related to peroxidases; both promote H2O2 oxidation by mechanisms that involve ferryl intermediates (Deisseroth and Dounce, 1970; Dawson, 1988). However, catalases differ from peroxidases, with the exception of chloroperoxidase and myeloperoxidase, in that they have the ability to utilize H2O2 as both an electron acceptor and donor that yields a disproportionate reaction. Due to this catalytic activity, the catalases are believed to be involved in the protective destruction of H2O2 that is generated in respiring cells (Fita and Rossmann, 1985; Ikeda-Saito et al., 1985; Shaffer et al.,1987).

Glutathione (GSH) has been detected virtually in all cell compartments such as cytosol, chloroplast, endoplasmic reticulum, vacuole, and mitochondria. GSH represents as one of the major source of non-protein thiols in most plant cells. The chemical reactivity of the thiol group of GSH makes it particularly suitable to serve a broad range of biochemical functions in all organisms. The nucleophilic nature of the thiol group is also important in the formation of mercaptide bond with metals and for reacting with selected electrophiles. In plants, GSH is involved in a plethora of cellular processes, including defense against ROS (Foyer and Noctor, 2005; Mullineaux and Rausch, 2005), sequestration of heavy metals (Cobbett and Goldsbrough, 2002; Freeman et al., 2004) and detoxification of xenobiotics (Dixon et al., 1998). Several studies have indicated that exposure of plants to high level of heavy metals induces ROS, either directly or indirectly by influencing metabolic processes. GSH participate in the control of H2O2 level of plant cells (Foyer and Noctor, 2005;Shao et al., 2005). Phytochelatins (PCs) are a set of novel heavy metal-binding peptides. PCs are synthesized inductively by exposure to not only Cd, but also by other heavy metals such as Hg, Cu, Zn, Pb and Ni. During the exposure of plants to such metals, PCs are synthesized from GSH by phytochelatin synthase (PCS) activity. Thereafter, numerous physiological studies have indicated their role in heavy metal detoxification as well as in the maintenance of ionic homeostasis (Zenk,1996; Hirata et al., 2005). Thus increased value of glutathione in contaminated soil clearly shows the role of GSH in plants during heavy metal stress exposure is the quenching of ROS and also GSH acts as a precursor for the synthesis of phytochelatins (PCs).

Conclusion

Thus in the present study it is clearly indicated that the plant Cleome Gynandra have highest values of total phenolics , percent antioxidant activity and Enzymatic antioxidant which helps the plant towards the oxidative stress caused by the sublethal and half of sublethal concentration of cadmium and copper. Among the two metals cadmium affects the plant to a greater extent than copper and the plant cleome gynandra can be used for the phytoextraction of both the metals.

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