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Effect of Chromium and Zinc Accumulation on Antioxidant Enzymes during Phytoremediation in Amaranthus Viridis and Parthenium Hysterophorous

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Abstract

Background/Objectives: The study investigate the variation in antioxidant response in experimental plant to overcome heavy metal stress and higher level of antioxidant enzymes may be associated with tolerance capacity of the weeds to defend the plant from oxidative damage. **Methods/Statistical Analysis:** Pot culture experiments were completed in greenhouse and the effect of chromium and zinc on antioxidants enzymes were investigated using UV- Spectrophotometer. A strong positive correlation were revealed in between concentration of heavy metals in various parts of plants and soil. Heavy metal stress enhanced the activity of CATalase (CAT) and Ascorbate PeroXidase (APX). **Findings:** Present study concluded that *Parthenium hysterophorous* and *Amaranthus viridis* has considerable ability to accumulate zinc and chromium and induced higher catalase and ascorbate peroxidase activity. **Application/Improvement:** The study describes a strategy and methodology that these two plants have ability to survive in stressed conditions and enhancing their environmental significance.

Keywords: Antioxidant Enzymes, Heavy Metals, Reactive Oxygen Species and Phytoremediation

1. Introduction

The use of vegetation (without delay or not directly) to remediate infected soil or water is referred to as phytoremediation. Heavy metals remain in the environment for a long term because they are non biodegradable. Toxicity of heavy metal are related to bioaccumulation of heavy metal in food chains show one of the principal environmental and health hassle. Food chain infected with heavy metal is the very essential assets of heavy metals access into human and other living organisms[1]. Ecosystems heavy metal pollution is a substantial universally quandary[2,3] due to the major consequences for human wellbeing, biodiversity and environment balance[4-7]. Heavy metal contaminants once to be had an excessive concentrations are intended as environmental pollution for the reason that of their detrimental consequences on human fitness[8,9]. Bioaccumulation of substantial heavy metals inside environment and their potential fitness risks are of outstanding subject. Poisonous factors launched via on fire of fossil fuels, industrialization as well as industrial effluents are constantly cumulating in the surroundings. Heavy metal infection of soil as well as groundwater poses a vital threat to the environment globally and may cause serious health risks. Such a bundle of studies had been conceded out to estimate the ecological consequences of the heavy metal Zn within soil-plant structures, which suggests to facilitate plant yield decreases because of poisonous impact of Zn on the surroundings[10,11]. Sophisticated level of Zn in soils polluted by using mining and smelting activities, in farming soils handled by means of sewage sludge and in urban and peri-city soils enriched through anthropogenic inputs of Zn is the fundamental basis of Zn toxicity in plants[12,13]. In worldwide soils, whole

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Zn content levels among 60 and 100 mg kg^{-1}; and in soil solution range[14] between 20 and 570 µg L^{-1}. In soil solution, Zn occurs as both loose and complexed ions which include $Zn^{2+,}$ $ZnNO_3$+, $ZnOH^+$, $ZnHCO_3^+$, $ZnSO_4$ and Zn (HPO_4), which are the mainly familiar and free Zn species in soil. Zn-organic species may additionally arise in soil solution[15]. Water contamination by means of Zn is greater prominent near enterprise zones because of waste discharges. Leaching of Zn can happen for the reason that of its mobility in soil, growing water contamination[16,17]. Sign of zinc toxicity consists of decreased yields and stunted growth, Fe-deficiency triggered chlorosis by reductions in chlorophyll synthesis and chloroplast degradation and interference with P, Mg and Mn uptake[18]. In human beings, reviews of Zn-toxicity had been usually in reply to food poisoning incidents. Outcome of lengthy time period and excessive zinc intakes (ranging from a hundred and fifty mg/day to at least 1-2 g/day) reasons nausea, vomiting, hypochromic anaemia, leukopenia, epigastric pain, abdominal cramps and diarrhoea[19,20]. Water pollution with the aid of chromium (Cr) is of huge concern, as it's far one of the maximum toxic heavy metal that attenuates the environment[21]. The incidence of Cr in environment is broadly spoken because of anthropogenic activities, in particular because of their enormous use in industries together with electroplating, leather tanning, metal finishing, nuclear power plant, textile, steel production, catalyst, pigment manufacturing, metal corrosion inhibitor in addition to chromate preparation. Their amplified concentrations have formed an environment that is unsafe to human beings and plant existence[22]. Chromium (Cr) is one of the most toxic Heavy Metal (HM) and in nature is to be found in two stable forms like Cr (III) and Cr (VI). The hexavalent form of Cr is mentioned to be most deadly and a robust oxidant as possesses excessive redox ability which accounts for fast generation of ROS[23]. The toxicity of Cr in plant life outcomes in decreased growth, damaging to roots, brought on chlorosis, disturbed photosynthesis and in the end results in plant demise. Hexavalent chromium in ground water has generally been assumed to be anthropogenic (man-made) contamination, in view of the fact that it's far utilized in a number of industrial packages, inclusive of electroplating, tanning, industrial water cooling, paper pulp production and petroleum refining. Chromates of barium, lead and zinc provide the pigments of lemon chromium, chromium yellow, chromium red, chromium orange, zinc yellow and zinc green glass.

The far two decades have seen the improvement of several remediation techniques[24,25] . These strategies have been evolved to reduce the full or to be had metal concentration in soils and in flip their accumulation within the food chain[26,27]. The phytoremediation potential of a plant is determined not best throughout its capability to take in too much metallic concentration, however in addition by its capability to take in excessive metallic concentration, however also by way of its capability to translocate the metal from roots to aerial components and convey simultaneously a excessive biomass. Environmental stresses, along with abiotic stresses such as drought, salinity, water-logging, extremes of temperature, radiation, mineral deficiency or excess and biotic stresses that occurs due to harm carried out to vegetation with the aid of other residing organism, consisting of bacteria, viruses, fungi, parasites, useful and dangerous insects and weeds[28]. ROS are generated from the physiological movements of dwelling gadget such as peroxidation reaction of lipids, alterations in the as nucleic acids, amino acid structures and changes in the chemical structure of the sugars[29]. Maximum crucial results of stresses on plants is formation of reactive oxygen species (ROS), singlet oxygen ($^1O_2$), superoxide ($O_{2-),}$ hydroxyl radical ($OH^·$) and hydrogen peroxide (H_2O_2) which are extraordinarily reactive in nature and they could engage with a number of different molecules and metabolites such as DNA, pigments, proteins, lipids and different crucial mobile molecule which result in a series of unfavourable tactics[30,31]. Excessive quantities of ROS may be harmful because they can provoke bio-molecular oxidations which lead to cell harm and demise and create oxidative strain[32]. ROS in plant life are produced in normal growth conditions[33]. ROS in a plant in the course of strain are generated through pathways which includes photorespiration, mitochondrial respiration and from the photosynthetic apparatus. Underneath strain condition, the balance among formation of ROS and its usage is destroyed. Diverse environmental stresses result in improved manufacturing of ROS[34]. As an instance, stomatal closure results from osmotic pressure limits CO_2 availability for photosynthetic carbon assimilation, thereby, causing excessive assimilation of superoxide in chloroplast which can cause photo inhibition and photo oxidation damage.

2. Materials and Methods

2.1 Pot Culture Experiment

Pot culture experiments had been executed in greenhouse. For this motive, the soil was surpassed through a 1 mm sieve and 7.5kg soil was placed into each pot. Various concentration of chromium 10 to 160 mg/kg and Zinc 200 to 1400 mg/kg had been supplied as Potassium dichromate ($K_2Cr_2O_7$) and Zinc sulphate ($ZnSo_4$) respectively from its stock solution along with control.

2.2 Antioxidant Enzyme Assay

For the estimation of antioxidant enzyme, 0.5gm of plant components were homogenized in mortar and pestle with 5.0ml of 100mM potassium phosphate buffer at pH 7.0 in ices frozen condition. Homogenate changed into was centrifuged at 15,000g for 20 minutes and supernatant turned was used for evaluation of antioxidative enzyme.

2.2.1 Catalase Assay

Catalase activity was decided according to technique described by way of Aebi, 1984[35]. About 0.5 g fresh leaf samples was homogenised in 5 mL of cold 200 mM sodium phosphate buffer (pH 7.8), the use of chilled mortar and pestle. The homogenates were centrifuged at 10,000×g for 20 min at 4 °C, and the supernatant was assayed for catalase activity by the usage of UV–visible spectrophotometer. The response aggregate (2.8 mL) contained 1.5-mL 200-mM sodium phosphate buffer (pH 7.8), 1.0-mL deionised water and 0.3-mL 0.1-M H_2O_2 prepared afresh prior to its use. The response combination was then brought with 0.5 mL enzyme extract and the enzyme activity was measure with the aid of tracking the decrease in absorbance at 240 nm because of H_2O_2 consumption. One unit of catalase activity was defined as change in absorbance of the mixture at 240 nm[36].

2.2.2 Ascorbate Peroxidase Assay

Ascorbate peroxidase activity was measured using the technique of Nakano and Asada (1981)[37]. It catalyzes the reduction of H_2O_2 with the usage of substrate ascorbate. It is found in chloroplasts, cytosol, and vacuole and apoplastic space of leaf cell in excessive concentrations. One mole of H_2O_2 oxidizes one mole of ascorbate to provide one mole of dehydroascorbate. The rate of oxidation of ascorbate was followed via means of diminish in absorbance at 290 nm. Three (3.0) ml of the response mixture consisting of 1.5 ml phosphate buffer, 300 µl ascorbate, 600 µl H_2O_2 and 600 µl enzyme extract was taken and the decrease in absorbance became recorded at 290 nm.

3. Statistical Analysis

Information supplied in this study is the mean values of three replicates. Statistical analysis becomes completed by SPSS v20.0 statistical software. Pearson correlation and ANalysis Of VAriance (ANOVA) was used to evaluate the connection among doses of heavy metal and among various parts of plants.

4. Results and Discussion

4.1 Effect on Antioxidant Defence System of *Amaranthus viridis* Due to Uptake of Chromium

In ninety days vintage flora, Cr-induced phytotoxicity was apparent in terms of reduced shoot length and number of leaves of *Amaranthus viridis* facing Cr strain when in comparison to control plants. Catalase is the maximum everyday universal oxidoreductase, which scavenges H_2O_2 to O_2 and H_2O. CAT activity increases by means of the increasing the metal ions concentration in root, shoot, leaf and flower contaminated plants compared with control (manage). The vital purpose of CAT is to metabolize the peroxidase liberated inside peroxisome following the conversion of glycolate all through photorespiration[38]. Ascorbate peroxidise lets in the scavenging of small amounts of H_2O_2 especially components of the cell similar to chloroplasts and mitochondria[39]. Ascorbate and glutathione are found in plant tissues in millimolar concentrations and in stress conditions, their levels increases[40]. Ascorbate is the basic primary antioxidant reacting straight by means of ROS (OH, O_2^- and $^1O_2$), it additionally acts as a secondary antioxidant with the aid of reducing the oxidized form of α- tocopherol and preventing membrane harm. As compared with Cr(VI)-untreated *Amaranthus viridis,* Cr(VI)-exposed plants revealed a greater activity of catalase (Table 1) and APX enzymes (Table 2). Further, Cr(VI) treated plants confirmed greater activity of catalase and APX in stem and leaves than in root and flower. Positive significant correlation was analysed among various doses of heavy metal and flower ($p=0.684$, $r=0.01$) in total activity of catalase.

Based totally on analysis of variance, there is enormous difference ($p \leq 0.05$) in between catalase and APX activity in all parts of plants.

4.2 Effect on Antioxidant Defence System of *Parthenium Hysterophorous* Due to Uptake of Zinc

Zinc is a vital micronutrient for vegetation and is a crucial component of many enzymes and acts as a stabilizer and protector of proteins[41]. The antioxidant properties of Zinc may additionally modulate loose radicals[42]. Zn is essential for plant growth but excessive concentrations of Zn can be toxic to vegetation[43]. Total activity of CAT and APX was increased when the concentration of heavy metals were increased (Table 3 and 4). Maximum activity of enzymes was investigated in leaves as compared with the alternative parts of the plants. Positive significant correlation was found among different doses of heavy metals and leaves ($p=0.789$, $r=0.01$) in total activity of catalase.

Table 1. Catalase activity in different bioparts of *Amaranthus* viridis with different concentration of chromium.

S.No	Doses of Cr (mg/kg)	Concentration in Root (Units/min/g FW)	Concentration in Stem (Units/min/g FW)	Concentration in Leaves (Units/min/g FW)	Concentration in Flower (Units/min/g FW)
1	0	245±5.1	256±11.3	167±7.8	105±8.5
2	10	1326±21.7	1097±17.7	1488±14.6	1133±11.2
3	20	1423±15.9	1263±19	1576±21.5	1279±12.3
4	30	1466±27.8	1597±15.4	1745±27.8	1331±7.7
5	40	1673±12.9	1632±27.9	1815±31.5	1414±8.8
6	50	1805±35.7	1602±15.8	1910±27.8	1427±10.9
7	60	1896±22.5	1715±13.5	2011±17.8	1506±14.8
8	70	1932±28.6	1792±19.8	2225±11.8	1563±17.8
9	85	2004±27	1835±24.7	2267±16.3	1610±10.3
10	100	2068±31	2492±27.9	2310±9.8	1693±11
11	120	2103±19.8	2576±21.6	2401±12.7	1739±12.3
12	140	2137±11.4	2621±18.7	2498±11.9	1799±10
13	160	2189±22	2691±23.6	2587±18.8	1821±9.7

Table 2. Ascorbate peroxidase activity in different bioparts of *Amaranthus* viridis with different concentration of chromium.

S. No	Doses of Cr (mg/kg)	Concentration in Root (Units/min/g FW)	Concentration in Stem (Units/min/g FW)	Concentration in Leaves (Units/min/g FW)	Concentration in Flower (Units/min/g FW)
1	0	2.3±0.02	2.9±0.07	2.3±0.004	3.2±0.005
2	10	6±0.3	7.7±0.6	7.3±0.5	6.7±0.8
3	20	6.3±0.2	7.5±0.4	7.7±0.6	7.7±0.4
4	30	6.9±0.8	8.6±0.7	8.4±0.4	7.9±0.5
5	40	7.2±0.5	9.1±0.5	8.8±0.8	8.2±1.4
6	50	7.9±0.8	9.9±0.7	9.6±0.9	8.9±1.2
7	60	8.5±0.4	10.4±1.1	10.9±0.5	9.7±0.8
8	70	9.3±0.6	10.9±1.3	11.8±1.0	10.6±2.1
9	85	10.1±0.9	11.2±0.5	12.5±1.2	11.5±1.6
10	100	11.7±0.6	12.9±0.6	13.4±0.9	12.3±1.2
11	120	12.7±1.2	13.2±1.0	14.9±1.3	13.7±1.7
12	140	13.5±0.7	14.2±1.4	15.5±1.5	14.3±1.1
13	160	14.1±0.9	14.9±1.2	16.6±2.0	15.2±1.2

Table 3. Catalase activity in different bioparts of Parthenium hysterophorous with different concentreation of Zinc.

S.No	Doses of Zn (mg/kg)	Concentration in Root (Units/min/g FW)	Concentration in Stem (Units/min/g FW)	Concentration in Leaves (Units/min/g FW)	Concentration in Flower (Units/min/g FW)
1	0	193±1.5	178±2.7	335±1.5	275±1.5
2	200	1904±12.5	2274±10.9	5030±11.3	2116±11.8
3	400	2496±11	4141±14.8	7835±10.9	2226±12
4	600	2525±10.8	6251±12.8	9233±12	3155±13.8
5	800	2923±9.8	7582±14	9689±14	3331±11
6	1000	3026±13.7	9784±11	10494±9.8	4993±8.9
7	1200	3988±14.5	10823±10.9	13155±14.6	5074±7.9
8	1400	4443±12.9	10990±9.8	13616±12.6	5302±11

Table 4. Ascorbate peroxidase in different bioparts of Parthenium hysterophorous with different concentration of Zinc.

S.No	Doses of Zn (mg/kg)	Concentration in Root (Units/min/g FW)	Concentration in Stem (Units/min/g FW)	Concentration in Leaves (Units/min/g FW)	Concentration in Flower (Units/min/g FW)
1	0	0.6±0.05	0.56±0.08	2.2±0.1	2.3±0.7
2	200	1.6±0.1	1.2±0.09	6.6±0.5	3.4±0.9
3	400	2±0.4	2.7±0.4	11.8±1.0	4.6±0.3
4	600	4.2±0.2	3.9±0.3	13.5±0.9	5.5±0.7
5	800	5.7±0.2	5.8±0.2	16.9±1.2	7.1±0.4
6	1000	7.5±0.6	7±0.4	18.9±0.8	8.3±0.5
7	1200	8.9±0.3	8.3±0.6	20.8±1.3	9.6±0.4
8	1400	9.3±0.8	8.9±0.6	22.3±1.1	10.2±0.4

Positive significant correlation was analysed in between root and stem ($p = 0.834$, $r = 0.01$). Based on analysis of variance, there is significant difference ($p \leq 0.05$) among catalase and APX activity in all parts of plants.

5. Discussion and Conclusions

Vegetation grown in metallic enriched substrata absorbs metal ions in varying levels. Two plant species, *Parthenium hysterophorous* and *Amaranthus viridis* had the potential to deal with metal stress using effective oxidative stress defense mechanisms and had higher average relative growth rate. These consequences had been additionally discovered in lots of different flora[43-45]. Changes in CAT and APX confirmed a clear correlation with Zinc and chromium concentrations. A great increment was proven in activities of major antioxidant enzymes which are involved in detoxification of ROS. Reactive Oxygen Species (ROS) are main part of free radicals, which can cause oxidative stress. ROS can assault lipids, proteins, pigments and nucleic acid, which cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting cell viability[46]. Catalase is an antioxidant enzyme extensively distributed in all animal and plant tissues. An elevated concentration of hydrogen peroxide inside of biological cell can increase an oxidative stress, which may additionally bring about serious complications in the cells. Hydrogen peroxide if not eliminated from the cell, could convert into a hydroxyl radical. Hydroxyl radicals are the most deadliest free radicals, that have ever been observed[47,48]. Catalase decomposes hydrogen peroxide and protects the tissues or cells from reactive hydroxyl radicals. Hence, catalase enzyme can play a pivotal part in keeping the redox reaction within the cells. Ascorbate peroxidase are regarded to play a considerable role in oxidative stress conditions and it has been shown that peroxidase activity can be used as

a potential biomarker for sublethal metal toxicity in plant species .The prevailing study found that both chromium and zinc induces higher activity of CAT than APX, suggesting that CAT provide a better defense mechanism in opposition to metal ion and can cause oxidative damage in both the plant.

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