A Study on Accumulation of Heavy Metal and Antioxidant Responses in *Amaranths dubius*

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ABSTRACT: *In the present study was explored that the effects of metal contaminated soil on the antioxidant response of Amaranths dubius. The soil pH and electrical conductivity were noted as 6.77±0.52 and 1.82±0.37 dSm-1 respectively and the moister content (23.26 ±0.87%) and organic matter (2.82± 0.21%) were noted in the metal contaminated soil. The heavy metal concentration of A. dubius leaf was analysed. The highest heavy metal concentration was found in a Fe (162.3±1.3 mg/kg dw), the moderate accumulation was noted in the Zn (45.3±1.2 mg/kg dw), and followed by Ni (23±0.5 mg/kg dw) and lowest concentration was observed in the Cd (18±0.2 dw), Cu (17.1±0.7 dw) and Pb (12.2±0.5 dw). The effect of heavy metal stress on antioxidant activity in A. dubius was investigated. The enzymatic activity such as Superoxide dismutase, catalase, polyphenol oxidase, peoxidase and phenylalanine ammonia lyase were estimated. Among this, the CAT activity was more 27.5±1.4 Ug^{-1} in the stress conditions when it compared to other activities. The non enzymatic antioxidant like proline was showed the 12.3±1.1 (mg g^{-1}) and 19.2±1.3 (mg g^{-1}) in untreated and treated conditions of A. dubius. In the present study, all the enzyme activities were much higher in the metal treated Amaranths dubious when compared to control.*

Key Words: *Amaranths dubius, Phytoremediation, Heavy metals, Antioxidant and Proline*

Introduction

The agricultural and industrial revolutions in the last few decades have resulted in increased concentration of toxins in our environment that are the major causes of toxicity in plants and animals. Among different toxins, increasing levels of salts, heavy metals, pesticides and other chemicals are posing a threat to agricultural as well as natural ecosystems of the world. Human activities have dramatically been changing the composition and organisation of the soil on earth. Industrial and urban wastes, in particular the uncontrolled disposal of waste and the application of various substances to agricultural soils, have resulted in the contamination of our ecosystem.

Heavy metals of soil and water are of serious concern to the environment due to their non-degradable state. All plants have the ability to accumulate essential metal from the soil solution. Plants need different concentration for growth and development. This ability allows the plants to accumulate other non-essential metals (Al, As, Au, Cd, Cr, Hg, Pb, Pd, Pt, Sb, Te, Ti and U) which have no known biological function (Djingova and Kuleff, 2000). The capacity of plants to concentrate metals has usually been considered a detrimental trait since some plants are directly or indirectly responsible for a proportion of the dietary uptake of toxic heavy metals by human (Chaney *et al*., 1997). Plants often accumulate heavy metals to concentrations exceeding their levels in soil by several folds, where from they enter the food chain. The capacity of plants to accumulate such metals and tolerate their high concentrations is a species-specific trait. Plants ideal for phytoremediation should grow fast, have high biomass and tolerate or accumulate a wide range of heavy metals in their harvestable parts.

Plants contain high concentrations of numerous redox-active antioxidants such as polyphenols, flavanoids, carotenoids, tocopherols, glutathione, ascorbic acid and enzymes with antioxidant activity, which fight against hazardous oxidative damage of plant cell components. Phenolics are antioxidants, which allow them to act as reducing agents, hydrogen donators and singlet oxygen quenchers (Pietta 2000). They also have metal chelation properties. To overcome heavy metal toxicity, plant cells are equipped with enzymatic mechanisms to eliminate or reduce their damaging effects. The anti-oxidant enzymes system, mainly including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) has the ability to scavenge reactive oxygen species and, thereby, prevent oxidative damage.

The plant used in the phytoremediation technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time. Many families of vascular plants have been identified as metal hyperaccumulator (Reeves and Baker, 2000; Prasad and Freitas 2003), and many of them belong to Brassicaceae and Amaranthaceae. These hyperaccumulators are metal selective, having slow growth rate, produce small amounts of biomass and can be used in their natural habitats only (Kamnev and van der Lelie, 2000). *Amaranthus* belongs to the family *Amaranthaceae* with approximately 60 species that are recognized (Anjali *et al.,* 2013). Amaranths have been domesticated as leaf vegetables, fodder, potherbs or as ornamentals. Amaranth species are characterized by a high level of diversity and wide spectrum of adaptability to diverse environmental conditions. Information on genetic as well as nutritional diversity among the species and their wild relatives is essential for efficient utilization of plant genetic resources such as crop improvement. In the present study, it is aimed to analyse the impact of metals on the antioxidative response in *Amaranths dubius*.

Materials and Methods

Preparation of Soil

Top soils (5-10cm) were collected from Garden of Marudupandiyar College, Thanjavur, Tamil Nadu, India. The soils were thoroughly mixed by a mechanical mixer and passed through 4 mm sieve to remove fibre and non soil particulate in the sample (Spirochova *et al.,* 2003). The following physio chemical properties of experimental soils were assessed.

pH and Conductivity

A soil suspension was prepared with soil and deionized water in 1:5 ratio (20 g of soil and 100 mL of water) and allowed to stand for one hour. Soil pH and electrical conductivity were measured using a portable combination probe (Hanna Instruments, United Kingdom) calibrated in accordance with the manufacturer's instructions.

Moisture content

About 10 g ± 0.001 g soil was weighed into a clean pre-weighed tarred porcelain crucible and placed in an oven at 105°C overnight. The sample was then placed in a desiccator using tongs, allowed to cool and then weighed to a constant weight. The moisture content is expressed as a percentage via the following algorithm.

Moisture content = [(mass of air-dried soil – mass of oven-dried soil)/mass of air-dried soil] x100 (Watts and Lyndsay, 1996).

Organic matter content by loss on ignition analysis

A clean dry porcelain crucible was placed in an oven at 100 °C for an hour then allowed to cool before taking the weight of the crucible (W1). About 5 g of 2 mm sieved soil was weighed in the pre-weighed crucible (W2) and dried in an oven at 105 °C for 24 hours. The pre-ignition weight after oven drying at 105 °C was measured and calculated (DW105). The crucible was placed in the oven at 550 °C for 4 hours. The post ignition weight was taken and calculated as DW550 after heating the soil at 550 °C (Heiri *et al*., 2001 and Ribeiro *et al*., 2011).

Cultivation conditions

This soil sample was uniformly saturated with the concentration of 50 mg/kg of Cd, 100 mg/kg of Ni, 150 mg/kg of Pb, 300 mg/kg of Cu, 500 mg/kg of Fe and 1000 mg/kg of Zn were added. *Amaranths dubius* was grown in pot filled with 2 kg of soil samples saturated with corresponding concentration of metals. Deionised water (300ml) was added twice a week during the first month. All the pots to place on 500 μmols of photo synthetically active radiation at the plant top with a 12:12 hr photoperiod at 22± 2° C for 30 days. After 30 days of acclimatization, the heavy metal application was performed three times with an interval of 2 days and one time a day in the early hours of the day.

Analysis of metal accumulation in the soil and plants samples

The concentrations of Cu, Cd, Ni, Pb, Zn and Fe were analysed in the leaves of *Amaranths dubius* after experimental periods. The leaves of *Amaranths dubius* was washed in a tap with distilled water and dried at 105°C. 0.15-g portion of dried plant material was treated with 5 ml of concentrated nitric acid and left for 24 h. Next, the samples were digested at 110°C until complete mineralization was achieved. After mineralization, the samples were diluted with deionized water to a volume of 10 ml. Concentration of Cu, Cd, Ni, Pb, Zn and Fe were measured using inductively coupled plasma-atomic emission spectroscopy. The concentration of various heavy metals were computed and expressed as mg Kg^{-1}

Antioxidant activity

Enzymatic antioxidant

Crushed plant parts were homogenized in a 100 mM phosphate buffer (pH6.8) and centrifuged at 12,000×g for 20 min. The supernatants were used to determine the enzyme activity levels. The whole procedure was carried out at 4 °C. The activity of Superoxide dismutase (SOD) was assayed spectrophotometrically by measuring its ability to inhibit the photochemical reduction of Nitro blue Tetrazoilum (Beauchamp and Fridovich, 1971). One unit of SOD is the amount of extracts that gives 50% inhibition in the rate of NBT reduction. Catalase activity (CAT) was determined by consumption of H_2O_2 and was monitored spectrophotometrically at 240 nm for 3 min (Luck, 1974). For Polyphenol oxidase activity, catechol was used and the activity was expressed as changes in absorbance at 495 nm min-1 g -1 fresh weight of tissue (Esterbauer et al., 1977), For Peroxidase assay (POD) the increase in absorbance due to oxidation of guaiacol (extinction coefficient 26.6 mM^{-1} cm^{-1}) was monitored at 470 nm (Putter, 1974). Phenylalanine ammonia lyase activity was estimated by the method of Brueske (1980).

Non- enzymatic antioxidants

Proline was analysed spectrophotometerically at 520 nm using toluene for a blank as per Bates *et al* (1973). The acid–ninhydrin method was used to determine the proline content. The plant material (0.5 g) was homogenized in 10 mL of sulfosalicylic acid (3 g per 100 mL) and the homogenate was filtered through Whatman No. 2 filter paper. The reaction mixture containing 2 mL of homogenate, 2 mL of acid ninhydrin and 2 mL of glacial acetic acid was incubated at 100 °C for 1 h. The reaction mixture was placed on ice and extracted with 4 mL of toluene. The absorbance was read at 520 nm using toluene as the blank. The proline content expressed in micromoles proline per gram fresh weight was calculated.

Result and Discussion

Physico-chemical analysis of soil

Soil quality can be monitored by a set of measurable attributes termed indicators. These indicators can be broadly grouped as physical and chemical indicators and one can assess overall soil quality by measuring changes in these indicators (Dalal and Moloney 2000). In the present study various physico-chemical characteristics of the metal contaminated soil was analysed. The soil pH and electrical conductivity were noted as 6.77±0.52 and 1.82±0.37 dSm-1 respectively. This low pH enhances solubility and mobility of heavy metals (Akan *et al.,* 2013) and the presence of humic acid which is the major acid in soil organic matter. Heavy metal mobility decreases with increasing soil pH, hence most of the sites with low pH had relatively high concentration of selected heavy metals. The moister content (23.26 ±0.87%) and organic matter (2.82± 0.21%) were noted in the metal contaminated soil (Table.1). presences of many organic waste residues which add more organic matter after their decay. Values of soil organic matter and organic carbon content obtained were higher than the control sites. This observation corroborated Oyedele *et al.,* (2008) who reported that polluted sites had significant higher soil organic matter and organic carbon as compared to the control site. Apart from this accumulation and subsequent decomposition of plant residues also result in building organic matter.

Heavy metal analysis

Soil was contaminated with mixture of heavy metals such as Cd, Cu, Pb, Ni, Fe and Zn. After experimental periods, the leaves of *Amaranths dubius* were extracted with concentrated nitric acid and analysed the heavy metal concentration from this plant leaves. The highest heavy metal concentration in leaves of *Amaranths dubius* was found in a Fe (162.3±1.3 mg/kg dw), the moderate accumulation was noted in the Zn (45.3±1.2 mg/kg dw), and followed by Ni (23±0.5 mg/kg dw) and lowest concentration was observed in the Cd (18±0.2 dw), Cu (17.1±0.7 dw) and Pb (12.2±0.5 dw) (Table.2). The variable accumulation in plant tissues at different sites may be interpreted in terms of decrease in soil pH, increased solubility of metals in spoils and their mobility in the plant tissue (Kumar *et al.,* 2009). Not only the vegetative parts but also the ultimate seeds followed the same sequence of the extent of accumulation of metal in reference. The reason of high uptake of Fe may be due to presence of Fe in the form of iron pyrites lowering pH of the spoil and thus rendering soluble metals more available for plant uptake (Pandey *et al.,* 2008). Another reason for increased metal accumulation at low pH may be the metal binding properties of the organic matter (Pandey *et al.,* 2006b).

Antioxidant activity

The antioxidant enzyme and non enzyme activity of the *Amaranths dubious* were analyzed under the heavy metal accumulated conditions (Fig.1). The activity of SOD was recorded as 10.3±0.2 Ug^{-1} in control plants and 16.1±0.5 Ug^{-1} in metal stressed plants. The increase Superoxide dismutase activity can be considered as an indirect evidence for enhanced production of free radicals. In earlier study reported that the metal stressed plants showed the higher SOD during oxidative damage (Chongpraditnum *et al.,* 1992). Catalase activity in *Amaranths dubious* leaf was 16.2±1.2 Ug^{-1} in untreated conditions. The leaf of metal treated plant showed higher catalase activity (27.5±1.4 Ug^{-1}) when compared to control. In the previous study, *Sinapis arvensis* L. at the highest concentration of heavy metals, the activity of CAT was higher in Cd treatment (Mostafa and Semin, 2001).

The activity of the enzymes PPO and POD were higher in the leaf of *Amaranths dubious.* Generally, these activities were higher in metal stresses plants than in the control. The PPO activity was 0.5±0.1 Ug^{-1} and 1.8±0.1 Ug^{-1} respectively in control and treated plant. The POD activity (14.2±1.5 Ug^{-1}) was also higher in the metal treated plants when compared to untreated plants. Saffar (2009) reported that in *Arabidopsis thaliana* PPO activity might be the result from prolonged heavy metal stress. The highest Phenylalanine Ammonia Lyase (PAL) activity (0.04±0.2 Ug^{-1}) was recorded in metal stressed Amaranths *dubious* leaf when compared to control plants (0.01±0.1 Ug^{-1}). Phenylalanine ammonia lyase (PAL) is the first committed enzyme involved in the plant phenylpropanoid pathway. The PAL activity increased in *Jatropha curcas* L. under heavy metal stress in all bioparts (Devi Chinmayee *et al*., 2014).

Proline can play an important protective role against heavy metal stress. The proline content of Amaranths *dubious* leaf was 12.3±1.1 (mg g^{-1}) and 19.2±1.3 (mg g^{-1}) in untreated and treated conditions. Free proline accumulation under heavy metal exposure seems to be widespread among plants (Costa and More, 1994). Among this, the CAT activity was more 27.5±1.4 Ug^{-1}in the stress conditions when it compared to other activities.

Table. 1 Physio chemical properties of soil

S.No	Physico-chemical properties	Values
1	pH	6.77±0.52
2	Electrical conductivity (dSm-1)	1.82±0.37
3	Moister content (%)	23.26 ±0.87
4	Organic matter (%)	2.82± 0.21

Table. 2 Heavy metal accumulation of *A.dubius* leaves

S.No	Heavy metal	Concentrations (mg/kg)	*A.dubius* leaves (mg/kg dw)
1	Cd	50	18±0.2
2	Ni	100	23±0.5
3	Pb	150	12.2±0.5
4	Cu	300	17.1±0.7
5	Fe	500	162.3±1.3
6	Zn	1000	45.3±1.2

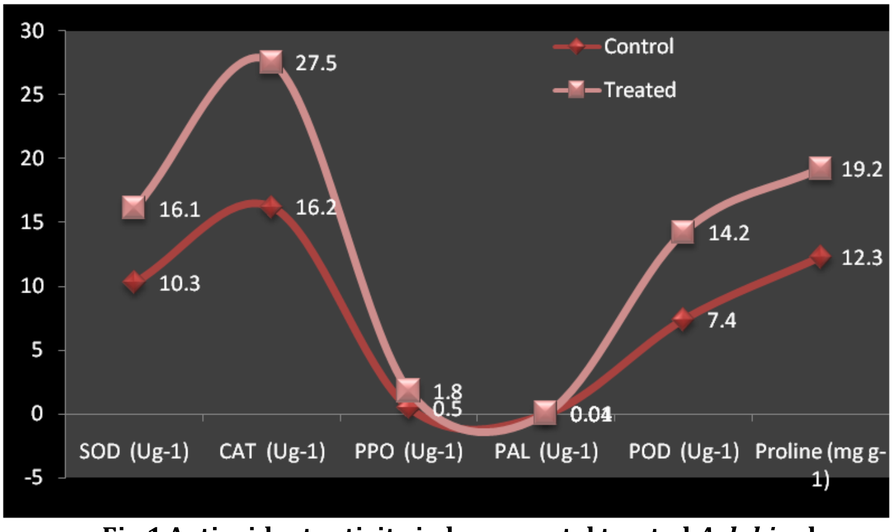

Fig.1 Antioxidant activity in heavy metal treated *A.dubius* leaves

Conclusion

The metal accumulations in plant tissues increased with increasing metal concentration as well as the plant growth period. Application of Fe and Zn led to a significant increase in these metals concentration detected in plants. They are enhanced the antioxidant defence. In the present study concluded that the

Amaranths dubious had ability in metal stress depends on oxidative stress defense mechanisms. The enzymatic activities showed the depending on the concentration of heavy metals. In the present study, all the enzyme activities were much higher in the metal treated *Amaranths dubious* than in the control plants. *Amaranths dubious* were able to protect against multi-metal stress that serves as an important component in antioxidant defense mechanisms.

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