ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN 0976-9595

Research Article

PHYTOREMEDIATION POTENTIAL AND BIOCHEMICAL RESPONSE OF OIL CROPS TO SOIL HEAVY METALS - A CASE STUDY WITH *ARACHIS HYPOGAEA* L. (FABACEAE)

Prameela T S, Anil Kumar V S*

Department of Botany, University college, Thiruvananthapuram, Kerala, India

**Corresponding author: vsanilbotany@gmail.com*

ABSTRACT

The objective of the present study was to investigate the phytoremediation ability of oil seed crop, *Arachis hypogaea* L. A pot experiment was conducted with different concentrations of lead (Pb), cadmium (Cd) and chromium (Cr) as lead nitrate (Pb $(NO_3)_2$, Cadmium chloride ($CdCl_2$) and potassium dichromate ($K_2Cr_2O_7$) based upon the threshold level for 30 and 60 days. Generally, the value of heavy metal was found to be in the decreasing order Cd > Pb > Cr. The obtained values were used to evaluate the Bio Concentration Factor (BCF) and Translocation Factor (TF), indicating the ability of phytoextraction and transport of heavy metals in the plant. Results showed that the BCF value of Cd and Cr was greater than 1(BCF>1) in root and shoot after 30 and 60 days of interval while Pb was higher than 1 in the root only after 60 days of interval. This indicates that the plant act as a Pb, Cd and Cr hyperaccumulator. The TF value greater than 1 (TF>1) for Cd, only after 30 days of interval indicates the metal accumulation and transport to different plant parts from root. In order to reduce the oxidative stress with the production of Reactive Oxygen Species (ROS), the enzymatic and non enzymatic antioxidant response of *A. hypogaea* were also evaluated. Pb induced oxidative stress causes a significant increase in the activity of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC.1.11.1.6), peroxidase (POX; EC.1.11.1.7), ascorbate peroxidase (APX; EC 1.11.1.11), polyphenol oxidase (PPO; EC.1.14.18.1), ascorbic acid and proline than Cd and Cr treated plant.

Keywords: Phytoremediation, *Arachis hypogaea* L., Reactive oxygen species, Heavy metals

1. INTRODUCTION

A variety of organic and inorganic pollutants have been reported to cause environmental pollution and severe health hazards in living beings [1, 2]. Among them, heavy metals (HMs) are highly notorious pollutants. Due to their high abundance and non-biodegradable persistent nature in the environment, they cause soil/ water pollution and induce toxic, genotoxic, terato-genic, and mutagenic effects in living beings [3, 4]. The concentrations of heavy metals in the environment increase from year to year [5].

Heavy metals may cause oxidative stress by forming reactive oxygen species (ROS) [6]. To overcome this oxidative stress, plant cells have developed antioxidant defense mechanism which is composed of enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxi-dase (GPX) and glutathione reductase (GR) and non enzymatic antioxidants like ascorbic acid, glutathione (GSH), carotenoids, alkaloids, tocopherols, proline and phenolic compounds (flavonoids, tannins and lignin) that act as the scavengers of free radicals [7-9]. The redistribution of metals within the plant is metal specific [10]. The elevation of non-essential metals like Pb, Cd and micronutrients such as Zn, Cu and Ni may be the cause of several negative aspects of oxidative stress [11, 12]. Therefore, the effectiveness of a plant's antioxidant defense may be crucial for elucidating its tolerance mechanisms to the common heavy metal contaminants of soil. Low molecular weight antioxidants such as proline, ascorbic acid and glutathione detoxify oxygen free radicals. Non-protein compounds rich in-SH groups, are capable of binding metal ions and forming non-toxic complexes with metals. They are also involved in determining a plant's tolerance to heavy metal ions [13-16].

According to Environment Protection Agency (EPA), the eight most common heavy metal pollutants are As, Cd, Cr, Cu, Hg, Ni, Pb and Zn [17]. Several biotic and abiotic factors such as temperature, soil pH, soil

aeration, moisture, type of plants- their size and root systems, competition between the plants and the accessibility of elements in soil highly influence metals uptake rates in plants. There is a direct relationship between soil chemical characteristics and heavy metals' concentration to the morphological as well as biochemical responses of plants. Metabolic and physiological responses of plants to heavy metal concentration can be viewed as potential adaptive changes during stress [18]. Plants accumulate considerable amount of toxic metals and could tolerate them by inducing different enzymes, stress proteins and enzymatically synthesized phytochelatins [19]. Even though plant growth can be inhibited by metal absorption in heavy metal polluted soils, some plant species are still able to accumulate large amounts of heavy metals without showing any stress symptoms [20].

The competitive advantage of Fabaceae members for phytoremediation is their ability to obtain additional nitrogen through symbiotic relationship with nitrogen fixing rhizobia [21]. Therefore, present study examines the potential of *Arachis hypogaea* for thriving in the heavy metal contaminated soil through the plant's biochemical responses and the effect of these metals uptake on the survival of the plant species.

2. MATERIAL AND METHODS

2.1. Experimental design

Seeds of *Arachis hypogaea* L. were surface sterilized for 20 min. in 1 % (v/v) sodium hypochlorite, and then washed with distilled water. Air dried garden soil (Bulk density- 1.11 g/cm3; p^H- 7.8; EC- 0.27 and organic carbon- 3.15%) was used as control. The sterilized seeds were planted in pots containing 2 Kg garden soil saturated with different concentrations of $Pb(NO_3)_2$ (75,150 mM), $CdCl_2$ (75, 150 mM), K_2Cr_2O7 (6.25, 12.5 mM). Unsaturated soil was used to raise the control plants. The observations were recorded after 30 and 60 days of treatment. The experiments were carried out in a randomized design with five replicates. On the 30^{th} and 60^{th} days, plants were harvested and washed thoroughly with distilled water, frozen and stored at -80°C for further analysis.

2.2. Plant and soil metal analysis

The harvested plants were washed with running tap water and distilled water to remove dust particles. The plant samples were cut into root and shoot. These parts (root and shoot) were air dried and then placed in a de hydrator for 2-3 days and further dried in an oven at 100°C. Dried plant parts were ground into a fine powder using mortar and pestle and stored in polythene bags until used for acid digestion.

2.3. Preparation of plant samples Nitric-per-chloric acid digestion [22]

A 1g of sample was placed in a 250 ml digestion tube and 10 ml of Con: HNO_3 was added. The mixture was boiled gently for 30-45 min. After cooling, 5 ml of 70% $HClO_4$ was added and the mixture was boiled gently until dense white fumes appear. After cooling, 20 ml of distilled water was added and the mixture was boiled further to release any fumes. The solution was cooled, filtered through Whatman no.42 filter paper and transfer quantitatively to a 25 ml volumetric flask by adding distilled water. The filtrate was analyzed for metal content using Atomic Absorption Spectro-photometer (AAS).

2.4. Soil sampling

The soil samples were air- dried in the laboratory to constant weight, after which the samples were crushed and passed through a 2mm sieve to get fine fractions for chemical analysis. One gram of each sample was digested according to the conventional nitric -perchloric acid digestion [23, 24]. The solution was analyze for concentrations of Pb, Cd and Cr using an Atomic Absorption Spectrophotometer and the concentrations of heavy metals were computed and expressed as mg kg^{-1}.

2.5. Metal accumulation efficiency

The metal accumulation efficiency in plants can be calculated by Bioconcentration Factor (BCF) and Translocation Factor (TF). BCF is defined as the ratio of metal concentration in the roots to that in soil, and TF is the ratio of metal concentration in shoots to the roots [25].

For evaluating whether a particular plant is a metal hyperaccumulator, both BCF and TF have to be considered. Therefore, plants with both BCF and TF greater than one (BCF >1, TF>1) have the potential to be used in phytoextraction. Whereas, plants with bioconcentration factor greater than one and trans-location factor less than one (BCF >1 and TF <1) have the potential to be used in phytostabilization [26].

2.6. Analysis of chlorophyll, protein , ascorbic acid and proline

2.6.1. Chlorophyll content

One (1) gm of leaves were homogenized with 20 ml of 80 % acetone. The extract was centrifuged at 5000-10000 rpm for 5 min. The supernatant was transferred into a dry volumetric flask. The procedure was repeated till the residue become colorless. The absorbance was measured at 645 and 663 nm respectively, for chlorophyll "a" and chlorophyll "b" against the solvent (acetone) blank. Chlorophyll content was expressed as mg/g fw (fresh weight) [27].

2.6.2. Protein content

The protein concentration was measured by the method of Bradford [28] using bovine serum albumin (BSA) as standard.

2.6.3. Ascorbic acid

Ascorbic acid content of the sample was done according to Rao and Deshpande [29] by 2,6-dichlorophenolindophenol (DCPIP) titration method.

2.6.4. Proline measurement

Proline was measured spectrophotometrically at 520 nm according to Abraham *et al*., [30]. 500 mg of sample extracted with 3% aqueous 5-sulphosalycilic acid, centrifuged at 10000 rpm for 1 min. Supernatant were used for the proline assay and read the absorbance at 520 nm. Proline content was expressed as µg/g fw.

2.7. Enzymatic activity

2.7.1. Superoxide dismutase activity (SOD; EC.1.15.1.1)

SOD activity was estimated according to the modified method of Zhang *et al*. [31]. One unit of SOD enzyme activity was defined as the quantity of SOD enzyme required to produce a 50% inhibition of reduction of Nitroblue Tetrazolium (NBT).

2.7.2. Catalase (CAT; EC.1.11.1.6)

CAT activity was determined according to the method of Rao *et al* [32] following the consumption of H_2O_2 (extinction coefficient, 9.4 Mm-1cm-1) at 240 nm for 2 min.

2.7.3. Peroxidase activity (POX; EC.1.11.1.7)

Peroxidase activity was assayed by the method of Putter [33]. The increase in absorbance due to oxidation of guaiacol (extinction coefficient =26.6 $M^{-1}cm^{-1}$) was monitored at 470 nm.

2.7.4. Polyphenol oxidase activity (PPO; EC.1.14.18.1)

For PPO activity, catechol was used and the activity is expressed as change in absorbance at 495 nm min^{-1} g^{-1} fresh weight of tissue [34].

2.7.5. Ascorbate peroxidase (APX; EC 1.11.1.11)

Ascorbate peroxidase activity was estimated according to the method of Nakano and Asada [35]. One mole of H_2O_2 oxidises one mole of ascorbate to produce one mole of dehydroascorbate. The rate of oxidation of ascorbate was followed by decrease in absorbance at 290 nm.

3. RESULTS AND DISCUSSION

3.1. Metal content in soil remediated with *Arachis hypogaea* L.

Table 1 shows the concentration of heavy metals in soil remediated with *Arachis hypogaea*. The concentrations of heavy metals obtained after harvesting *Arachis hypogaea* after 30 and 60 days of time interval were 1.73, 21.88 and 0.72 mg/kg after 30 days for soil treated with Pb,Cd and Cr respectively and 31.8,3.0 and 2.0 mg/kg after 60 days for soil treated with Pb, Cd and Cr respectively. After 30 and 60 days of heavy metal treatment, the metals showed lesser concentration when compared with initial amount of heavy metal in soil remediated with *Arachis hypogaea.* This indicates the plant's phytoextraction potential in removing heavy metals from polluted soil. Previous studies reported accumulation of lead in larger amounts (75mg Pb/g dw) in the roots of *Phaseolus vulgaris* [36] and more than 60 ppm of heavy metal like Cd and Cu by *Lathyrus sativus* [37]. The phytoremediation potentials of various crops have already been evaluated for different heavy metals [38-42].

3.2. Metal in harvested parts of Arachis hypogaea L.

Table 2 shows the concentration of heavy metal in different parts of *Arachis hypogaea*. The concentration of heavy metal after 30 days of interval in root and shoot of plant are 1.51, 20.2 and 0.86 mg/kg for Pb, Cd and Cr respectively in root and 0.54, 27.47 and 0.28 mg/kg for Pb, Cd and Cr respectively for shoot.

The concentration of heavy metal after 60 days of interval in roots are 32.6, 24.4 and 50.3 mg/kg and in shoot, 29.0, 22.7 and 5.4 mg/kg for Pb, Cd and Cr respectively. Higher levels of heavy metals were present in roots compared to the shoot. The root system

provides a large surface area that absorbs and accumulate water and nutrients that are essential for growth, but also absorbs other non-essential contaminants [43] such as Pb. There exist two basic strategies by which plants respond to increased concentrations of heavy metals in the environment-exclusive mechanism in which plants avoid excessive uptake and transport of metals and the other is accumulation and sequestration mechanism in which large amount of metals are taken up and transported to the plant roots [44]. Higher levels of Pb accumulation has been reported in the roots than in leaves [45]. However, the phytoextraction potential of *Medicago sativa* has been attributed to the plant's potential of accumulating Pb (43,300 $mgkg^{-1}$DW) in the shoot region [46]. Higher concentrations of Cr were found in roots than shoot in six peanut cultivars. The Cr concentrations in the roots ranged from 2.08 (Huagu 22) to 9.96 mgkg-1 (Qinghua 6) for 10 µmol L-1 treatment and from 12.78 (Huayu 22) to 14.90 mgkg-1 (Qinghua 6) for 100 µmol L-1 [94]. Scientists [27] reported Cd phytoextraction in *Medicago sativa* L. Further, the accumulation efficiency and tolerance of *Prosopis juliflora* to Cd and Cu has also been investigated [47].

3.3. Bioconcentration factor (BCF) and Translocation factor (TF) of heavy metals in *Arachis hypogaea* L.

Table 3 shows the Bioconcentration Factor (BCF) and Translocation Factor (TF) of Heavy metals in *Arachis hypogae*. The greater is the coefficient, the greater will be the uptake of heavy metal.

The BCF varied under different heavy metals in the soil. It was in the range of 1.184 to 2.178 after 30 days and 1.937 to 27.85 after 60 days of heavy metal treatment. The BCF value of *Arachis hypogaea* was highest in Cd (2.178) after 30 days of interval and after 60 days of interval the BCF value highest for Cr (27.85). In the present study the BCF >1 in heavy metal treated plant indicating the metal accumulation capability of the plant. Previous reports suggest that A. hypogaea is suitable for Cd phytoextraction [48] and Phytoextraction of Pb [49] reported that the BCF value of alfalfa was highest in Cd5 treatment (22.67) suggesting the better ability of Cd bioaccumulation.

Table 1: Heavy metal accumulation (mgkg-1) in soil after 30 and 60 days of treatment

Sample	Heavy metals	Conc.	Time interval	
			30 days	60 days
Soil	Control	0	1.67	2.9
	Pb	75 mM	1.73	31.8
	Cd	75 mM	21.88	3.0
	Cr	6.25 mM	0.72	2.0

Table 2: Heavy metal analysis (mg kg^{-1}) in plant samples after 30 and 60 days of treatment

Sample	Heavy metals	Conc.	30 days		60 days	
			Root	Shoot	Root	Shoot
Plant	Control	0	1.25	0.54	2.3	1.33
	Pb	75 mM	1.51	0.54	32.6	29.0
	Cd	75 mM	20.2	27.47	24.4	22.7
	Cr	6.25 mM	0.86	0.28	50.3	5.4

Table 3: Bio concentration Factor (BCF) and Translocation Factor (TF) of *Arachis hypogaea L.* under heavy metal stress

Days	Heavy metals	BCF		TF
		Root	Shoot	
30	Pb	0.87	0.31	0.35
	Cd	0.92	1.25	1.35
	Cr	1.19	0.38	0.32
60	Pb	1.02	0.91	0.88
	Cd	8.13	7.56	0.93
	Cr	25.15	2.7	0.10

TF>1 is an indicative of metal accumulation and transport into the different plant parts, and TF< 1 for the storage of metal in roots. Here the TF value of Cd is greater than 1(TF >1) in *A. hypogaea* at 30 days of time interval is an indication of translocation and storage of metal in aerial parts. Plants with both bioconcentration factor and translocation factor greater than 1 (BCF and TF>1) have the potential to be used in phytoextraction. Whereas, plants with bioconcentration factor greater than 1 and translocation factor less than 1 (BCF >1 and TF<1) have the potential for phytostabilization [50]. Here *A. hypogaea* is suitable for phytoextraction and phytostabilization of heavy metals.

3.4. Biochemical response of *Arachis hypogeae* L.

SOD activity in *Arachis hypogaea* showed significant increase in cadmium treated plant at T1 concentration after 30 days, 65.44 U/mg protein and after 60 days increase in SOD activity in Pb-treated plant at T2 concentration *i.e.* 85.19 U/mg protein. These findings support the earlier works carried out in maize [51] and *Arachis hypogaea* [52] suggesting that an increase in the activity of SOD was observed in Cd treated soil. SOD activity was increased during water deficit stress during pod development stage and highest increase (75%) was observed in ICGS 44 and TAG 24 cultivar of *Arachis hypogaea* [53]. In *Vicia faba*, the SOD activity in leaf exposed to 50 µM Cd for 3-6 days were observed to be significantly high (P<0.05) when compared with the control [54]. Similar results were observed in the roots and shoots of pigeonpea (*Cajanus cajan* L.) under Zn and Ni stress shows higher values of SOD in cv.ICPL87 [55]. SOD activity decreased in all other heavy metal concentration compared to control (Fig. 1). It was reported that at 12 mg/kg of Cd concentration, there is a decline in SOD by 45% in pea nut (*Arachis hypogaea*) with FengHua3, HuaYu20 and LuHua 12 cultivars [56]. SODs are the group of enzymes that accelerate the dissociation of superoxide radicals to H_2O_2 [57]. There are reports that SOD activity gets stimulated under a variety of stressful conditions including Cu, Al, Mn, Fe and Zn toxicity [58, 59].

CAT catalyzes hydrogen peroxide to water and oxygen through the transfer of electrons [60]. CAT activity increased in Pb-treated plant at T1 *i.e.* 1142.6 µmol min^{-1} mg $protein^{-1}$ after 30 days and 2869.58 µmol min^{-1} mg $protein^{-1}$ in T2 (150mM), as compared with the control. The Pb and Cd at T1 treatment and Cd and Cr at T2 treatment caused a decrease in catalase activity as compared with the control (Fig. 2). At 12 mg/kg cadmium concentration for pod setting stage, for harvest stage and harvest stage of FengHua 3 cultivar of peanut, there is a decline in CAT by 62 % [61]. CAT catalyzes the decomposition of H_2O_2, decreases with increasing concentration of Cd in pea [62, 63]. In the roots and leaves of both horse gram (*Macrotyloma uniflorum* (Lam.) Verdc. Cv VZM1) and bengal gram (*Cicer arietinum* L. cv Annogiri), higher concentration of Pb (800 ppm) resulted 2- to 3- fold increase in CAT activity [64].

POD is an antioxidant enzymes involved in the elimination of active oxygen species. In this study, the activity of POD was higher in Pb and Cd treated plants than normal growth conditions. Pb and Cd treated plant shows maximum peroxidase activity at T1 concentration (75mM) *i.e.* 14.46×10^{-3} U g^{-1} after 30 days and at T2 concentration (150 mM) *i.e.* 12.59×10^{-3} U g^{-1} after 60 days respectively (Fig. 3). It is reported that the activity of POD increased with increasing concentration of cadmium in *Arachis hypogeae* [65]. In *Vicia faba,* the POD activity ranged from 9.35 -182.4 in leaves and 976-236.3 µmol tetra-guaiacol min^{-1} $mg\ protein^{-1}$ in stem [66] and enhancement of POD in soybean plant in Cd contaminated soil [67]. Thus, the higher activity of POD reduces the accumulation of ROS and the effect of oxidative stress on the treated plants and contributes for developing metal tolerance. The major scavenger SOD catalyzes the dismutation of superoxide (O2) to hydrogen peroxide (H_2O_2) and (O_2). Since H_2O_2 is toxic to cell, it gets further detoxified by CAT or POD to water and oxygen [68].

APX is the major ROS scavenging enzymes which catalyze the reduction of H_2O_2 to prevent cellular damage. In the present study Pb activity induced more APX activity at T1 concentration compared to Cd and Cr ie. 0.347 µmol min^{-1} mg $protein^{-1}$ after 30 days and 1.329 µmol min^{-1} mg $protein^{-1}$ after 60 days when compared with the control (Fig. 4). It was reported that the cultivar ICGS44 and TAG 24 of *Arachis hypogaea* showed maximum increase (63%) in APX activity at pegging stage during water deficit stress condition, whereas ICGS 44 showed maximum increase (37%) of APX activity [69] and increase in APX activity in leaves and roots during the period of 10-25 days with increase in Cd concentration [65]. The induction of APX activity was also reported in Cu treated *Phaseolus vulgaris* [70] and *Ceretophyllum demersum* treated with Cu [71].

PPO activity was induced in Pb-treated plant at T2

concentration (150 mM) of 68.413 min^{-1} mg^{-1} of protein after 30 days of plant growth and after 60 days the maximum activity was also observed in Pb-treated plant at T1 concentration (Fig. 5). Previous study reported that in *Vigna radiata* (L.) Wilczek under Co stress the PPO activity increased with increase in metal concentration (250 $mgkg^{-1}$) when compared with control [72]. Similarly, in 2 pigeonpea cultivars (*Cajanus cajan* cv. LRG-41 and Yashoda-45), the PPO activity increased in response to both heavy metal (Cd and Cr) and water stress [73].

A common response of plants to heavy metal stress is the accumulation of proline [74]. In the present study, at 30 days of plant growth, increased concentration of proline occur in Pb-treated plant at T1 *i.e.* 24.4 µg g^{-1} FW while after 60 days, the maximum proline was recorded in Cr treated plant at T2 concentration is 236.0 µg g^{-1} FW when compared to the control (Fig. 6). Vineeth *et al* [75] reported that under heavy metal stress the proline content significantly increased (0.815±0.009 mg/g) in *Vigna radiata* when compared with control. The higher level of proline content was found in leaves and stems of *Vicia faba* grown in Zn and Ni contaminated soil [66]. So the increase in proline content in plant leaves under heavy metal stress indicates proline's metal tolerance ability [76].

Ascorbic acid is a non enzymatic antioxidant and under heavy metal stress, a significant increase in ascorbic acid content was noted [77]. The maximum ascorbic acid was observed in Pb-treated plant at T1 is 378.7 mg/100g after 30 days and 256.41 mg/100g in Cr treated plant at T2 after 60 days, when compared with the control (Fig. 7). Nareshkumar *et al*. [78] reported that groundnut cultivar K6 showed higher ascorbic acid content in response to Pb stress. Zengin and Munzuroglu, (2005)[79] reported that the ascorbic acid content in bean (*Phaseolus vulgaris* L.) seedlings increased in a dose dependent manner in Pb, Cu, Cd and Hg treatments.

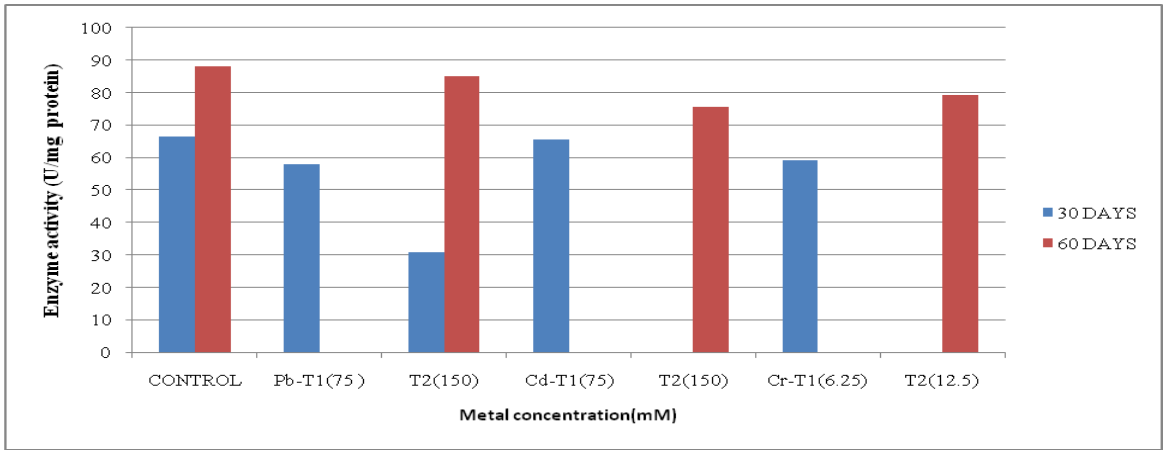

Fig. 1: Superoxide dismutase activity in *Arachis hypogeae* L. under heavy metal stress

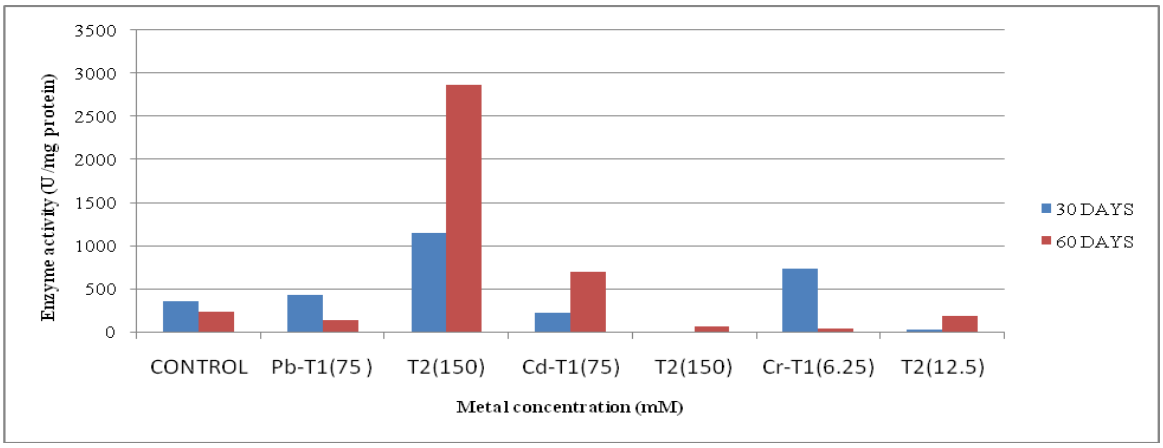

Fig. 2: Catalase activity in *Arachis hypogeae* L. under heavy metal stress

The ascorbic acid perform diverse roles under abiotic stress. Ascorbic acid has an ability to donate electrons so it acts as a strong ROS scavenger [80]. The tolerance of plant may be due to increased amount of ascorbic acid in stress condition.

Chlorophyll a -Maximum chlorophyll a was observed in Pb-treated plant at T1 after 30 days ie.1.55mg g^{-1} FW and after 60 days the maximum chlorophyll content was observed in Cr treated plant at T2 is 0.90 mg g^{-1} FW when compared with control (Fig. 8). Chlorophyll b-Maximum chlorophyll b content (1.04 mg g^{-1} FW) was observed in Cr-treated plant at T1 after 30 days and after 60 days maximum chlorophyll content (0.45mg g^{-1} FW) was observed in Pb-treated soil at T1 (Fig. 9). Total chlorophyll-The maximum mean value for total chlorophyll (2.12 mg g^{-1} FW) was observed in Cr treated soil at T2 after 30 days interval and after 60 days the maximum value (1.33mg g^{-1} FW) was also observed in Cr treated soil at T1 (Fig. 10).

In the present study, the total chlorophyll content varied with heavy metal stress. A remarkable decrease in total chlorophyll content was observed with increase in the heavy metal concentration after 60 days. Previous studies reported that under Mn stress, an increase of 22% in total chlorophyll content at 10-20 µM treatment and there after a significant decrease of 30-43 % at 80-60 µM in *Vicia faba* [81]. The decrease in chlorophyll content may be due to reduce synthesis of chlorophyll due to inhibition of enzyme activity such as δ-aminolevulinicacid dehydratase (ALAdehydratase) [82] and protochlorophyllide reductase [83], replacement of Mg with heavy metals in chlorophyll structure [84], decrease in the essential elements such as Fe^{2+} and Zn^{2+} for chlorophyll synthesis [83, 85] or inhibition in the activity of some enzymes in Calvin cycle [86, 87].

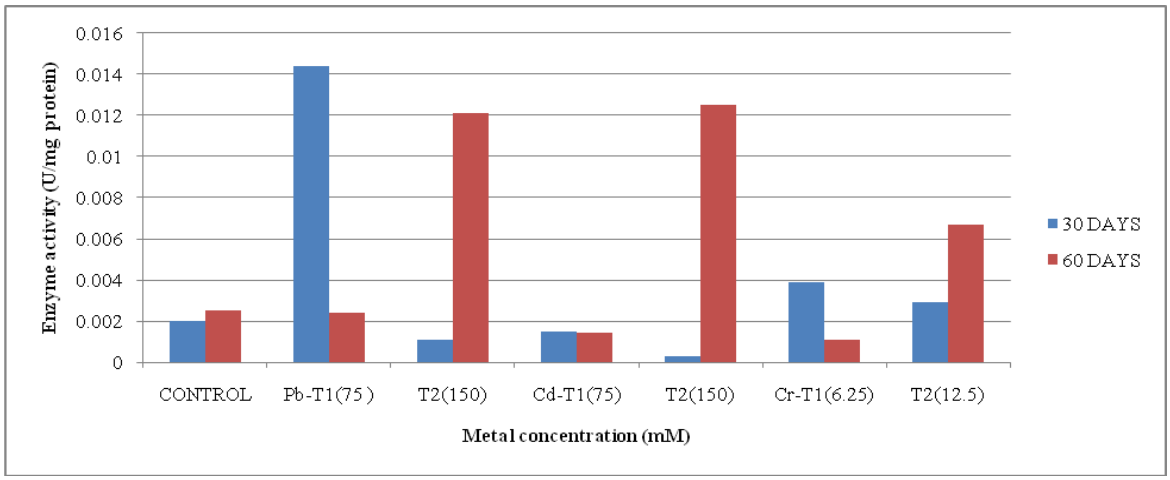

Fig. 3: Peroxidase activity in *Arachis hypogeae* L. under heavy metal stress

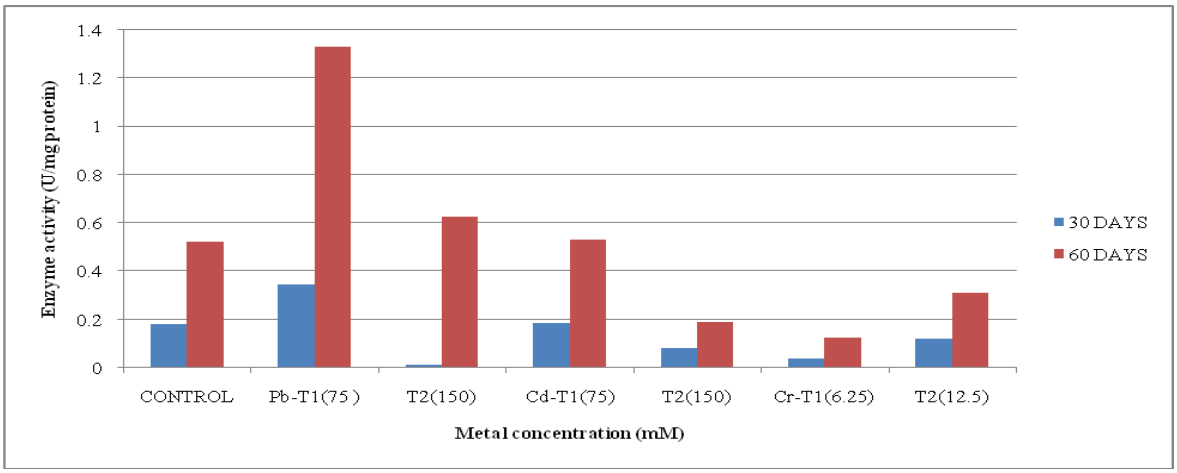

Fig. 4: Ascorbate peroxidase activity in *Arachis hypogeae* L. under heavy metal stress

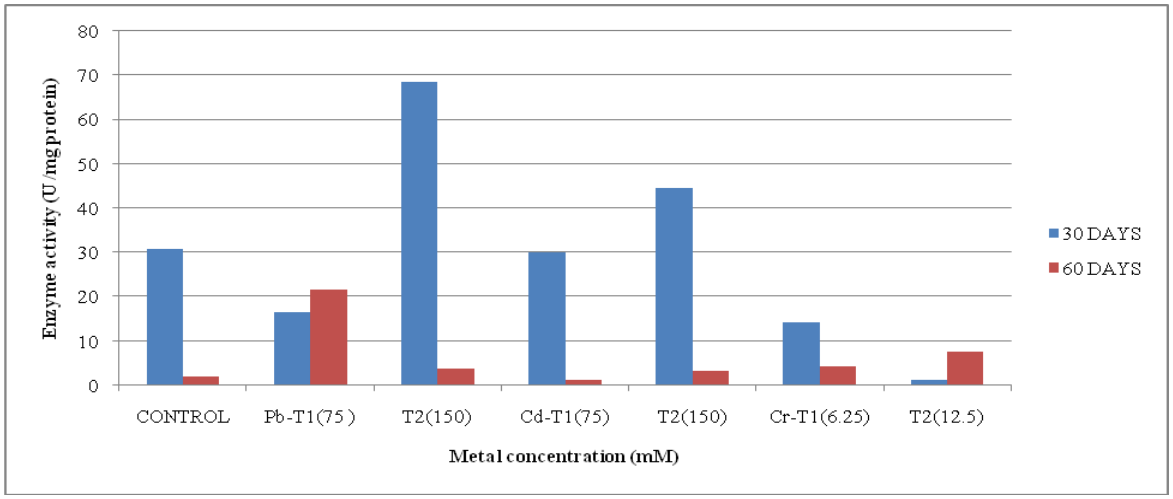

Fig. 5: Polyphenol oxidase activity in *Arachis hypogeae* L. under heavy metal stress

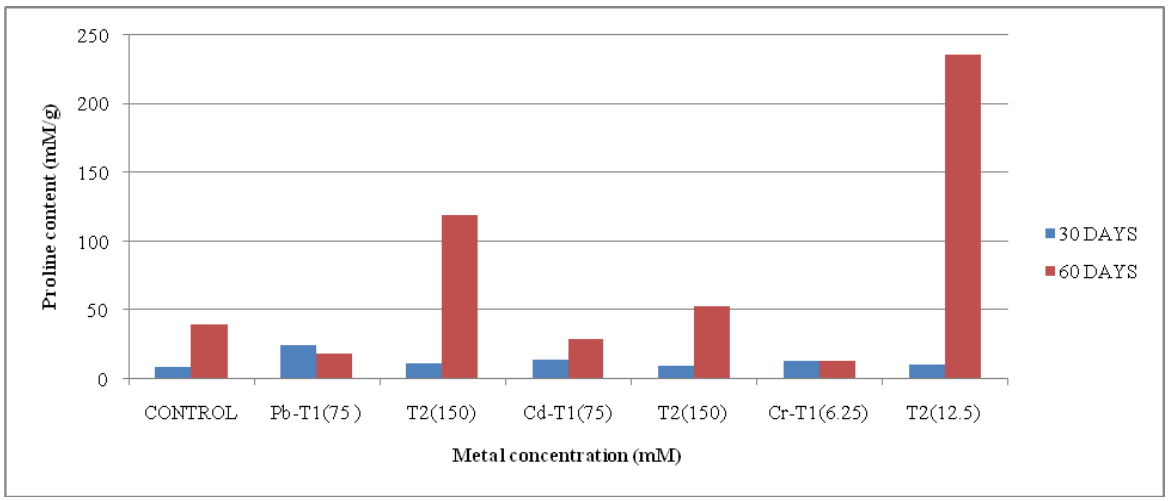

Fig. 6: Proline content in *Arachis hypogeae* L. under heavy metal stress

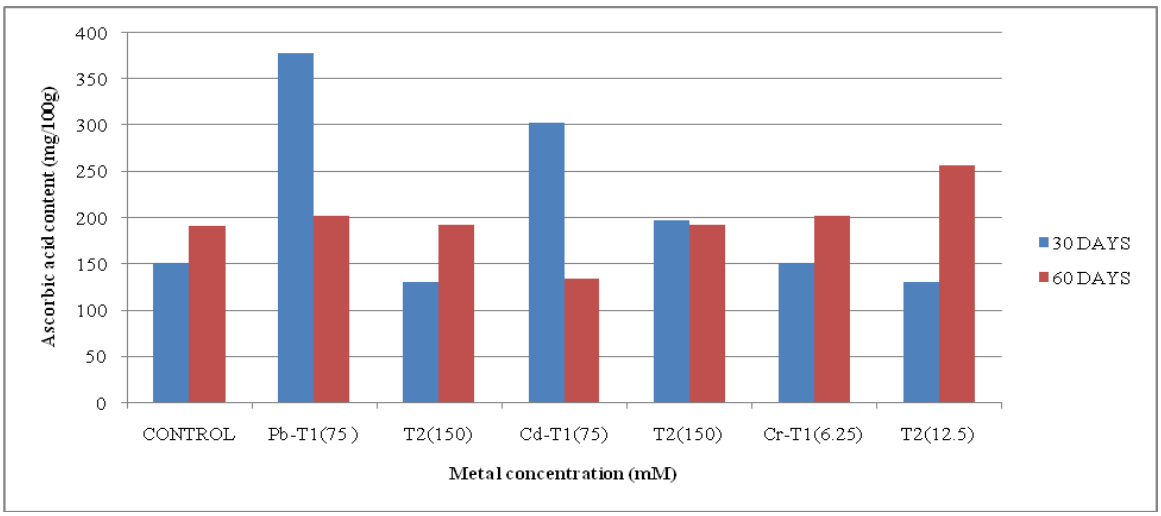

Fig. 7: Ascorbic acid content in *Arachis hypogeae* L. under heavy metal stress

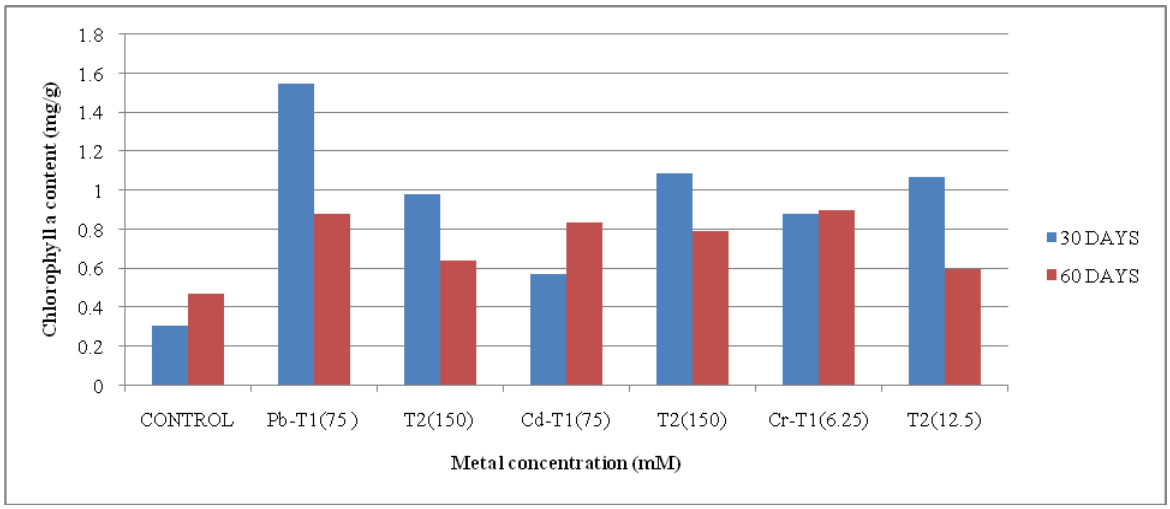

Fig. 8: Chlorophyll a content in *Arachis hypogeae* L. under heavy metal stress

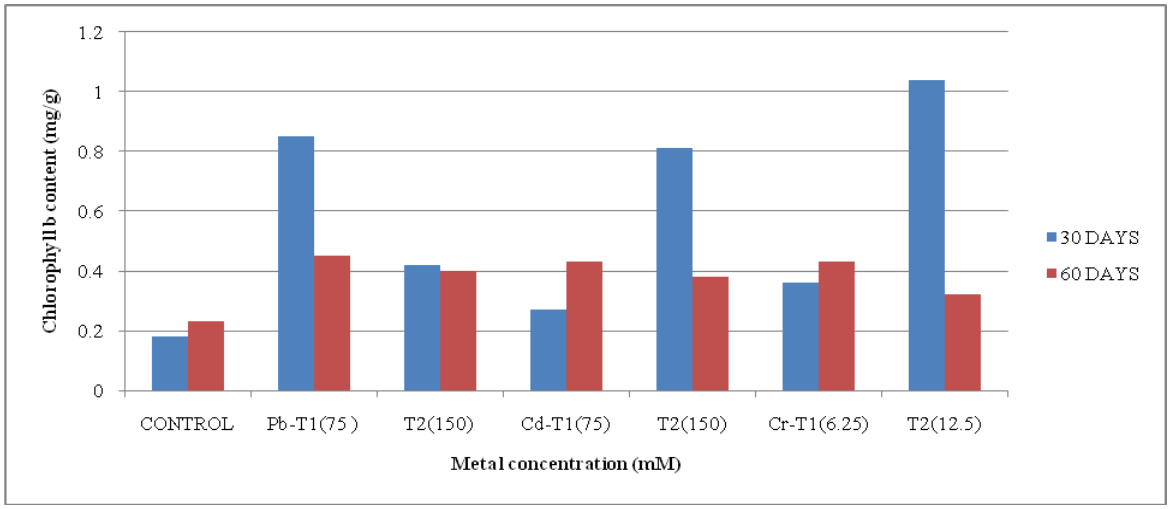

Fig. 9: Chlorophyll b content in *Arachis hypogeae* L. under heavy metal stress

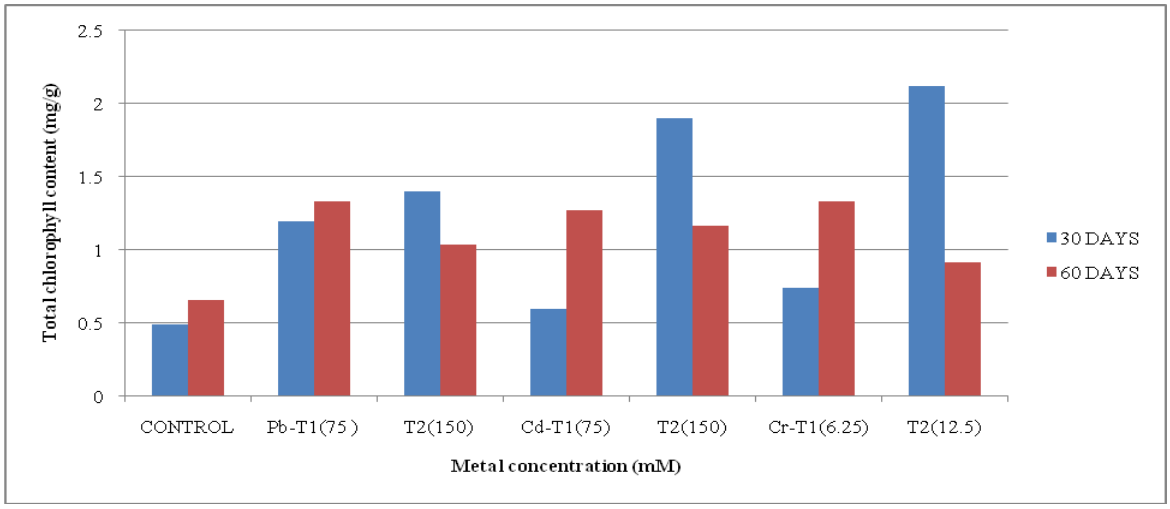

Fig. 10: Total chlorophyll content in *Arachis hypogeae* L.under heavy metal stress

Fig. 11 shows maximum value of protein content in Cd treated soil (0.727 mg g -1 FW) at T2 after 30 days interval and after 60 days, the maximum protein content was also observed in Cd treated soil at T1 when compared with the control. In the present study, the protein content increased with increasing concentration of heavy metal after 30 days of interval but finally decreases with increasing concentration of heavy metal after 60 days. It is reported that the level of nitrate reductase protein was decreased by about 80% after 7 days of Cd exposure and only 15% after 1 day in *Phaseolus vulgaris* L. [88] and in *Vigna mungo*, the protein content decreases with increase in Zn concentration [89]. When the heavy metal toxicity crosses the threshold limit, the protein level decreases due to the reduced incorporation of free amino acid into the protein [90]. The decrease in protein concentration may be due to degradation of protein [91]. Heavy metals may alter the structure of plant proteins and cause toxic effects [92, 93]. During the process of folding, proteins are more susceptible to heavy metals [94].

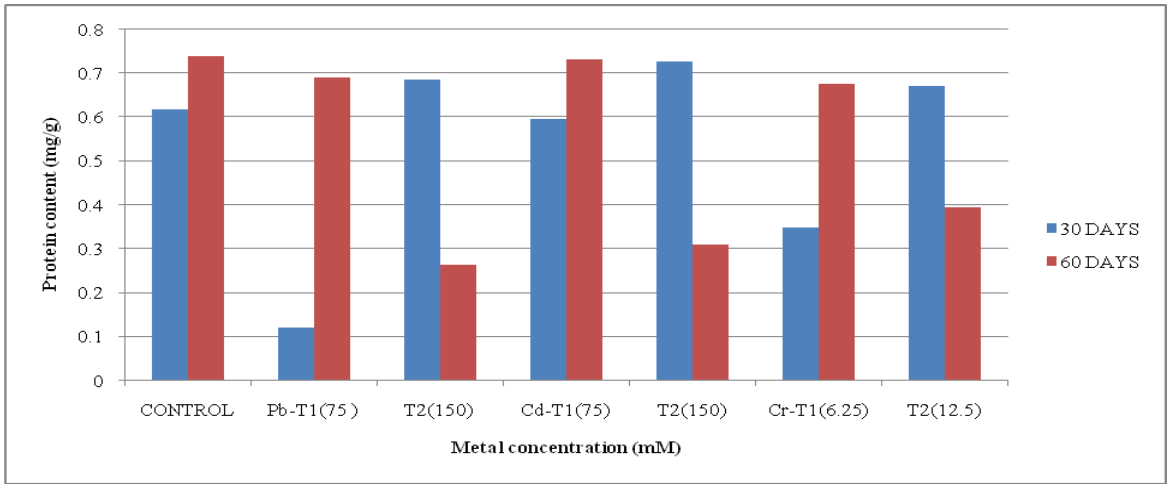

Fig. 11: Protein content in *Arachis hypogeae* L. under haevy metal stress

4. CONCLUSION

The present study evaluates the heavy metal tolerance and the biochemical changes under heavy metal stress in *Arachis hypogaea* L. Different antioxidants respond differently to various heavy metals, ensuring the survival of the plant. The variation in activity of different detoxifying enzymes may be due to different threshold tolerance to the stress conditions. The increase in antioxidant enzymes in some treatments showed that there is an active role in the tolerance of this plant against various heavy metals. Different factors such as proteins, enzymes and phenolic compounds are involved in the tolerance towards heavy metal stress. The elevated BCF and TF values are indications of hyper accumulation properties. In the present study, BCF value is greater than one (BCF >1) in *A. hypogaea*, indicative of its potential to be used in phytoextraction and also as phytostabilizer, where BCF >1 and TF <1. The results recommend the practical application of *Arachis hypogaea* L. in polluted soil for phytoremediation.

5. ACKNOWLEDGEMENT

The authors are thankful to the Director, CEPC Lab Kollam for providing facilities for the work. The first author acknowledge the Principal, University college, Thiruvananthapuram, Kerala for providing facilities and University of Kerala for financial assistance. The corresponding author acknowledges The Director of Collegiate Education, Government of Kerala for providing a platform for performing the work.

6. REFERENCES

1. Maszenan AM, Liu Y, Ng WJ. *Biotechnol Adv,* 2011; **29 (1):**111-123.
2. Saxena G, Bharagava RN. Organic and inorganic pollutants in industrial wastes, their ecotoxicological effects, health hazards and bioremediation approaches. In: Bharagava RN (ed). *Environmental pollutants and their bioremediation approaches*, 1 st edn. CRC Press, Boca Raton, 2017, 23-56.
3. Dixit R, Wasiullah MD, Pandiyan K, Singh UB, Sahu A, Shukla R. *et al. Sustainability,* 2015; **7:**2189-2212.

4. Sarwar N, Imran M, Shaheen MR, Ishaq W, Kamran A, Matloob A *et al*. *Chemosphere,* 2017; **171:**710-721.
5. Govindasamy C, Arulpriya M, Ruban P, Francisca LJ, Ilayaraja A. *Int J Environ Sci*, 2011; **2:**145-153.
6. Jaishankar M, Tseten T, Abbalagan N, Mathew BB, Beeregowda KN. *Interdiscip Toxicol*, 2014; **7(2):**60-72.
7. Sharma P, Jha AB, Dubey RS, Pessarakli M. *J Bot*, 2012; **37:**1-26.
8. Michalak A. *Pol J Environ Stud*, 2006; **15(4):**523-530.
9. Rastgoo L, Alemzadeh A, Afsharifar A. *POJ,* 2011; **4(7):**377-383.
10. Page W, Feller U. *Ann Bot*, 2005; **96(3):** 425-434.
11. Zengin F, Munzuroglu O. *Acta Biol Crac,* 2005; **47 (2):**157-164.
12. Meng Q, Zou J, Jiang W, Liu D. *Acta Biol Crac*, 2007; **49(1):**95-101.
13. Wei Z, Woonchung J, Chen D. *Microchem J*, 2003; **74:**207-213.
14. Sharma SS, Dietz KJ. *J Exp Bot,* 2006; **57(4):**711-726.
15. Sun RL, Zhou QZ, Sun FH, Jin CX. *Environ Exp Bot*, 2007; **60:**468-476.
16. Xu J, Yin HX, Li X. *Plant cell Rep*, 2009; **28(2):**325-333.
17. Chaney RX, Hundermann, Palmer WT, Small RJ, White MC, Decker AM. *Proc. Natn Conf. Composting municipal residues and Sludge information Transfer Inc. Rockvilla, M.D*, 1978; 96-97.
18. Preeti P, Tripathi AK. *Int J Environ Sci*, 2011; **1(5).**
19. Ali BM, Vajpayee P, Tripathi RD, Raj UN, Singh SN, Singh SP. *Bull Environ Contam Toxicol*, 2003; **70 (3):**462-469.
20. Oliver MA. *Eur J Soil Sci,* 1997; **48:**573-592.
21. Olson PE, Castro A, Joem M, Du Teau NM. *J Environ Qual,* 2007; **36:**1461-1469.
22. AOAC[a] (*Association of Official Analytical Chemists*) Arlington, Virginia, 1990; 84-85.
23. AOAC[b] (*Association of analytical chemists*) (15th edition). USA, VA. 1990; 1-34.
24. Hseu ZY. *Bioresource Technol*, 2004; **95(1):**53-59.
25. Malik RN, Husain SZ, Nazir I. *Pak J Bot*, 2010; **42(1):**291-301.
26. Kabata-Pendias A. Trace elements in soils and plants. Boca Raon: Chemical Rubber Company Press, Boca Raton, 2011.
27. Arnon D. *Plant Physiology*, 1949; **24(1):**1-15.
28. Bradford MM. *Anal Biochem*, 1976; **72:**248-254.
29. Rao B, Deshpande V. Experimental biochemistry Tunbridge Wells, Kent:Anshan, 2006.
30. Abraham E, Hourton-Cabassa C, Erdei L and Szabados L. *Plant stress tolerance*, 2010; **639:**317-331.
31. Zhang HY, Jiang YN, He ZY, Ma M. *J Physiol*, 2005; **162(9):**977-984.
32. Rao MV, Paliyath G, Ormrod DP. *Plant Physiol*, 1996; **110(1):**125-136.
33. Putter J. Peroxidase in Bergmeyer Hu (Ed), Methods of Enzymatic Analysis. "*Weinhan*, *Verlag Chemie*, 1974, 685-690.
34. Esterbaurer H, Schwarzl E, Hayn M. *Anal Biochem*, 1977; **77(2):**486-494.
35. Nakano Y, Asada K. *Plant Cell Physiol.*, 1981; **22:**867-880.
36. Piechalak A, Tomaszewska B, Baralkiewicz D, Malecka A. *Phytochemistry*, 2002; **60(2):**153-162.
37. Nagati VB, Koyyati R, Marx P, Chinnapaka VD, Padigya PRM. *Int J Pharm Tech Res,* 2015; **7(3):**528-534.
38. Garg N, Singla P, Bhandari P. *Turk J Agric For,* 2015; **39:**234-250.
39. Kambhampati MS, Vu VT. *Bull Environ Contam Toxicol*, 2013; **91(3):**310-313.
40. Wani PA, Khan MS. *Bacteriology J,* 2012; **2(4):**66-78.
41. Dasgupta S, Satvat PS, Mahinrakar AB. *IJTES*, 2011; **2(3):**338-341.
42. Wani PA, Khan MS, Zaidi A. *Australian J Exp Agric,* 2007; **47(6):**712-720.
43. Arthur EL, Rice PJ, Anderson TA, Baladi SM, Henderson KLD, Coats JR. *Crit. Rev. Plant Sci.*, 2005; **24:**109-122.
44. McGrath SP, Shen ZG, Zhao FJ. *J Plant and Soil*, 1997; **188(1):**153-159.
45. Boominathan R, Doran PM. *Biotechnol Bioeng*, 2003; **83(2):**158-167.
46. Koptsik GN. *Eurasian Soil Sci*, 2014; **47:**923-939.
47. Senthilkumar P, Prince WSPM, Sivakumar S, Subbhuraam CV. *Chemosphere,* 2005; **60(10):**1493-1496.
48. Su G, Li J, Liu C, Shi G. *Plant Soil,* 2013; **365:**337-345.
49. Zhi-xin N, Li-na S, Tie-heng S, Yu-shuang L, Hong W. *J Environ Sci,* 2007; **19(8):**961-967.
50. Yoon J, Cao X, Zhou Q, Ma LQ. *Sci. Total Environ*, 2006; **368(2-3):**456-464.

51. Ekmekci Y, Tanyolac D, Ayhana B. *J. Plant physiol*, 2008; **165(6):**600-611.
52. Shan S, Liu F, Wan S. *J. Agric Sci,* 2012; **4(6).**
53. Chakraborty K, Singh AL, Kalariya KA, Goswami N, Zala PV. *Acta Bot Croat*, 2015; **74(1):**123-142.
54. Zhang S, Zhang H, Qin R, Jiang W, Liu D. *Ecotoxicology,* 2009; **18(7):** 814-823.
55. Rao KVM, Sresty TVS. *Plant Sci,* 2000; **157(1):**113-128.
56. Li C, Yan C, Liu Y. *African J Biotechno,* 2012; **14:**1151-1157.
57. Fernandez-Ocana A, Chaki M, Luque F, Gomez-Rodriguez MV, Carreras A, Valderrama R. *et al. J Plant Physiol*, 2011; **168(11):**1303-1308.
58. Prasad KVSK, Pardha SP, Sharmila P. *Environ Exp Bot*, 1999; **42:**1-10.
59. Cakmak I, Horst WJ. *Physiol Plant*, 1991; **83(3):**463-468.
60. Wang Z, Zhang Y, Huang Z, Huang L. *Plant Soil*, 2008; **310:**137-149.
61. Li C, Yan C, Liu Y. *African J Biotechno,* 2012; **14:**1151-1157.
62. Romero-Puertas MC, Corpas FJ, Rodriguez-Serrano M, Gomez M, del Rio LA, Sandalio LM. *J Plant Physiol*, 2007; **164(10):**1346-1357.
63. Dixit V, Pandey V, Shyam R. *J Exp Bot*, 2001; **52(358):**1101-1109.
64. Reddy AM, Kumar SG, Jyothsnakumari G, Thimmanaik S, Sudhakar C. *Chemosphere,* 2005; **60:**97-104.
65. Dinakar N, Nagajyothi PC, Suresh S, Udaykiran Y and Damodharam T. *J Environ Sci*, 2008; **20(2):**199-206.
66. Nadgorska-Socha A, Kafel A, Kandziora-Ciupa M, Gospodarek J, Zawisza-Raszka A. *Environ Sci Pollut Res,* 2013; **20(2):**1124-1134.
67. Shamsi K, Wie K, Zhang GP, Jilani GH, Hassan MJ. *Biol Plant,* 2008; **52:**165-169.
68. Zhu Z, Wei G, Li J, Qian Q, Yu J. *Plant Sci,* 2004; **167(3):**527-533.
69. Chakraborty K, Singh AL, Kalariya KA, Goswami N, Zala PV. *Acta Bot Croat*, 2015; **74(1):**123-142.
70. Gupta M, Cuypers A, Vangronsveld J, Clijsters H. *Physiol Plant*, 1999; **106(3):**262-267.
71. Devi R, Prasad MNV. *Plant Sci,* 1998; **138:**157-165.
72. Jaleel A, Jayakumar K, Chang-xing Z, Azooz MM. *Plant Omics Journal*, 2009; **2(3):**120-126.
73. Battana S, Ghanta RG. *J Sci Inno Res,* 2014; **3(1):**72-80.
74. Parmar P, Dave B, Sudhir A, Panchal K, Subramanian RB. *Int J Curr Res*, 2013; **5(1):** 80-89.
75. Vineeeth D, Venkateshwar C, Unnisa SA. *IJCR,* 2015; **7(8):**18936-18942.
76. Nayyar H, Walia DP. *Biol Plant,* 2003; **46(2):** 275-279.
77. Qadir S, Qureshi MI, Javed S, Abdin MZ. *Plant Sci*, 2004; **167:**1171-1181.
78. Nareshkumar A, Nagamallaiah GV, Pandurangaiah M, Kiranmai K, Amaranathareddy V, Lokesh U. *et al. Agric Sci,* 2015; **6:**1283-1297.
79. Zengin F, Munzuroglu O. *Acta Biol Crac,* 2005; **47(2):**157-164.
80. Mishra S, Srivastava S, Tripathi RD, Kumar R, Seth CS, Gupta DK. *Chemosphere*, 2006; **65(6):**1027-1039.
81. Arya SK, Roy BK. *J Environ Biol*, 2011; **32(6):**707-711.
82. Padmaja K, Prasad DDK, Prasad ARK. *Photosynthetica*, 1990; **24(3):**399-405.
83. Van Assche F, Clijsters H. *Plant Cell Environ*, 1990; **13(3):**195-206.
84. Kupper H, Kupper F, Spiller M. *Photosyn Res,* 1998; **58:**123-133.
85. Kupper H, Kupper F, Spiller M. *Exp Bot,* 1996; **47(2):**259-266.
86. Baryla A, Carrier P, Franck F, Coulpmb C, Sahut C, Havaux M. *Planta*, 2001; **212(5-6):**696-709.
87. Benavides MP, Gallego SM, Tomaro ML. *Braz J Plant Physiol*, 2005; **17:**21-34.
88. Gouia H, Ghorbal MH, Meyer C. *Plant Physiol Biochem*, 2000; **38:**629-638.
89. Pavadi P, Dhanavel D, Vijayarengan P, Seetharaman N, Selvaraju M. *Plant Arch*, 2004; **4(2):**475-478.
90. Chettri DR, Surpratin M, Ahmad S. *Environment and ecology*, 2004; **22(1):**27-33.
91. Davies KJ. *J. Biol Chem*, 1987; ***262(20):*** 9895-9901.
92. Tan YF, O'Toole N, Taylor NL, Millar AH. *Plant Physiol*, 2010; **152(2):**747-761.
93. Luque-Garcia JL, Cabezas-Sanchez P, Camara C. *Trends Anal Chem*, 2011; **30:**703-716.
94. Sharma SK, Goloubinoff P, Christen P. *Biochem Biophys Res Commun*, 2008; **372:**341-345.