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Role of phytochelatin and metallothionein in phytoremediation of heavy metals by *Aloe vera* L. (*Aloe barbadensis* Miller)

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Plants, though sessile, have various enzymatic and non-enzymatic antioxidant systems which help them in combating metal toxicity and various other abiotic stresses. Metallothioneins (MTs) as well as phytochelatins (PCs) act as metal chelators. In the present investigation, a comparative study on the role of MTs and PCs in combating zinc induced oxidative stress in *Aloe barbadensi* was carried out. Seedlings were exposed to various sub-lethal doses of zinc and were grown for a period of one month. Relative expression of these genes was studied by RT and Q PCR and the presence of major secondary metabolites and phytochelatins was measured by High Performance Liquid Chromatographic (HPLC) technique. In both the cases, it was observed that 800 μM of zinc treatment is the highest dose that the plants can withstand. A sharp decrease in the expression of the genes was observed under high level of metal toxicity. Results of this study proves that phytochelatin and metallothionein function in a coordinated way to chelate, detoxify and play significant role in bioremediation of transition metals such as zinc.

Keywords: Zinc (Zn), *Aloe barbadensis*, zinc, phytoremediation, phytochelatin, metallothionein.

Introduction

One of the major concerns of the modern era is heavy metal toxicity (HM) and its implications in global environment. In developing countries such as ours like India, HMs are not only affecting our ecosystem but also causing huge negative impact on the ecosystem. Rapid and unregulated urbanization and various anthropogenic activities are contributing to this problem day in and day out[1]. Smelters, effluents from galvanization, paint and other industries are the rich source of Zn pollution[2]. Fe, Mn, Zn, Co, Mo are few important essential heavy metals whereas Cd, Pb, Ur, Tl, Cr, Ag, Hg are considered as non essential heavy metals[3]. Plants and the metals in the soil have had a long and intimate evolutionary association that has resulted in many complex interactions. Zinc is an essential micronutrient for plant. Its act as the co factors of various important enzymes and hence controls their activity also; apart from that it is an integral part of zinc finger motifs which plays important role in gene expression. Zn is not directly associated with oxido-reduction reactions but an excess of it is capable of producing reactive oxygen species (ROS) that affect both metabolism and health of plants and animals including the human being[4]. Among the several mechanisms by which plants can cope with the ill-effects exerted by heavy metals is the activation of the genes responsible for metallothionein (MT) and phytochelatin (PC) production[5]; they can bind to a variety of metals like zinc, cadmium by forming bonds. Plants with help of MTs can detoxify the metals by chelating and compartmentalizing the heavy metals in few cases in the cytosol and in most cases either in the vacuoles or in the apoplasts of the cell[6].

On the other hand PCs are non-protein, cysteine-rich peptides which are biosynthesized enzymatically from the precursor glutathione (GSH). Zn have positive impact on the biosynthesis of PCs. MT combats transition metal induced cytological toxicity; PCs on the other hand, are involved in nullifying further negative impact[7]. *Aloe vera* is a shrubby, perennial, xerophytic[8]. This plant is a rich source of minerals, sugars, antoquinones, fatty acids, hormones etc. Due to rapid urbanization and industrialization the amount of agriculture land is decreasing day by day and farmers are now

growing vegetables in small hamlets nearer to the suburban areas. Unfortunately the soil of those areas is highly contaminated with heavy metals including zinc due to various industrial and anthropogenic activities. Now a day's people are planting *Aloe vera* as ornamental plants as well as due its medicinal value. This study opens a new dimension in the use of this plant in an entirely different way. This present study is aimed to understand how this plant can mitigate Zn induced toxicity by enhancing expression of important genes of MT and PC biosynthesis pathways and hence by higher production of MT and PC which are capable of quenching the heavy metals.

Results and discussion

(A) Chlorophyll and carotenoid content:

About 1.3 and 1.3 fold increase in chlorophyll a content was recorded in plants treated with 500 and 800 μM solution of $ZnCl_2$. A simillar pattern was also noticed in case if chlorophyll b. Significant increases of 1.34 and 1.22 fold in the total chlorophyll contents in 500 and 800 μM Zn treated seedlings and an insignificant decrease in 1000 μM Zn treated seedlings were recorded (Fig. 1).

Statistically significant increments in the carotenoid content has been observed in seedlings treated with low doses however, 1000 μM Zn treated plants show an even more significant decrease in carotenoid content (Fig 2).

(B) Amount of lipid peroxidation:

Statistically significant increases of 1.2, 1.35 and 1.18 fold have been observed treated sample compared to the control untreated ones (Fig. 3).

(C) Total antioxidant activity:

A steady increase of 1.3, 1.5 and 1.8 fold was recorded in the treated samples (Fig. 4).

(D) DPPH scavenging activity:

There has been a significant increase in the radical scavenging activity in the all the Zn treated plant samples (Fig. 5).

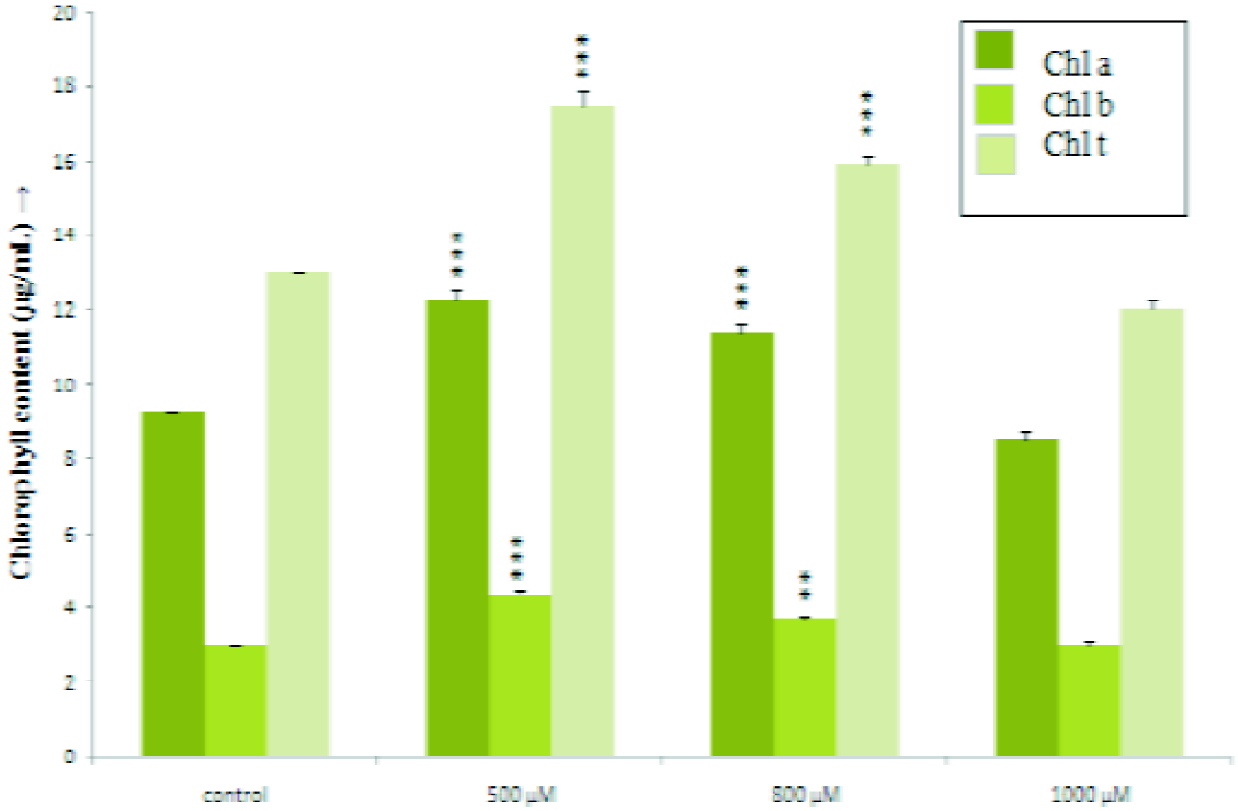

Fig. 1. Amount of chlorophyll.

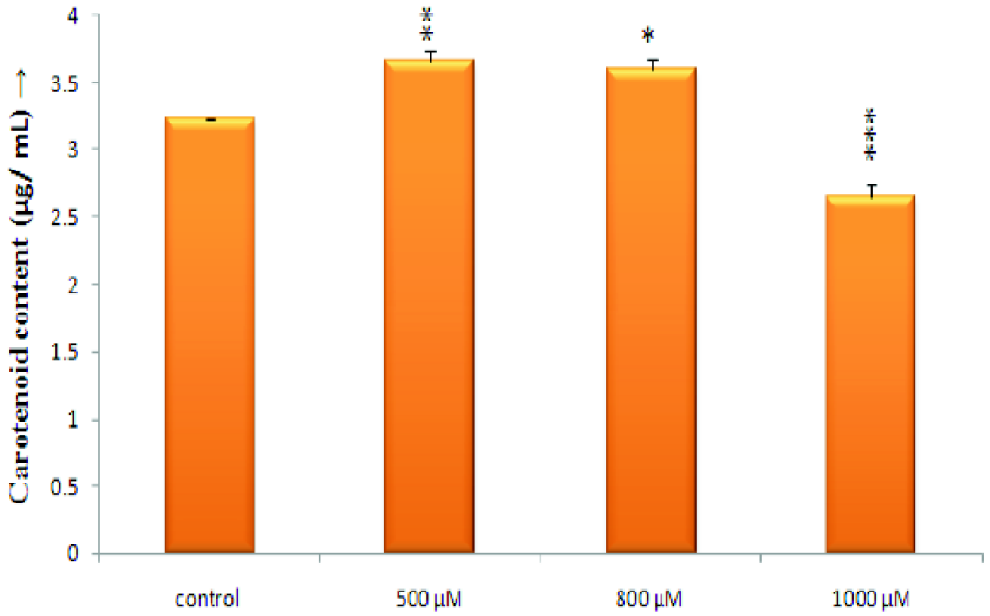

Fig. 2. Amount of carotenoid.

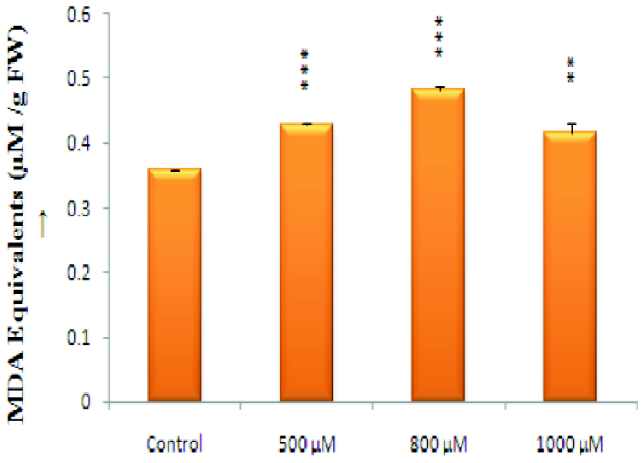

Fig. 3. Change in lipid peroxidation.

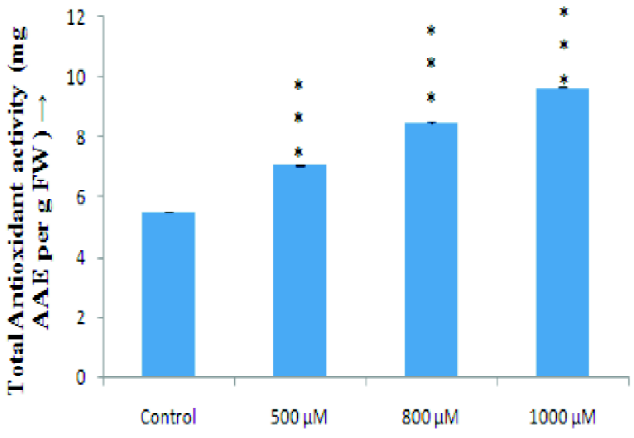

Fig. 4. Change in total antioxidant activity.

(E). RT and Q PCR analysis:

From the gel pictures and densitometric analysis of RT PCR products it can be concluded that 1.3, 1.4 and 1.7 fold increment in MT2 expression occurred in plants treated with lower to higher doses of Zn (Fig. 6 and Fig. 7). A similar trend was recorded in case of Q PCR analysis also. The similarity in MT2 expression in both the methods confirms the pattern of induction of MT2 under Zn stress (Fig. 8).

(F) Amount of different phytochelatins (by HPLC):

The results obtained from HPLC (Fig. 9) clearly implied that zinc treatment stimulated the biosyntheses of phytochelatin 3, 4, 5 and 6 in the plants. 800 µM Zn treated samples showed the highest content of all the phytochelatins; phytochelatin 3, being the exception showed maximum concentration in 500 µM Zn treated samples (Fig. 10).

Zinc is an essential trace element used as a micronutri-

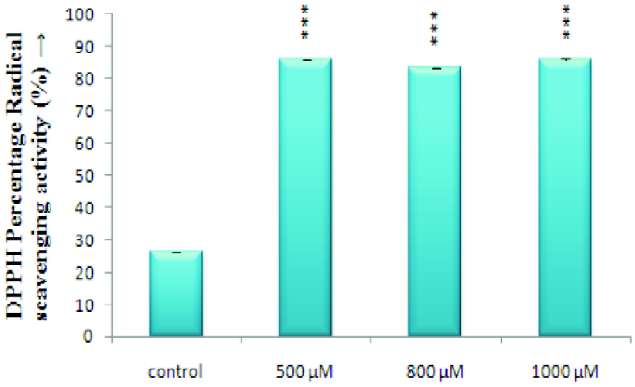

Fig. 5. Change in DPPH scavenging activity.

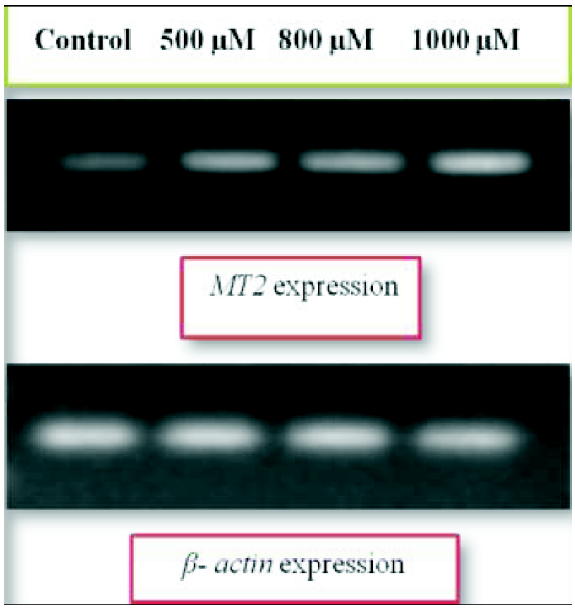

Fig. 6. Gel electrophoresis.

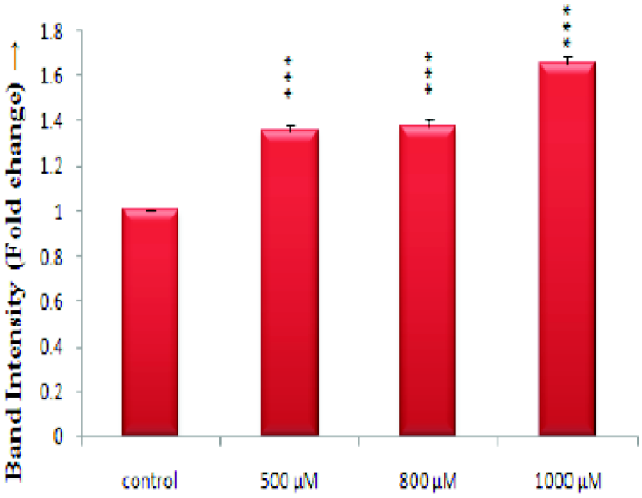

Fig. 7. Alterations in MT2 gene expression.

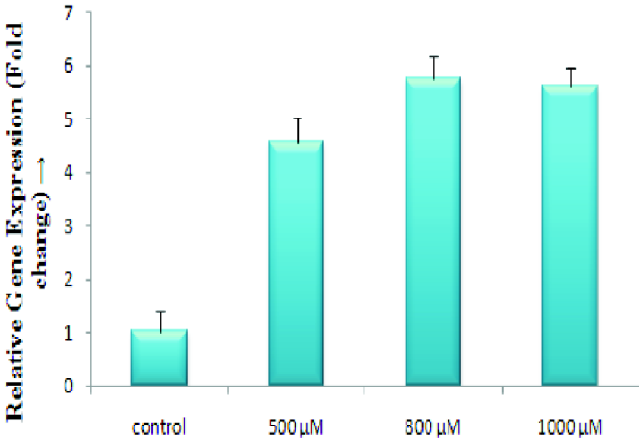

Fig. 8. Q PCR analysis of MT2 gene.

ent. Metal toxicity affects plant metabolism, growth and development at various levels both directly and indirectly. Several recent studies have documented that treatment with zinc has resulted in significant increases in the chlorophyll and carotenoid contents (Gonzalez *et al*. 2012). The chlorophyll and carotenoid content shows that excess zinc in the soil is one of the causes of oxidative stress. The increased total antioxidant and DPPH radical scavenging activities show that the plant is trying to combat the oxidative stress with the help of its various antioxidant systems and conferring tolerance to zinc. Plant metallothionein is a stress-inducible protein, with antioxidant activities. It is quite evident from the biochemical assays that the zinc treatment has caused oxidative stress in *Aloe* plants. Metallothionein with the help of its antioxidative activity can protect the seedlings from the zinc induced oxidative stress. The increase in the MT2 gene expression in both semi-quantitative and quantitative PCR techniques proved that the protein MT2 in is protecting the plant against the oxidative stress induced by zinc. 800 µM zinc is the highest dose that the plants can withstand. On the other hand further increasing the dose to 1000 µM, a sudden drop in the expression of MT2 gene was observed. PCs are more efficient heavy metal chelators and detoxifiers compared to the MTs in higher plants. These results clearly showed MT and PC work in a coordinated manner to detoxify

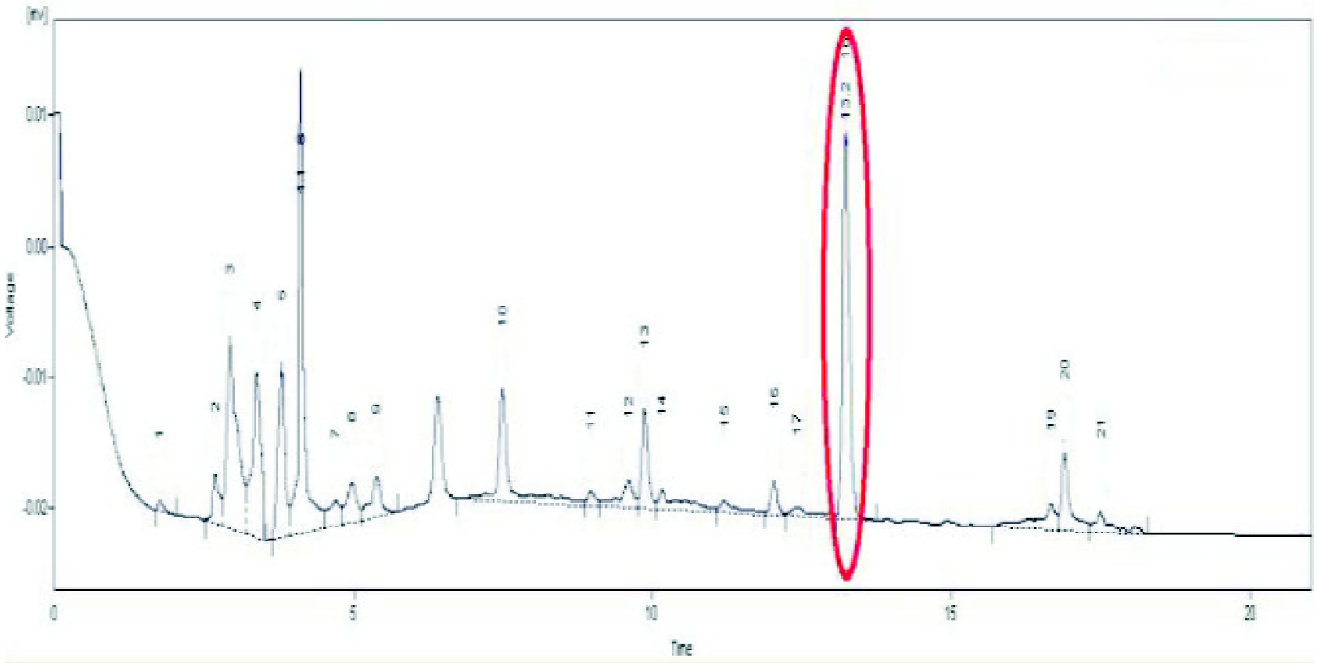

Fig. 9. HPLC chromatogram of phytochelatin.

heavy metals such as zinc in *Aloe*. The outcome of this study in one hand would give a clear idea about the mechanism phytoremediation of heavy metals by plants and also would be beneficial for understanding their medicinal importance.

Experimental

(A) Plant materials and treatments:

Aloe barbadensis were used for this study. $ZnCl_2$ solution of 500, 800 and 1000 μM was used for treatment. LD_{50} was measured and it was found to be 1100 mM for Zn treatment. Hence all doses were taken lesser than the LD_{50} value.

(B) Estimation of chlorophyll and carotenoids content:

Amount of chlorophyll was measured according to the method of Lichtenthaler (1987)[9] with little modifications. 500 mg plant tissue was crushed in 100% acetone solution. The homogenate was centrifuged at 12000 rpm for 15 min. OD was recorded and with the help of the following formula content was estimated:

Total chlorophyll

= OD at 663 nm×7.15 + OD at 646 nm ×18.71

(C) Estimation of extent of lipid peroxidation:

Heath and Packer (1968) procedure with little modifications[10] was adapted. 500 mg tissue was homogenized in 4 ml of trichloroacetic acid and thiobarbituric acid mixture. The solution was centrifuged at 15000 rpm (10 min). It was then boiled for 40 min and kept for 1 h in room temperature. OD was taken at 532 nm.

(D) Preparation of plant extract:

Brolis *et al*. (1998) was followed[11] with little modifications[12]. 1 g tissue was crushed using ethanol and centrifuged for 10 min at 10000 rpm. The supernatant was stored in – 20°C temperature for future use.

(E) Total antioxidant assay:

Prieto *et al*. (1999) was followed[13]. 0.5 ml of extracted plant sample and 5 ml of phosphomolybdate was mixed and heated at 95°C for 1 h 30 min. It solution was kept at room temperature for 2 h and then OD was measured at 695 nm.

(F) DPPH radical scavenging assay:

Brand-Williams *et al*. (1995) was followed[14], with few modifications[12]. 0.7 ml plant extract was mixed with 2.3 ml of reagent solution and incubation was done in dark condition for two hours. The extent of radical scavenging was measured as –

Percentage activity =

[(OD of control – OD of sample)/OD of control]×100%

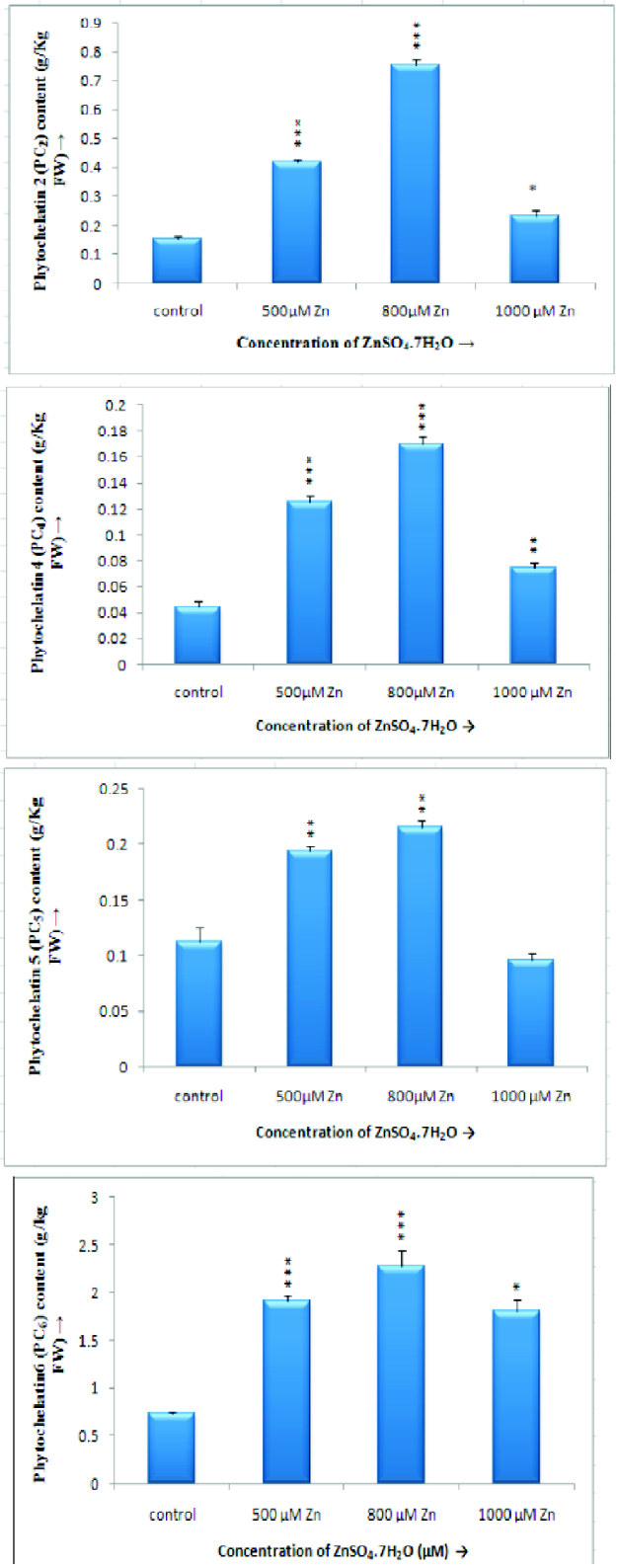

Fig. 10. Amount of PC3, PC4, PC5, PC6 respectively under different dosed of zinc treatment.

(G) RT and Q PCR analysis:

Table 1. Primer-Forward and Reverse

	Primer-Forward (5′ to 3′)	Primer- (5′- 3′)
MT 2 Actin	5′ATGTCCTGCA CGGAGCACT3′	5′CTAAATGCAAT CGCATGCATTG3′
	5′ATCATGACGT GTGGTTGACT3′	5′ACCTATCGTCA CGCTGTTCTG3′

Table 2. Thermal cycler condition for RT PCR

	Temperature (°C)	Time (min)	
Reverse transcription	50	30	
Initial denaturation	95	15	
Denaturation	94	1	40 Cycles
Annealing	53	1	
Elongation	72	.5	
Final extension	72	10	

Table 3. Thermal cycler condition for Q PCR - Livak and Schmittgen 2001[15]

	Initial hold	Denaturation	Annealing elongation
Temp. (°C)	95	95	60
Time (min)	10	.5	1
		40 cycles	

(H) Determination of phytochelatin content by HPLC:

Mobile phase was made up of solution A: Methanol, solution B: Acetonitrile and C18 reverse phase column was used.

(I) Statistical analysis:

Data analysis was done by an analysis of variance (ANOVA) and Student's t test.

Conclusion

This work on metal toxicity in one hand would give a clear idea about the mechanism phytoremediation of heavy metals by plants and also would be beneficial for human health. Hence this present study totally adds a new dimension to the conventional and traditional uses of *Aloe vera*, apart from its medicinal use the present study signifies this plant's immense importance in phytoremediation of toxic heavy metals.

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