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Comparative Physiological, biochemical and transcriptomic analysis suggests ultraviolet radiation is more toxic than cadmium for *Anabaena*.

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Abstract- Toxic effect of ultra violet radiation-B and cadmium stress was examined on the Anabaena sp. PCC7120, a diazotrophic cyanobacterium, with respect to growth behavior, pigment composition(chlorophyll, carotenoid, phycocyanin), physiological parameter(PSI, PSII, respiration, whole chain, ATP, NADPH), enzymatic and non-enzymatic antioxidants(catalase, superoxide dismutase, ascorbate peroxidase, GSH, thiol content), peroxide production, lipid peroxidation, phytochelatin content and transcript analysis. Cadmium, a non-redox active metal having ranked 7 (out of 275) in the priority list of hazardous metal, produced more prominent effect on the energy state, GSH, phytochelatin and total thiol content in the organism. Ultra violet radiation-B was found to increase 1.6-fold in carotenoid, 4.3-fold in TBARS content and 1.95-fold in peroxide production more significantly than cadmium while 1.53- fold decline in chlorophyll and 2.85-fold in phycocyanin pigments was observed in it. Enzymatic activity of superoxide dismutase and catalase registered a maximum increase1.48-fold and 1.62-fold in ultra violet radiation-B as compared to cadmium while ascorbate peroxidase and glutathione reductase were found to be highest 1.89-fold and 1.35-fold respectively in response to cadmium. Enzymatic activity was further validated by transcript analysis.

Keywords: Anabaena, abiotic stress, qRTPCR, antioxidant, catalase

I. INTRODUCTION

Diazotrophic species like *Anabaena* can fix atmospheric nitrogen into the fields enhance crop growth and productivity. *Anabaena* sp.PCC7120, used in this study is a filamentous diazotroph with a completely sequenced genome. Abiotic stresses cause extensive loss to agricultural production worldwide can be classified as naturally occurring and anthropogenic [1]. While naturally occurring stresses like heat and UV-B are due to change in abiotic factors which cannot be regulated, anthropogenic stresses like heavy metal and metalloid are being added to our environment raising a more serious concern of bioaccumulation.

Cyanobacteria constitute the largest, most diverse and widely distributed group of photosynthetic prokaryotes. It is a group of Gram-negative phototrophic bacteria and oxygenic photosynthesis emerged on earth with cyanobacteria that changed the atmosphere and defined the biosphere as we know it today [2, 3]. Cyanobacteria are adapted to a wide range of environmental conditions; they colonize most ecosystems on earth [4-7]. In their natural environments, some cyanobacteria are often exposed to the highest rates of UV irradiance available on the globe [8]. They are important primary producers of organic material and play significant roles in biogeochemical cycles of carbon, nitrogen, and oxygen [9]. Cyanobacteria account for 20-30% of Earth´s photosynthetic productivity and convert solar energy into biomass-stored chemical energy at the rate of ~450 TW [10].

Anabaena is the organism used for the studies in this work. It is a heterocystous cyanobacterium, lacking the ability to form hormogonia like closely related cyanobacterium *N. punctiforme.* Although a large number of cyanobacteria including species of

Anabaena are known to fix nitrogen, the test cyanobacterium *Anabaena* sp. PCC 7120 is the first filamentous cyanobacterium to have its genome sequenced (6.4 Mb), and the complete sequence is now available [11] and known to tolerate environmental stresses [12]. Approximately 58% of the genome of *Anabaena* sp. PCC7120 is known to encode hypothetical proteins whose function can give insight to important metabolic pathways. Since it has ability to form heterocyst and genetic techniques have long been available for this strain, it has been used extensively for study of photosynthesis, nitrogen fixation, cell differentiation and heterocyst pattern formation.

Anything that poses a challenge or a threat to our well-being is a stress. Stress of living organisms is an adverse force or influence that tends to inhibit normal system from functioning. Abiotic stress is defined as the negative impact of non-living factors on the living organisms in a specific environment. Unfavorable climates such as drought, flood, chilling, salinity and radiation are known abiotic stresses responsible for most agricultural losses. Abiotic stress is the most harmful factor for the growth and productivity of crops worldwide [13]. Being motionless organisms, a plant's defense to adverse conditions is limited to physiological and biochemical responses. In response to regulated changes in gene expression, central and secondary metabolisms are redirected to cope with the undesired effects of the hostile situation. This is achieved by up-regulating the synthesis of proteins and metabolites involved in protection (i.e., compatible solutes, antioxidant enzymes and compounds, heat-shock proteins, etc.). Whenever these defensive mechanisms are overcome by the intensity of the adverse condition, the plant is under environmental stress. Although different types of nutritional and environmental adversities have their own features and display idiosyncratic responses, they all have in common a significant perturbation of the electron transfer network of the stressed cells, leading to electron derivation to non-productive routes, breakage of redox homeostasis, and eventually to the generation of reactive oxygen species (ROS) such as singlet oxygen, the superoxide and hydroxyl radicals, and hydrogen peroxide. When a change in the environment exceeds a certain threshold level, the activities of some enzymes are inhibited or abolished and those of others are enhanced or induced. Abiotic stress signal is sensed and transduced to the target genes by two component signal transduction systems [14]. The stress specific genes thus upregulated lead to synthesis of specific proteins involved in operation of mechanisms for acclimation to stress condition. Some of these proteins, in turn, participate in the synthesis of certain stress-specific metabolites. The proteins and metabolites that are synthesized de novo in response to stress are important for the acclimation of an organism to the new environment. Now a day increased abiotic stresses like salinity, temperature, drought, pH, heavy metal and metalloid contamination in environment have high priority research topics. Rice yield is adversely affected by indirectly arresting the proliferation of nitrogen fixing cyanobacteria in these abiotic stresses.

Thinning of ozone layer due to accumulation of pollutants such as freons, nitrogen oxides, and increasing green house gases [15] facilitates the penetration of UV-B radiation (280-320nm) in to the earth's atmosphere [16]targeting terrestrial and aquatic ecosystems. Photosynthetic organisms including cyanobacteria are highly prone to UV-B tempted damage due to their requirement of light as energy source. Increasing the solar UV-B radiation might be detrimental to cyanobacterial communities, which in turns may affects the productivity of higher plants [17]. UV-B is known to causes oxidative damage to protein, nucleic acid and lipids [17-20]. UV-B is reported that it not only affects the motility and photo orientation of cyanobacteria [21] but also affects a number of biochemical and physiological processes such as growth, survival, pigmentation and protein profile [22]. Knowledge about the effects of UV-B radiation at biochemical and molecular levels in cyanobacteria is very limited however it is reported that there is reduction in nitrogenase, nitrogenase reductase, and glutamine synthetase by UV-B radiation [23, 24]. Cyanobacteria have variety of mechanisms to cope with UV-B radiation for example avoidance (movement), antioxidative defense enzymes, protective sheath and it is also reported synthesis microsporin like amino acids (MAAs), scytonemanin, increase in protective pigments such as carotenoids, echineone and myxoxanthophylls [25]. Proteomic analysis of UV-B provoked changes in protein profile of *Synecchocystis* sp. PCC6803 and *Anabaena* shows that proteins showing significant alterations belong to functional categories such as amino acid biosynthetic pathways, photosynthesis, respiration and energy metabolism etc. [26, 27].

Similar to UV-B, heavy metals stress has raised serious health and productivity issues. Heavy metals are concentrated over a specific area causing more damage and arousing a more serious concern. Cd ranked 7 in the priority list of hazardous metal by the Comprehensive Environmental Response, Competion and Liability Act (CERCA) [28] is added to the environment anthropogenically 25000 tones/year [29-31]. Cd is considered as non-essential metal having no role in biological systems while its bioaccumulation through food chain has been a matter of concern. Though Cd is not redox active metal its effect on electron transport chain and photosynthetic efficiency results in an oxidative burst thus leading to oxidative damage. Cd induces the antioxidative defense systems [32, 33]. Cd is reported to induce morphological variation on organisms such as *Phormidium*, *Anabaena flos-aquae* and *Nostoc muscorum* [34-36] etc. along with exerts toxic effect at various physiological and biochemical processes such as photosynthesis, respiration, nitrogen fixation, and uptake and distribution of nutrients [37-40] which ultimately effects the survival of the organism. Both UV-B and Cd have been established to stimulate the formation reactive oxygen species at various points of the photosynthetic and respiratory electron transport chain and induces lipid peroxidation [41-43]. The present paper is an attempt to evaluate the comparative affects of UV-B and Cd in the cyanobacterium *Anabaena* sp. PCC7120 by using biochemical and transcriptomic approaches.

II. MATERIALS AND METHODS

A. Organism And Growth Conditions

The cyanobacterium *Anabaena* sp. PCC 7120 was grown photoautotrophically in BG-11 medium buffered with Tris/HCl at 25 ± 2 ^{0}C under day light fluorescent tubes emitting 72 µmol photon m^{-2} s^{-1} PAR (photosynthetically active radiation) light intensity with a photoperiod of 14:10 h at pH 7.5. The cultures were shaken by hand 2–3 times daily for aeration.

B. Experimental setup and treatment

The LC_{50} dosage for Cd and UV-B were 10µM ($CdCl_2$) and 30 min respectively for *Anabaena* as determined by the plate colony count method [44]. A 100 µM stock solution of $CdCl_2$ was used for salt treatment. The UV-B treatment was given by a UV-B lamp (CAT No. 34408, Fotodyne Inc., and New Berlin, WI, USA). UV-B dose of $2.34x10^6$ µE.cm^{-2} was obtained by adjusting the distance between the UV-B source and the sample. The UV-B irradiance was measured with a Black-Ray J-221, longwave ultraviolet intensity meter (UVPInc., San Gabriel, CA, USA). All the samples (treated and control) were grown under similar conditions in triplicate to ascertain reproducibility of the results.

C. Growth Measurement

Growth was estimated by measuring the absorbance of the cyanobacterial culture at 750 nm in a UV–VIS spectrophotometer (Systronics, India) on every third day up to 15 days.

D. Pigment estimation

The control and treated *Anabaena* cells, harvested by centrifugation, dried and subjected to chlorophyll extraction for overnight in 80% acetone. Chlorophyll and carotenoid were measured at 663 nm and 480 nm respectively in UV/Vis spectrophotometer and the concentration was determined using specific absorption coefficient [45]. Phycocyanin content was estimated following the method of Seigelman and Kycia [46]. The pellet obtained was crushed in 50mM potassium phosphate buffer (pH 6.8) and absorbance was measured at 615 nm and 652 nm.

E. Measurement of physiological parameter

The rate of respiration was determined by measuring the total O2 consumed in the dark for a given time period minus nonspecific O2 uptake [47] and electron transport activities were measured according to the method of Tripathi and Mohanty [48]. The nitrogenase activity was determined by acetylene reduction assay [49] by using a Varian CP-3800 gas chromatograph equipped with a Porapak R column and a flame ionization detector. The size of ATP pool was measured by the method of Larson and Olsson [50]. Control and treated cells incubated for a known period were withdrawn, centrifuged and treated with trichloroacetic acid (TCA). The sample was diluted with Tris buffer to a final TCA concentration of <0.01%. The ATP content was measured by Luciferin-Luciferase assay using LKB 1250 luminometer. The NADPH/NADH content was measured by extraction in Tris–Cl (pH 8.0) using ultrasonicator. This was subjected to centrifugation at 6500 rpm for 15 min at 4^{0}C and the supernatant was collected. The NADPH/NADH content was measured at 340 nm [51].

F. Estimation of TBARS, total peroxide, GSH, thiol and phytochelatin content

Oxidative damage of lipid was measured in terms of the total content of 2-thiobarbituric acid reactive substances (TBARS) using the method of Cakmak and Horst [52]. The total peroxide was measured according to Sagisaka [53]. Total thiol content was estimated according to the method of Ellman [54]. Total GSH content was measured according to method of Anderson [55]. Phytochelatin content was estimated by Bhargava et al. [56].

G. Enzymatic assays

For assay of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase cell extract was prepared by sonication in lysis buffer under ice cold condition. The cell lysis buffer contained 1 mM EDTA and 1% (w/v) poly vinyl pyrrolidone (PVP) with the addition of 1 mM ascorbate in case of ascorbate peroxidase activity assay. The sonicated sample was centrifuged at 4ºC and the resulting supernatant was used for the assay of antioxidant enzymes. Superoxide dismutase activity was assayed by monitoring the inhibition in reduction of nitro blue tetrazolium (NBT) according to the method of Gianopolitis and Ries [57]. Catalase activity was determined by measuring the consumption of H_2O_2 (extinction coefficient 39.4 mM^{-1} cm^{-1}) at 240 nm for 3 min [58]. Activity of glutathione reductase was determined as per the method of Schaedle and Bassham [59]. Ascorbate peroxidase activity was determined by measuring the decrease in absorbance at 290 nm (extinction coefficient 2.8 mM^{-1} cm^{-1}) for 1 min in 1 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM Ascorbic acid, 0.1 mM H_2O_2, and 200 µl of enzyme extract [60]. Total soluble protein was measured by the method of Bradford [61].

H. Transcript analysis

The *Anabaena* was grown in BG-11/BG-11medium exposed to LC_{50} dose of Cd and UV-B in an orbital shaker (200 rpm) at 37ºC. Primer was designed for transcript analysis of antioxidant genes such as *kat*, *sodA, ahpC, apx, gst, pcs, prx* and *gor* by using primer 3 software (Table 2). RNA was isolated by using QIAGEN RNeasy plant mini kit for expression analysis. qRT-PCR were performed as described previously [62].

III. RESULTS

A. Growth measurement and pigment estimation

Figure1 Shows the growth behaviour of control and treated *Anabaena* at LC_{50} doses of Cd and UV-B. A decline of 30% and 41.2% in specific growth rate was observed in Cd and UV-B respectively with respect to control. Figure 2 demonstrates

changes in pigment content in control and treated samples. A decline of 1.42 and 1.53fold for Cd and UV-B respectively was observed in chlorophyll as compared to control. Similarly, a decline of 1.25 and 2.85 fold was observed for phycocyanin. However, carotenoid content was decreased 1.25 fold in Cd and increase of 1.6 fold in UV-B was recorded.

B. Physiological responses

Physiological responses in term of PSI (photosystem I), PSII (photosystem II), respiration, whole chain, ATP, NADPH and nitrogenase activity in *Anabaena* treated with Cd and UV-B are presented in Table 1. A significant decline in PSI, PSII, whole chain activity, nitrogenase and ATP content was observed in both of the treatment. In contrast to this, the rate of respiration and NADPH showed marked increment. An increase of 1.74 and 1.59 fold in rate of respiration and 1.33 and 1.67 fold in NADPH level was recorded for UVB and Cd respectively. Nitrogenase activity registered drastic reduction although it was less severe under cadmium treatment as compare to UV-B.

C. Total peroxide, TBARS (lipid peroxidation), GSH, and thiol content

Figure 3 compiles data on thiol, GSH, phytochelatin, TBARS content (lipid peroxidation) and total peroxide in control and treated *Anabaena* cells. An increase of 1.45 and 1.19 fold in thiol and 1.83 and 1.25 fold in GSH content was recorded for Cd and UV-B respectively as compared to control. Further 1.7 fold increased phytochelatin content was observed in Cd and 1.24 fold in UV-B. Similarly, increase of 1.65 and 1.95 fold in total peroxide and 2.2 and 4.3 fold in TBARS content was recorded for Cd and UV-B respectively.

D. Enzymatic assays

All tested enzymes showed a significantly enhanced activity in Cd and UV-B treatments as compared to control. Superoxide dismutase showed an increase of 1.21 and 1.48 fold in Cd, and UV-B treatments with respect to control. Maximum catalase activity of 1.62 fold was recorded in UV-B followed by 1.23 fold in Cd. A similar trend in activity was observed for both ascorbate peroxidase and glutathione reductase. A 1.89 and 1.51 fold increase in ascorbate peroxidase and 1.35 and 1.20 fold increase in glutathione reductase was observed in Cd and UV-B respectively (Fig. 4).

E. Expression of various antioxidant genes in Anabaena under UV-B and Cadmium stresses

Figures 5 presents data on the expression profile of various antioxidant genes measured by qRT-PCR under UV-B and Cd. Abiotic stressors used in this study possess a common mode of action e.g. disruption of redox homeostasis and generation of reactive oxygen species (ROS) which impaire key metabolic, regulatory and dissipative pathways. Expression of ROS scavenging and stress responsive genes like *kat*, *sodA, ahpC, apx, gst, pcs, prx* and *gor* investigated by using qRT showed appreciable increase in their transcripts under UV-B and cadmium both stresses.

IV. DISCUSSION

The results of the biochemical and transcriptomic data suggest a more pronounced damaging effect of UV-B in comparison to Cd [63]. In the present study Cd and UV-B were applied individually on *Anabaena.* Interestingly most of the biochemical parameters including growth behavior indicate that UV-B toxicity appeared more severe than Cd.

A more pronounced decrease in chlorophyll was observed in UV-B than Cd (Fig. 2). This finds support from the study of Bhargava et al., [64]. Chlorophyll degradation in Cd may be due to inhibition of chlorophyll biosynthesis pathway [65]. In comparison to Cd, carotenoid content was found to be highest in UV-B (Fig. 2). Carotenoids act as filter for UV-B radiation [66]. They are effective singlet oxygen quenchers having ability to eliminate activated oxygen radicals hence provide protection against photooxidative damage therefore their induction in UV-B stress is quite logical. The enhanced carotenoid in UV-B could further be linked to the induction of 3-hydroxy-3 methyl glutaryl reductase RNA for the synthesis of carotenoids [67]. Phycocyanin content was maximally inhibited in UV-B alone as it is extremely sensitive to UV radiation in comparison to Cd[68].

In this study the oxidative stress indicators like TBARS and peroxide content were significantly more evident in case of UV-B suggesting UV-B alone produce more redox imbalance in comparison to Cd (Fig.3). In UV-B stress, production of toxic oxygen radicals may oxidized the fatty acids leading to lipid peroxidation and disturb the ratio of saturated/unsaturated fatty acid and hence membrane fluidity [69]. In contrast to this Cd induced lipid peroxidation is an indirect consequence caused by hydrogen peroxides accumulated due to inhibition of various metabolic pathways [70]. It is worthwhile to mention that increased generation of reactive oxygen species was associated with enhanced activities of ROS scavenging enzymes like superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase.

A significant increase in activity as well as upaccumulation of transcript of Sod in the treatments, maximum in UV-B indicate scavenging of oxygen free radicals and dismutation of free hydroxyl radicals by the formation of hydrogen peroxide [71]. Further to detoxify the hydrogen peroxide, enhanced activities and transcript of catalase (*Kat*) and APX in both the stresses is reasonable (Fig. 4, 5). Catalase activity was found to be significantly higher in case of UV-B alone suggesting removal of H_2O_2 and toxic peroxides. Glutathione reductase (*gor*), which catalyses the NADPH-dependent reduction of oxidized glutathione, showed significant increase in activity and expression in term of mRNA like other enzymes and can be directly corroborated with APX involved in the Halliwel-Asada pathway. Glutathione reductase and ascorbate peroxidase(apx) activity as well as transcript was maximally observed in case of Cd (Fig. 4,5).

AhpC and Prx(*alr4642*) both are member of novel peroxiredoxin family have potential to detoxified all type of peroxide either organic or inorganic produced during abiotic stresses showed there upaccumulation under both stresses but higher in case of UV-B than cadmium (Fig.5).

Glutathione homeostasis is essentially regulated by glutathione-S-transferase activity, and the glutathione redox status is critical for various biological events. Thiols and GSH are known to play a role in maintenance of redox status as well as in the detoxification of metals [72]. GSH acts as an antioxidant, quenching the ROS generated in response to various abiotic stresses [73]. The high GSH content in Cd in contrast to UV-B stress is probably due to Upaccumulation of glutathione-S-transferase transcript (Fig.5) and transcriptional activation of *gsh*1 and *gsh*2 genes coding for GSH [74], which is required for phytochelatin synthesis and Cd sequestration. In addition to this, due to the presence of thiol groups, GSH can also act as a defence compound against oxidative stress. The sensitivity of GSH to UV-B could be because it absorbs in the range of 200-300 nm [75], leading to its oxidation. Similarly high thiol content in Cd (Fig. 4) might be attributed to its role as an antioxidant and in protecting membranes against free radical damage [76,77].

Phytochelatins are oligomers of glutathione and act as chelators, and are important for heavy metal detoxification produced by the enzyme phytochelatin synthase [78-81]. Elevated phytochelatins (PCs) content in cadmium finds support by enhanced transcript accumulation in cadmium than UVB (Fig.3, 5).

All antioxidants enzymes such as – superoxide dismutase (*Sod*), catlase(*Kat*), ascorbate peroxidase(*Apx*), glutathione reductase(*Gor*), alkylhydro peroxide reductase (*AhpC*) and peroxiredoxin(*Prx*) alongwith phytochelatin synthase(*Pcs*) and glutathione-S-transferase(*Gst*) showed increase (Fig. 5) which reflects their key role in cell survival and tolerance in Cd and UV-B stresses [82].

Thus, an overall assessment of biochemical and transcriptomic data in this study suggests that a negative effect of UV-B is more pronounced then cadmium.

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Figures legend

Fig. 1 Growth behaviour of *Anabaena* PCC 7120 subjected to conditions (i) Control (ii) Cd (iii) UV-B after 24 hours. Value is given as mean±SE
Fig. 2 Chlorophyll, phycocyanin and carotenoid content (in mg/ml) in control and treated *Anabaena* cells
Fig. 3 Measurement of Thiol (in nM/mg protein), GSH (in nM/mg protein), phytochelatin, TBARS (in mM/mg protein) and total peroxide (in mM/mg protein) content in control and treated *Anabaena*.
Fig. 4 Enzymatic activity assays of SOD (U enzyme/mg protein), CAT (µM/min/mg protein), APX (nM/min/mg protein) and GR (mM/min/mg protein) under control and treatments of UV-B and cadmium
Fig. 5 qRTPCR showed expression pattern of selected ROS scavenging genes under the control and treatments

Table 1 Physiological attributes of *Anabaena* spp. under UV-B and Cd treatment. All values are mean±standard deviation. Values in parenthesis indicate % decrease (−) and increase (+), respectively,with
respect to control

Physiological parameter	Control	UV-B	Cadmium
PS-I activity (µmol O2 consumed µg−1 protein h−1)	17.01 ±1.02	4.50±1.02	5.72±1.02
PS-II activity (µmol O2 evolved µg protein−1 h−1)	11.51±1.02	3.56±1.02	5.25±1.02
Respiration(µmol O2 consumed mg protein−1 min−1)	11.23±1.02	19.52±1.02	17.81±1.02
Whole chain(µmol O2 evolved µg protein−1 h−1)	12.02±1.02	7.30±1.02	9.62±1.02
NADPH (mM NADPH mg protein−1)	0.27±1.02	0.36±1.02	0.45±1.02
ATP assay (ng ATP mg°protein−1)	75.31±1.02	29.25±1.02	45.92±1.02
Nitrogenase activity (nm C2H2 mg protein−1 h−1)	92.03±1.02	12.04±1.02	19.71±1.02

Table. 2 List of genes and their primer sequences for qRTPCR

Serial No.	Gene	Primer Sequence	
1	*ahpc*	**AHPF**	CCCTGCTGACTATACCCCAG
		AHPR	TGGGGTAGTTGAGTGTGGTG
2	*prx*	**PRXF**	GGAACACAAGAAAGCCTCCG
		PRXR	CGTGGGGCAAACAAAGGTAA
3	*gor*	**GORF**	TGGTGATGTTACAGACCGCT
		GORR	GACTGTAGAGGCTTGTGGGT
4	*gst*	**GSTF**	GGCTAGTGGTCAAGTTCCCT
		GSTR	GGTGTCCTGAGCTTTTGAGC
5	*apx*	**APXF**	TGCGATGGAGACGGCAATAT
		APXR	AGCTGTTTCTATGTCTTGCCAG
6	*kat*	**KATF**	TTCTGGTTTAGGTGCGTTGC
		KATR	CCTGGGTCGTTGCTCATTTC
7	*sodA*	**SODF**	ACAGATTGTCAGCACTCCCA
		SODR	GCTTGGGTGCGTCTGTTAAT
8	*pcs*	**PCSF**	CCCGAAGTAGTAGCTCGTCA
		PCSR	TGACAAAGTTTCCGTCCTGC

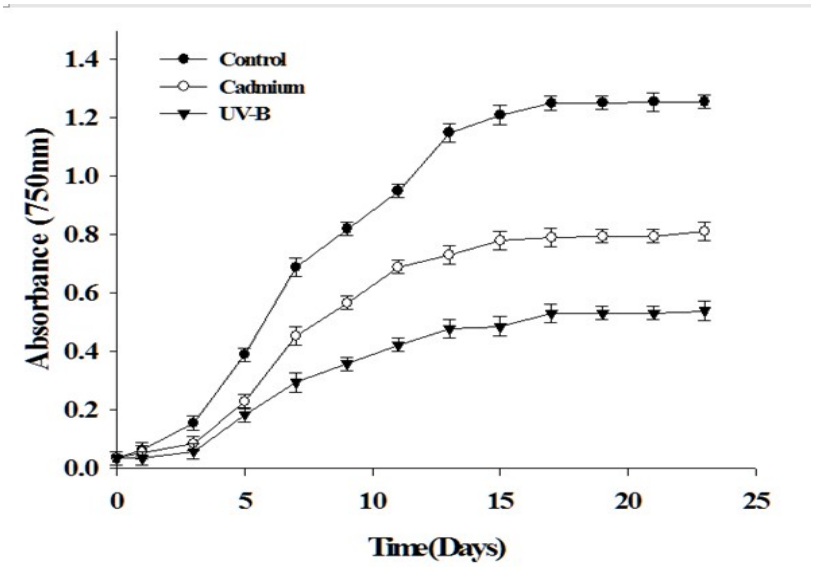


Figure 1

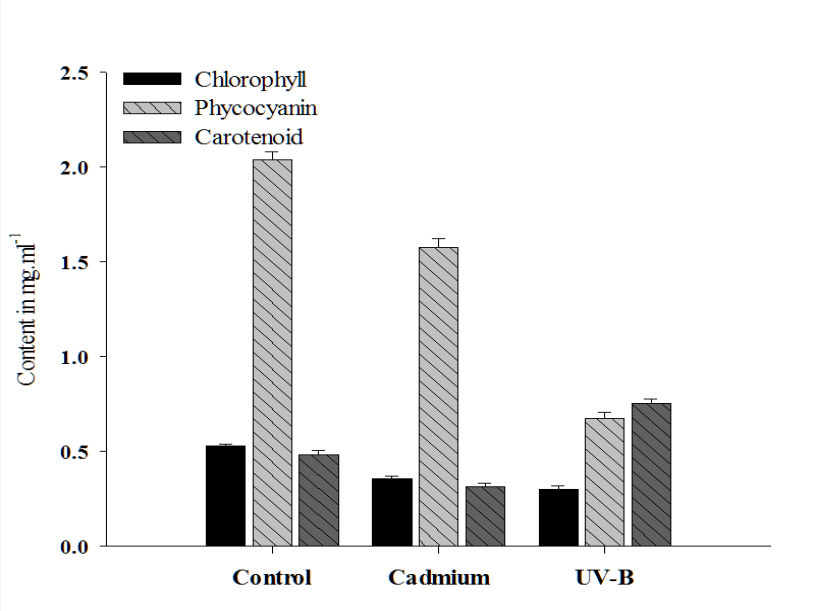


Figure 2

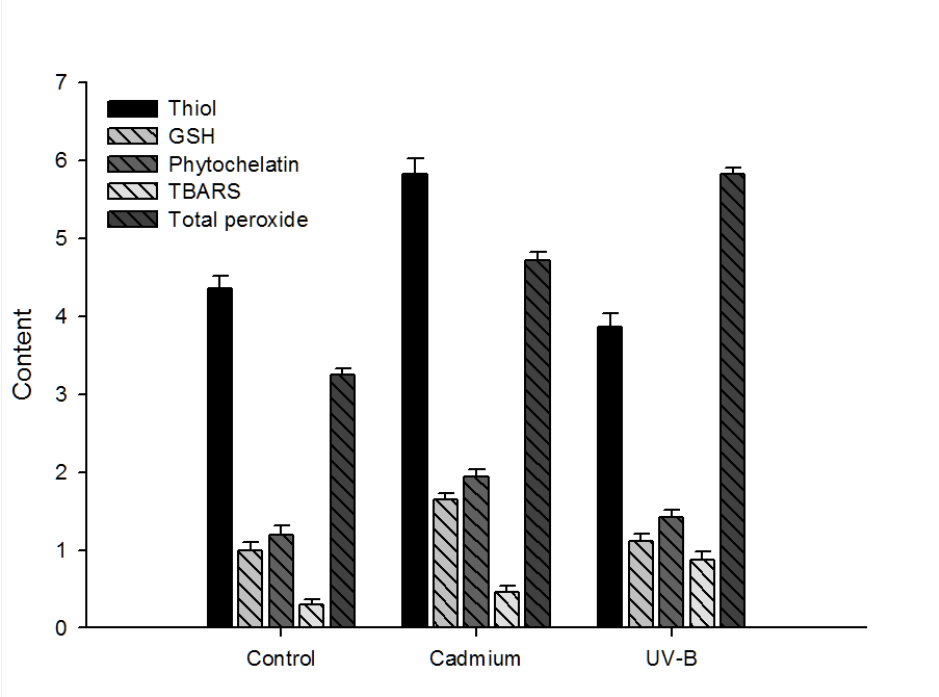


Figure 3

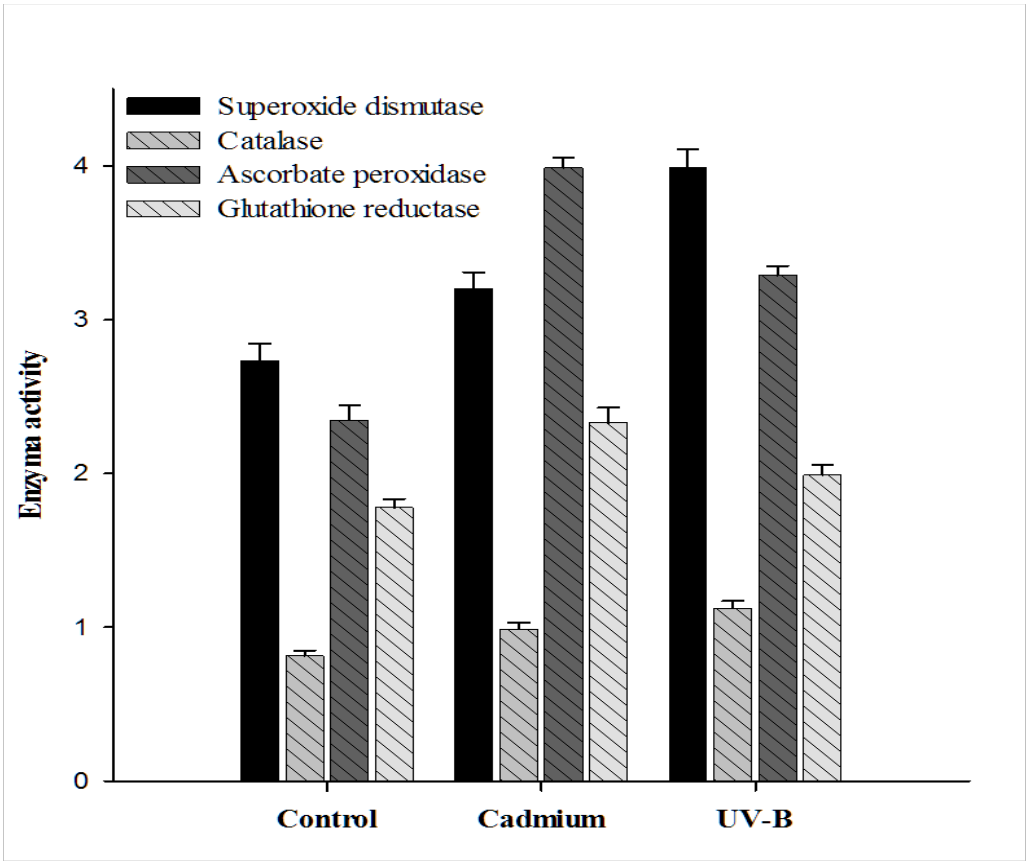
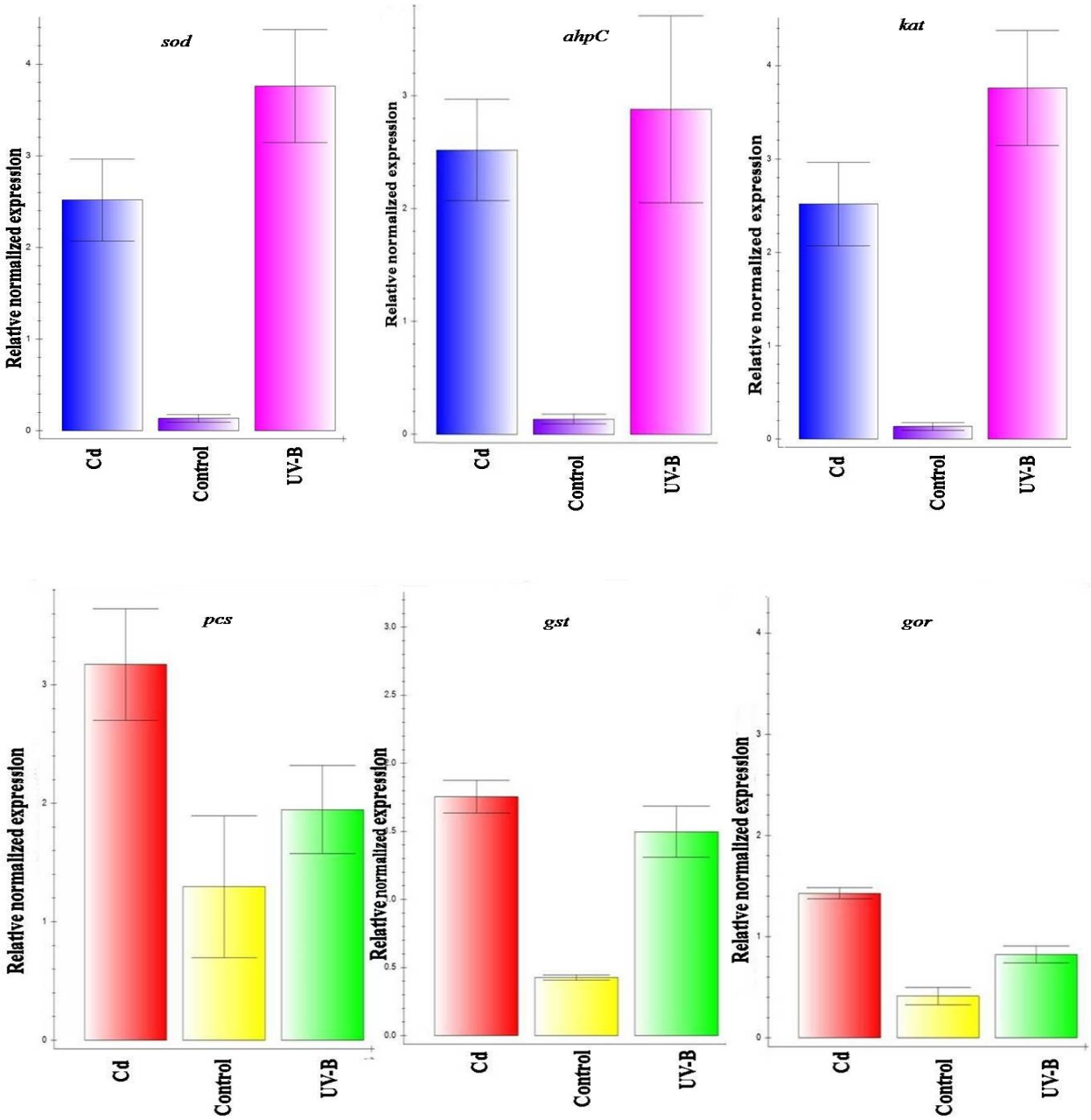

Superoxide dismutase
Catalase
Ascorbate peroxidase
Glutathione reductase
Enzyme activity
0
1
2
3
4
Control
Cadmium
UV-B

Figure 4


sod
ahpC
kat
pcs
gst
gor
Relative normalized expression
Cd
Control
UV-B

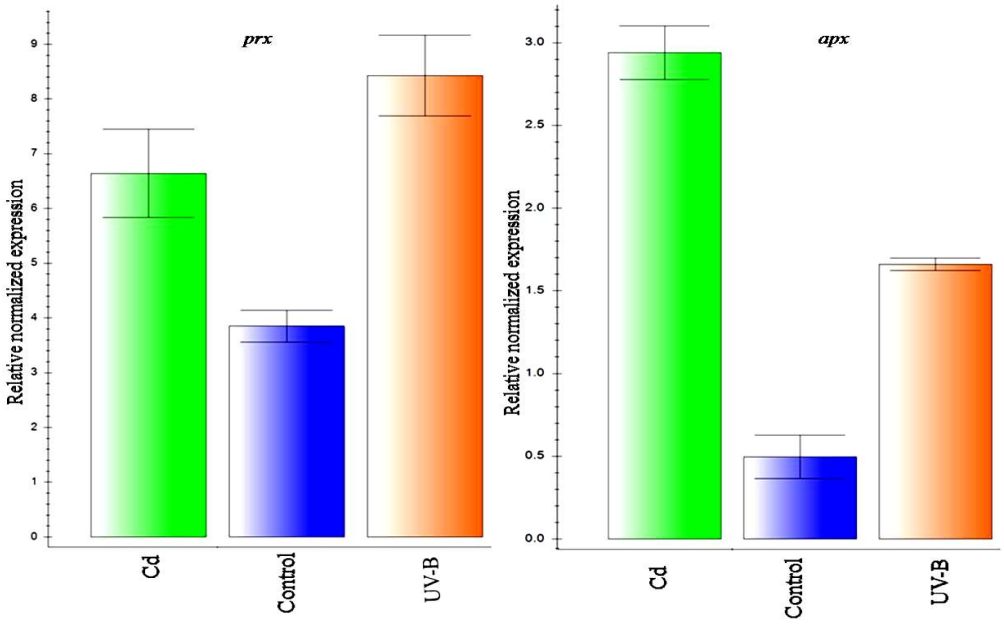

prx
Relative normalized expression
0
1
2
3
4
5
6
7
8
9
Cd
Control
UV-B
apx
Relative normalized expression
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Cd
Control
UV-B

Figure 5