Effect of heavy metal pollution on protein expression, enzyme activity, pigments and phytohormones in some plants growing in Wadi Alargy wetlands, Taif, Saudi Arabia

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Abstract: Heavy metals accumulated by *Amaranthus hybridus, Chenopodium ambrosioides, Mentha longifolia* and *Typha domingensis* have been reported in the present study to affect on some physiological parameters. The study proved that total protein contents in the contaminated and noncontaminated shoot samples of *A.hybridus* and *M.longifolia* were found to be eight proteins while expressed proteins were four in the other two test species. Comparing protein intensities in shoots of the test species showed more protein intensity in most shoots of the contaminated samples. The current work proved the effect of heavy metals on catalase (CAT), glutathione peroxidase (GP) and glutathione reductase (GR) activities. CAT recorded maximum value in shoots of contaminated *T.domingensis* samples amounting to 114.22 µg g^{-1} FW. The effect of heavy metals on concentrations of chlorophyll a and b, xanthophylls and carotene pigments in the target species were estimated. The recorded values for Chlorophyll b, xanthophyll and carotene in contaminated *A.hybridus* were 189.25, 82.91 and 65.22 mg g^{-1} FW. Indole-3-acetic acid (IAA) showed greater values ranged between 1110 and 1449 µM in the noncontaminated plant samples as compared to those of contaminated samples which ranged between 960 and 1089 µM, while Gibberellin (GA_3), recorded values ranged between 864 and 1068 µM in the contaminated plant samples of the test species.
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1. Introduction:

In recent years there is increasing interest in studies of the plant response to heavy metal contamination in wetland ecosystems. Wetlands have been widely used as sites where effluents are discharged and metallic industrial wastes are dumped (Peters *et al*., 1997). Heavy metals are not subjected to degradation processes and remain almost indefinitely in the environment, then tend to accumulate in living systems (Tanhuanpää *et al.*, 2007; Rai and Tripathi, 2009; Malik *et al*., 2010). Many researchers have reported the phytoremediation or the utilization of plants to absorb, transport and concentrate metals from contaminated soil into different plant organs (Marchand *et al.*, 2010; Soda *et al*., 2012; Farrag and Manal, 2012; Zhang *et al*., 2013). Heavy metals decrease growth rate of plants by affecting metabolism and mineral uptake (Barceló and Poschenrieder, 1990), membrane function (Hernández and Cooke, 1997; Mansour, 2013; Chen *et al.*, 2014), enzyme activities (Tamá *et al*., 2006), oxidation and cross-linking of proteins (Ortega-Villasante *et al.,* 2005), cell division (Fusconi *et al*., 2006), induction of DNA damage (Gichner *et al*., 2004) increase oxidative stress (Romero-Puertas *et al.,* 2004) plant hormone (Khan *et al*., 2009) and pigment degradation (Shanker *et al.,* 2009). Yet, this type of studies in Saudi Arabia is under worked area. Moreover, our previous studies proved the phytoremediation potentiality for *Amaranthus hybridus* (Amaranthaceae), *Chenopodium ambrosioides* (Chenopodiacea), *Mentha longifolia* (Labiatae) and *Typha domingensis* (Typhaceae), naturally growing in Wadi Alargy wetlands located in Taif area (Farrag 2012; Farrag *et al*., 2014). In addition, the same studies revealed that the phytoremediation potentiality of the studied species can be arranged in the order of *T. domingenesis*> *A. hybridus*> *M. longifolia*> *C. ambrosioides* and nominate the accumulation organs in the following order; stem> root> leaves. However, there is lack of information on the effect of the heavy metals on the physiological aspects of the studied species.

Consequently, the present work aimed at studing the impact of heavy metals, *viz*., lead, chromium, nickel, copper, cobalt, iron, arsenic, cadmium and molybdenum on the protein expression, enzyme activities of three enzymes; Catalase (CAT), Glutathione peroxidase (GP) and Glutathione reductase (GR) in shoots of the four tested species. We investigated also the effect of heavy metals on plant pigments (chlorophyll a, chlorophyll b, xanthophyll

and carotene), phytohormones like Indole-3-acetic acid (IAA) and Gibberellin (GA_3) in the target species.

2. Material and methods

2.1. Plant materials

Plant specimens of the studied species; *A. hybridus, C. ambrosioides, M. longifolia* and *T. domingensis*, were collected from two different sites at Wadi Alargy, Taif province, KSA. One site was a wetland contaminated with industrial wastewater (21^0 $19^/$ N, and E 40^0 $32^/$, and altitude of 1591) and the other was noncontaminated wetland near Seesed park (21^0 $17^/$ N and 40^0 $29^/$ E and altitude of 1595m). Plant samples washed several times by deionized water to remove extraneous and salts, then separated to individual organs of leaves, stems (culms in case of *T. domingensis*) and roots. Plant samples then divided into two categories; fresh one for the enzyme activity, pigment and hormone analyses, and the other category kept at -70 ^{o}C for the total protein analysis.

2.2. Total protein analysis

Leaves were taken for the purpose of protein analysis and was washed by distilled water several times and kept at -70 ^{o}C until use. Cytoplasm proteins were extracted and purified from the test species for SDS-PAGE analysis based on Mechin *et al.* (2003). Leaves of each test species were frozen in a liquid nitrogen and grind for about 30 second in a mortar with 3 ml of buffer D/g of tissue. Filter through muslin, and centrifuge for 15 minutes in a microfuge then dilute to about 2 mg protein / ml, ensuring that the final protein solution contains about 2% (w/v) SDS, 0.002% (w/v) bromophenol blue, and at least 6% (w/v) sucrose. Aliquots separated by SDS-PAGE on 10% non-denaturing polyacrylamide gels and electrophoresed at 40 V for 6 hours at 4 ^{o}C (Laemmli, 1970). The analysis was carried out in Agriculture Genetic Engineering Research Institute (AGERI) and in Genetic Engineering Lab, Giza, Egypt.. The gels were run in a mini-protein gel (Bio-Rad).

2.3. Determination of enzyme activity

All enzyme activity data were related to plant fresh weight (FW). About 1.0g of leaf sample (culm in case of *T. domingensis*) was ground in an ice-cooled mortar with 5ml of ice-cooled 50 mM Na-phosphate buffer (pH 7.8, containing 0.1 mM EDTA) and polyvinyl polypyrrolidone (PVPP). The homogenate was centrifuged at 10000 rpm for 10 min. at 4 ^{o}C and the supernatant used for enzyme activity determinations. CAT activity was determined using a method described by Cao *et al.* (2004) while GP and GR were determined as described by Wu and von Tiedemann (2002) and all enzyme activities expressed as µg/g FW. This analysis was carried out in the Soils, Water and Environment Research Institute (SWERI) which belongs to Agricultural Research Center (ARC), Giza, Egypt.

2.4. Determination of pigments

Exact of 400 mg fresh leaf (culm in case of *T. domingensis*) sample was extracted with aqueous 80% acetone in a mortar with the aid of clean Fontainebleau sand with absorbances A= 470, 520, 647 and 663 nm for Carotene, xanthophylls, chlorophyll a and chlorophyll b; respectively, by using spectrophotometer (Thermo Spectronic Helios α). All these analyses were carried based on the method described by Lichtenthaler, (1987) and the results were expressed as chlorophylls and xanthophylls content in the tissue (mg g^{-1} FW). This analysis was carried out in the Soils, Water and Environment Research Institute (SWERI) which belongs to Agricultural Research Center (ARC), Giza, Egypt.

2.5. Determination of phytohormones

Two important phytohormones were selected for the determination in fresh leaves (culm in case of *T. domingensis*); Indole-3-acetic acid (IAA) and Gibberellin (GA_3) in the target species. Concentration of (IAA) was carried out according to the method described by Czerpak and Bajguz (1997), while (GA_3) was estimated according to Czerpak *et al.* (2006). This analysis was carried out in the Soils, Water and Environment Research Institute (SWERI) which belongs to Agricultural Research Center (ARC), Giza, Egypt.

2.6. Analysis of data

Data were analyzed using Statistical Package for the Social Sciences (SPSS ver.12). Means were separated using Duncan's multiple range test at a P value of 0.05.

3. Results

3.1. Total protein expression

Total protein profile of *A. hybridus* and *M. longifolia* was presented in (Fig. 1) and the data was given in (Table 1), while the total protein profile of *C. ambrosioides* and *T.domingensis* was illustrated in (Fig. 2) and the data given in (Table 2).

Total protein contents in the contaminated and noncontaminated shoot samples of *A. hybridus* (Lane 2 and 3 in Fig.1) were found to be eight proteins. Comparing protein intensities in the two shoots showed more protein intensity in most shoots of the contaminated samples. For example, band 4 recorded intensities of 46.481 and 47.594 KDa in shoots of noncontaminated and contaminated *A. hybridus* samples; respectively (Fig. 1 and Table 1). On the contrary, the R.F. values of contaminated *A. hybridus* shoot samples were less than those in shoots of noncontaminated ones (Table 1). Similarly, protein intensity and R.F. values of contaminated and noncontaminated *A. hybridus* root samples, gave more

intensity and less R.F. in contaminated samples (Lane 4 and 5, Fig.1 and Table 1). In addition, protein expression of shoot and root *M. longifolia* samples growing in contaminated and noncontaminated sites were represented by lanes 6-9. In most *M. longifolia* samples and opposite to the previous case, protein intensity and R.F. values of contaminated *A. hybridus* root samples, gave less intensity and more R.F than those of noncontaminated ones. For example, protein intensities were 72.036 and 75 KDa in contaminated shoot and root samples of *M. longifolia*; respectively, and these values relatively increased to 72.993 and 77.133 KDa for the parallel noncontaminated samples (lanes 6-8, Fig.1 and Table 1).

Total protein expression in shoots and roots of *C. ambrosioides* and *T. domingensis* demonstrated formation of four or five protein bands with intensities ranged between 14.825 (lane 1, band 5, Fig.2 and Table 2) and 42.545 KDa (lane 8, band 1, Fig. 2 and Table 2) in the noncontaminated *T. domingensis* root and in the contaminated shoot samples of *C. ambrosioides*; respectively. Generally, the recorded values of protein intensities in either shoot or root contaminated samples were greater than noncontaminated samples. Moreover, recorded R.F. values in contaminated shoot or root samples gave less than contaminated samples (Table 2). In general, expression of proteins in contaminated plant samples of the present study was significantly increased or decreased at the level of protein intensity as compared to noncontaminated plant samples.

3.2. Enzyme activities

Investigation of the effect of heavy metals on CAT, GP and GR activities was given in Table 3. Comparing these data in the target species growing in contaminated and noncontaminated habitats showed elevated levels of enzyme activity in all contaminated plant samples as compared to those of noncontaminated plant samples. In other words, heavy metals increase activity of the selected enzymes. The recorded CAT activities for example, were found to be 45.65, 25.29, 30.79 and 52.37 µg g^{-1} FW in noncontaminated shoots of *A. hybridus, C. ambrosioides, M. longifolia* and *T. domingensis*; respectively, and these values significantly different ($P< 0.05$) and nearly doubled into 88.47, 38.56, 42.67 and 114.22 µg g^{-1} FW in contaminated shoot samples of the same species on succession. Moreover, among different measured enzymes, CAT recorded maximum values in shoots of contaminated and noncontaminated *T. domingensis* samples amounting to 114.22 and 52.37 µg g^{-1} FW; respectively. Shoots of *C. ambrosioides*, recorded the minimum enzyme activity among the four enzymes belonging to GR which are 6.25 and 9.65 µg g^{-1} FW for noncontaminated and contaminated samples; respectively.

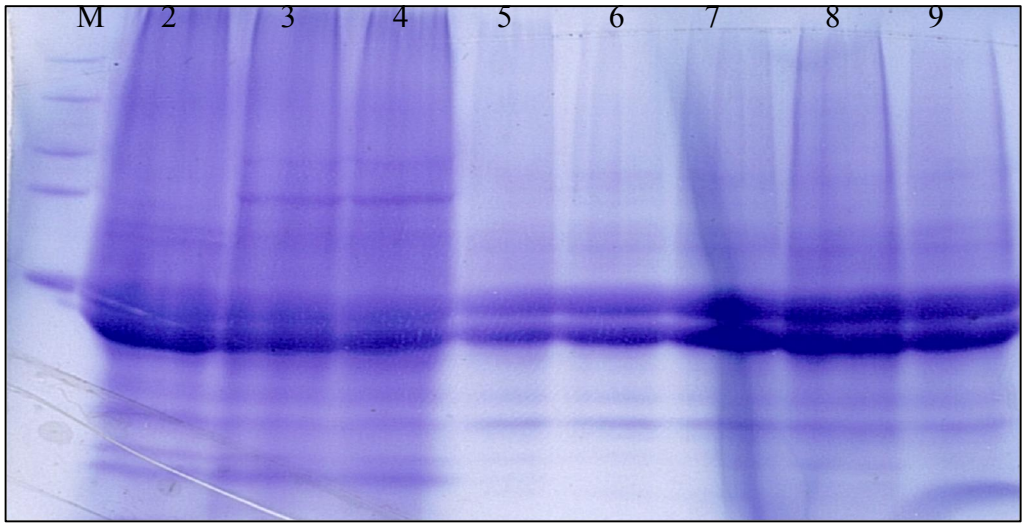

Figure1. SDS-PAGE of cytoplasmic proteins extracted from shoots and roots of *A.hybridus* and *M.longifolia*. Lane 1, corresponds to marker; (lanes 2 and 3) correspond to extracted proteins from contaminated shoot samples of *A.hybridus* (Acsh), noncontaminated shoot samples of *A.hybridus* (Ansh); (lanes 4 and 5) correspond to extracted proteins from contaminated root samples of *A.hybridus* (Acr), noncontaminated root samples of *A.hybridus* (Anr); (lanes 6and 7) correspond to extracted proteins from contaminated shoot samples of *M.longifolia* (Mcsh), noncontaminated shoot samples of *M.longifolia* (Mnsh); (lanes 8and 9) correspond to extracted proteins from contaminated root samples of *M.longifolia* (Mcr), noncontaminated root samples of *M. longifolia* (Mnr). Molecular masses (KDa) are indicated.

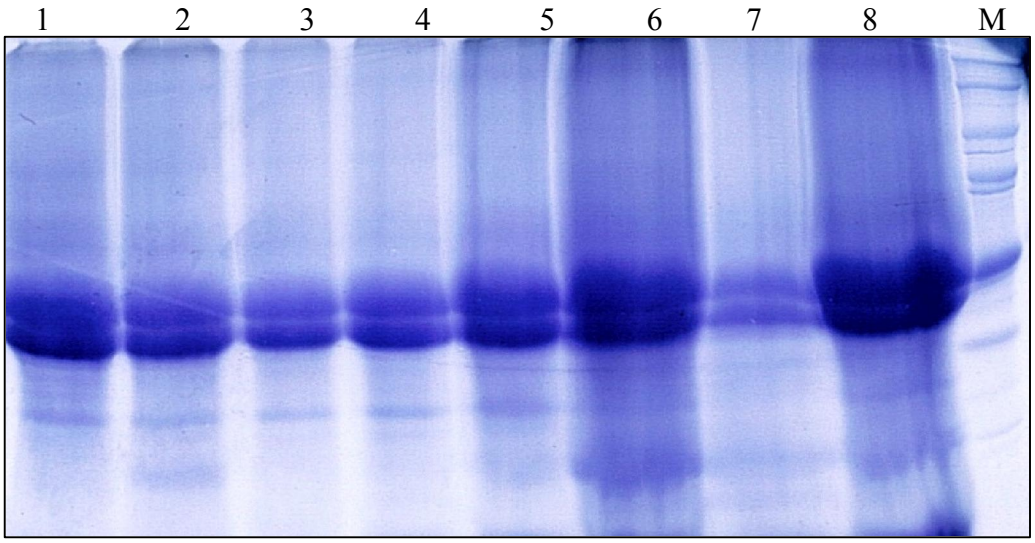

Figure2. SDS-PAGE of cytoplasmic proteins extracted from shoots and roots of *C.ambrosioides* and *T.domingensis*. Lane 1 and 2 corresponding to extracted proteins from noncontaminated root samples of *T.domingensis* (Tnr), noncontaminated shoot samples of *T.domingensis* (Tnsh); (lanes 3 and 4) correspond to extracted proteins from contaminated root samples of *T.domingensis* (Tcr), contaminated shoot samples of *T.domingensis* (Tcsh); (lanes 5 and 6) correspond to extracted proteins from noncontaminated root samples of *C.ambrosioides* (Cnr), noncontaminated shoot samples of *C.ambrosioides* (Cnsh); (lanes 7 and 8) correspond to extracted proteins from contaminated root samples of *C.ambrosioides* (Ccr), contaminated shoot samples of *C.ambrosioides* (Ccsh). Lane 9 corresponds to marker. Molecular masses (KDa) are indicated.

Table1. Molecular weight (KDa) and R.F. values of the different protein specimens extracted from contaminated shoot samples of *A.hybridus* (Acsh), contaminated root samples of *A.hybridus* (Acr), noncontaminated shoot samples of *A.hybridus* (Ansh), noncontaminated root samples of *A.hybridus* (Anr), contaminated shoot samples of *M.longifolia* (Mcsh), contaminated root samples of *M.longifolia* (Mcr), noncontaminated shoot samples of *M.longifolia* (Mnsh), noncontaminated root samples of *M. longifolia* (Mnr). Lane one refers to marker (M).

Treatment →	***M***	***Acsh***	***Ansh***	***Acr***	***Anr***	***Mcsh***	***Mnsh***	***Mcr***	***Mnr***
M.W.(KDa)	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9
Band1	225.000	95.914	91.935	95.935	95.935	97.288	98.643	98.643	98.643
Band2	150.000	70.212	67.695	69.345	72.993	72.036	72.993	75.000	77.133
Band3	100.000	55.755	54.242	54.242	54.242	53.721	54.808	55.755	55.755
Band4	75.000	47.594	46. 481	47.594	47.412	47.229	47.594	47.412	47.229
Band5	50.000	43.672	43.342	43.546	43.342	43.748	43.748	43.546	43.546
Band6	35.000	36.314	36.095	36.532	36.751	36.095	35.876	35.876	36.532
Band7	25.000	33.950	33.800	33.000	33.600	34.000	33.200	32.600	32.400
Band8	22.000	29.800	30.000	30.000	30.000	29.800	29.200	29.200	29.800

Treatment →	***M***	***Acsh***	***Ansh***	***Acr***	***Anr***	***Mcsh***	***Mnsh***	***Mcr***	***Mnr***
R.F.Values	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9
Band1	0.076	0.275	0.285	0.275	0.275	0.272	0.268	0.268	0.268
Band2	0.159	0.348	0.358	0.351	0.338	0.341	0.338	0.331	0.325
Band3	0.265	0.430	0.447	0.447	0.447	0.454	0.440	0.430	0.430
Band4	0.331	0.496	0.566	0.566	0.570	0.573	0.566	0.570	0.573
Band5	0.520	0.636	0.639	0.636	0.639	0.632	0.632	0.636	0.636
Band6	0.768	0.748	0.752	0.745	0.742	0.752	0.755	0.755	0.745
Band7	0.864	0.788	0.788	0.801	0.791	0.785	0.798	0.808	0.811
Band8	0.902	0.854	0.851	0.851	0.851	0.854	0.864	0.864	0.854

Table2. Molecular weight (kDa) and R.F. of the different protein specimens extracted from contaminated shoot samples of *C.ambrosioides* (Ccsh), contaminated root samples of *C.ambrosioides* (Ccr), noncontaminated shoot samples of *C.ambrosioides* (Cnsh), noncontaminated root samples of *C.ambrosioides* (Cnr), contaminated shoot samples of *T.domingensis* (Tcsh), contaminated root samples of *T.domingensis* (Tcr), noncontaminated shoot samples of *T.domingensis* (Tnsh), noncontaminated root samples of *T.domingensis* (Tnr). Lane nine refers to marker (M).

Treatment →	***Tnr***	***Tnsh***	***Tcr***	***Tcsh***	***Cnr***	***Cnsh***	***Ccr***	***Ccsh***	***M***
M.W.(KDa)	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9
Band1	40.001	40.600	41.223	40.909	40.909	41.544	41.544	42.545	225.000
Band2	35.662	35.888	36.116	36.348	36.821	37.308	38.068	37.557	150.000
Band3	27.024	26.553	26.396	26.240	26.396	26.553	26.553	26.553	100.000
Band4	20.787	20.789	20.965	20.965	20.789	21.491	21.140	21.140	75.000
Band5	14.825		14.825				14.825		50.000
Band6									35.000
Band7									25.000
Band8									22.000

Treatment →	***Tnr***	***Tnsh***	***Tcr***	***Tcsh***	***Cnr***	***Cnsh***	***Ccr***	***Ccsh***	***M***
R.F.Values	Lane1	Lane2	Lane3	Lane4	Lane5	Lane6	Lane7	Lane8	Lane9
Band1	0.536	0.529	0.522	0.525	0.525	0.519	0.519	0.508	0.081
Band2	0.593	0.590	0.586	0.583	0.576	0.569	0.559	0.566	0.190
Band3	0.753	0.763	0.766	0.769	0.766	0.763	0.763	0.763	0.247
Band4	0.878	0.878	0.875	0.875	0.878	0.864	0.871	0.871	0.312
Band5	0.993		0.993				0.993		0.447
Band6									0.603
Band7									0.797
Band8									0.811

Table3. Catalase, Glutathione peroxidase (GP) and Glutathione reductase(GR) activities (µg/g FW) in shoots of *Amaranthus hybridus* (AMHY), *Chenopodium ambrosioides* (CHAM), *Mentha longifolia* (MELO) and *Typha domingensis* (TYDO), growing in noncontaminated wetland (I) and contaminated wetland (II) located at Wadi Al-Argy, Taif. (Values are the mean + SD of three replicates)

Type of species and soil	Enzyme activity of		
	(CAT)	(GP)	(GR)
AMHY			
I	45.65±8.42^{a}	19.32±1.13^{a}	8.37±1.04^{a}
II	88.47±6.29^{b}	62.27±2.16^{ab}	13.56±1.48^{a}
CHAM			
I	25.29±3.24^{a}	23.87±3.38^{a}	6.25±0.52^{a}
II	38.56±5.69^{a}	55.28±6.89^{b}	9.65±1.28^{a}
MELO			
I	30.79±6.56^{ab}	38.12±8.42^{a}	6.86±0,87^{a}
II	42.67±4.22^{a}	66.33±7.87^{a}	12.76±1.06^{a}
TYDO			
I	52.37±7.51^{a}	42.43±5.67^{ab}	10.43±1.56^{a}
II	114.22±10.96^{a}	96.67±8.29^{b}	18.76±2.56^{a}
	*	**	n.s.

* $p \leq 0.05$, ** $p \leq 0.01$, n.s. = not significant**.**

Table4. Chlorophyll a, chlorophyll b, xanthophylls and carotene pigments (mg g^{-1} FW) and gibberellin (GA_3), indole-3-acetic acid (IAA) phytohormones (µM) of *Amaranthus hybridus* (AMHY), *Chenopodium ambrosioides* (CHAM), *Mentha longifolia* (MELO) and *Typha domingensis* (TYDO) growing in the noncontaminated soil (I) and contaminated soil (II). (Mean values are given ± SD)

Species	Chlorophyll a		Chlorophyll b		Xanthophyll		Carotene		$GA3_3$		IAA	
	I	II	I	II	I	II	I	II	I	II	I	II
AMHY	159.47±5.62	133.2±3.56	135.4±7.33	189.25±3.21	20.7±0.08	82.9±6. 6	33.42±0.98	65.2±1.1	946±11.35	1068±33.6	1110±71.35	1089±43.1
CHAM	393.76±11.72	305.6±2.11	123.25±2.68	150.41±4.27	35.03±3.12	49.6±0.8	32.1±1.88	65.4±2.7	1418±36.64	1034±66.2	1449±55.34	1016±28.9
MELO	85.5±6.98	69.3±0.67	46.2±0.55	34.52±0.96	56.54±0.84	83.5±2.1	12.7±0.063	25.8±0.3	1154±55.12	864±19.6	1246±36.33	1005±37.1
TYDO	86.57±14.2	50.6±1.05	77.39±1.36	144.35±6.84	55.46±2.68	84.6±1.6	36.21±1.08	63.1±2.6	1342±33.78	1024±25.5	1167±22.67	960±11.3

3.3. Plant pigments and hormones

The effect of heavy metals on concentrations of chlorophyll a and b, xanthophylls and carotene pigments were estimated and the results are summarized in Table 4. The recorded pigment concentrations in the contaminated plant samples were more than that of noncontaminated ones.

The recorded values for Chlorophyll b, xanthophyll and carotene in contaminated *A. hybridus* were 189.25, 82.91 and 65.22 mg g^{-1} FW and these values comparatively decreased into 135.4, 20.7 and 33.42 mg g^{-1} FW in the noncontaminated samples for the same test species. On contrary to all measured pigments, chlorophyll a gave more values in the noncontaminated plant samples as compared to contaminated plant samples. The recorded values for chlorophyll a were 393.76 and 305.6 mg g^{-1} FW for noncontaminated and contaminated *C. ambrosioides* plant samples; respectively (Table 4).

The presented data in Table 4 proved the effect of heavy metals on the studied phytohormones. This effect varies according to type estimated phytohormones and type of plant species. Gibberellin (GA_3), recorded values ranged between 864 and 1068 µM in the contaminated plant samples of the test species and by comparison these values increased to be ranged between 946 and 1418 µM in the noncontaminated plant samples. In other words, heavy metals induce the inhibition of this phytohormone. On the contrary, Indole-3-acetic acid (IAA) showed greater values ranged between 1110 and 1449 µM in the noncontaminated plant samples as compared to those of contaminated samples which ranged between 960 and 1089 µM (Table 4). In this case, heavy metals enhance the increase of IAA secretion in plant tissues of the target species.

4. Discussion

The present study showed that protein expression in the contaminated plant samples was significantly increased or decreased in the level of number and protein intensity as compared to noncontaminated plant samples. These results were confirmed by findings of Nath *et al*. (2005). Moeover, Shalini Sharma (2009) in his study on *Brachythecium populeum*, proved the impact of heavy metals on some physiological parameters like total chlorophyll, sugar, protein content and activity of catalase and peroxidase enzymes. Similarly, Pearson and Rengel, 1997, mentioned that "heavy metal caused carbohydrate and protein metabolism impairment". In addition, increase in heavy metal concentrations in the contaminated plant samples causes parallel increase of enzyme antioxidant defenses which are used as biomarkers of oxidative damage (Regoli and Principato, 1995). Moreover, prolonged exposure of plants to environmental pollution with heavy metal causes depletion of the antioxidant enzymes as a result of oxidative damage to biological molecules, such as lipid peroxidation, protein and DNA damage (Bebianno *et al*., 2005).

The current investigation showed elevated levels of CAT, GP and GR enzyme activities in all contaminated plant samples as compared to those of noncontaminated plant samples, this can be explained by Hao *et al*. (2006), who proved the direct relation between heavy metal and increase in oxidative stress as a result of an enhanced level of lipid peroxidation and hydrogen peroxide production in both roots and leaves of stressed plants. In addition, increase of heavy metals especially Mn in all plant samples in the contaminated site would have been one of the reasons for increased GP activity (Shalini Sharma, 2009). Similarly to our results, heavy metal increased the CAT and peroxidase activities in alfalfa and also in maize (Ortega-Villasante *et al*., 2005; Rellán-Álvarez *et al*., 2006). Moreover, increase the magnitude of Hg induced oxidative stress (Zhang and Tyerman, 1999).

Comparing measured pigments in contaminated and noncontaminated plant samples of the target species showed general increase in carotene concentration and decrease in the total chlorophyll and xanthophyll in the former case (stressed plants) to provide protection against the formation of free radicals (Ferrat *et al*., 2003). The obtained results were in accordance with those obtained by Ngayila *et al*., 2007. Current results also showed that heavy metal induce the inhibition of (GA_3) and greater (IAA) values in stressed (contaminated) plant samples. These results can be explained by Vachirapatama *et al*. (2011), who proved the inhibition of heavy metals to the plant growth. Moreover, Potters *et al*. (2007), suggested that metal stress induced alteration in levels of phytohormones and result in reduced cell elongation

and, therefore reduced root growth. The current results were in accordance with Jain and Khurana (2009).

5. Conclusion

In conclusion, the current study investigated the stress of heavy metals accumulated by *A.hybridus, C.ambrosioides, M.longifolia* and *T.domingensis* on some physiological parameters of these plants. The present work proved that expression of proteins in contaminated plant samples was significantly increased or decreased at the level of protein intensity as compared to noncontaminated plant samples. Heavy metals in contaminated plant samples, increased activities of catalase, glutathione peroxidase and glutathione reductase. The study also measured pigments in contaminated and noncontaminated plant samples of the target species and showed general increase in carotene concentration and decrease in the total chlorophyll and xanthophyll in the former case. Contaminated samples of the test species showed general increase in carotene concentration and decrease in the total chlorophyll and xanthophylls as compared to noncontaminated samples.

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