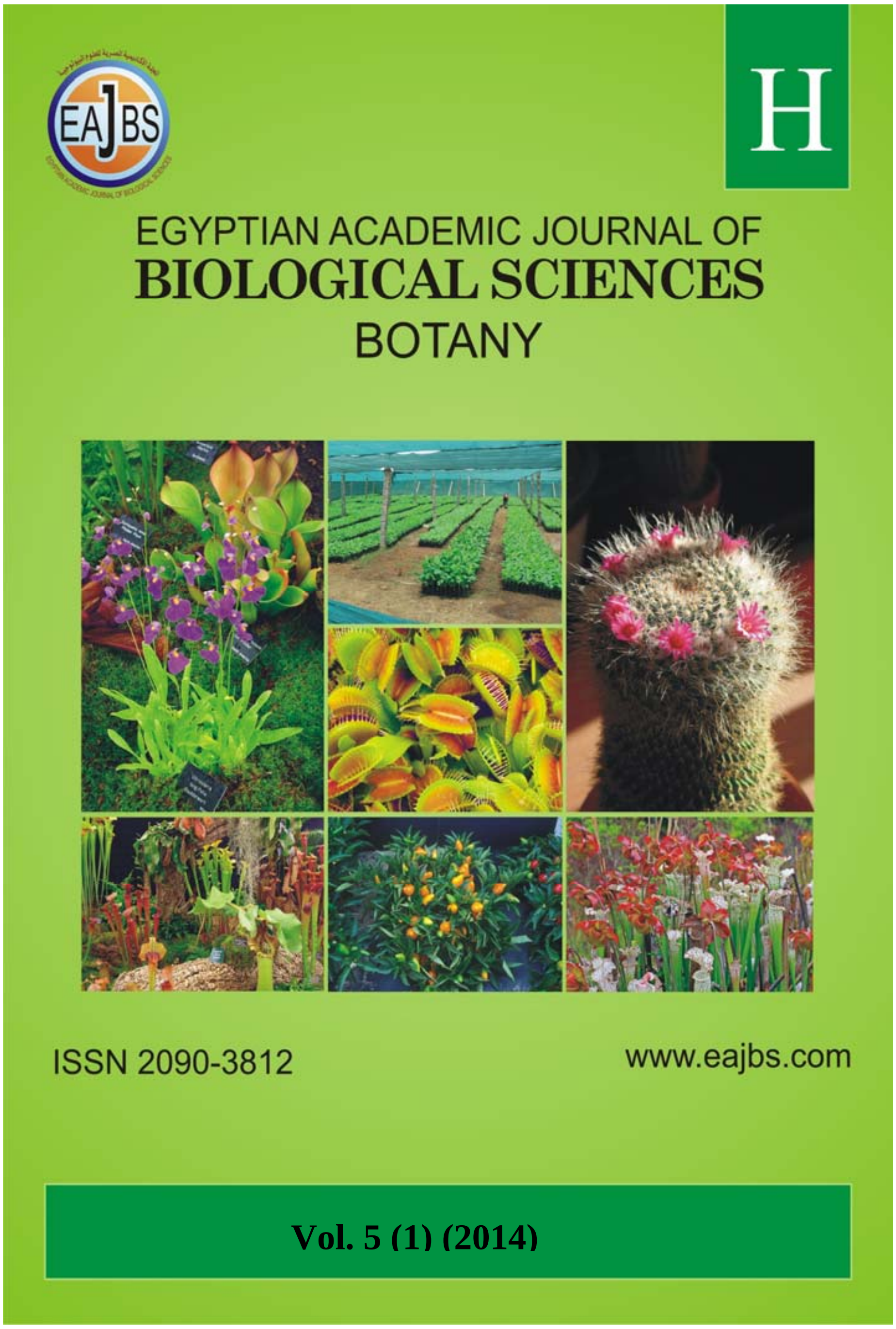

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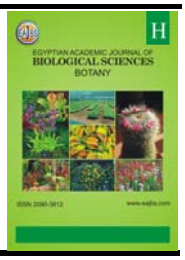

Physiological responses of flax (*Linum usitatissimum*) and canola (*Brassica napus*) to cadmium and lead stresses

Hend A. El-khawaga
Botany and Microbiology Department, Faculty of science-Al-Azhar University- Cairo, Egypt

ABSTRACT

A pot experiment was conducted to study the effects of cadmium and lead on some metabolic products of flax (*Linum usitatissimum* Giza 5) and canola (*Brassica napus* Serw 4) at two growth stages; vegetative and flowering stages. The soil enriched with different levels of Cd as Cd $(NO_3)_2$ (25, 50, 100 and 150 mg/kg soil) and Pb as Pb $(NO_3)_2$ (250, 500, 1000 and 1500 mg/kg soil) in addition to control. The results showed that a significant reduction in pigment fractions while proline, total soluble sugars, thiols, lipid peroxidation, ascorbic acid and activity of catalase as well as peroxidase increased significantly in both plants with increasing concentration of Cd and Pb. The results also showed that there was an increase in pigment fractions, proline, ascorbic acid and total soluble sugar while lipid peroxidation, thiols and enzymatic activity were decreased as plants proceeded from vegetative to flowering stages.

Keywords: Cadmium, Lead, Canola, Flax, Pigment, Free thiol, MDA, Antioxidant.

INTRODUCTION

Heavy metal toxicity is one of the main current environmental health problems, and potentially harmful because of bioaccumulation through the food chain and plant products for human consumption. Therefore, heavy metal contaminations of soils and plants have become an increasing problem especially by industrial effluents and agricultural improvement. Heavy metal contents of food plants can be affected by the anthropogenic factors such as the application of fertilizers, sewage sludge or irrigation with wastewaters (Frost and Ketchum, 2000). Amongst the heavy metals, cadmium and lead, cadmium is a trace pollutant toxic for plants, animals and humans. Exposure of plants, to Cd causes inhibition of growth and even plant death owing to its influence on photosynthesis, respiration, water and nutrient uptake (Sanità di Toppi and Gabbrielli, 1999). In several species Cd is easily taken up by roots and readily translocated and accumulated in shoots (Wagner, 1994). At the molecular level, Cd injury has been attributed to *1*) blocking of essential functional groups in biomolecules (Schützendübel and Polle, 2002), 2) displacement of essential metal ions from biomolecules (Rivetta, *et al.* 1997) and 3) production of reactive oxygen species (ROS) by autoxidation and Fenton reaction (Chaoui, *et al.* 1997).

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Lead is one of the most widely distributed heavy metals and is very toxic to plants (Kosobrukhov *et al*., 2004). Lead can affect photosynthesis, mesophyll cells and pigment content. It interferes with nutritional elements of seedlings and plants, thus causing deficiencies or adverse ion distribution within the plant (Trivedi and Erdei, 1992) as well as inhibition of growth (Malkowski *et al*., 2002).

Excess concentrations of heavy metal may induce significant toxic effect by altering the protein function and enzyme activity (Hansch and Mendel, 2009).

Toxicity may result from the binding of metals to sulfhydryl groups in the protein, leading to the inhibition of activity or disruption of protein structure (Morelli and Scarano, 2004). In addition, excess heavy metal concentrations are said to generate oxidative stress due to an increase in the levels of reactive oxygen species (ROS) within subcellular compartments. ROS include the superoxide radical (O_2-•), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•), all of which affect mainly lipids, proteins, carbohydrates, and nucleic acids (Brahim and Mohamed, 2011). ROS are also known to damage cell membranes by inducing lipid peroxidation; causing membrane damage, and inactivation of enzymes and alteration of DNA activity (De Vos *et al*., 1993). Under normal conditions, the ROS molecules are scavenged by various antioxidative defense mechanisms. The equilibrium between the production and the scavenging of ROS may be perturbed by various abiotic stress factors such as heavy metals. These disturbances in equilibrium lead to sudden increase in intracellular levels of ROS. To overcome this, cells are equipped with enzymatic and non-enzymatic mechanisms to eliminate or reduce their damaging effects. The importance of antioxidant enzymes is their ability to scavenge ROS and thereby prevent oxidative damage. The antioxidant system comprises several enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidases (PODs) enzymes which play a crucial role to protect plant from oxidative damage, and it is believed that their amount and activities determine the degree of tolerance in plant (Teisseire and Guy, 2000). In addition to the chain of enzymatic reactions (Asada, 1994), a direct radical scavenging activity performed by non enzymatic chemical species which cooperate in the remova1 of the active forms of oxygen (Rama Devi and Prasad, 1998), such as ascorbate (AsA), glutathione (GSH), carotenoids and some low molecular weight compounds containing sulfhydryl groups (Lombardi and Sebastiani, 2005). Plants synthesize and accumulate several organic solutes like sugars, polyols, betaines, proline and sulfur-containing indole derivatives in response to copper stress. Proline plays an important role in osmoregulation, protection of enzymes and stabilization of the machinery of protein synthesis (Choudhary *et al*., 2007). It also acts as an effective singlet oxygen quencher (Alia and Matysik, 2001). Additionally, sugars represent the major accumulating solutes, reserve in the seeds and maintained the osmotic regulation of cells (Bewlay and Black, 1994). There are several reports on carbohydrate accumulation during various abiotic stresses in the temperate grasses and cereals where long term carbohydrate storage occurs during reproductive development (Meier and Reid, 1982). Accumulation of sugars in different parts of plants is enhanced in response to the variety of environmental stresses (Prado *et al*., 2000). The main objective of the current study was to investigate the effect of cadmium and lead stress on chlorophylls, proline, total soluble sugars, free thiols, lipid peroxidation, catalase, peroxidase and ascorbic acid in *Linum usitatissimum* and *Brassica napus* at vegetative and flowering stages.

MATERIALS AND METHODS

A pot experiment was carried out during winter season of 2013-2014 at a fenced area under the prevailing environmental conditions, Faculty of Agriculture Al Azhar University at Nasr City, Cairo, Egypt to investigate the effect of cadmium and lead on some metabolic products of flax (*Linum usitatissimum* cultivar Giza 5) and canola (*Brassica napus* cultivar Serw 4).

Different levels of cadmium and lead were added as nitrate solution at the rate of 25, 50, 100 and 250 mg for cadmium and 250, 500, 1000 and 1500 mg lead per each kg soil in addition to the control (without adding heavy metals). These heavy metals were added to the soil before cultivation.

Planting was done in plastic pots (25 cm in diameter and 24 cm in height), each pot was filled with 7 kg of sandy loam soil.

The pots were designed as a complete randomized pattern with five replicates for each treatment. Seeds of flax (*Linum usitatissimum*) and canola (*Brassica napus*) were obtained from the Agricultural Research Center, Giza, Egypt. Ten seeds were sown in each pot. The seeds were directly planted at depth of 1.5 cm from soil surface. After complete emergence (two weeks from sowing), seedling were thinned to 5 identical ones in each pot. Irrigation was monitored at 60% of water holding capacity (WHC). Canola plant was fertilized with NPK at the rate of 150 kg N/ Fadden (as ammonium nitrate 33.5%), 100 kg/ Fadden P_2O_5 (as super phosphate 15.5%) and 50 kg/ Fadden K_2O (as potassium sulphate 20.5%), while flax plant was fertilized in the same manner as canola plant without addition of K_2O, as recommended dose of Agriculture Ministry.

The soil used for this experiment was obtained from cultivated farmland at El-Monifea governorate. Some physical, chemical analyses and heavy metals content of the studied soil are presented in Table (1).

Table 1: Some physical, chemical properties and total metal ions in the studied soil.

Physical properties	Particle size %	Total Sand	70.75
		Silt	10.25
		Clay	19.00
	Texture class		Sandy loam soil
Chemical properties	pH		8.03
	EC mm hos/cm		0.87
	Soluble cations meq/L	Ca^{++}	12.1
		Mg^{++}	6.48
		Na^{+}	10.5
		K^{+}	3.14
	Soluble anions meq/L	CO_3^{-}	0.00
		HCO_3	2.23
		Cl^{-}	4.8
		SO_4^{--}	0.44
Heavy metal ion con. µg/g		Cd	0.001
		Pb	0.004

Air-dried soil samples were ground, passed through a 2 mm sieve and mixed thoroughly according to Piper (1947). Physical and chemical properties of the used soil were determined according to standard methods of Page *et al*. (1982) and Clark *et al*. (1986). The pH was measured using a pH meter in soil water suspension (1: 2.5), total soluble salts (ECe) were determined in the saturated soil paste. Cd and Pb were determined by Inductively Coupled Plasma Spectrometry (Ultima 2 JY Plasma), as described by Soltanpour and Schwab (1977).

Two samples for shoots were taken to represent the vegetative and flowering stages; after 30 and 60 days from planting, respectively, the following chemical analyses were carried out.

Photosynthetic pigments (chlorophyll a, b and carotenoids) were determined according to Metzner *et al*. (1965) and expressed as mg/g f.wt, Proline content was estimated following the method of Bates *et al*. (1973) and expressed as mg/g f. wt. Total soluble sugars were determined based on the method of phenol-sulfuric acid (Dubois *et al*., 1956) its content was expressed as mg/g f. w., lipid peroxidation was determined by measuring the level of malondialdehyde by a modification of the method of Zhou (2001), it expressed as nmol MDAg^{-1} f. wt. Free thiol was estimated by Ellman (1959), the results were expressed as µM/mg f. wt. Ascorbic acid content was determined according to Oser (1979) and expressed as µg/g f. wt. Activities of catalase and peroxidase were assayed in fresh shoot tissue extract using a modification method of Zhou (2001) and Zhang (1990). One unit of catalase activity was defined as amount of enzyme that reduce 50% of H_2O_2 in 60 sec. at 25C^o expressed as mM/ml/hour, while one unit of peroxidase activity was defined as amount of enzyme that catalyze the conversion of one micromole of H_2O_2 per minute at 25C^o expressed as units /sec/mg (Kong *et al*., 1999).

Statistical analysis of data was done using ANOVA program. The differences among means for all treatments for significance at 5% levels were compared by using Duncan (1955) new multiple range test as described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

1. Photosynthetic pigments:

Data presented in Table (2) show the effect of various concentrations of heavy metals used on photosynthetic pigments during vegetative and flowering stages.

There was an increase in the concentration of pigment fractions as plant proceeded to maturity. Data also, showed that there was a consistent and gradual reduction in chlorophyll a. chlorophyll b and carotenoid contents in both plants as the concentrations of the different heavy metals used were increased.

Table 2: Effect of Cd and Pb (mg/kg soil) on pigment fractions (mg/g f. wt.) of flax and canola plants at vegetative and flowering stages.

Pigment fractions	Growth stage	Flax									Canola									LSD 5%
		Control	Cadmium				Lead				Control	Cadmium				Lead				
			25	50	100	150	250	500	1000	1500		25	50	100	150	250	500	1000	1500	
Chlorophyll a	Veg.	0.670	0.612	0.466	0.449	0.350	0.660	0.621	0.419	0.388	0.878	0.650	0.592	0.489	0.466	0.795	0.720	0.582	0.485	0.0016
	Flow.	0.754	0.744	0.663	0.581	0.538	0.690	0.641	0.550	0.390	1.031	0.803	0.690	0.551	0.520	0.931	0.841	0.779	0.620	0.0042
Chlorophyll b	Veg.	0.339	0.316	0.260	0.220	0.202	0.327	0.221	0.125	0.090	0.832	0.383	0.378	0.345	0.212	0.476	0.421	0.284	0.124	0.0042
	Flow.	0.404	0.392	0.362	0.235	0.210	0.368	0.314	0.291	0.191	0.497	0.451	0.381	0.368	0.260	0.491	0.482	0.396	0.290	0.0016
Total Chlorophyll	Veg.	1.009	0.928	0.726	0.669	0.552	0.987	0.842	0.544	0.478	1.710	1.033	0.970	0.834	0.678	1.271	1.141	0.866	0.609	0.0042
	Flow.	1.158	1.136	1.025	0.816	0.748	1.058	0.955	0.841	0.581	1.528	1.254	1.071	0.919	0.780	1.422	1.323	1.175	0.910	0.0016
Carotenoid	Veg.	0.244	0.212	0.171	0.111	0.093	0.232	0.201	0.150	0.139	0.325	0.271	0.241	0.114	0.094	0.268	0.198	0.154	0.140	0.0042
	Flow.	0.266	0.258	0.253	0.235	0.104	0.255	0.247	0.210	0.152	0.440	0.379	0.330	0.272	0.253	0.280	0.201	0.169	0.160	0.0027

In case of control plants, total chlorophyll content was lower in flax than in canola in both growth stages.

At the highest concentrations of heavy metals Cd and Pb chlorophyll a contents, at vegetative stage, were reduced by 47%, 42.1% for flax and 47%, 44.8% for canola, respectively, as compared to corresponding control. In the same order, chlorophyll (b) contents were reduced by 40.4%, 73.5% for flax and 75%, 85.1% for canola respectively, while, the contents of carotenoids were depressed by 61.9, 43.0% for flax and 71.7%, 57.0% for canola in the same respect.

The loss in chlorophyll content could be due to peroxidation of chloroplast membranes or replacing of magnesium in chlorophyll molecule by heavy metal (Mal *et al*., 2002).

A common response of plant to metal stress is a decrease of photosynthetic pigments (chlorophylls and carotenoids) in leaves of plants (Monteiro *et al*., 2009). The reduction of total chlorophyll, chlorophylls a, b and carotenoids on exposure to heavy metals has been observed in many species treated with different metals (Ekmekci *et al*., 2008). Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis (Cadmium was reported to affect chlorophyll biosynthesis and inhibit protochlorophyll reductase and aminolevulinic acid synthesis (Stobart *et al*., 1985).

Heavy metals affect the function of PSI and PSII and it was stronger with the latter (Yang *et al*., 1989). The chlorophyll proteins, which took protons for photosynthesis in PSII, were decomposed and decreased under Cd stress.

The sub-microstructure of chloroplast was changed and the membrane system was destroyed. Therefore, the capacity of taking protons declined and photosynthesis function was influenced (Peng and Wang, 1991). Khider (1996) showed that there was a consistent and gradual reduction in Chl a, Chl b and carotenoid concentrations of wheat plants as the concentrations of Zn, Cd or Pb were increased. Mostafa (2003) reported similar results with cotton plants.

2. Proline, free thiol and total soluble sugars:

Proline concentration in shoots of flax and canola was significantly increased due to the increase in concentrations of heavy metals at both studied stages i.e. vegetative and flowering one. There was an increase in proline content by plant age (Table 3).

These results align with those obtained by Schat *et al*. (1997) who determined the accumulation of free proline in response to Cu, Cd and Zn in non-tolerant and metal-tolerant *Silen vulgaris* (Moench) Garcke the results showed that constitutive proline concentration in leaves was 5 to 6 times higher in the metal-tolerant ecotype than in the non-tolerant one.

Accumulation of free proline in response to heavy metal exposure seems to be wide-spread among plants. The functional significance of this accumulation would lie in its contribution to water balance maintenance (Costa and Morel, 1994). These authors also suggested that proline mediated alleviation of water deficient stress could contribute to cadmium tolerance of the plant. Proline increases the stress tolerance of the plant through such function as osmoregulation, the protection of enzymes against denaturation, and the stabilization of protein synthesis (Kuznetsov and Shevyakova, 1997).

The concentration of free thiol in shoot tissue of flax and canola grown under various concentrations of Cd and Pb as well as the control tend to decrease with progress of plants towards maturity (Table 3). It is clear that the increasing concentrations of heavy metals; Cd and Pb significantly increased free thiol in both plants, this is true at vegetative and flowering stages. Free thiol values reached to maximum level at high concentrations of Cd and Pb, the rate of increment was higher in canola than in flax. In this concentration, Aly and Mohamed (2012) reported that level of thiol compound was significantly increased by increasing Cu level in growth media in two cultivars of *Zea mays*. Metal stress has been reported to enhance the sulfur reduction pathway by affecting not only the sulfur uptake and transport but also by inducing the enzymes of the pathway (Rausch and Wachter, 2005; Herbette *et al*., 2006). Such change in levels of sulfhydral compounds may be indicative of transient disturbance of metabolism.

Variations in total soluble sugars in flax and canola plants as affected by heavy metal; Cd, Pb supply at vegetative and flowering stages are presented in Table 3. There was an increase in total soluble sugars as plants proceeded from vegetative to flowering stages. It is clear that the increasing concentrations of heavy metals Cd and Pb significantly increase the total soluble sugars in both plants.

High concentration of heavy metal caused a conspicuous increase in total soluble sugars in both plants and flax plant accumulates total soluble sugars than canola plant during vegetative and flowering stages.

In this concern Verma and Dubey (2001) studied the effect of cadmium stress (100mm µM and 500 µM Cd) on the content of total soluble sugars in two rice cultivars during a 5 to 20 days exposure in the growth medium, an increase in the content of total soluble sugars was observed. The authors indicated that, an increase of soluble sugar accompanied with increased activity of acid invertase and sucrose synthesis. In addition to the role of sugars in osmoregulation, the soluble sugars allow the plants to maximize sufficient carbohydrates storage reserves to support basal metaboslim under stressed environment (Hurry *et al*., 1995; Dubey and singh, 1999). On the other hand, excess heavy metal may interfere with the biosynthesis of photosynthetic machinery and may modify the pigment and protein components of photosynthetic membranes (Maksymiec *et al*., 1994).

Table 3: Effect of Cd and Pb (mg/kg soil) on proline content (mg/g f. wt.) free thiol (µM/g f. wt.) and total soluble sugars (T.S.S.) (mg/g f. wt.) of flax and canola plants at vegetative and flowering stages.

	Growth stage	Flax									Canola									LSD 5%
		Control	Cadmium				Lead				Control	Cadmium				Lead				
			25	50	100	150	250	500	1000	1500		25	50	100	150	250	500	1000	1500	
Proline	Veg	0.512	1.37	1.66	2.33	3.09	1.28	2.09	2.99	4.31	1.11	2.42	3.49	5.16	7.60	1.47	1.86	2.50	3.90	0.069
	Flow	8.92	10.60	11.03	12.20	13.45	9.32	10.80	11.60	12.92	7.91	30.40	32.20	36.30	55.90	63.80	32.90	40.70	46.50	0.164
Free thiol	Veg	0.411	0.661	0.782	0.873	0.942	0.523	0.632	0.799	0.889	0.913	1.21	1.89	2.11	2.90	1.42	1.56	1.87	2.09	0.04
	Flow	0.210	0.342	0.570	0.662	0.724	0.332	0.431	0.587	0.680	0.754	0.78	0.91	1.14	1.46	0.945	1.33	1.53	1.69	0.009
T. S. S.	Veg	65.7	70.3	75.4	80.6	83.7	72.9	77.4	80.5	83.4	62.6	73.6	77.9	82.3	87.1	64.4	70.6	72.1	74.2	0.16
	Flow	75.8	80.4	85.5	87.9	90.4	82.8	87.3	91.4	93.7	72.7	80.5	84.4	91.4	93.6	73.6	76.8	79.9	83.4	0.17

3. Lipid peroxidation:

Results recorded in Table (4) show that the level of malondialdehyde (MDA) in shoot tissues of studied plants grown under various concentrations of Cd and Pb as well as control tend to decrease with progress of plant towards maturity. The results also show that there was a significant increase in content of (MDA) in shoots with increasing the concentration of Cd and Pb. This was true at both studied stages (i.e. vegetative and flowering stages). Malondialdehyde values reached to maximum level at 150 mg Cd and 1500 mg Pb treatment in both plants but the rate of increment was higher in canola than flax. The content of MDA in plant is an indicator of lipid peroxidation (Cheng, 2003). The high rate of tissue autoxidation in leaves, which indicated lipid peroxidation of cellular membranes, was stimulated by endogenously active oxygen radical (Luo, 1999). Huang and Hong (1997) reported that the accumulation of MDA in the plant seedling was related to the concentration of Cd. It can be concluded that membrane peroxidation by overabundant free radicals is one of the serious impact of heavy metals such as Cd and Pb on plants.

The present results showed an increase in the level of lipid peroxide with increasing concentrations of Cd & Pb indicating that Cd & Pb induce oxidative stress in studied plants (flax and canola). Similar to our observations, some report showed that MDA content increased significantly with increasing copper concentrations in germinating rice seeds exposed to 0.2 to 1.5 mM Cu (Ahsan *et al*., 2007) and in roots of *Brassica junicea* treated with 8 µM Cu (Wang *et al*., 2004). Increasing concentrations of MDA, which is a product of lipid peroxidation, is an indicator of oxidative stress after heavy metal dosing; the increase correlated with the increase of metal concentrations.

Table 4: Effect of Cd and Pb (mg/kg soil) on lipid peroxidation (nmol/g f. wt.) of flax and canola plants at vegetative and flowering stages.

	Growth stage	Control	Flax								Control	Canola								LSD 5%
			Cadmium				Lead					Cadmium				Lead				
			25	50	100	150	250	500	1000	1500		25	50	100	150	250	500	1000	1500	
L. peroxidation	Veg	1.48	1.67	2.40	2.55	3.80	1.84	3.00	3.50	4.00	1.87	2.52	3.31	3.75	4.33	1.90	2.40	3.03	4.55	0.077
	Flow	1.24	1.33	2.17	2.24	3.51	1.57	2.59	2.81	3.16	1.61	2.36	2.81	2.89	3.55	1.76	2.15	2.60	3.15	0.017

4. Antioxidants:

Concerning the effect of heavy metals Cd and Pb on catalase and peroxidase activity in shoots of studied plants at vegetative and flowering stages, results presented in Table 5 clearly show that the activity of antioxidant enzymes [peroxidase (POX) and catalase (CAT)] increased significantly in response to Cd and Pb in both growth stages. The highest Cd level (150 mg/kg) and Pb level (1500 mg/kg) caused maximum increase in enzyme activity at both growth stages. Enzyme activity decreased with the growth advancement from vegetative to flowering stages. Canola possessed more enzyme activities than flax at both growth stages. So in canola the higher activity of antioxidative enzyme offers a greater detoxification efficiency which provides better resistant to a plant against heavy metal induced oxidative stress (Mohamed *et al*., 2012).

These results align with those obtained by Irfan *et al*. (2014) who reported that catalase, peroxidase and superoxide dismutase increased significantly in two varities of *Brassica Juncea* under Cd treatments. Catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) are important enzymes for plants adapted to environmental stress; they are called the plant protective enzymatic system. The harmonious interactions of three enzymes make the balance of free radical production and elimination, and keep the level of free radicals in plants low to prevent the injury of cells by free radical (Cheng, 2003).

Ascorbic acid behavior had the same trend as observed for enzyme activities. At the vegetative stages, ascorbic acid was increased by 48.3, 29.3% & 20.7, 28.8% as compared with the control. While, at the flowering stage such increase was 45.5%, 25.9% & 14.1%, 27.7% for canola and flax plants grown under the highest concentrations of Cd and Pb respectively (Table 5).

Table 5: Effect of Cd and Pb (mg/kg soil) on catalase (mMole/ml/ hour), peroxidase (unit/sec/mg) and ascorbic acid (µg/g) of flax and canola plants at vegetative and flowering stages.

Antioxidants	Growth stage	Control	Flax								Control	Canola								LSD 5%
			Cadmium				Lead					Cadmium				Lead				
			25	50	100	150	250	500	1000	1500		25	50	100	150	250	500	1000	1500	
Catalase	Veg.	65.8	79.0	99.3	129	153	93.4	137	187	201	76.8	148	178	206	240	164	309	345	375	1.46
	Flow	46.0	60.8	81.5	98.3	116	61.8	94.0	132	164	60.2	94.5	112	149	168	102	211	260	310	1.35
Peroxidase	Veg.	46.8	48.1	51.7	52.2	54.5	55.2	56.3	58.4	60.3	44.1	49.8	52.7	56.3	65.4	56.5	57.6	59.7	62.0	0.17
	Flow	45.3	47.8	51.3	51.6	54.1	54.4	55.3	56.3	58.7	46.4	48.2	51.3	54.5	61.5	54.5	54.9	56.6	58.4	0.165
Ascorbic acid	Veg.	6.69	15.0	32.6	40.1	50.3	20.4	30.1	39.3	42.3	50.3	121	130	140	152	180	265	520	643	0.79
	Flow	5.89	10.0	28.1	35.3	48.4	15.2	27.1	37.5	40.6	45.5	119	122	135	148	173	260	515	617	1.57

Increasing ascorbic acid content due to different heavy metals treatment have been reported in *Albizia procera* seedlings (Pandy and Tripathi, 2011). Ascorbate is an ubiquitous soluble antioxidant in photosynthetic organisms, and the most important reducing substrate for H_2O_2 detoxification (Singh *et al*., 2005). It has been suggested that pollutants produce oxyradicals in plants; these radicals cause widespread damage to membranes and associated molecules including chlorophyll pigments (Zengin and Munzuroglue, 2005). Ascorbic acid maintains the stability of cell membranes during pollution stress and scavenges cytotoxic free radicals (Pandey and Tripathi, 2011).

CONCLUSIONS

It could be concluded that, canola plant showed better responses to cadmium and lead stress than flax at vegetative and flowering stages. In addition, canola showed higher photosynthetic pigments, proline, free thiols, lipid peroxidation, enzyme activities and ascorbic acid. On the other hand, content of total soluble sugars were lower in canola than in flax. All the above mentioned biochemical parameters might be played an important role in the response of plant to Cd and Pb stress. In addition, these parameters may serve as important biochemical markers for heavy metal tolerance trait in plants. Given that such stress impose considerable constraints on crop production.

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ARABIC SUMMARY

الإستجابات الفسيولوجية لنباتى الكتان والكانولا لإجهاد الكادميوم والرصاص

هند أحمد يونس الخواجه
قسم النبات والميكروبيولوجى- كلية العلوم- جامعة الأزهر- فرع البنات

أجريت تجربه أصص خلال فصل الشتاء موسم ٢٠١٣-٢٠١٤ على نباتي الكتان صنف جيزة ٥ و الكانولا صنف سرو ٤ بمنطقه محمية بكلية الزراعة جامعه الأزهر بمدينه نصر القاهرة وذلك لدراسة تأثير بعض العناصر الثقيلة (كادميوم - رصاص) على بعض النواتج الايضيه خلال مرحلتين من مراحل النمو، الأولى تمثل المرحلة الخضرية والأخرى تمثل مرحلة تكوين الأزهار وقد أضيف كل من الكاديميوم و الرصاص في صورة محلول نترات وكانت التركيزات المستخدمة للكادميوم ٢٥ ، ٥٠ ، ١٠٠ ، ١٥٠ مللي جرام ، اما بالنسبة لعنصر الرصاص فقد استخدم ٢٥٠، ٥٠٠، ١٠٠٠، ١٥٠٠ مللي جرام لكل كيلو جرام من التربة وقد تمت إضافة هذه العناصر قبل الزراعة بالإضافة إلى التربة المقارنة (بدون أضافه عناصر ثقيلة). أدت زيادة التركيزات للعناصر الثقيلة إلى نقص تدريجي في محتوى كلورفيل أ ، ب وكذلك محتوى الكاروتين، بينما زاد تركيز البرولين ،السكريات الكلية الذائبة ،الكبريت الحر والمالون داى اللدهيد و نشاط إنزيمي الكتاليز و البيروكسيديز وكذلك حمض الاسكوربيك زيادة معنوية في المرحلتين الخضرية وتكوين الأزهار تحت زيادة التركيزات العالية للعناصر الثقيلة المستخدمة وقد وجد عموما في مرحلة تكوين الأزهار زيادة كلورفيل أ، ب والكاروتين وكل من البرولين وحمض الاسكوربيك و السكريات الكلية الذائبة بينما زاد كل من الكبريت الحر والمالون داى اللدهيد وكذلك نشاط الإنزيمات (الكتاليز والبيروكسيديز) في المرحلة الخضرية.