9 Egypt . J. Soil Sci . 43 , No. 1, pp. 125 - 141 (2003)

Influence of Cadmium on The Growth and Metabolic Contents of *Ambrosia maritima*

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THE EFFECT of cadmium on the growth and some metabolic contents of *Ambrosia maritima* L. was studied. All morphological features of the plant were affected by the increase of Cd concentration from 0.0 to 3 mM. All measured growth parameters decreased by Cd treatment. The obtained results showed that the pigments contents of *Ambrosia maritima* leaves decreased by Cd increasing in the soil.

Disaccharides as well as polysaccharides decreased with Cd treatments. In contrast to monosaccharides increased mainly due to the effect of Cd on enzymes activities. Protein nitrogen and total nitrogen were significantly decreased by the increase of Cd in the nutrient solution, but soluble nitrogen increase. Ambrosin and damsin contents decreased by Cd treatment. The ABA content of the shoot and root extract of *Ambrosia maritima* increased by Cd treatment.

Keywords: Cadmium, *Ambrosia maritima.*

Cadmium is considered as one of the most potentially toxic metal. Higher plants grown on Cd-containing substrates show disturbed water balance. Cd effects stomatal function, water transport and cell wall elasticity. Cd may inhibit leaf cell expansion growth through alteration of water balance (Poschenrieder *et al.*, 1989). Also, Chugh *et al.* (1991) demonstrated that Cd effects on development of chloroplasts, chlorophyll biosynthesis and photosynthetic pigments of pea seedlings, and also Cd cause perturbations in various plant process.

There are different sources of Cd in environment for example burning of fossil fuels such as coal or oil and the incineration of municipal waste materials, smoking and application of phosphate fertilizer or sewage sludge.

Ambrosia maritima L. is an annual, sometimes perennial, herbaceous weed, belonges to family compositae. It is distributed in different regions in Egypt (Tackholm, 1974). Also, *Ambrosia maritima* is an important medicinal plant used as molluscicidal plants (Geerts *et al.*, 1991) and the molluscicidal activity is mainly due to the presence of sesquiterpene lactones (Sherif and El-Sawy,1962). Not only but also used as a drug against urinary schistomiasis (Kloos *et al.*, 1982) and as antispasmodic, a diuretic or in kidney stones and used to treat inflammation (Vassiliades and Diaw, 1980).

Costa and Spitz (1997) reported that Cd have toxic effect on most metabolic content, *i.e* carbohydrate content. In vitro cultured *Lupinus albus* Cd cause a decrease in total soluble carbohydrate in contrast to some monosaccharides which are increased such as mannose, raffinose and hydroxyproline. Moreover, Skoryznska polit and Baszynski (1995) found that Cd cause an inhibitory effect on the acceptor side of PSII of primary leaves and this effect is slightly decreased by the increase of plant age.

Pal *et al.* (1996) reported that Cd cause reduction in protein and total nitrogen contents of *Cicer arietinum*. Cd treatment influences activities of key enzymes and leads to marked perturbations in nitrogen metabolism such as nitrate reductase, glutamate syntheses, glutamate dehydrogenase and aminotransferases (Van Assche and Clijster,1983).

This paper reports detailed examination of the effect of a range of Cd concentrations on some growth criteria and metabolic contents throughout different growth stages of *Ambrosia maritima* to show how Cd modified the growth and metabolic content of the plants.

Material and Methods

The experimental plant used in this investigation was *Ambrosia maritima* L. in the bioassay of growth regulators activities involving abscisic acids, wheat (*Triticum aestivum* var. Giza 155) was used. Pure strains of grains and seeds of these plants were obtained from the Ministry of Agriculture and Land Reclamation, Dokki, Egypt. The early physiologists used wheat (*Triticum aestivum* var. Giza 155) in abscisic acids bioassay because this plant showed high rate of straight coleoptile growth length.

The experiment was carried out in the botanical green house of Botany Dept., Faculty of Science, Zagazig University, Egypt, in plastic pots 5 kg soil capacity filled with soil of sandy loam type have 40% water holding capacity.

Ambrosia maritima seeds of similar size were sown at a depth of 0.5 cm from soil surface and at equal distance apart in each pot (10 seeds in each pot) on 20 December 1999. All pots were supplied with equal amounts of water after 13 days from sowing, germination of *Ambrosia maritima* seeds appeared on the soil surface at 10°C in 2 January 2000. Ten days later thinning was done at 12 January 2000, so that five uniform plants were left in each pot for experimentation. The plants were exposed to normal day length with normal illumination.

Plant treated with different Cd concentrations started on 20 January 2000, where the seedling 3 cm with two foliage and two cetyledonary leaves. During the treatment pots were divided into four separate sets, first set treated with water free of Cd as control, second set with 0.5 mM Cd. The third with 1.5 mM Cd and the fourth one with 3 mM of Cd. Water irrigation in different sets was adjusted regularly using a balance along the life cycle of cach plants group to avoid changes of Cd concentration until five months old.

Growth measurements were carried out on four different stages during the experimental period as follows stage 16 weeks, stage II 11 weeks, stage III 15 weeks and stage IV 20 weeks after Cd application.

Growth measurements involving the following criteria: 1-Shoot length, 2-Number of lateral branch, 3- Number of leaves, 4- Root length.

-The pigments (chlorophyll a, chlorophyll b and carotenoids) were determined according to the method described by Metzner *et al.* (1965).

-Estimation of carbohydrate fraction according to Nelson (1944) and modified by Naguib (1964).

-Estimation of total nitrogen by conventional micro-kjeldal (Allen, 1953), and total soluble nitrogen determined according to the method of Hassanein (1977).

-The method of extraction and estimation of sesquiterpene lactones described by Mabry (1970).

Extraction, separation and bioassay of growth regulating substances carried out. Since extraction was essentially similar to that of Gazit and Blumenfeld (1970) and as described by Mostafa *et al.* (1990). Separation was carried out according to Nitsch and Nitsch (1955) as described by El-Araby (1987). Bioassay of abscisic acid carried out according to method described by Bently and Hously (1954) and Zeevart (1971).

Results and Discussion

The results obtained in Tables 1,2,3 & 4 showed that Cd cause decreases in all morphological growth criteria of *Ambrosia maritima* plants (*i.e.* shoot length, number of lateral branches, number of leaves and root length). According to Poschenrieder *et al.* (1989), Cd induced alteration of cell wall properties, decrease of the relative water content, decrease cell expansion and decrease turger pressure in bean plants. Also, Arduini *et al.* (1995) found that heavy metal Cd and Cu decrease cell elongation and cell division of *Pinus pinea* and *Pinus pinaster*. Thus, it can be concluded that Cd decrease cell division, cell elongation which leads to decreasing in all growth criteria and this was confirmed by Quzounidou (1997) in his study on the effect of Cd on wheat plants and Cieslinski *et al.* (1998) in his study on the effect of Cd on straw berry (*Cultivars rainier*).

TABLE 1. Effects of different cadmium concentrations (mM) on shoot lenght of *Ambrosia maritima* plant (cm).

Stages / Cd Conc.	Stage I	Stage II	Stage III	Stage IV
0.0	12.56 ± 0.01	36.8 ± 0.02	55.6 ± 0.06	67.9 ± 0.04
0.5	09.14 ± 0.01	29.4 ± 0.01	48.7 ± 0.02	52.3 ± 0.02
1.5	08.12 ± 0.00	21.8 ± 0.01	40.5 ± 0.00	44.0 ± 0.01
3.0	07.38 ± 0.00	20.1 ± 0.00	33.2 ± 0.00	37.1 ± 0.01

Each listed value is a mean of 10 replicates ± SE.

TABLE 2. Effects of different cadmium concentrations (mM) on number of lateral branches per *Ambrosia maritima* plant (cm).

Stages / Cd Conc.	Stage I	Stage II	Stage III	Stage IV
0.0	0.0	15.8 ± 1.54	21.6 ± 1.73	21.6 ± 1.73
0.5	0.0	12.0 ± 0.62	19.0 ± 0.92	19.0 ± 0.92
1.5	0.0	10.4 ± 0.27	13.2 ± 1.34	13.2 ± 1.34
3.0	0.0	9.2 ± 0.48	9.0 ± 0.54	9.0 ± 0.54

Each listed value is a mean of 10 replicates ± SE.

TABLE 3. Effects of different cadmium concentrations (mM) on the number of leaves per *Ambrosia maritima* plant (cm).

Stages / Cd Conc.	Stage I	Stage II	Stage III	Stage IV
0.0	9.2 ± 1.50	40.0 ± 1.64	79.2 ± 1.26	79.2 ± 1.26
0.5	8.0 ± 0.82	32.8 ± 1.53	69.2 ± 1.38	69.2 ± 1.38
1.5	7.2 ± 1.33	26.2 ± 0.96	51.2 ± 1.72	51.2 ± 1.72
3.0	6.4 ± 0.17	20.8 ± 1.44	40.4 ± 0.22	40.4 ± 0.22

Each listed value is a mean of 10 replicates ± SE.

TABLE 4. Effects of different cadmium concentrations (mM) on root length of *Ambrosia maritima* plant (cm).

Stages / Cd Conc.	Stage I	Stage II	Stage III	Stage IV
0.0	7.06 ± 0.32	18.9 ± 0.48	39.5 ± 1.78	46.9 ± 1.62
0.5	6.38 ± 1.54	16.5 ± 1.27	33.0 ± 1.64	41.7 ± 0.45
1.5	4.16 ± 0.83	12.2 ± 1.96	24.5 ± 0.88	36.0 ± 1.49
3.0	3.4 ± 0.76	11.0 ± 0.35	22.2 ± 1.57	29.0 ± 1.23

Each listed value is a mean of 10 replicates ± SE.

Result obtained in Table 5 showed that cadmium had a marked influence on pigment contents since Cd cause decreasing in chlorophyll a, chlorophyll b and carotenoid. Also, DeFilippis *et al*. (1981) found that heavy metals Zn, Cd and Hg decrease chlorophyll of *Euglena gracilis* and reported that heavy metals inhibit final reductive steps of chlorophyll formation. Also, Chugh *et al* .(1991) demonstrated the effect of Cd on various plant processes and developments of chloroplasts, chlorophyll biosynthesis and potosynthetic pigments of pea seedlings. They reported that Cd causes perturbations in various plant processes and leads to decreasing photosynthetic pigments.

Concerning effect of Cd on carbohydrate content of *Ambrosia maritima*, our result in Table 6 showed that Cd decreases disaccharide and polysaccharides and total carbohydrate while Cd causes a marked increase in monosaccharide content in the plant.

Van Duijendij *et al.*(1975) reported that the inhibitor action of Cd on donner side of photosystem II in isolated chloroplasts leads to decrease the photosynthetic rate which leads to decrease carbohydrate contents. Ahmed (1978) found that treatment of corn plants with heavy metals increases respiration rate of their organs and reduced photosynthetic rate. Also, Bazinsky *et al.* (1980) reported that Cd inhibits net photosynthesis by inhibiting PS-II related electron transport. These results confirmed by Mohamed (1986), Biddappa and Bopaiah (1989), Vangronsveld and Clijster (1992) and Moya *et al.* (1993).

TABLE 5. Effects of different levels of cadmium concentration (mM) on pigment contents of *Ambrosia maritima* plant (mg/g) throughout four stages of growth.

Pigments / Cd Conc.	Chlorophyll. a.	Chlorophyll. b.	Chlorophyll. (a+b)	Carotenoids.	Total pigments
Stage I.					
0.0	1.120±0.01	0.343±0.00	1.463±0.01	0.358±0.00	1.821±0.23
0.5	0.945±0.01	0.210±0.00	1.155±0.05	0.247±0.03	1.402±0.12
1.5	0.798±0.00	0.175±0.01	0.973±0.12	0.196±0.07	1.169±0.15
3.0	0.675±0.02	0.156±0.05	0.831±0.09	0.114±0.04	1.945±0.11
Stage II.					
0.0	1.297±0.01	0.584±0.02	1.881±0.13	0.369±0.01	2.250±0.11
0.5	0.985±0.03	0.506±0.04	1.491±0.14	0.256±0.06	1.747±0.18
1.5	0.834±0.09	0.437±0.05	1.271±0.14	0.203±0.02	1.474±0.23
3.0	0.801±0.11	0.319±0.08	1.120±0.16	0.129±0.08	1.249±0.27
Stage III.					
0.0	1.501±0.01	0.663±0.03	2.164±0.01	0.435±0.00	2.599±0.08
0.5	1.230±0.00	0.602±0.15	1.832±0.13	0.312±0.00	2.144±0.04
1.5	0.957±0.05	0.581±0.08	1.538±0.16	0.287±0.05	1.825±0.09
3.0	0.948±0.12	0.413±0.07	1.361±0.15	0.214±0.09	1.575±0.16
Stage IV.					
0.0	0.945±0.01	0.310±0.00	1.255±0.01	0.299±0.00	1.554±0.03
0.5	0.683±0.01	0.237±0.00	0.920±0.02	0.257±0.05	1.177±0.08
1.5	0.649±0.07	0.146±0.12	0.795±0.09	0.204±0.01	0.999±0.06
3.0	0.534±0.09	0.035±0.11	0.569±0.13	0.118±0.07	0.687±0.13

Each listed value is a mean of 3 replicates ± SE.

TABLE 6. Effects of different concentration of cadmium (mM) on carbohydrate contents of *Ambrosia maritima* leaves throughout four stages (I, II, III, IV) of growth. Values listed are mg glucose/lg dry weight of leaves.

Cadmium concentration (mM)	Mono-saccharides	Disaccarides	Polysaccarides	Total carbohydrates
Stage I				
0.0	2.01±0.11	6.95±0.18	60.8±0.24	69.76±1.97
0.5	2.95±0.18	6.21±0.32	52.4±1.10	61.56±1.31
1.5	3.11±0.13	5.38±0.25	35.6±0.35	44.06±0.74
3	4.32±0.00	5.01±0.40	20.1±0.74	29.43±2.23
Stage II				
0.0	12.1±0.38	25.6±0.61	89.3±2.58	127.00±1.90
0.5	13.7±0.00	23.7±0.43	85.4±3.40	122.80±2.73
1.5	17.3±0.45	22.8±0.87	61.5±3.01	101.6±3.21
3	18.2±0.16	20.9±0.73	49.8±1.89	88.9±1.58
Stage III				
0.0	17.8±0.25	32.03±0.48	125.1±3.10	174.93±0.90
0.5	18.4±0.19	30.15±0.94	109.6±2.27	158.15±1.63
1.5	20.5±0.45	29.2±0.67	90.0±1.18	139.7±4.10
3	22.7±0.30	27.3±1.38	83.7±0.78	133.7±2.30
Stage IV				
0.0	13.6±0.51	22.3±0.27	88.1±1.30	124.0±3.72
0.5	14.3±0.13	21.8±0.79	49.4±1.98	115.5±2.45
1.5	15.4±0.08	19.7±0.58	75.2±2.37	110.3±1.69
3	17.1±0.16	18.2±1.50	61.6±3.50	96.9±1.38

Each listed values is a mean of 3 replicates ± SE

Mohamed (l994) suggested that the increase in monosaccharides by heavy metals treatment occured due to the enhancement of the amylolytic activity which consequently leads to increase soluble sugars. The same result was confirmed by Costa and Spitz (1997).

Data in Table 7 showed that Cd decrease protein nitrogen and total nitrogen but total soluble nitrogen increase by Cd treatments. These results are in agreement with Mohamed (1986) who reported that heavy metals play a direct role in oxidation-reduction in the process of protein biosynthesis. Also, Bourdman (1975) demonstrated that heavy metals prevent N-assimilation.

Also, heavy metal effect on enzymes which involved in nitrogen metabolism were reported by Biddappa and Bopaiah (1989) and Van Assche and Clijster (1983).

TABLE 7. Effects of different concentration of cadmium (mM) on nitrogen contents of *Ambrosia maritima* leaves throughout four stages (I, II, III, IV) of growth. Values listed are mg nitrogen/g dry. wt.

Cd concentration (mM)	Total soluble nitrogen	Protein nitrogen	Total nitrogen
Stage I			
0.0	35±1.91	55±1.13	90±2.37
0.5	37±1.32	48±1.31	85±2.25
1.5	42±0.92	40±0.81	82±1.18
3	48±1.40	29±1.73	77±2.43
Stage II			
0.0	39±1.13	74±1.91	113±3.51
0.5	44±1.43	57±1.67	101±3.17
1.5	51±0.51	45±1.13	96±2.10
3	69±1.71	27±1.10	96±2.38
Stage III			
0.0	53±1.09	104±1.77	157±2.78
0.5	65±1.27	86±1.18	151±2.19
1.5	72±0.91	71±2.10	143±2.10
3	78±1.45	57±0.87	135±1.81
Stage IV			
0.0	37±0.91	65±2.80	102±3.11
0.5	43±0.27	51±3.70	94±4.10
1.5	49±0.45	39±1.60	88±1.82
3	58±0.31	27±1.10	85±1.61

Each listed values is a mean of 3 replicates ± SE

The increase of total soluble nitrogen by Cd treatments may be due to deamination of amino acids to ammonia and protein hydrolysis to soluble nitrogen (Rudolph, 1963).

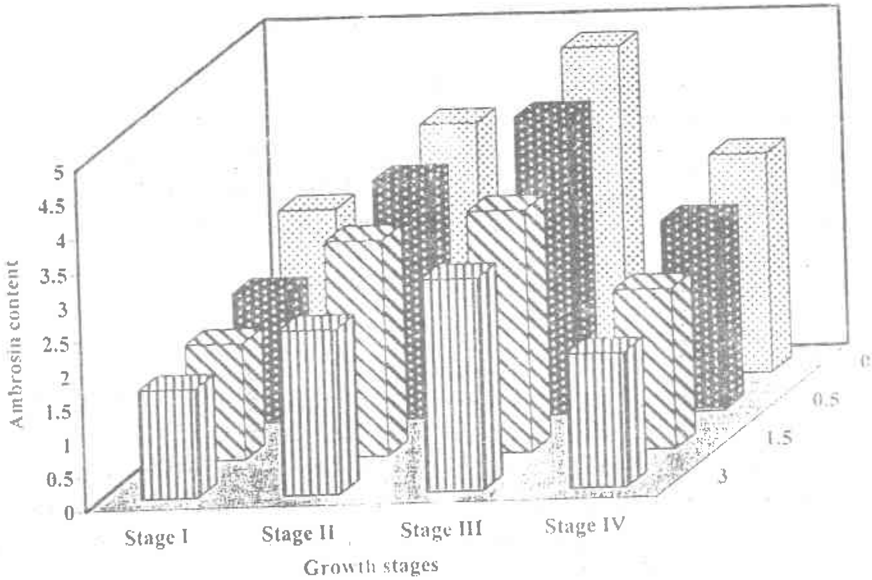
In the same respect, Daif (1998) demonstrated that heavy metals cause on increase of soluble and ammonia-nitrogen in leaves of tomato plants due to the mineralization of nitrogen that enhanced the accumulation of ammonia and soluble nitrogen.

The obtained results in Fig. 1a,b showed that Cd treatment decreases sesquiterpene lactones (ambrosin and damsin) in *Ambrosia maritima* L. leaves and these results are in agreement with Pasquale *et al.* (1995). They reported that Cd decreases pharmacological active constituents of the medicinal plant *Coriandrum sativum* L. Also, Zheljazkov *et al.* (1996) reported that heavy metal reduce active constituents of medicinal plants from genera *Artemisia, Draccocephalum, Inula, Ruta* and *Symphytum*.

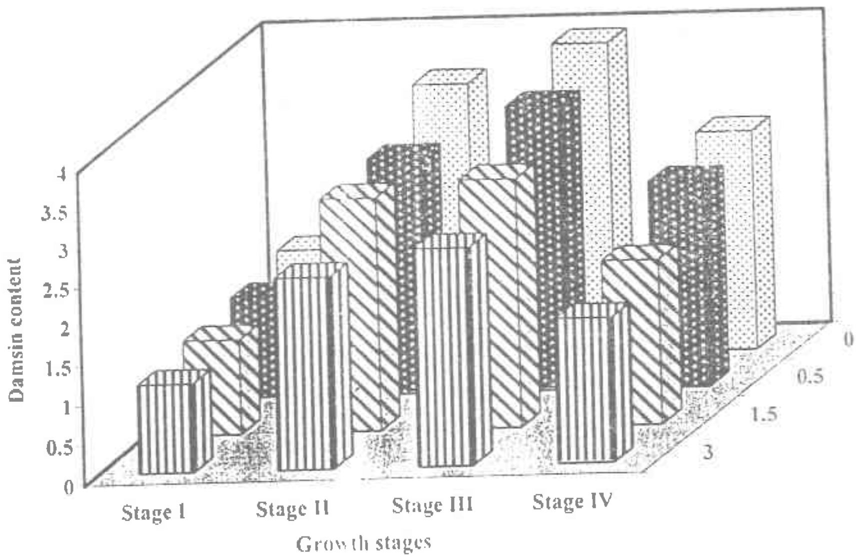
The values in Fig. 2a,b showed that treating plants with Cd causes an increase in abscisic acid content in shoot and root extracts of *Ambrosia maritima* and these results are in agreement with that of Poschenrieder *et al.* (1989) who showed that Cd addition causes increasing in abscisic acid content in bean plant. Also, Rubino *et al.* (1994) show that Cd increases ABA contents in rice plants. The same result obtained by Talanova *et al.*(1999) who reported that Cd usually cause an increase in ABA and proline contents in cucumber seedlings which is adaptive response to Cd treatments.

Conclusion

The results indicate that *Ambrosia maritima* can tolerate cadmium up to high concentrations, as well as on growth criteria and metabolism of the plant. Since all morphological features were affected and metabolic contents changes to overcome the toxicity of cadmium. These data showed another importance of *Ambrosia maritima* which may be used in phytoremediation despite of being an important medicinal plants.



a. Ambrosin contents (mg/g dry weight) of *Ambrosia maritima* leaves.



b. Damsin contents (mg/g dry weight) of *Ambrosia maritima* leaves.

Fig. 1a-b. Effects of different cadmium concentrations (mM) on sesquiterpenes lactones contents (amprosin, damsin) *of Ambrosia maritima* leaves during 4 stages of growth.

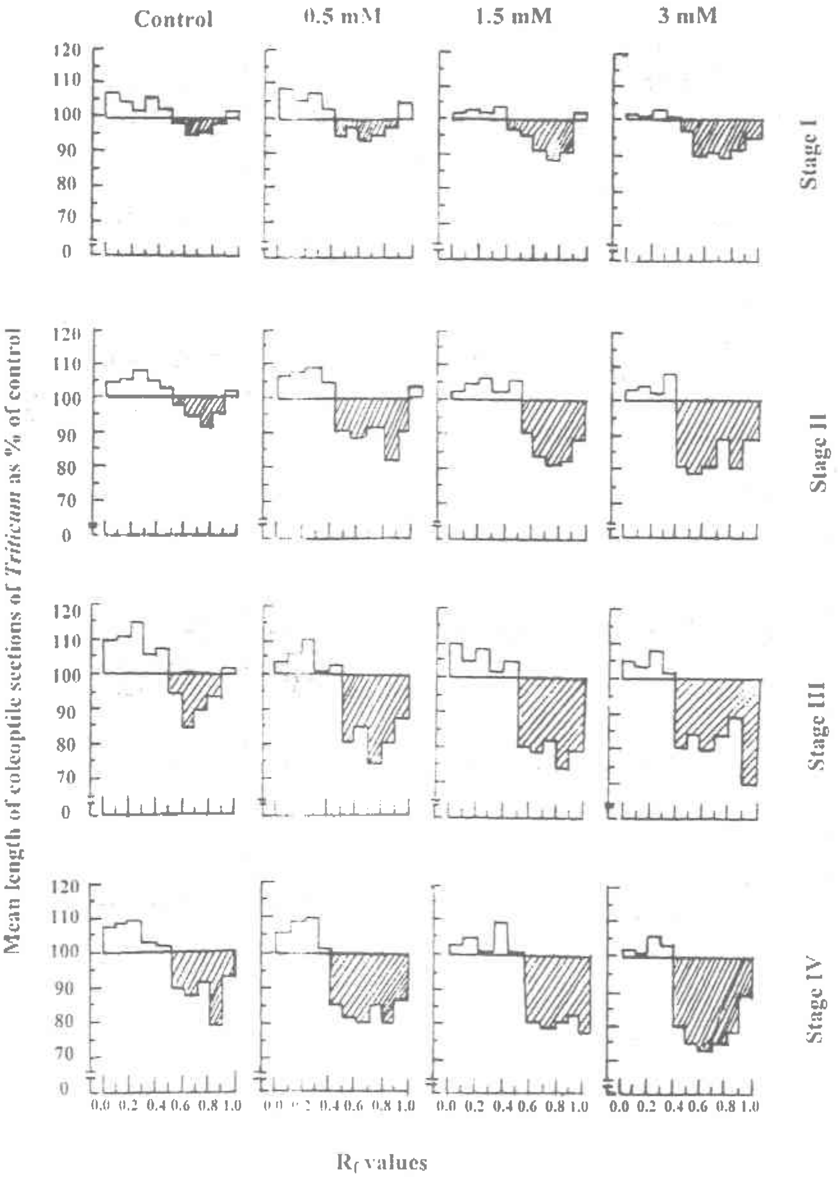


Fig. 2-a. The changes of abscisic acid contents of the fractionated shoot extracts of *Ambrosia maritima* plants grown at different cadmium concentration (mM) throughout four different stages of growth.

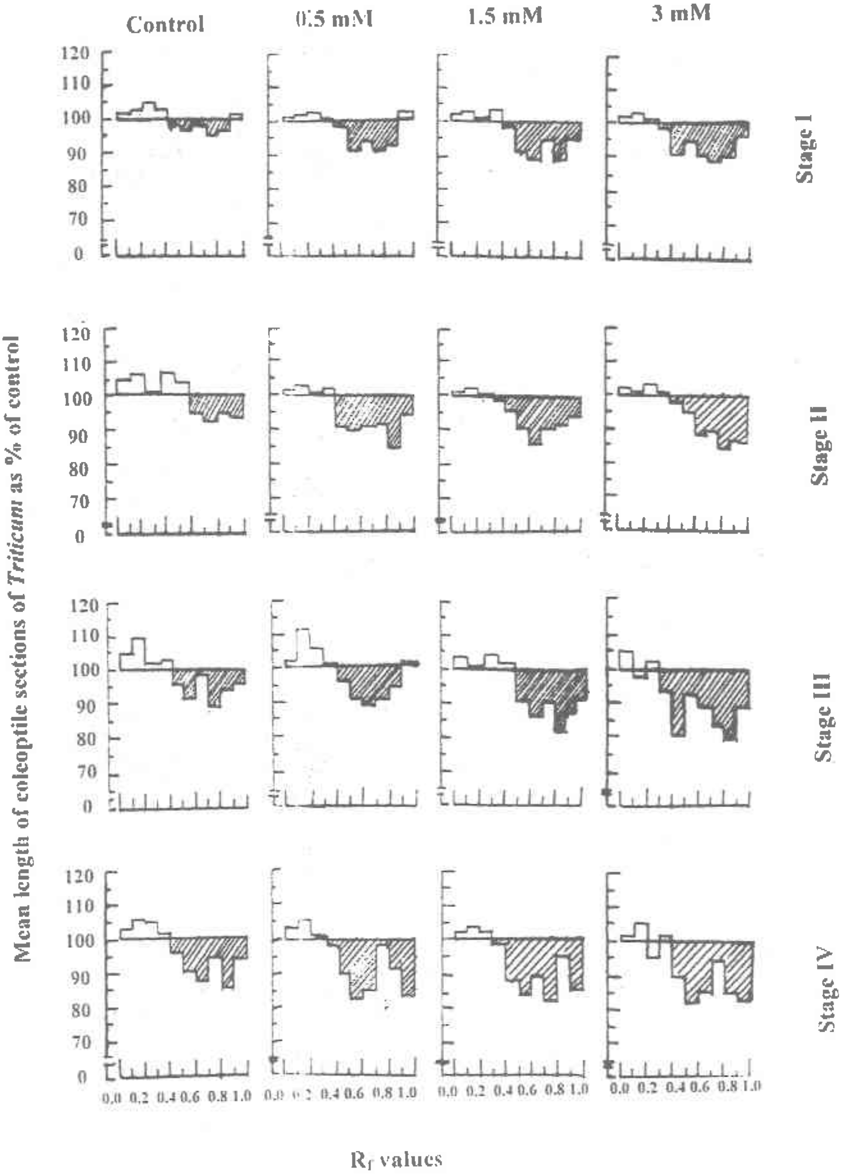


Fig. 2-b. The changes of abscisic acid contents of the fractionated root extracts of *Ambrosia maritima* plants grown at different cadmium concentration (mM) throughout four different stages of growth.

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(*Received* 7 / 2001)

تأثير عنصر الكادميوم على نمو وأيض نبات الدمسيسة

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يهدف هذا البحث إلي دراسة تأثيرعنصر الكادميوم على نمو وأيض نبات الدمسيسة وأظهرت النتائج أن كل المظاهر المورفولوجية لنبات الدمسيسة تتأثر بزيادة عنصر الكادميوم المضاف إلي التربة.

نقصت كل العوامل المقاسة من طول النبات وعدد الأفرع الجانبية وعدد الأوراق وطول الجذر بزيادة عنصر الكادميوم من صفر إلي ٣ملي مول. كما نقص محتوى أوراق نبات الدمسيسة من الصبغات النباتية نتيجة إضافة عنصر الكادميوم إلي التربة.

أوضحت النتائج أيضا أن محتوى نبات الدمسيسة من المواد السكرية الثنائية والكلية تقل بزيادة عنصر الكادميوم بينما زاد محتوى النبات من السكريات الأحادية الحرة.

كما زاد محتوي نبات الدمسيسة من النيتروجين الذائب بينما نقص محتوى النبات من النيتروجين الكلي والبروتيني الخام مع زيادة عنصر الكادميوم في التربة.

أدت الزيادة من إضافات الكادميوم إلى نقص الأهمية الإقتصادية لنبات الدمسيسة متمثلة في نقص كمية السيسكوتربينات (الامبروزين والدامسين).

صاحب نقص مظاهر النمو المورفولوجية زيادة في محتوى النبات من حمض الابسيسك بزيادة عنصر الكادميوم في التربة.