17

Egypt. J. Soil Sci. **43** , No. 2, pp. 243-264 (2003)

Effects of Cadmium on The Nitrate and Potassium Uptake in Guar

S. A. M. El-Sayed

Soil and Water Science Dept., Faculty of Agric., Al-Azhar University, Assiut, Egypt.

THE NET uptake, distribution and assimilation of NO^-_3 were studied in guar plants subjected to either long-term continuous Cd treatment for 252hr (9 or 45 *uM* Cd) or short-term treatment for (84hr) with 45 *uM Cd.* In the latter treatment, the effects of transferring the plants to a Cd- free nutrient solution for "recovery period" of 108hr were also studied. All these treatments were compared with " controls" plants which received no Cd. In both experiments, the reduction in fresh weight was associated with a decrease in th content (%) of shoot and root water and in transpiration rates as Cd concentration increased. The concentration of NO^-_3 in the shoots and sap decreased dramatically and net NO^-_3 uptake was severely inhibited, effects associated with a loss of shoot nitrate reductase (NR) activity . In the sort-term Cd treatment . net NO^-_3 uptake was almost completely inhibited after 36hr. but recoveresd after the transfer of plants to a Cd- free nutrient solution. Similarly , a dramatic decrease in the shoot NR activity was observed . The uptake, distribution and tissue partitioning of K was also studied. which is considered to be the major counterion of NO^-_3. Potassium uptake was similarly affected by Cd, as inferred from the ratio NO^-_3 uptake, which was ca. 9. The ratio K/NO^-_3 tissue content increased in the shoot concmitantly to Cd in both long-term and short-term metal supply. These parameters showed a tendency of k similar to that observed for NO^-_3, although its realative tissue distribution was not affected by Cd.

Keywords : Cadmium, Nitrate, Guar, Uptake, Distribution, Assimilation.

Abbreviations : $LDS_{0.05}$ = Least significant differences with 0.05 of probability. NR=nitrate reductase, g Fw-fresh weight.

Many different phytotxic effects have been observed induced by the exposure of higher plants to Cd (Van Assche & Clijsters, 1990 and El-Kassas, 1999). Alterations to fundamental physiological processes of plant metalobism have been attributed to Cd including photosynthesis (Krupa *et al.*, 1993) , cell respiration (Greger *et al.*, 1991) and plant water relationships (Barcelo and Poschenrieder, 1990).

Several authors found that NO_3^- assimilation was affected by the presence of Cd in the growing medium of hig her Plants ; in particular, NO_3^- content in tissues was reduced in broad bean (Khater *et al.*, 1991 and EL Sayed, 1999a), cucumber (Burzynski, 1988 and El-Sayed ,1998a) and sugar beet (Petrovic *et al.*,1990 and El-Sayed, 1998b). Cudmium accumulation in plant tissues resulted also in the loss of NR activity (Burzynski, 1989, Nussbaum *et al.*, 1988; Pertrovic *et al.*, 1990 and El-Shebiny , 1998). In maiza plants, NR activity decreased when Cd was added to the nutrient solution, which was related to a decrease in NO_3^- content in tissues. However, in maize scutella, the presence of Cd (upto 45*uM*) had no effect on in vitro NR activity nor on the de novo synthesis of the enzyme (Ghaly and El Sayed, 1997). Mathys (1975) observed that the presence of Cd (> 5 *u* M) and other-heavy metals in the incubation medium, inhibited NR activity of *Silene cucubalus* plants . However, Chugh *et al.* (1992) showed that *Pisum sativum* NR activity was not affected by exposure to concentration of Cd below 45 *uM* and Burzynski (1988) found that NR activity of cucymber plants was only inhibited above 100*uM* Cd . Therefore, depending on the stability of the NR extracted from different plants. Cd might alter NR activity *in vitro*. Nevertheless, there is little information available about the putative mechanisms involved in the alteration of NO_3^- absorption and assimilation under Cd exposure *in vivo*.

In the present work, guar pants were supplied with varying amounts of Cd. suplied in a long-and short-term experiments (maximum exposition for 25hr and 84hr, respectively), to evalute its effect on the uptake and distribution of NO_3^- . I have also studied the uptake and distribution of K, which is considered to be the major counterion in the processes of NO_3^- uptake and tissue distribution (Rufty *et al.*, 1986, Tremblay *et al.*, 1988 and El-Sayed, 1999b).

Material and Methods

Plant material

Guar seeds (*Cyamopsis tetragonoloba* L. Taub) variety Giza 1 were germinated on moistened paper for 4 days. Seedlings were cultivated on plastic

grids suspended in nutrient solution (macronutrients (*mM*) : 2.0 Ca $(NO_3)_2$, 1.5 KNO_3,1.0 Mg $(NO_3)_2$, 1.0 KH_2PO_4, 0.5 Mg SO_4, 0.1 NaCl, and micronutrients [*uM* element] : 45.0 Fe (EDDHA), 18.0 $MnSO_4$, 3.0 $CuSO_4$, 6. $ZnSO_4$, 2.0 Mo_7O_{24} $(NH_4)_6$. $4H_2O$, 23.5 H_3BO_3, pH 6.0) in greenhouse.

Long -term exposure to Cd

Fifteen selected seedlings suspended in plastic grids were treated with absence (control, 0.0), 9 or 45 *uM* Cd ($3CdSO_4$. H_2O], for 7 days in plastic pots containing 1.9L of nutrient solution in plants were then transferred to glass jars containing 320 mL of the same nutrient solution and sealed to avoid evaporation losses. After 0, 36, 60 and 84hr of treatment the volume of solution last through transpiration was replaced, to avoid any concentration effects, and an aliquot of the nutrient solution (15 mL) was taken to determine the concentration of the remaining NO_3^- and K. In NO_3 vitro NR activity was measured in one plant per glass jar at each havest . After 84 hr (accounting for a total Cd-exposure of 252hr), plants were harvested to determine: fresh weight of plant (shoot, root and seed). shoot and root . The content of NO^-_3 and K in the plant tissues and NO^-_3 concentration in sap.

Short-term-addition of Cd

In the short term treatment, 15 selected guar seedlings were grown in control nutrient solution for 7 days in the plastic pots described above. Then, cultivated in identical conditions as described above, plants were transferred to control or 45 *uM* nutrient solutions. In vitro NR activity , and K-and NO^-_3 -uptake were measured after 0,36,60 and 84hr of treatment. Plants were sampled after 84hr exposure to Cd to determine (Van Erp and Van lune, 1989), the same parameters as indicated above. Another bacth of plants was transferred to control nutrient solution in the recovery period and the same parameters measured after 36, 60, 84 and 108hr following transfer.

Determination of in vitro NR activity

The in vitro NR activity assay was done according to Ramon *et al.* (1989). Fresh tissue (0.5g) was quickly frozen in liquid N_2 and homogenized in extraction buffer (25 *mM* KH_2 PO_4 / K_2 HpO_4, 5*mM* EDTA. 1% W/V polyvinylpyrrolidone and 1 *mM* cysteine , pH 7.7). The extract was centrifuged at 10000 xg for 15 min at 4^0C. To avoid the putative hydrolysis of the solubilized

NR by proteases, the supernatant was kept at ice -cold temperature to slow down the protein degradation and its activity was immediately determined. In previous experiments the extraction buffer was supplemented with 2% (W/V) casein, as unspecific substrate for proteases, and activity measurements demonstrated that under the described assay conditions no significant difference in activity was detected. NR was assayed by adding 0.1 mL of 100 *mM* KNO_3, 0.5 mL of reaction buffer (100*mM* KH_2PO_4/ K_2HpO_4, 1 *mM* EDTA. pH 7.5) and 0.1 mL of 1mg/mL NADH as reductant substrate. The NR reaction was started by adding 0.1 mL of NR extract and incubated for 15 min at 20°C. NO_2^- was analyzed using freshly prepared colorimetric reagent comprising sulphanilamide (1% W/V) in 3 *M* HCL and 0.02% (W/V) N-(1-naphthyl) ethylenediamide dihydrochloride, in a 1:1 ratio. The strongly acidic colorimetric reagent denaturated all proteins present in the reactions medium and , before the absorbance was measured, the tubes were centrifuged at 1000 xg for 15 min to obtain a clear supernatant. The absorbance was read at 540 nm.

NO_3^- determination in plant tissues

The NO^-_3 content of plant tissues was determined essentially as described by Gojon *et al.* (1991). Dried sample (100 mg) was digested with 100 *mM* HCL (5mL) for 30 min at 70°C, the extract filtered and made to 25mL. with water . NO^-_3 was analyzed by using a Tecnicon. Acta 11 colorimetric autoanalyzer, with a Cd/Cu reducing column. The sample was diluted in reduction buffer (10g/L NH_4CL, pH8.5), reacted with the colorimetric reagent (1% WV sulphanilamide, 14 *M* H_3PO_4, 0.05% W/V N-(1-naphthyl) ethylendiamide dihydrochloride and 0.5 mg/L Brij 35) and the absorbance read at 520 nm.

The NO^-_3 content of sap was determined by extracting stems sap according to Cottenie *et al.* (1982); Page *et al.* (1982); Evenhuis and De Waard (1978). FAO (1978), with monor modifications. Stems were chopped. placed in plastic flask, immersed in diethyl ether and stored at -25°C. Sap was extracted under pressure and the aqueous phase isolated using a separating funnel. An aliquot (0.1 mL) was diluted in 1 mL of 2.5 mg /mL sulphosalycilic , centrifuged at 1500 xg and NO_3^- analyzed in the protein-free supernatant as described above.

The net uptake of NO^-_3 was measured from the amount of NO^-_3 that was lost from the nutrient solution, after its volume was corrected with deionised H_2O to take into account the losses due to plant transpiration .

K determination

Plant tissues were dried at 70°C to constant weight. After homogenization. samples were digested with a mixture of acids; HNO_3: H_2SO_4: $HClO_4$ (5:1:2). at low temperature (< 200°C) to avoid evaporation losses and formation of foam. Samples was diluted with deionized water , thoroughly vortexed and after filtration. The volume was made to 25 mL. Potassium was analyzed by atomic emission spectrophotometry (Perkin-Elmer 4000). with acetylene-air flame as described (Garte *et al.*, 1993). Negligible losses of melas by filtering were detected by using internal standards, and accuracy and precison of atomic spectropotometry methods were checked with high-pure commercial available standards (Merck and Carlo-Erba) diluted to appropriated concentrations in the acidic matrix used . The concentration K in the nutrient solution was determined in an aliquot of 5ml diluted with 1mL of mixture of acids, and analyzed as described above. Potassium net uptake was determined as described above for NO^-_3 .

Data analysis

Data are the average of at least three independent experiments. Significance of differences was determined by using the test of Duncan for analysis of variance. In the tables, data significantly different to the control at 0.05 and 0.02 probability are indicated with the corresponding asterisks. In figures, the least significant difference at 0.05 of probability ($LSD_{0.05}$) is given; so if the difference between two vlaues is larger than the $LSD_{0.05}$ both are significantly different .

Results

The fresh weight and size of the whole plant, shoots and roots declined as the concentration of Cd in the nutrient solution increased following 252hr of total Cd-exposure (Table 1), in agreement with previous results of guar plants treated with Cd (Leita *et al.*, 1993; El-Sayed , 1999 C). In the short-term experiment after 84hr of treatment with 45 *uM* Cd, only the weight of root was affected but during the recovery period , the fresh weight of shoots was also reduced (Table 1). Those results might indicate that the roots were affected first by the presence of Cd. and that those phytotoxic effects were irreversible.

TABLE 1. Fresh weight of the whole plant, shoot and root (g/plant) and the shoot (SWC) and root (RWC) water content (%) of guar plants, treated with control, 9 or 45 *uM* Cd in the long-term experiment, and treated with control or 45 *uM* Cd in the shoot-term experiment. Plants were sampled at the end of the Cd exposure (84hr) and at the end of the recovery period 108hr after transfer to a Cd-free nutrient solution. (Supply Cd, n=9, Cd -recovery period n = 6).

	Fresh weight (g/plant)				
	Plant	Root	Root	SWC(%)	RWC(%)
Long - term					
Control	0.86	0.28	0.30	88.51	92.76
9 *uM* Cd	0.62**	0.17**	0.20**	86.78**	91.32**
45 *uM* Cd	0.36**	0.05**	0.05**	81.66**	88.37**
Short-term addition of Cd					
Control	0.98	0.31	0.38	88.89	93.91
45 *uM* Cd	0.89**	0.28	0.30**	88.40	92.79**
Recovery					
Control	1.89	0.70	0.72	89.05	94.15
45*u*M *Cd*	1.19**	0.31**	0.31**	85.65**	9158**

* P< 0.05 (treatment vs. control).

** p < 0.02 (treatment vs. control).

In both experiments, Cd-treated plants suffered a reduction in their relative water contents (Table1). This reduction was concomitant with a decrease in transpiration rate, measured by the water consumption of the nutrient solution (Fig. 1). As daily, less volume of water was needed to restore the original volume of the nutrient medium of Cd-treated plants. Also, the total consumption of water decreased consistently as the concentration of Cd increased. Thus, Cd treated plants exhibited the characteristics of water -stress, as observed with bean plants (Poschenrieder *et al.*, 1989 and Darwish & Ahmad, 1997).

The net absorption of NO_3^- from the nutrient solution was reduced dramatically in the long-term presence of Cd (Fig. 2A) . After 60hr, NO_3^- uptake of plants treated with 9 *uM* Cd was less than a third of that found in control plants and this loss was even greater in plants treated with 45 *uM* Cd. The concentration of NO_3^- in all tissues also decreased (Table 2). In the shoot, NO_3^- declined sharply in plants treated with 45 *uM* Cd, whereas in the root there was only a decrease of ca. 50% the concentration of the control. In addition, the concentration of NO_3^- found in sap was reduced as the concentration of Cd increased, the values

observed being of the same order of magnitude as those obtained in the shoot (Table 2). Similarly, net K uptake completely inhibited in plants treated with 45*uM* Cd and plants supplied with 9 *uM* Cd showed an identical trend than that observed for net NO^-_3 uptake (Fig. 2B). The concentration of K in plant tissue decreased when Cd accumulated in plants. Both shoot and root tissue were equally affected (Table 2).

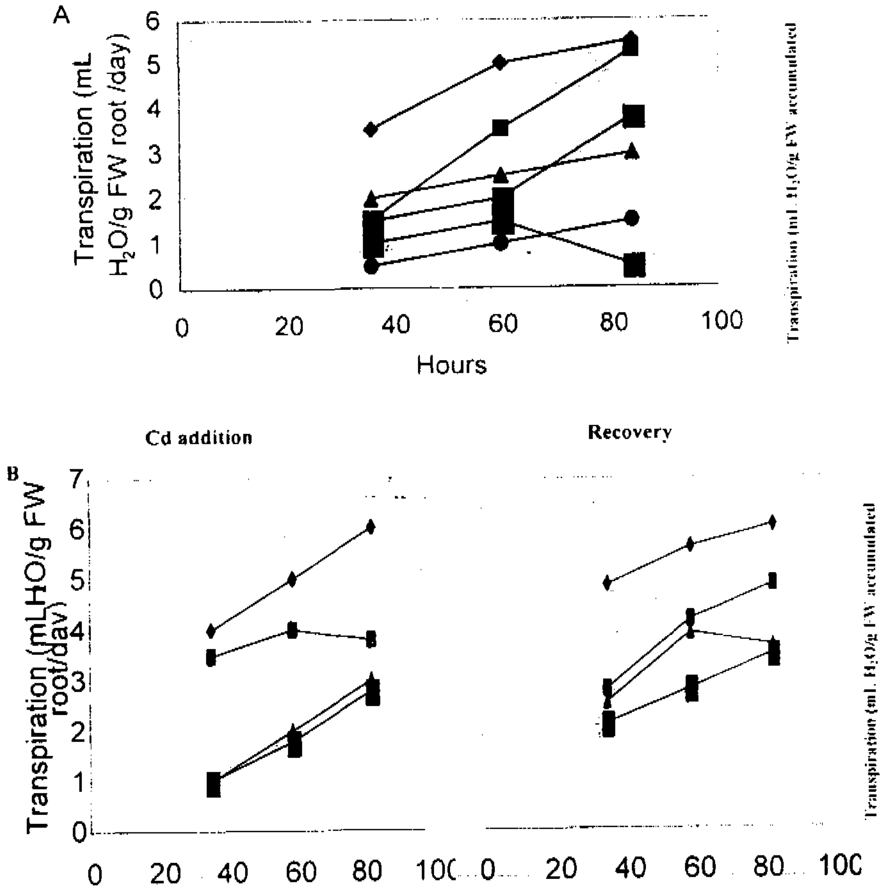

Fig.1. Transpiration of guar plants (ml H_2O g FW / root/day) after 36, 60 and 84 from the beginning of the long-term experiment (A. $LSD_{0.05}$:0.39) and of the short-term experiment (B,$LSD_{0.05}$: 1.03). Control (¦), (¦) or 45 *uM* Cd(¦). The accumulated volume of H_2O transpirated (ml H_2O g FW / root) is represented in the right - hand Y axis. Control (?) . 9 (¦) or 45 *uM* Cd (?) Supply Cd, n=9 ; Cd -recovery period n=6.

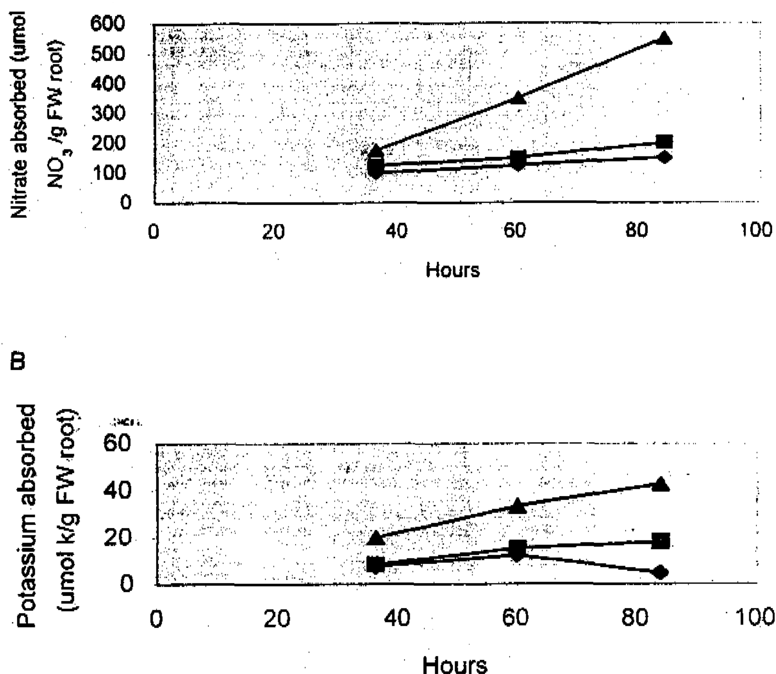

Fig.2. Long-term experiment concentration of NO^-_3 (A, $LSD_{0.05}$: 15.2) and K (B , $LSD_{0.05}$: 8.8) absorbed from the nutrient solution after 0.36.60 and 84 hr from the beginning of the experiment (umol nutrient g FW/root). Control ( ), 9 ( ), or 45 *uM* Cd( ),. (n=9).

In the short term experiment, the net uptake of NO^-_3 from the nutrient solution of treated plants declined sharply after only 36hr from the addition of Cd, and only resumed when plants were transferred to Cd-free nutrient solution (Fig. 3A). During Cd supply, less NO^-_3 accumulated in the shoots, whereas the concentration of NO^-_3 in the roots and the sap was not significantly different from that in the control plants (Table 2). In the recovery period, Cd-treated plants only accumulated ca. 33% of the NO^-_3 found in the control, whereas in the roots the concentration of NO^-_3 in treated plants was significantly greater than that observed in control plants (Table 2). Also, a significant decrease (ca. 45% of the control) was found in the sap NO^-_3 content (Table 2) . In parallel, K net uptake was completely inhibited after 36hr of short-term treatment with 45 *uM* Cd. During the recovery period following Cd removal from the nutrient solution, the absorption of K increased although values were ca. 5 times less than those

observed for control plants (Fig. 3B). Consecutively, shoot and root K concentration declined after Cd supply, whereas no significant differences were observed following Cd removal (Table 3) . Potassium uptake was similarly inhibited in birch plants under Cd stress with a decrease of ca. 45% when plants were expeosed to 5 *uM* Cd (Asp *et al* ., 1994; El-Gendi *et al.*, 1999).

In a previous work, I have observed that in vitro NR activity correlates ($r^2 > 0.90$) with the endogenous content of NO^-_3 in plant tissues (Hernandez *et al.*, 1995 and El-Sayed , 1999d). However, little or no in vitro activity was detected in roots by using the procedure described above. It is possible that guar root NR was more labile than that of shoots under the conditions used for the in vitro NR assay. Nevertheless, the data presented of shoot NR activity were valid and were used as an index for the shoot NO^-_3 nutritional status of plants treated with Cd. Thus, the reduction of shoot NO^-_3 content observed in Cd-treated plants was associated with the loss of NR activity (Table 3). Similarly, in the short-term experiments, the decrease in shoot NO^-_3 concentration was accompanied by a sharp decrease in the in vitro NR activity of Cd-treated plants (Table 3). After transferring the plants to Cd-free nutrient solution, there was no significant recovery of NR activity (Table 3).

Although K and NO^-_3 absorption were similarly affected by the presence of Cd in the long-and short-term treatments, their partitioniong between root and shoot was different. Thus, while NO^-_3 accumulated mainly in root of Cd-treated plants, K distributed equally in root and shoot under the long-term exposure to Cd or was not affected after the supply of 45 *uM* Cd in the short-term experiment (Table 2). Therefore, the ratio K/ NO^-_3 of root content remained constantly at ca. 2 in both experiments (Table 2). However, this ratio increased almost 10-fold in the shoot of 45 *uM* Cd long-term treated plants. Similarly, the ratio K/NO^-_3 increased in plants subjected to the short-term Cd exposure (ca. 6-fold after the recover period). This might indicate that NO^-_3 content was dramatically reduced in shoot in the presence of Cd, due to its assimilation and/or reduced transport from the root, whereas K shoot content was less afftected.

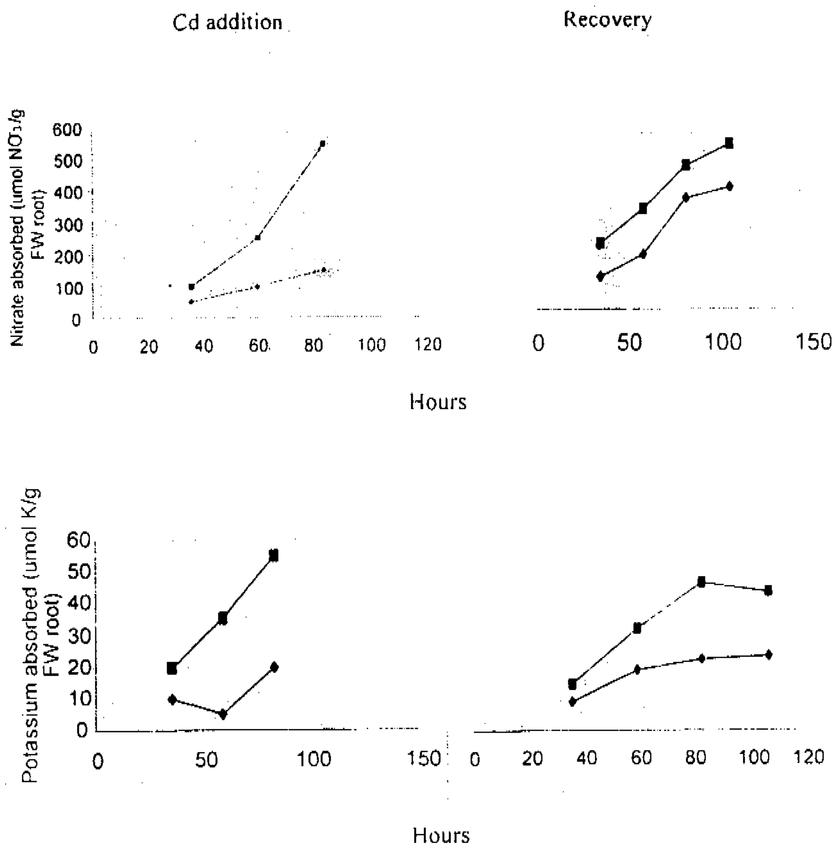

Fig. 3. Short-term experiment. Concentration of NO^-_3 (A,$LSD_{0.05}$: 8.6) and K (B, LSD 0.05 : 11.7) absorbed from the nuutrient solution after 0.36, 60 and 84hr from the beginning of the short-term addition of Cd, and after recovery at 36, 60, 84 and 108hr from the transfer of plants to a Cd-free nutrient solution (umol nutrient g FW /root) . Control (¦) or 45 *uM* Cd (¦). Supply Cd, n=9; Cd-recovery period, n=6.

Table 4 shows an estimated balance of K and NO^-_3 uptake. There was higher requirement of NO^-_3 than of K, and the ratio NO^-_3 /K uptake fluctuate in all samples around 10. Thus, regardless to Cd treatments, plants withdraw similar relative amounts of both nutrients from the growing solution, indicating that the effects of Cd on their uptake was similar. On the other hand, the ratio of total plant content of nutrient vs. their uptake revealed different behavior between

NO^-_3 (NCNU) and K (KCKU; Table 4). In the long -term Cd exposure experiment, UCNU increased only slightly in Cd-treated plants (from 2to 4), whereas KCKU was ca. 60 times higher in 45 *uM* Cd treated plants . Similar pattern was observed in the sort-term Cd supply. Therefore, in Cd-treated plants, much more K was accumulated in the tissue than absorbed from the nutrient solution. It is possible that NO^-_3 tissue content was more dependent on its uptake, because it is assimilated into organic N, and a continuous extracellular replenishment is required (Hoff *et al.,* 1992 ; Tischner *et al.,* 1993, Eissa and El-Kassas, 1999) This is demonstrated in the recovery period , following the 108hr transfer to Cd-free nutrient solution, where NCNU was below 1, indicating that more NO^-_3 was absorbed than accumulated, probably due to its assimilation.

TABLE 2. NO^-_3 and K concentration (Umol/gFW) of shoot, root and sap, and ratio between K and NO^-_3 tissue content (RKN) of guar plants treated with control. 9 or 45*uM* Cd in the long-term experiment, and of guar plants with control or 45 *um* Cd in the short-term experiment. Plants were sampled at the end of the Cd exposure (84hr) and at the end of the recovery period. 108hr after transfer to a Cd-free nutrient solution. (Supply Cd, n=9, Cd-recovery period n=6).

	NO^-_3 concentration			K Concentration		RKN	
	Shoot	Root	Sap	Shoot	Root	Shoot	Root
Long - term							
Control	37.33	44.51	38.53	103.86	91.11	2.7	2.0
9 *uM* Cd	18.16**	45.77	20.91**	67.25	67.90**	3.6*	1.4
45 *uM* Cd	1.83**	22.49**	1.29**	60.89**	59.13**	32.9**	2.5
Short-term addition of Cd							
Control	40.19	42.96	37.11	83.52	76.66	2.0	1.7
45 *uM* Cd	18.76**	42.19	31.91	69.30	62.95*	3.6*	1.4
Recovery							
Control	30.30*	29.41	35.18	52.55	55.08	1.6	1.8
45 uM Cd	9.04**	31.67**	11.00**	52.03	59.66	5.7*	1.8

* $P < 0.05$ (treatment vs. control).

** $P < 0.02$ (treatment vs. control)

Discussion

To my knowledge, these results are the first to describe the effects of Cd on the NO^-_3 and K uptake and tissue distribution in guar plants. The long-term experiments reveal that plants suffered a mild-stress and strong-stress due to their exposure to 9 and 45 *uM* Cd respecrively, according to the reduction observed in fresh weight (Table1).

TABLE 3. Shoot in vitro NR activity (Umol NO^-_2 / hr) of guar plants, treated with control, 9 or 45 *uM* Cd in the long-term experiment (n=6), and of guar plants treated with control of 45 uM Cd in the short-term experiment (Supply Cd n=9, Cd-recovery period n=6).

	Exposure to Cd (hours)				Recovery (hours)			
	0	36	60	84	36	60	84	108
Long-term								
Control	0.80	1.11	1.35	1.44				
9 *uM* Cd	0.38**	0.29**	0.18**	0.20**				
45 *uM* Cd	0.06**	0.08**	0.04**	0.10**				
Short-term								
Control	0.99	1.45	1.79	1.98	2.44	2.37	2.51	2.67
45 *uM* Cd	0.90	0.78**	0.18**	0.05**	0.10**	0.08**	0.19**	0.20**

* $P < 0.05$ (treatment vs. control).
** $P < 0.02$ (treatment vs. control)

Nitrate absorption was affected by the exposure of plants to Cd (Fig. 2A and 3 A). This was in agreement with the lower tissue concentration of NO^-_3 obseved in sugar beet (Petrovic *et al.*, 1990), cucumber (Burzynski, 1988)and maize (El-Sayed, 1995) treated with Cd. The short-term exposure to 45 *uM* Cd reflected a quick response of guar plants to the presence of a high concentration of Cd. This was demonstrated by the severe decrease in NO^-_3 uptake, even after 36hr of Cd supply (Fig. 3A), which was assoaciated with a little response of the fresh weight (Table 1) . Therefore, the reduction in NO^-_3 depletion from the nutrient solution in the presence of Cd is not exclusively due to a decrease in plant size . Nitrate uptake is mediated by transportes in the plasma membrane of root cells and the synthesis of these transporters is activated by the presence of in NO^-_3 in the growing medium (Agüera *et al.*, 1990; Henriksen & Spanswick, 1993 and El-Sayed, 1995). It is possible that the inhabitation of NO^-_3 uptake could be explained by an alteration in transporter function arising from a decrease in the trans-plasma membrance potential , responsible for the facilitated uptake, of NO^-_3 (Aidid and Okamoto, 1992; Meharg & Blatt, 1995 and Tahoun *et al.*, 1999). The transmembrane potential is mainly due to plasma membrane-bound Mg^{+2}-ATP as activity, which is the presence of Cd (100 *uM*) is inhibited in wheat and sunflower root (Fodor *et al.*, 1995; El-Sayed 1998b), and in guar root after 36hr of exposure to 45 *uM* Cd. The decline in NO^-_3 uptake of plants subjected to the Cd short-term treatement (Fig. 3A) could be explanined in this context.

TABLE 4. Balance of NO_3^- and K uptake and tissue accumulation in the long-and short treatments with Cd. The rations between NO_3^- and K-uptake is shown in the brackets , The rations (NUNC) and (KUKC) between NO_3^- and K uptake and their respective total plant concentration are also shown.

Total nutrient uptake (umole/ plant) VS. hours								Ratios a	
	36	60	84	36	60	84	108	NCNU	KCNU
NO_3^-									
Long – term experiment									
Control	0.808(8.8)	4.529(11.1)	12.980(12.5)					1.8	
9 uM Cd	0.374(12.8)	0.965(8.3)	3.214(11.2)					3.9	
45 uM Cd	0.041(10.4)	0.110(6.8)	0.364(16.5)					3.9	
Sort – term experiment				Recovery					
Control	0.801(6.2)	3.431(8.9)	11.143(9.9)	2.454(9.3)	13.300(8.1)	38.805(8.2)	84.814(9.3)		
45 uM Cd	0.333(5.1)	0.743(7.1)	2.009(6.0)	0.768(12.3)	2.997(5.7)	12.485(14.3)	24.073(14.4)		
								Control 0.5[b] + Cd 0.5[b]	8.6[c] 21.5[c]
K									
Long – term experiment									
Control	0.090	0.400	1.016						57.3
9 uM Cd	0.028	0.114	0.281						93.7
45 uM Cd	0.003	0.015	0.021						319.7
Sort – term experiment				Recovery					
Control	0.127	0.377	1.103	0.249	1.608	4.619	8.936		50.8
45 uM Cd	0.063	0.102	0.328	0.061	0.513	0.859	1.644		121.2

a NCNU- Ratio of NO_3^- total plant content vs. NO_3^- uptake when plants were collected,
KCNU - ratio of K total plant content vs. K uptake when plants were collected.
b Ratio calculated for the recovery period.
c Ratio calculated for the recovery period.

Similar decrease in NO^-_3 uptake due to a modification of the plasma membrane NO^-_3 permeability was observed in the presence of Al (Cakmak and Horst, 1991; Durieux *et al.*, 1993; Ghaly and El-Sayed, 1997). In this sense, Pandolfini *et al.* (1992) observed that in wheat roots exposed to Ni a leakage of K occurred, which was associated to the peroxidation of membrane lipids. This finding is in agreement with my results of K uptake (Fig. 2B and 3B), where a severe loss of K uptake was observed, indicating that a general alteration of absorption processes in root cells may occur in the presence of Cd. This would reflect a general loss of plant plasma membranes integrity (Meharg, 1993; Eissa and El-Kassas, 1999).

In the shoots, the increase in Cd nutrient solution concentration was accompanied by a decrease in NR activity , which was inhibited dramatically by the greatest concentration of Cd supplied in the long-term experiment (Table 3). This loss of NR activity in shoot was associated with a similar reduction in NO^-_3 content (Table 2), symptoms also observed in maize plants under similar concentrations of Cd (El-Sayed, 1999c). Likewise, several authors reported a decrease in NR activity of different plant species when Cd was supplied to the nutrient solution (Burzynski, 1988; Chugh *et al.*, 1992; Nussbaum *et al.*, 1988; Petrovic *et al.*, 1990; Darwish and Ahmad, 1997). After 36hr exposure to Cd in the short-term treatment, the NR shoot activity was ca. 45% of that in the control shoot and was almost totally inhibited after 84hr (Table 3). This loss of NR activity was concomitant to a severe restricition in net NO^-_3 uptake, and its extreme accumulation of NO^-_3 in roots .

The largest proportion of Cd was found in the soluble fraction (consisting mainly of vacuolar and cytosolic contents), according to the subscellular distribution of Cd in guar plants. However, Cd was associated to proteins or polypeptides upto ca. 85% of the total Cd concentration in this fraction (El-Gendi *et al.*, 1999) . It is possible that Cd is released from cellular storage pools by the tissue homogenization needed for the solubilization of the NR. This might provoke its inhibition due to the interaction of Cd with the enzyme. However, as indicated, many other proteins are expected to form complexes with Cd, in particular phytochelatins (Gupta and Goldsbrough, 1991), which suggests that Cd does not interact alone with NR. Therefore, it is unlikely that the *in vitro* NR. inhibition is primarily due to an artifact. Moreover, shoot *in vivo* NR activity (determined) as described by Hernandez *et al.* (1995) of sunflower plants was

severely inhibited (from 1.14±0.16 to 0.08±0.02 umol NO^-_3 /g FW shoot/hr) when treated with 15 *uM*Cd for 7 days, results that support those presented here (Table 3) .

The accumulation of NO^-_3 in the roots might indicate that the xylem transport of NO^-_3 was limited in the presence of Cd in the growing medium. The long-term exposure to Cd caused a severe reduction in the shoot and sap NO^-_3 content, but in the roots the decrease was observed only in plants treated with 45 *uM* Cd (Table 2). The results of the short-term treatment also support this hypothesis, where after 84hr the shoot concentration of NO^-_3 declined to ca. 50% of the control values, whereas in the roots the NO^-_3 content was not effected and increased after the recovery (Table 2). Nitrate long distance transport to the shoot via xylemis mainly controlled by the transpiration (Barthes *et al.*, 1996; El-Sayed 1998 a; El-Kassas, 1999) The exposure of soybean plants to Cd provoked a reduction in their sap flow , transpiration and water uptake (Leita *et al.*, 1995). Therefore, the loss of transpiration observed in the presence of Cd (Fig. 1), an effect also described in bean (Poschenrieder *et al.*, 1989) and sugar beet (Greger and Johansson, 1992), might explain partially the decrease in NO^-_3 transpot.

De movo synthesis of NR is induced by the concentration of NO^-_3 in the cytosol (Hoff *et al.*, 1992; Tischner *et al.*, 1993 and El-Sayed, 1999a) which represents a minor fraction compared to the storage pool of NO^-_3 . This major proportion is located in the vacuole (Martinoia *et al.*, 1981, Zhen *et al.*, 1991; El-Sayed , 1999b), and is available to NR only under conditions of external NO^-_3 deprivation (Grandstedt and Huffaker, 1982; Hernandez *et al.*, 1995; El-Shebiny, 1998). The concentration of cytosolic NO^-_3 in aerial parts is mainly controlled by the xylem flux, and by the concentration of incoming NO^-_3 from the xylem (Mcknown *et al.*, 1983, Soussana *et al.*, 1989 and El-Sayed, 1999d). Therefore, the loss in uptake and transport of NO^-_3 due to exposure of guar plants to Cd would lead to a decrease in the cytosolic NO^-_3 concentration. This effect could partially explain the inhibition of NR activity observed in those plants by a decrease in NO^-_3 availability. This hypothesis is supported by the complete inhibition of net NO^-_3 uptake and transport to assimilating tissues observed after 36hr of short-term Cd treatment, effect also observed onwards (Fig. 3A, Table2). Furthermore, the almost complete inhibition of water transpiration (Fig. 1B) which would probably affect the transport of NO^-_3 via xylem (Barthes *et al.*, 1996; Tahoun *et al.*, 1999), confirms that hypothesis.

Conclusion

It is possible that the inhibition of NO^-_3 and K uptake and the loss of water transpiration reflect a general Cd phytotoxic effect on plasma membranes integrity. As a result, NO^-_3 content and assimilation is severely reduction in shoot.

References

Agüera, E; De La Haba, P., Fonter, A. G. and Maldonado, J.M. (1990) Nitrate and nitrite uptake and reduction by intact sunflower plants . *Planta*, **182**, 149.

Aïdid, S.B. and Okamoto, H. (1992) Effects of lead, cadmium and zinc on the electric membrane potential at the zylem / symplast interface and cell elongation of *Impatients balsmina. Environ . Exp. Bot.* **32**,439.

Asp, H., Gussarsson, M., Adalsteinsson, S. and Jensen, P. (1994) Control of potassium influx in roots of birch (*Betula pendula)* seedlings exposed to cadmium . *J. Exp. Bot.* **45**, 1823.

Barcelo, J. and Poschenrieder, C. (1990) Plant water relationship as affected by heavy metal stress: a review. *J. Plant Nutr.* **13**,1.

Barthes, L., Deleens, E., Bousser, A. Hoarau, J. and Prioul, J.L. (1996) Xylem exudation is related to nitrate assimilation pathway in detoped maize seedlings: use of nitrate reductase and glutamine synthetase as tools. *J.Exp. Bot.* **47**, 485.

Burzynski, M. (1988) The uptake and accumulation of phosphorous and nitrates and the activity of nitrate reductase in cucumber seedlings treated with pb and Cd. *Acta Soc. Bot. Pol.* **57**, 349.

Cakmak, I. and Horst, W.J. (1991) Effect of aluminium on net efflux of nitrate and potassium from root tips of soybean (*Glycine max* L.) *J. Plant Physiol.* **138** , 400.

Chugh, L.K., Gupta, V.K. and Sawhney, S.K. (1992) Effect of cadmium on enzymes of nitrogen metabolism in pea seedlings . *Phytochemistry*, **31**, 395.

Cottenie, A., Verloo, M., Kiekens, L., Velghe, G. and Camerlynch, R. (1982) *Chemical Analysis of Plants and Soils.* Hand book, pp. 1-63.

Darwish, O.H. and Ahmad, F.F. (1997) Availability, movement and distribution of sludge-borne elements in soils. *Alex. Sci. Exch.* **18** (4) , 505.

Durieux, R.P., Jackson, W.A., Kamprath, E.J. and Moll, R.H. (1993) Inhibition of nitrate uptake by aluminum in maize. *Plant Soil,* **151**, 97.

Eissa, A.M. and El-Kassas, H.I. (1999) Impact of heavy metaly on soil, plant and water at Abou-zaabal area. *Egypt. J. Soil . Sci.* **39** (3) , 351.

El-Gendi, S.A., Abu-Sinna, S.A.H.M. and Kandil, N.F. (1999) Fractionation and accumulation of some heavy metals in soils and plants irrigated with sewage effluent. *Egypt. J. Soil Sci.* **39** (2) , 211.

El-Kassas, H. I. (1999) Lead, cadmium and nickel released from different fertilizers and their implication on fennel plants growth in sandy soils. *Egypt. J. Soil Sci.* **39** (3) , 337.

El-Sayed, S.A.M. (1995) Sewage sludge as a phosphorus amendment in Egyptian soil. *Proceeding of the second conference of on-farm irrigation agroclimatology.* pp. 653-667. January 2-4, 1995.

El-Sayed. S.A.M. (1998a) Use of sewage sludge and farmyard-manure as N- fertilizer in New Valley Governorate . *Egypt. J. Soil Sci.* **38** (1-4) , 1.

El-Sayed, S.A.M. (1998b) Denitrification by guar rhizobia strains in New Valley Govenorate. *Egypt. J. Agric. Res.* **76** (2) , 451.

El-Sayed, S.A.M. (1999a) Effect of trace elements of soil-plant and root-shoot relationship in some forage legumes. *Egypt. J. Soil Sci.* **39** (1) , 85.

El-Sayed. S.A.M. (1999b) Effect of uptake of trace elements by rice on a gypsum amended sodic soil in New Valley Governorate. *Egypt. J. Soil. Sci.* **39** (3) , 281.

El-Sayed, S.A.M. (1999c) Forage cowpea growth as influenced by soil amendments of sewage sludge and heavy metals in field. *J. Agric . Sci. Mansoura Univ.*, **24** (9), 5119.

El-Sayed S.A.M. (1999d) Evaluation of nitrogen availability in sewage sludes at different sites Behera Governorate. *Third Conference of on Farm Irigation and Agroclimatology*, Vol. **1**(No.2.). paper No. 57, pp 780-793. January 25-27, 1999.

El-Shebiny, G.M. (1998) The influence of cadmium increments on the yield and cadmium accumulation in tomato plants. *Alex. Sci. Exch.* **19** (3) , 405.

Evenhuis, B. and De Waard, P.W.F. (1978) *Simplified Methods for Foliar Analysis.* Royal Tropical Institute Amsterdam, Holland.

F A O. (1980) Soil and plant testing and abalysis. FAO. *Soils Bulletin* **38** / 1.FAO. Rome, Italy.

Fodor, E., Szabo, A., Nagy and Erdei, L. (1995) The effects of cadmium on the fluidity and H-ATP ase activity of plasma membrane from sunflower and wheat roots. *J. Plant Physiol.* **147**, 89.

Garate, A., Ramos, I ., Manzanares, M. and Lucena, J.J. (1993) Cadmium uptake and distribution in three cultivars of *Lactuca* sp. *Bull Environ. Contam. Toxicol.* **50** , 709.

Ghaly, A.A. and El-Sayed, S.A.M. (1997) Effect of soil sodicity on growth yield chlorophyll and some biochemical parameters in guar. *J.Agric. Sci. Mansoura Univ.*, **22** (4) , 1187.

Gojon, A., Wakrim, R., Passama, L. and Robin, A. (1991) Regulation of NO^-_3 assimilation by anion availability in excised soybean leaves. *Plant Physiol* . **96**, 398.

Grandstedt, R.C. and Huffaker, R.C. (1982) Identification of the leaf vacuole as a major nitrate storage pool. *Plant Plysiol.* **70**, 410.

Greger, M., Brammer, E., Lindberg, S. and Idestam- Almquist, J. (1991) Uptake and phsiological effect of cadmium in sugar beet (*Beta vulgaris)* related with mineral provision. *J. Exp. Bot.* **42**, 729.

Greger, M. and Johansson, M. (1992) Cadmium effects on leaf transpiration of sugar beet (*Beta vulgaris*). *Physiol Plant* **86,** 465.

Gupta, S.C. and Goldsbrough, P.B. (1991) Phytochelatin accumulation and cadmium tolerance in selected tomato cell lines. *Plant Physiol.* **97,** 306.

Henriksen, G.H.R.M. and Spanswick (1993) Investigation of the apparent inducation of nitrate uptake in barley (*Hordeum vulgare)* using NO^{-}_{3} - selective microelectrodes. *Plant Physiol.* **103,** 885.

Hernandez, L.E, Ramon, A.M., Carpena-Ruiz, R.O. and Garate, A. (1995) Evalutation of nirate nuitrition indexes in maize leaves: metabolic nitrate, total nitrate content and nitrate reductase activity. *J. Plant Nutr.* **18,** 869.

Hoff, T., Stummann, B.M. and Henningsen, K.W. (1992) Structure, function and regulation of nitrate reductase in higher plants . *Physiol Plant,* **84,** 616.

Khater, A., Abou-Seeda, M., Soliman and Salem, N (1991) Growth, nodule formation, chlorophyll content and the uptake of some nutrients by broad bean plants as affected by cadmium application. *Agrochim.* **35,** 434.

Krupa, Z., Oquist, G. and Huner, N.P.A. (1993) The effects of cadmium on photosynthesis of *Phaseolus vulgaris* -a fluorescence analysi. *Physiol. Plant* . **88,** 626.

Leita, L., Marchio, M., Martin, A., Peressett, G., Dellevedove and Zerbi, G. (1995) Transpiration dynamies in cadmium-treated soybean (*Glycine* max L.) *Plants.J. Agron. Crop. Sci.* **175,** 153.

Leita, L, Nobili, De M Modini, C. and Baca-Garcia, M.T. (1993) Response of leguminosae to cadmium exposure. *J. Plant uutr.* **16,** 2001.

Martinoia, E., Heck, V. and Wiemcken. A. (1981) Vacuoles as storage compartments for nitrate in barley leaves. *Nature.* **289**, 292.

Mathys, W. (1975) Enzymes of heavy-metals -resistant and non-resistant populations of *Silene cucubalus* and their interactions with some heavy metals in vitro and in vivo. *Physiol. Plant* **33,** 161.

Mcknown, C.T., Jackson, W.A. and Volk, R.J. (1983) Partitioning of previously accumulated nitrate to translocation, reduction and efflux in corn roots. *Planta*. **157**, 8.

Meharg, A.A. (1993) The role of the plasmalemma in metal tolerance in angiosperms. *Physiol. Plant* **88,** 191.

Meharg, A.A. and Blatt, M.R. (1995) NO^-_3 transport across the plasma membrane of Arabidopsis thaliana root hairs : Kinetic control by pH and membrane voltage. *J. Membrane Biol.* **145,** 49.

Nussbaum, S., Schmutz, D. and Brunold, C. (1988) Regulation of assimilatory sulphate reduction by cadmium in *Zea mays* L. *Plant Physiol.* **88,** 1407.

Page, A.L., Miller, R.H. and Keeney, D.R. (1982) *Methods of Soil Analysis*. Part 2. *Chemical and Microbiological Properties* . American Society of Agronomy . Madison, Wisconsin, USA.

Pandolfini, T.R., Gabriellei, R. and Comparini, C. (1992) Nickel toxicity and peroxidase activity in seedlings of *Triticum aestivum* L. *Plant Cell Environ.* **15**, 719.

Petrovic, N., Kastori, R. and Rajcan, I. (1990) The effect of cadmium on nitrate reducase activity in sugar beet *(Beta vulgaris)*. In :*Plant Nutrition-Physiology and Applications*. M.L. van Beusichem (Ed.). p. 107. Kluwer Academic Publishers, Dordrecht.

Poschenrieder, C., Guinse, B. and Barcelo, J. (1989) Influence of cadmium on water relations, stomatal resistance and abcisic acid content in expanding bean leaves. *Plant Physiol* . **90,** 1365.

Ramon. A.M., Carpena-Ruiz, R.O. and Garate, A. (1989) *In vitro* stabilization and distribution of nitrate reductase in tomato plant. Incidence of boron deficiency. *J. Plant Physiol.* **135**. 126.

Rufty. T.W., Thomas, J.F., Remmler, J.L.. Campbell, W.H. and Volk, R.J. (1986) Intercellular localization of nitrate reductase in roots. *Plant Physiol.* **82** . 675.

Soussana, J.F., Gojon, A., Passama, L., Wakrim, R. and Robin, P. (1989) Critical evaluation of the in situ nitrate reductase assay, *Plant Soil* **120** , 243.

Tahoun, S.A., El-Garhi, I.A., Mohamed, I.R. and El-Falah, A.H. (1999) Assessment of some micronutrients in the soils of Abu Hammad, Egypt. *Egypt. J. Soil Sci.* **39** (3). 383.

Tischner, R.,Waldeck, B. Goyal, S.P. and Rains, W.D. (1993) Effects of nitrate pulses on the niutrate-uptake rate, synthesis of m-RNA coding for nitrate reductase, and nitrate reductease activity in roots of barley seedlings. *Planta* (189) 533.

Tremblay, N., Gasia, M.C.H., Ferauge, M.T.H., Gosselin, A. and Trudel, M.J. (1988) Influence of photosynhetic irradiance. On nitrate reductase activity, nutrient uptake and partitioning in tomato plants. *J. Plant Nutr.* **11**, 17.

Van Assche, F. and Clijsters, H. (1990) Effects of metals on enzyme activity in plants *Plant Cell Environ.* **13**, 195.

Van Erp, P. J. and Van Lune, P. (1989) A new method for determining the relation between soil-and plant-cadmium. *Plant and Soil* **116**, 119.

Zhen, R.G, Koyro, H.W., Leigh, R.A., Tomos, A.D. and Miller, A.J. (1991) Compartmental nitrate concenrtations in barley root cells measured with nitrate-selective microelectrodes and by single -cell sap sampling. *Planta*. **185**, 356.

(*Received* 6/ 2001)

تأثير الكادميوم على امتصاص كل من النترات والبوتاسيوم فى الجوار

سعيد عباس محمد السيد

قسم علوم الأراضى والمياه - كلية الزراعة بأسيوط - جامعة الأزهر - مصر .

تم دراسة محصلة الامتصاص وتوزيع وتمثيل النترات فى نبات الجوار لفترة طويلة ، حيث تم إضافة الكادميوم لمده ٢٥٢ ساعة بتركيزات ٩ر٥٤ ميكرومول ، تم إضافة الكادميوم لفترة قصيرة بتركيز ٤٥ ميكرومول لمدة ٨٤ ساعة . وفى المعاملة الاخيرة تم دراسة انتقال الكادميوم الحر فى المحلول المغذى إلى النباتات وسميت هذه الفترة بفترة " استرداد الكادميوم " وتم دراستها عند ١٠٨ ساعة. وتم مقارنة جميع هذه المعاملات مع الكنترول الذى يمثل النباتات التى لم يضاف اليها عنصر الكادميوم لوحظ فى كل من التجربتين أن هناك نقصا فى الوزن الطازج للنباتات يصاحبه نقص فى النسبة المئوية لمحتوى الماء فى كل من سيقان وجذور نباتات الجوار وكذلك فى معدل النتح عند زيادة تركيز الكادميوم .

وأوضحت النتائج حدوث نقص مفاجى فى تركيز النترات فى كل من سيقان وعصارة نباتات الجوار، حيث حدث تثبيط كبير لامتصاص النترات وصاحب هذا التأثير فقد للنترات بواسطة نشاط إنزيم Reductase كذلك أوضحت النتائج أنه فى الفترة القصيرة من معاملة الكادميوم كانت المحصلة النهائية لامتصاص النترات وهو حدوث تثبيط لها بعد ٣٦ ساعة . كذلك حدث نقص مفاجئ فى نشاط إنزيم Reductase .

تم دراسة امتصاص البوتاسيوم وتوزيعه فى أنسجة نباتات الجوار حيث لوحظ حدوث تضاد للبوتاسيوم فى وجود النترات كذلك تأثير امتصاص البوتاسيوم لوجود الكادميوم وذلك من معرفة نسبة NO^-_3/K حيث كانت ١٠ .أ وضحت النتائج أن نسبة K/NO^-_3 فى أنسجة نباتات الجوار تزداد فى السيقان مع زيادة الكادميوم المضاف فى كل من الفترة الطويلة والقصيرة . أوضحت هذه الدراسة أن سلوك البوتاسيوم يشابة سلوك النترات بالرغم من عدم تاثر التوزيع النسبى للكادميوم فى أنسجة النباتات.