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The influence of cadmium on the kinetics of NO_3^- uptake in wheat seedlings (*Triticum aestivum* L.)

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ABSTRACT

Uptake of NO_3 in the presence of cadmium was studied in wheat (*Triticum aestivum* L. cv. Giza 157). It was found that Cd^{2+} inhibited NO_3 uptake more severely at low (1 mM) than at high (5 mM) NO_3 concentration. The presence of Cd^{2+} did not eliminate the characteristic induction pattern of nitarte uptake upon first exposure of nitrogen-depleted seedlings to the cadmium. Removal of Cd^{2+} after 12 hours of induction resulted in recovery within 3h to the rates of nitrate uptake that had been induced in the absence of Cd^{2+}. Furthermore, seedlings grown in the absence of Cd^{2+}, rapidly restricted the rate of nitrate uptake to the level of seedlings grown in the presence of Cd^{2+}, indicating that Cd^{2+} inhibited the activity of nitrate transport to a greater extent than the induction process. Data of NO_3 uptake at low NO_3 concentration in the nutrient solution showed a typical Michaelis-Menten hyperbolic curve. Cadmium increased the apparent K_m and decreased the apparent $V_{max.}$ Cd^{2+} also inhibited ATPase activity at 15 and 20 μM, and the activity of nitrate reductase (NR) enzyme in the roots and shoots.

KEYWORDS: *Triticum aestivum*, Cadmium, kinetics, NO_3^- uptake

INTRODUCTION

Nitrate (NO_3) and ammonium (NH_4) are the predominant forms of available nitrogen and can be utilized either separately or in combination. However, the ability of the plants to absorb and metabolize these ions varies greatly, and the physiological responses of the plants to these ions are very different. The majority of studies have been devoted to the metabolism of nitrate in plants subsequent to its absorption, but little is known about the actual uptake. NO_3 uptake in higher plants is well characterized on a kinetic level (Clarkson & Luttge 1991). Most kinetic experiments on the NO_3^- uptake of various plants have been based on measurements of chemical depletion rates of NO_3^- from nutrient solution i.e. the determination of NO_3^- net flux (Warner & Huffaker 1989; Aslam *et al.* 1992). There is general agreement in several studies that the dependence of NO_3^- uptake on the induction concentration of nitrate $[NO_3^-]_o$ can be resolved into at least two kinetically distinct systems. The first is a high affinity transport system (HATS), which operates in a linear fashion at $[NO_3]_o$ > 1mM (Glass & Siddiqi 1995). In the second system, nitrate can be taken up together with H^+ (McClure *et al.* 1990) or through a 2 NO_3^- OH^- exchange as proposed in maize roots (Wijk & Prins 1993). The temporary ability of plants to absorb NO_3^- from culture solution is markedly affected by (a) the availability and concentration of NO_3^- ions (Redinbaugh & Campell 1991), (b) changes in external conditions, such as the pH and temperature of the exogenous solution (Vessey *et al.* 1990). However, there is some evidence of a transport system for nitrate induced by its substrate. We know that inhibitors of protein and RNA synthesis may prevent indirectly the uptake of nitrate in wheat, while nitrate uptake in barley is decreased by inhibitors of respiration and oxidative phosphorylation (Rao & Rain 1976). Nitrate uptake is also metabolically controlled. Nitrate has to overcome the potential difference which opposes anion entry (Salsac *et al.* 1987).

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However, when the plants are first exposed to nitrate, the capacity of their roots to absorb nitrate progressively increases for few hours above an initial constitutive rate (Durieux *et al*.1993). This pattern of induction shows that the rate of nitrate uptake at a given instant reflects: (1) the extent to which the uptake system has been induced, (2) the activity of ATPase enzyme associated with the plasma membrane, which has been postulated to function in ion transport, and (3) the effective operation of these transporters. Accordingly, the poor growth response of many plants can be attributed to a low capacity of the NO_3^- transport system. Therefore, inhibition of nitrate uptake by heavy metals may also be due to a restriction of the induction of transport activity.

As one of the most dangerous heavy metals to plants, cadmium is principally dispersed in natural and agricultural environments through human activities such as distribution of sewage sludge and atmospheric fallout from industrial and municipal activities (Wagner 1993). High concentrations of this metal are strongly phytotoxic, causing growth inhibition (Steffens 1990), membrane damage (Kennedy & Gonsalves 1987), alteration of enzyme activities (Van Assche & Clijsters 1990) and even plant death.

Accordingly, the first objective of the present study was to determine which effect is responsible for the inhibition of NO_3^- by cadmium. The second objective was to characterize the kinetics of the NO_3^- uptake system and translocation in the plant to determine which of these two potential effects is sensitive to inhibition by cadmium. The third objective was to determine the relative effects of cadmium on the activity of nitrate reductase, since the first step of nitrate assimilation (reduction of nitrate to nitrite) is catalyzed by the enzyme nitrate reductase (NR, EC:1.6.6.1). This is the rate-limiting step that regulates indirectly the process of nitrate uptake in the plants (Abdin *et al*. 1992). Finally, as already stated , the uptake of ions is dependent on energy. Thus ATPase activity of the plasma membrane has been also examined under cadmium treatment. In the experiments, nitrate uptake from the solutions was determined and apparent values were calculated for V_{max} and K_m.

MATERIALS AND METHODS

Plant material and growth conditions: This study was conducted in Research Lab. of Plant Physiology, Botany Dept., and partially in The Central Lab. Faculty of Science. Alexandria University, Alexandria, Egypt. Grains of wheat (*Triticum aestivum* L.Giza 157) were surface sterilized with 95% ethanol for 10 min followed by 0.1 % $HgCl_2$ for 2 min, then rinsed five times with distilled water and soaked in aerated distilled water for 24 h. Ten seeds were germinated in a plastic pots (15 cm diameter) containing autoclaved vermiculite. All pots were maintained in a growth chamber (2m ×1.0 m ×1.5m) for 7 days, at day and night temperatures 30±2^{o}C and 20±1^{o}C, respectively. Seedlings were grown with light / dark regimes of 16 / 8 h, with a light intensity of 800-1200 mol m^{-2} s^{-1} (PAR) at the surface of the pots and a relative humidity of 60 to 70%. Seeds were regularly supplied with a 20 % solution of the nitrogen free Hoagland nutrient solution modified by Johnson *et al.* (1957). On the 8th day, seedlings were carefully removed from vermiculite, then washed with distilled water and subjected to uptake rate measurements and further studies. Uptake rate measurements

The roots of intact seedlings were placed in 100 ml glass tubes each containing 75 ml of nitrogen-free nutrient solution. Four experiments were then conducted. In experiment 1, the uptake of NO_3^- was measured at N concentrations of 1 and 5 mM and a single dosage of either 0, 10 and 20 μM Cd^{2+}. In experiment 2, KNO_3 was added into the incubation solution at initial concentrations of 0.05, 0.1, 0 .2, 0.3, 0.4, 0.5, 1.0, 2.0, and 3.0 mM in the presence of either 0, 10 or 20 μM Cd^{2+} .The tubes were left in the growth chamber for 6 h with three

replications. The solutions were continuously aerated to maintain optimal oxygen supply and to prevent formation of boundary layers at the roots surfaces.

At the appropriate time, seedlings were removed and the root fresh and dry weights determined. Nitrate uptake, over a 6 hour period was assayed by measuring the loss of NO_3^- from the ambient solution and was expressed as μmol NO_3^- g^{-1} root fwt. h^{-1}. The data presented are the average of four cultures. Nitrate was determined as described by Woolley *et al*.(1960). Kinetic parameters K_m and V_{max} were calculated by fitting $v = V_{max} . S / (K_m+S)$ via the least squares methods (Epstein 1972).

In experiment 3, the rapid response to Cd^{2+}withdrawal or addition was examined by initially exposing cultures to 1 mM $NO_3^- \pm$ 20 μM Cd^{2+}. After 12 hours, three of the cultures were transferred from cadmium to cadmium-free solution while another three cultures remained in cadmium. Similarly, three of the cultures that had been in cadmium-free solution were transferred to cadmium and another three remained in cadmium-free solution. The rate of nitrate uptake was measured hourly for 12 hours.

Experiment 4, studied the effect of high cadmium concentration (20 μM Cd^{2+}) applied over different time periods (2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 h) either before or after nitrate addition was also studied

At the appropriate time samples were taken and extracted. An aliquot from the extract was taken for estimation of accumulated nitrate, and an aliquot of the nutrient solution was taken for the determination of the final concentration of NO_3^- from which the uptake can be deduced . The amount of reduced NO_3^- (metabolized) was calculated:-

$$NO_3^- \text{ reduced} = NO_3^- \text{ uptake} - NO_3^- \text{ accumulation}$$

Determination of nitrate reductase activity: At the end of experiment whole seedlings were harvested and the activity of nitrate reductase was determined.

Amino acid determination: Amino acids were determined colorimetrically as described by Sugano *el al*. (1975). The colour developed was estimated at 570 nm. Leucine was used as the standard.

Protein determination: Protein content in the tissue extracts was determined by the method of Bradford (1976), with bovine serum albumin as the standard.

ATPase assay: Plasma membranes were prepared according to Hodges & Leonard (1974). The purified membrane pellets were diluted with the gradient buffer (1 mM $MgSO_4$, 1 mM dithiothreitol, 1 mM Tris-MES, pH 7.8) for ATPase assay. All steps were carried out at 4^oC. ATPase activity was measured by a modified procedure of Hodges & Leonard (1974) described by Watson *et al*. (1980). The activity was expressed in μmole Pi mg^{-1} protein h^{-1}.

Statistical analysis: The mean values ± SE are reported in tables and figures. The significance of differences between control and each treatment was evaluated by the *t*-test.

RESULTS AND DISCUSSION

Heavy metals have been implicated as a factor influencing the productivity of the plants. Several mechanisms have been proposed by which heavy metals can affect plant growth. For example exposure to high Cd^{2+} concentration results in an internal accumulation of ions in the root, stem and leaf (Tab. 1). Many authors have been reported that an excess of this metal ion may induce ion toxicity, and cause structural changes in chlorophyll biosynthesis (Somashekaraiah *et al.* 1992) and membrane damage (Kennedy & Gonsalves, 1987). Cd^{2+} also affects photosynthesis by inhibiting different reaction steps in the Calvin cycle (Siedlecka & Krupa 1996). Finally, Cd^{2+} can inhibit plant growth by contributing to nitrogen limitation (Ouariti *et al.* 1997).

Table 1: Cadmium concentrations in different parts of wheat plant. Values are the means ± SE (n=5). Means within column followed with the same letters are not significantly different at $P < 0.05$.

Cd^{2+}	Cd^{2+} concentration (μg g^{-1} dry wt.)		
Treatment (μM)	Leaf	Stem	Root
Control	12.86 ± 0.9 a	9.7 ± 0.8 a	14.42 ± 1.1 a
10	25.32 ± 1.2 b	12.6 ± 0.8 b	210.84 ± 9.9 b
20	39.43 ± 2.8 c	19.8 ± 0.9 c	365.98 ± 11.8 c

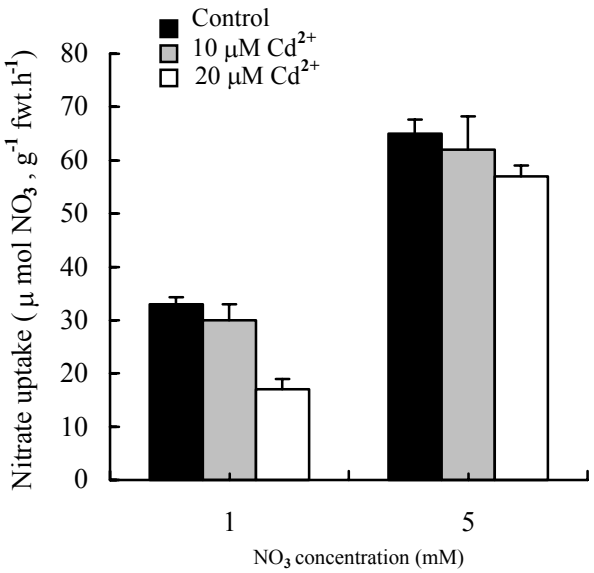
In this study, the inhibitory effect of Cd^{2+} was observed by the decrease in the fresh weight of shoot but whereas the fresh weight of root was unaffected by Cd^{2+} concentration up to 10 μM, the fresh weight of the root decreased at the highest level of Cd^{2+} (20 μM). Thus, the shoot:root ratio on the fresh weight basis decreased significantly in plants grown with 10 and 20 μM Cd^{2+} in nutrient solution (Table 2). By contrast dry weight of the plant organs were not affected by Cd^{2+}, indicating that the effect of Cd^{2+} was mainly on the water content of the plant.

Table 2: Effect of cadmium on fresh and dry weight of shoot and root of wheat plant.Values are mean ± SE (n=10). Values followed by the same letters are not significantly different at $P < 0.05$.

Treatment	Fresh weight (g)		Dry weight (g)		Shoot/root
	Shoot	Root	Shoot	Root	Ratio
Control	4.8 ± 0.3 a	4.3 ± 0.7 a	0.24 ± 0.01 a	0.26 ± 0.01a	1.1± 0.15 a
10 μM Cd^{2+}	2.9 ± 0.4 b	4.1 ± 0.4 a	0.26 ± 0.01ab	0.24 ± 0.01b	0.93 ± 0.03 ab
20 μM Cd^{2+}	2.8 ± 0.2 b	3.9 ± 0.2 b	0.22 ± 0.08 ab	0.28 ± .02ab	0.82 ± 0.04 c

Effect of Cd^{2+} on nitrate uptake: This study demonstrates that increasing Cd^{2+} concentrations inhibit the uptake of NO_3. At high concentration of Cd^{2+} (20 μM), nitrate uptake was reduced by approximately 48 and 16 % at 1 and 5 mM of NO_3^-, respectively. These results clearly showed that high cadmium concentration had a drastic effect on NO_3^- uptake when NO_3^- was added at low concentration by affecting the high-affinity mechanism. On the other hand, Cd^{2+} has little, if any, effect on nitrate uptake at high concentration of NO_3^- by enhancing the nitrate uptake through the low affinity mechanism.

Fig.1. Effect of cadmium and nitrate concentrations on NO3 uptake.Seedlings were grown two weeks in N-free nutrient solution, then exposed to NO3. Values are mean + SE (n=8)



Effect of Cd^{2+}exposure time: Inhibition of the rate of nitrate uptake occurred quickly when Cd^{2+} (20 μM) was added to wheat seedlings that had been were supplemented with nitrate (1mM) for 12 hours in the absence of Cd^{2+} (Fig. 2). The inhibition occurred within the first 2 hours and the rate of nitrate uptake continued at the inhibited level to the end of experiment (Fig.2A). Transfer of Cd^{2+}-treated wheat seedlings to Cd^{2+} free solution resulted in an increased in the rate of nitrate uptake over the next few hours almost to the rate of seedlings which had been exposed to Cd^{2+} free nutrient solution (Fig 2B).

The results presented in Fig. after induction with NO_3, a μm Cd^{2+} restricted nitrate u hours but not to the initial r contrast following removal induced transport mech became fully functional (Fi interpretation, therefore, the present in combination with Cd^{2+} was absorbed and was effective in the induction process. Also the transporters assembled in the presence of Cd^{2+} were capable of rapid restoration to a functional state with removal of Cd^{2+}. Thus Cd^{2+} appears to have a direct effect but only a loose interaction with the nitrate transporters. When the seedlings were exposed to Cd^{2+} for varying periods before or after addition of nitrate to the nutrient solution (Fig.3), it was found that high concentrations of Cd^{2+}used before the seedlings were given nitrate increased the rate of nitrate uptake, while exposure for 12 hr or more reduced the uptake process. Furthermore, exposure of wheat seedlings to 20 μM Cd^{2+} before given nitrate, significantly stimulated nitrate uptake, particularly during the first 12 hours of exposure time, while addition for longer than 12h reduced the uptake.

Effect of Cd^{2+} on the kinetics of NO_3^- uptake: From the account of passive diffusion of substances into cells it is clear that most metabolites could not enter the cell at an appreciable speed were it not for the existence of specific mechanisms which enable them to cross the plasmalemma. Edwards & Hassall (1980) have reported that in many cases the cooperation of a carrier has been demonstrated. Carrier are usually thought to be proteins or lipoproteins located in the cell membrane itself. They are analogous to enzymes in that they have a binding site which is specific for one metabolite or sometimes for a group of substances with some common chemical structure (Edwards & Hassall 1980).

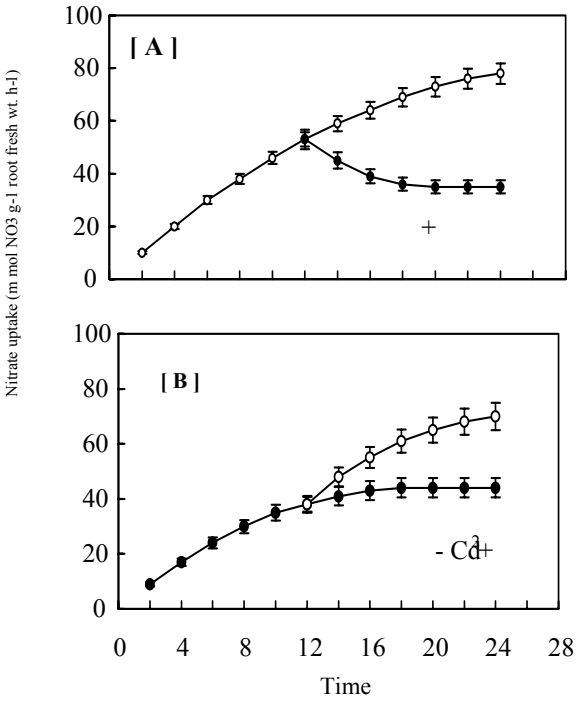


Fig.2. Effect of 20 mM Cd2+ on the rate of nitrate uptake by nitrogen-depleted wheat induced in 1 mM NO3 for 12 h [A] and the effect of Cd2+ withdrawal after 12 h when wheat seedlings had been induced in the presence of 20 m M Cd2+ [B]. Values are mean + SE (n=8)

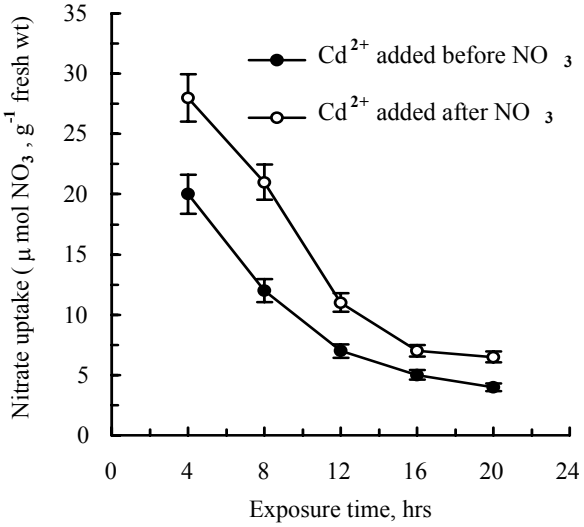


Fig.3. Effect of cadmium on nitrate uptake when it was added before or after nitrate addition. Values are mean + SE (n=5)

The substance to be carried becomes attached to the carrier on the outside of the cell and then by some rotation or other change in orientation of the carrier within the membrane the substance is transferred to the inside layer of the plasmalemma where it is released into the cell. This is known as carrier mediated transport. Transport kinetics resemble the kinetics described for the enzymes, where the substance transferred is regarded as the substrate.

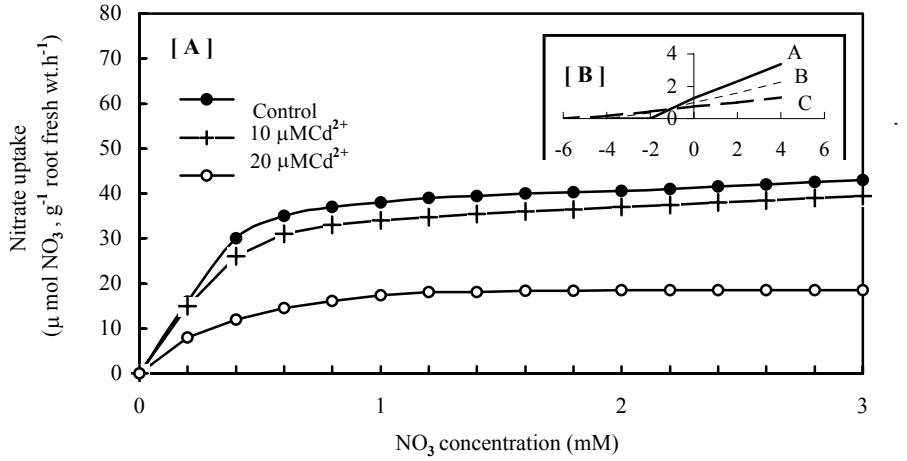


Fig.4. (A) Net uptake as a function of [NO-3] in the nutrient solution for wheat seedlings. (B), insets show Linweaver-Burk reciprocal plot of the same data of fig.4A, (A, 0; B, 10 m M and C, 20 m M of Cd2+ concentrations).

Thus, when the rate of nitrate uptake was expressed as a function of nitrate concentration, the nitrogen-depleted wheat seedlings exhibited the characteristic pattern of induction in nitrate uptake, defined as a dual pattern of ion uptake with different affinities showing Michaelis-Menten Kinetics (Rao *et al*. 1993). Thus, the rate of NO_3- uptake at low nitrate concentrations become saturated and fits well with Michaelis-Menten equation (uptake rate) as a rectangular hyperbolic function of concentration (Fig. 4A), whereas the uptake rate at high concentration (> 2 mM) are far from the line expected from the Michaelis-Menten equation (Fig.4A). However, the data obtained are also not compatible with a single saturatable component, suggesting that there seems to be at least two different uptake patterns within the concentration range tested in this study. The transition point between the two components was approximately around 1 mM (Fig. 4). In this connection, Rao *et al*.(1993) reported that in legumes and cereals, the transition point was around 1 mM nitrate. A double reciprocal plot (Lineweaver-Burk plot) of the same data of Figure 4 excluding the highest concentrations gave straight lines (Fig.4B, insert).

The kinetic constants V_{max} and K_m for NO3$^-$ uptake were calculated by measuring the depletion of NO_3^- from the external nutrient solution. V_{max} can be viewed as a function of the saturated uptake rate of the carrier and the concentration per surface area of root of active carrier sites, while K_m is a measure of the ability of each carrier system to bind with and take up substrate as a function of relative substrate concentration. In this study, V_{max} and K_m for NO_3^- were affected by the presence of Cd^{2+} At high concentrations of Cd^{2+}, V_{max} drastically declined, whereas K_m continued to increase (Table 3). Changes in the apparent V_{max} might be result from changes in membrane properties caused by the interaction between Cd^{2+} and the proteinaceous components such as the ATPase enzyme postulated to function in ion transport and apparently an integral component of the membrane (Watson *et al.*1980).

Table 3. Effect of cadmium on apparent V_{max} and K_m of NO_3^- uptake. Parameters obtained from data of Figure 4. Data are means ± SE (n=5). Values within the column followed with same letters are not significantly different at $P< 0.05$.

Cd^{2+} Concentration (μM)	V_{max} (μ mol NO_3^- g^{-1} fwt root h^{-1})	K_m (μM)
0	2.56 ± 0.32 a	4.00 ± 0.33 a
10	2.33 ± 0.18 ab	5.22 ± 0.22 b
20	1.61 ± 0.14 c	8.40 ± 0.81 c

Effect of cadmium on nitrate reductase

This experiment was designed to determine whether the decrease in net uptake of nitrate by heavy metals could be indirectly accounted for entirely by inhibition of the activity of nitrate reductase, or the direct effect of heavy metals on the nitrate uptake process. The results show that amount of nitrate absorbed by wheat was 38.9 μ mol NO_3^- g^{-1} f.wt. in the presence of cadmium (20 μM). Only 26% of this amount was reduced and the rest was accumulated (Fig.5). In the control, corresponding value was 58.9 μ mol NO_3^- g^{-1} f.wt. and 83%, indicating that untreated wheat seedlings absorbed more nitrate than those treated with cadmium, and also more of the NO_3^- (absorbed) was reduced. This result could be explained

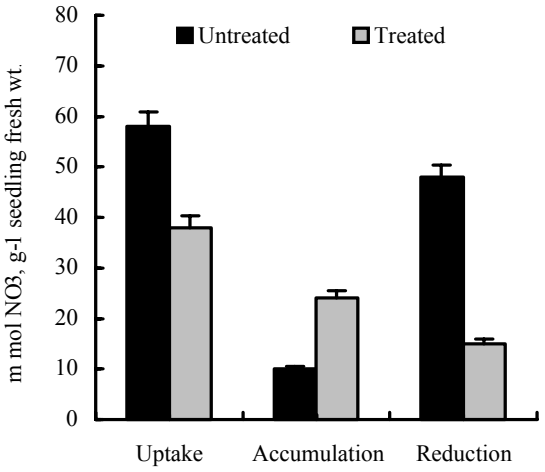


Fig.5. Uptake, accumulation and reduction of NO3 in the presence of Cd2+.Seedlings were 7days old and 24 h in NO3 and Cd2+ treatment. NO3 and Cd2+ concentrations were 1 mM and 20 mM respectively. Values are mean + SE (n=8)

that when nitrate is taken in, it passes in the tissues through a metabolic pool: the level of nitrate in this pool requires energy and reducing power or carbon skeleton (Aslam *et al*.1987) and depends on the activity of the nitrate reductase enzyme (NR). This means that the inhibitory effect of cadmium is on the uptake process as well as on the activity of nitrate reductase. For instance, addition of Cd^{2+} resulted in a great loss in activity of nitrate

Table 4. Effect of Cadmium on the activity of NR in leaves and roots of wheat seedlings. Values are means ± SE. (n = 5). Values followed by the same letters are not significantly different for $P < 0.05$

Seedlings parts	NR-activity (μ mol NO_2 mg $protein^{-1}$ hr^{-1})	
	Untreated	Treated
Leaves	2.18 ± 0.10 a	0.32 ± 0.02 a
Roots	0.95 ± 0.06 b	0.15 ± 0.01 b

reductase both in the leaves or roots of wheat seedlings (Tab.4). Conceivably, there could be a relationship between the inhibitory effects of cadmium and the inhibition of ATPase activity and hence the supply of energy needed for ion transport on the one hand, and inhibition of NR activity on the other hand. However, Wijk and Prins (1993) reported that if nitrate reduction was restricted, this could be due to decrease in nitrate uptake. Furthermore, the results presented in Fig 6 showed that plants receiving Cd^{2+} before nitrate had much lower levels of

NR activity than those plants receiving Cd^{2+} after nitrate, indicating that when NO_3^- was added first before Cd^{2+}, it induced increase in NR activity probably due to its *de novo* synthesis and increased stability. By contrast the reverse was true when Cd^{2+} was added before nitrate.

Enzyme activity could be reduced drastically if the exposure of cadmium occurred early enough prior to nitrate uptake, probably due to the inhibition of protein synthesis. . Thus, we suppose that the presence of cadmium in the induction medium could interfere with the distribution of nitrate from roots to shoots and/or with the products of its reduction within the cells, what in effect gives an indirect changes in NR activity. The amino acid content (Table 5) was reduced in plants exposed to cadmium. Exposure to only 2 hr of cadmium actually increased the amino acid content. Protein content was lower only when Cd^{2+} was present for the first 6 hr of the experiment (Table 5). The decrease in soluble protein content of seedlings is a typical response to salt stress and the response may be due to an alteration in the incorporation of amino acids into protein (Table 5). This conclusion is in agreement with results of Bourgeais-Challiou *et al.* (1992).

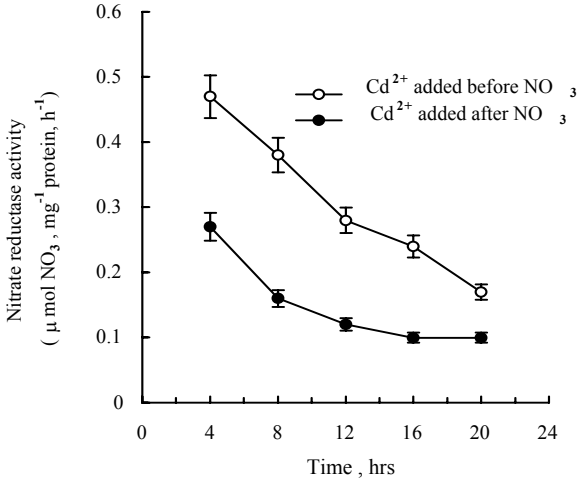


Fig .6 . Effect of cadmium on the activity of nitrate reductase. Cadmium was added before and after addition of nitrite. Values are mean + SE (n = 10)

Table 5: Effect of Cd^{2+} at high concentration (20 μM) on the average amino acids and water soluble protein content through exposure time. Values are means ± SE (n = 5).

Time of Exposure (hr)	Total amino acids (mg of amino N g^{-1} fresh wt.)	Water soluble protein (mg protein g^{-1} fresh wt.)
0	1.24 ± 0.04	31.22 ± 1.2
2	1.80 ± 0.02	32.2 ± 1.4
4	1.0 ± 0.02	34.4 ± 0.9
6	0.9 0 ± 0. 01	27.3 ± 0.5
8	0.78 ± 0.02	32.8 ± 0.9
10	0.82 ± 0.01	31.8 ± 1.2
12	0.64 ± 0.01	31.2 ± 1.1

Effect of Cd^{2+}on ATPase of plasma membranes: Isolated plasma membranes from the roots of wheat plant were treated with Cd^{2+} to determine its effect on ATPase activity.. Therefore, treatment of plasma membrane ATPase in wheat with different concentrations of Cd^{2+} (Table.6) resulted in a reduction in the activity of ATPase by 5, 37, 45 and 62, respectively at 5,10,15 and 20 mM Cd^{2+}, indicating that cadmium can alter the membrane structure through physicochemical interactions and by modulating the supply of ATP needed to energize the membranes for ion transport (Martinez & Lauchli 1994). It is well known that ATPase is associated with plasma membranes, requires magnesium ions and is stimulated by the K^+ ion. Accordingly, one may suggest that cadmium may act on the magnesium-ATP substrate or on the substrate-enzyme complex, preventing ATP hydrolysis rather than on the enzyme itself. Another explanation is the inhibition (of synthesis) of the nitrate carrier (Wijk & Prins1993) by cadmium.

Table 6. Effect of different concentrations of cadmium on ATPase activity of plasma membranes of wheat roots. Data are means ± SE (n=5).

Cd^{2+} concentration (μM)	ATPase activity μ mole Pi mg^{-1} protein. H^{-1}	Percent of control
0	63.9 ± 1.6	100
5	60.8 ± 2.1	95
10	40.3 ± 1.1	63
15	35.4 ± 2.2	55
20	24.6 ± 1.8	38

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الملخص العربى

تأثير الكاديوم علي ديناميكية إمتصاص أيون النترات في نبات القمح

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1- قسم العلوم البيولوجية ، كلية التربية ، جامعة الأسكندرية ، الأسكندرية ، ج .ع .م

2- قسم النبات ، كلية العلوم ، جامعة الأسكندرية ، الأسكندرية ، ج . ع . م

في هذا البحث تمت دراسة إمتصاص النترات في وجود أيون الكادميوم كأحد العناصر الثقيلة في بادرات القمح وأوضحت النتائج أن أيون الكادميوم قد ثبط كثيرا من إمتصاص النترات إذا ما أضيفت بتركيزات منخفضة (1 ملل مول) عن إذا ما أضيفت بتركيزات عالية (5 ملل مول) كما أوضحت النتائج أيضا أن وجود أيون الكادميوم لا يؤدي إلي تثبيط أو الي التخلص من نمطيه الحث المتميز والمعرف لإمتصاص النترات عند تعرض البادرات الناضبة نيتروجينيا للعناصر الثقيلة0 وأظهرت النتائج أيضا أن إزاله أيون الكادميوم بعد فترة من وجوده (12 ساعه) قد أدي إلي تحفيز إمتصاص النترات خلال فتره قصيرة قدرت بساعتين وبمعدل قد يساوي معدل الإمتصاص في حالة غياب أيون الكادميوم أصلا وعلى العكس حيث أدت إضافة أيون الكادميوم إلي نقص حاد وسريع في معدل الإمتصاص والذي يدنو من مستوي معدل الإمتصاص الناتج من وجود أيون الكادميوم منذ البداية مما يتضح جليا أن تأثير أيون الكادميوم علي تثبيط عملية إنتقال النترات كان أكثر وضوحا من تأثيره علي تثبيط عملية حث الأنتقال 0

وفي دراسة ديناميكية وحركة وإنتقال النترات في هذا البحث دلت النتائج أن وجود أيون الكادميوم قد زاد من ثابت Km (ثابت ميكائيل) وقلل من ثابت سرعة الامتصاص Vmax كما ثبط أيون الكادميوم وخاصة بالتركيز العالي نشاط إنزيم ATPase المرتبط بالغشاء البلازمي واللازم لدفع عملية إنتقال الأيونات عبر هذه الأغشية وأيضا كان لأيون الكادميوم الأثر المثبط لنشاط إنزيم إختزال النترات (نيترات ريداكتيز NR) هذا بالاضافة الي الأثر الضار لذلك العنصر الثقيل علي جميع دالات النمو والتى درست في هذا البحث 0