

BRIEF COMMUNICATION

Effects of pH and nitrogen on cadmium uptake in potato

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This study investigated the effects of pH and nitrogen form and concentration on cadmium (Cd) uptake by potato (*Solanum tuberosum* L.) grown in hydroponic culture. Potato plants grown in a pH-buffered nutrient solution for 10 d were exposed for 24 h to 25 nM CdCl₂ labelled with ¹⁰⁹Cd. Plants showed a significantly higher Cd uptake and accumulation at pH 6.5 than at pH 4.5 and 5.5. Nitrogen supplied as nitrate (NO₃⁻) generally resulted in a higher Cd uptake and accumulation than N supplied as ammonium (NH₄⁺). This effect was most pronounced at pH 6.5. The N concentration increasing from 6.5 to 26 mM resulted in a decreased Cd influx when either NO₃⁻ or NH₄⁺ was used. Cd translocation to the shoot was increased when NO₃⁻ was used as the sole N source. In conclusion, pH had a strong influence on Cd uptake by roots and N form is especially important for Cd translocation within the potato plant.

Additional key words: ammonium, Cd accumulation and translocation, nitrate, *Solanum tuberosum*.

High cadmium content in soil is mainly due to atmospheric deposition and the use of Cd-containing phosphate fertilizers and sewage sludge, as well as the natural occurrence of Cd in the soil. The recent recommendation of lowering the maximum weekly Cd intake to 2.5 µg(Cd) kg⁻¹(body mass) by the European Food Safety Authority (EFSA 2009) makes it important to study the impact of cultivation practices on Cd accumulation in major food crops, such as cereals, potatoes, and vegetables. Due to high consumption, potatoes contribute approximately 20 - 25 % of dietary intake of Cd in Scandinavia and the United Kingdom (Olsson *et al.* 2005).

In the potato plant, Cd is taken up *via* basal roots and transported in the xylem to the shoot, where it is partly loaded to the phloem and transported to the tubers (Reid *et al.* 2003). Due to the absence of functional xylem connections between basal roots and tubers (Kratzke and Palta 1986), direct Cd transport *via* roots to tubers is restricted. Cadmium may be taken up in the periderm but no further transport into the tuber occurs (Reid *et al.* 2003). The amount of Cd absorbed by plants is due to the availability of Cd in the soil which is influenced by pH, clay and organic matter content, and soil management (Eriksson and Söderström 1996, Mench 1998, Adams *et al.* 2004). Plant-available Cd generally increases at low

pH, *e.g.*, Öborn *et al.* (1995) reported a negative correlation between the Cd content in potato tubers and soil pH but the opposite has also been reported (McLaughlin *et al.* 1994). Due to these contradictory results, several hydroponic studies (avoiding the influence of soil) have been conducted to investigate the influence of pH on Cd uptake. Soybean grown in nutrient solution increases Cd uptake into all plant parts except the roots at pH 4.5 compared with 6.5 (Ohya *et al.* 2008). However, in lettuce and ryegrass grown in nutrient solution, the total Cd content increases when pH increases from 5.0 to 7.0 (Hatch *et al.* 1988). The form of N-fertilizer applied (NO₃⁻ or NH₄⁺) influences the pH in the soil solution which indirectly affects Cd availability and uptake. Since plants fertilized with NH₄⁺ have a lower content of other cations than plants fertilized with NO₃⁻ (Marschner 1995, Monsanto *et al.* 2010), the N form *per se* may also be important for Cd uptake and translocation in potato plants, mainly due to ion competition. A number of experiments in soil with various plant species, including potato examining the acidifying fertilizer (NH₄)₂SO₄, have reported an increase in Cd content in plants compared to the use of alkaline nitrate-based fertilizers (Eriksson 1990, Florijn *et al.* 1992, Willaert and Verloo 1992, Maier *et al.* 2002). In hydroponic studies with rice, plants supplied with (NH₄)₂SO₄ have

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been found to have a lower Cd content than plants supplied with NH_4NO_3 or $\text{Ca}(\text{NO}_3)_2$ (Hassan *et al.* 2008, Jalloh *et al.* 2009). Jalloh *et al.* (2009) suggested an antagonistic effect between NH_4^+ and Cd^{2+} . Xie *et al.* (2009) also found that the hyperaccumulator *Noccaea caerulescens* (formerly *Thlaspi caerulescens*) accumulates a higher amount of Cd when supplied with NO_3^- compared with NH_4^+ in nutrient solution. Irrespective of the growing system used, there is competition between cations at the root surface which influences the uptake of Cd^{2+} . The SO_4^{2-} ion is often left unbalanced in nutrient solution since it is considered to be of less importance. However, Cd- SO_4 complexes may be formed in solution, and according to McLaughlin *et al.* (1998), these complexes and Cd-Cl complexes may also be taken up by plants.

In order to grow high quality food in a commercial and environmentally safe way, it is important to know more about how N fertilization and pH influence Cd uptake in potato. Liming to raise the soil pH is believed to decrease Cd uptake due to decreased availability of Cd but experiments, in both soil and solution, have produced the opposite result showing the complex effects of pH on Cd uptake. In previous field experiments, we found that both $\text{Ca}(\text{NO}_3)_2$ and $(\text{NH}_4)_2\text{SO}_4$ increased the Cd content in tubers compared with a balanced fertilizer, and also that increased N application decreased the tuber Cd content (Larsson Jönsson and Asp 2011). The aim of the present study was to further investigate the influence of pH, N form and N concentration on Cd uptake in potato plants grown under controlled conditions at Cd concentrations relevant for uncontaminated agricultural soils.

Plugs with one shoot were taken from pre-sprouted potato tubers (*Solanum tuberosum* L. cv. Fakse) and grown in *Vermiculite* for 15 d. The potato plants (without tubers) were then transferred to plastic containers containing 25 dm³ of a continuously aerated nutrient solution. Potato plants used in Cd uptake studies were grown for 10 d in these containers whereas plants used in growth experiments (see below) were transferred to experimental conditions after 3 d. The cultivation solution (pH 5.6) consisted of macronutrients [mM]: 5.14 $\text{Ca}(\text{NO}_3)_2$, 1.29 NH_4NO_3 , 3.19 K_2SO_4 , 1.29 KH_2PO_4 , 2.06 MgSO_4 ; and micronutrients [μM]: 53.7 Fe-EDTA, 18.2 MnSO_4 , 1.5 ZnSO_4 , 1.6 CuCl_2 , 0.5 Na_2MoO_4 and 27.7 H_3BO_3 . Only half-strength solution was used for the first three days. The solution was changed twice a week. All experiments were carried out in a climate chamber with photosynthetically active radiation (PAR 400 to 700 nm) of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during a 16-h photoperiod, day/night temperatures of 20/18 °C and relative humidity of 70 %. Plants pre-grown in this way were used for three different experiments.

In the first experiment, plants were transferred from the cultivation solution to 250 cm³ of uptake solution for 24 h. These plants were treated with either NO_3^- or NH_4^+ in the same concentration at three different pH levels. The uptake solution was buffered with 10 mM 2-(N-morpholino) ethane-sulphonic acid (MES) and pH was adjusted to 4.5, 5.5, or 6.5 using NaOH. The NO_3^-

treatment solution consisted of macronutrients [mM]: 12.85 KNO_3 , 5.14 CaCl_2 , 1.29 KH_2PO_4 , 2.06 MgSO_4 ; and micronutrients [μM]: 53.7 $\text{Fe}(\text{NO}_3)_3$, 18.2 MnSO_4 , 1.5 ZnSO_4 , 1.6 CuCl_2 , 0.5 Na_2MoO_4 and 27.7 H_3BO_3 . The NH_4^+ treatment solution consisted of macronutrients [mM]: 6.43 $(\text{NH}_4)_2\text{SO}_4$, 6.43 K_2SO_4 , 5.14 CaCl_2 , 1.29 KH_2PO_4 , 2.06 MgSO_4 ; and micronutrients as above. Cadmium (25 nM) was added to the uptake solution as CdCl_2 labelled with $^{109}\text{CdCl}_2$ (555 kBq dm⁻³).

For each treatment, six replicates were used. After a 24-h uptake period in solution with ^{109}Cd , plants were transferred to a Cd-free desorption solution containing cultivation solution with 10-fold higher Ca concentration as CaCl_2 , and $\text{Fe}(\text{NO}_3)_3$ instead of Fe-EDTA. After 20 min of desorption, the plants were divided into roots and shoots, blotted between paper, and dried at 70 °C for two days.

In the second experiment, potato plants were subjected for 24 h to an uptake solution containing NO_3^- or NH_4^+ at one of three different concentrations (6.5, 13, or 26 mM) and one of two pH levels (4.5 and 6.5, buffered with MES). Nitrogen was added as KNO_3 or $(\text{NH}_4)_2\text{SO}_4$ to the uptake solution and potassium was balanced with K_2SO_4 . The rest of the uptake solutions were as in exp. 1 and the uptake experiment was performed in the same way.

In the third experiment, growth of plants was studied. After three days in cultivation solution, the plants were transferred to containers with NO_3^- (13 mM) or NH_4^+ (13 mM) in the nutrient solution and 0, 2, 25, or 250 nM CdCl_2 . The NO_3^- and NH_4^+ treatment solutions were as described in exp. 1. The pH was adjusted to 5.6 using NaOH. The solution was changed every second day, at which time plants were weighed and pH in the solution was measured. The plants were harvested after 10 d and dried at 70 °C for two days. For each treatment, five replications were done.

The dried root and shoot samples were wet-combusted in 10 cm³ of HNO_3 (65 %) using a microwave technique. Radioactivity was measured by liquid scintillation spectrometry (*PW4700*, Philips, Netherlands). The concentrations of Cd and other elements were analysed by inductively coupled plasma emission [ICP-MS; (*Elan DRC2*, Perkin Elmer, Sciex, USA) for Cd, Cu, Zn, and Fe; ICP-OES (*IRIS Advantage*, Thermo Jarell, Ash, USA) for Ca, K, Mg, Mn, P, and S]. Cadmium speciation in the nutrient solution was calculated using the software *Win HumicV* (Tipping and Hurley 1992).

Statistical analyses were carried out with the package *Minitab Statistical Software v. 15* (Minitab, Pennsylvania, USA) using a factorial design with the factors pH, N form, and N concentration, or using one-way ANOVA. The Tukey's test was used for pair-wise comparisons of means.

Due to availability factors, soil acidity is considered a major determining factor of Cd uptake in plants and decreasing pH is often connected to increasing Cd uptake (Öborn *et al.* 1995, Eriksson and Söderström 1996, Gray *et al.* 1999). The pH effect on Cd availability in soil and

Table 1. Effects of pH and nitrogen form on Cd accumulation in roots and shoots and total Cd uptake in potato plants exposed to 25 nM Cd for 24 h in a buffered uptake solution containing NO_3^- or NH_4^+ at pH 4.5, 5.5, and 6.5, respectively. Means \pm SE, $n = 6$. Values followed by different letters are significantly different at $P < 0.05$.

pH	N form	Cd accumulation in root [nmol g ⁻¹ (d.m.) d ⁻¹]	Cd accumulation in shoot [nmol g ⁻¹ (d.m.) d ⁻¹]	Cd uptake [nmol g ⁻¹ (root d.m.) d ⁻¹]
4.5	NO_3^-	3.14 \pm 0.31c	0.88 \pm 0.11ab	4.55 \pm 0.37c
4.5	NH_4^+	3.35 \pm 0.66c	0.73 \pm 0.08b	4.67 \pm 0.71c
5.5	NO_3^-	4.18 \pm 0.42c	1.23 \pm 0.20ab	6.20 \pm 0.6bc
5.5	NH_4^+	3.93 \pm 0.46c	0.70 \pm 0.15b	5.00 \pm 0.58bc
6.5	NO_3^-	9.09 \pm 0.94a	1.58 \pm 0.26a	12.10 \pm 1.35a
6.5	NH_4^+	6.61 \pm 0.38b	0.85 \pm 0.15b	7.46 \pm 0.54b

Table 2. Effects of N form and concentration on root Cd uptake in potato plants grown in a buffered uptake solution at pH 4.5 and 6.5. All solutions contained 25 nM Cd. The uptake solution contained 6.5, 13, and 26 mM N as NO_3^- or NH_4^+ . Means \pm SE, $n = 6$. Values followed by different letters were significantly different at $P < 0.05$. Plants exposed to NO_3^- had significantly higher Cd uptake compared to NH_4^+ exposed plants when all treatments within the buffered experiment were compared in a factorial design.

pH	N form	N conc. [mM]	Cd uptake [nmol g ⁻¹ (root d.m.) d ⁻¹]
4.5	NO_3^-	6.5	4.87 \pm 0.33cd
4.5	NO_3^-	13.0	3.97 \pm 0.28d
4.5	NO_3^-	26.0	3.71 \pm 0.46d
4.5	NH_4^+	6.5	3.76 \pm 0.77d
4.5	NH_4^+	13.0	2.92 \pm 0.52d
4.5	NH_4^+	26.0	2.14 \pm 0.54d
6.5	NO_3^-	6.5	9.52 \pm 0.85a
6.5	NO_3^-	13.0	8.75 \pm 0.49a
6.5	NO_3^-	26.0	8.12 \pm 0.54ab
6.5	NH_4^+	6.5	7.52 \pm 0.92abc
6.5	NH_4^+	13.0	7.86 \pm 1.08abc
6.5	NH_4^+	26.0	5.17 \pm 0.35bcd

the fact that different sources of N strongly affect soil pH make studies of the effects of pH and N form on the physiology of Cd uptake difficult to perform in soil. This may also be the reason for the contradictory results reported when Cd uptake at different soil pH values is studied (McLaughlin *et al.* 1994, Öborn *et al.* 1995).

The present study in nutrient solution demonstrated that the total root Cd uptake into potato plants was significantly higher at pH 6.5 than at pH 4.5 and 5.5, irrespective of N form in the solution (Table 1). Similar results have been reported for lettuce and ryegrass grown in nutrient solution where the Cd content in roots and shoots increased when solution pH was 7.0 compared with 5.0 (Hatch *et al.* 1988).

According to the Cd speciation in the solutions used in our experiments, the Cd^{2+} activity in the uptake solution was approximately the same at pH 4.5 and 6.5

(data not shown), indicating that the difference in Cd accumulation at different pH was not due to different availability of free Cd^{2+} ions. The higher Cd content in both the root and the shoot at pH 6.5 compared with pH 4.5 may have been due to cationic competition between H^+ and Cd^{2+} for binding sites at the root surface. In *Chlamydomonas reinhardtii*, Francois *et al.* (2007) suggested H^+ may also inhibit metal uptake by causing conformational changes in transport proteins. The increased Cd uptake in the potato plants with increasing pH was not dependent on the N form, but the increase at higher pH was more pronounced when NO_3^- was present compared with NH_4^+ . In particular, Cd accumulation in the shoots increased significantly when NO_3^- was used under all pH (Table 1). These results suggest that pH is a more important factor in Cd root uptake whereas N form is more important for Cd transport to the shoot. The increase in Cd uptake at pH 6.5 compared with 4.5 was seen at all N concentrations tested (Table 2).

In general, potato plants exposed to NO_3^- took up more Cd than plants exposed to NH_4^+ (Tables 1 and 2). This effect has also been observed in hydroponic studies with rice and the hyperaccumulator *Noccaea caerulescens* (Hassan *et al.* 2008, Jalloh *et al.* 2009, Xie *et al.* 2009). In our study, there was significantly higher root accumulation of Cd in the NO_3^- -fertilized potato plants compared with NH_4^+ -fertilized ones at pH 6.5, and Cd accumulation in the shoot increased significantly in NO_3^- -fertilized plants at both pH 5.5 and 6.5 when all N concentrations were considered (Table 1). These results are quite similar to those obtained in hydroponic studies with *Noccaea caerulescens*, where the Zn concentration is higher in NO_3^- -fertilized plants than in NH_4^+ -fertilized, and is also higher in roots at pH 6.5 compared with 4.5 (Monsant *et al.* 2010). Zinc and Cd are divalent cations with similar properties and may be absorbed through the same channels and/or carriers. The higher concentration of Cd^{2+} in both the roots and shoots of the potato plants fertilized with NO_3^- in our experiments (Tables 1, 2, and 3) and the higher concentration of Zn^{2+} in *Noccaea caerulescens* in the study by Monsant *et al.* (2010) could be the result of increased cation uptake to balance the increased anionic

Table 3. Increase in total fresh mass from the start of experiment and Cd content in roots and shoots of potato plants after 10 d of Cd treatments (0, 2, 25, and 250 nM). Means \pm SE, $n = 5$. Values followed by different letters are significantly different at $P < 0.05$.

Cd concentration [nM]	N form	Fresh mass increase [g]	Root Cd content [$\mu\text{g g}^{-1}(\text{d.m.})$]	Shoot Cd content [$\mu\text{g g}^{-1}(\text{d.m.})$]
0	NO_3^-	27.97 \pm 3.54a	0.06 \pm 0.00b	0.05 \pm 0.01c
0	NH_4^+	7.79 \pm 0.90b	0.05 \pm 0.03b	0.02 \pm 0.00c
2	NO_3^-	24.07 \pm 4.43a	0.30 \pm 0.03b	0.20 \pm 0.02c
2	NH_4^+	7.55 \pm 1.30b	0.20 \pm 0.07b	0.08 \pm 0.02c
25	NO_3^-	33.13 \pm 6.36a	1.69 \pm 0.21b	0.97 \pm 0.08c
25	NH_4^+	7.22 \pm 0.68b	1.27 \pm 0.48b	0.47 \pm 0.07c
250	NO_3^-	36.41 \pm 3.42a	17.83 \pm 1.47a	8.98 \pm 0.75a
250	NH_4^+	8.01 \pm 1.22b	14.27 \pm 3.83a	4.46 \pm 0.77b

charge due to NO_3^- uptake. A general increase in cation uptake in NO_3^- treatments has been reported (Marschner 1995). Furthermore, the membrane transport of cations is stimulated by NO_3^- (Kirkby and Knight 1977, Monsanto *et al.* 2010). In the present study, NO_3^- increased the accumulation of cations, including Cd^{2+} , in the shoot (Table 3) which may have been an effect of transport of NO_3^- to the shoot requiring cations to achieve charge balance (Taiz and Zeiger 2006). This 10-d study confirmed that the content of Ca, Mg, and K increased significantly in both the roots and shoots when the plants were grown in the nutrient solution containing NO_3^- compared with NH_4^+ (data not shown). The N form had a higher impact on Cd accumulation in the shoots than in the roots. The shoots of plants grown in the NH_4^+ solution had about half Cd of those grown in the NO_3^- solution. However, the nutrient content did not decrease equally, for example, Cd in the NH_4^+ -fertilized roots was about 80 % of that in the NO_3^- -fertilized roots whereas the Ca and Mg content was about 10 and 25 %, respectively, of that in the NO_3^- -fertilized roots. Plants fertilized with NO_3^- contain higher content of organic acids than those fertilized with NH_4^+ (Kirkby and Knight 1977). Organic acids may form stable complexes with Cd in vacuoles and also enhance translocation to the xylem and thus to the shoot, as it has been suggested for Zn (Rausser 1999, Monsanto *et al.* 2010).

Differing availability of Cd in the NO_3^- and NH_4^+ containing uptake solutions could be an alternative explanation for the results. The calculated activity values (calculated with *WinHum V*) for the different Cd forms in the solutions (data not shown) showed that the NO_3^- solution contained more free Cd^{2+} but the sum of Cd^{2+} , CdSO_4 , and CdCl^+ activities in the NH_4^+ solution was higher. The activity of different Cd-complexes was rather similar at pH 4.5 and 6.5. So if Cd^{2+} is considered to be the most important Cd form regarding uptake, higher availability of Cd may explain the increased uptake of Cd in NO_3^- -fertilized potato plants. However, some studies have shown that CdSO_4 and CdCl^+ complexes are equally plant-available as free Cd^{2+} (Bingham *et al.* 1986,

McLaughlin *et al.* 1997, 1998, Khoshgoftarmanesh *et al.* 2006).

Considering both NO_3^- and NH_4^+ , total Cd uptake decreased significantly when the N concentration in the pH-buffered uptake solution increased from the lowest to the highest values (Table 2). The MES buffer was able to keep the pH constant during the uptake period of 24 h. The plants supplied with NH_4^+ showed a significantly lower total Cd uptake than the plants supplied with NO_3^- (Table 2).

The potato plants grown for 10 d in nutrient solutions containing different Cd concentrations were significantly smaller when NH_4^+ was used as N source compared with the case when NO_3^- was used (Table 3). The Cd concentrations used in the uptake experiments did not inhibit growth. The low growth seen in the NH_4^+ -fertilized plants (Table 3) was probably due to low pH. The solution was changed every second day but in the absence of any additional buffer, the pH decreased to 3 in the NH_4^+ solution and increased to 7 in the NO_3^- solution.

Irrespective of N form, increased N concentration resulted in lower Cd uptake in the potato plants (Table 2). This has also been observed in field experiments, where potatoes fertilized with 6 g(N) m^{-2} contain a higher amount of Cd in tubers than potatoes fertilized with 16 g(N) m^{-2} (Larsson Jönsson and Asp 2011). Growth normally increases with increasing N fertilization and if the uptake rate of Cd is constant, increased growth rate leads to dilution of Cd in the plant.

We concluded that pH was the dominant factor influencing Cd uptake in the potato plants, irrespective of N form. N form also played an important role as Cd uptake at pH 6.5 was higher in the presence of NO_3^- . Nitrogen form also proved to be important for the accumulation of Cd in the shoots. Cd availability in a field increases with decreasing pH, most often leading to increased Cd uptake in plants. The absence of this increased uptake observed in some studies may be due to physiological effects on uptake and translocation by pH and N factors, as shown here in the hydroponic systems.

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