# The acquisition of cadmium by *Lupinus albus* L., *Lupinus angustifolius* L., and *Lolium multiflorum* Lam.

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Dedicated to Prof. Dr. *Günther Schilling* on his 70th birthday

Accepted 18 September 2000

## Zusammenfassung – Summary

Several plant species mobilize soil phosphate and cations like Fe and Al by excreting organic acid anions and protons. We investigated the Cd acquisition of mobilizing plant species (L. *albus* and L. *angustifolius* cultivars) and *Lolium multiflorum* as a non-mobilizing plant species. Two contrasting soils were utilized: an acid humic podzol and a calcareous loess subsoil. The Cd uptake into the shoots was 5 – 10 times higher in *Lolium multiflorum* compared with the of L. *albus* and L. *angustifolius*. There are several reasons for this result: (i) The root length/shoot weight ratio was 2–3 times higher in ryegrass compared with the lupin cultivars, (ii) the translocation of Cd from the root into the shoot is higher in ryegrass compared with lupin, (iii) the Cd uptake is lower in lupin than in ryegrass in the humic podzol and at P deficiency also in the loess soil.

In the acid podzol, the Cd concentration of the soil solution under lupin was lower than the control (pots without plants), whereas in the calcareous soil, the reverse relation was found. In white and blue lupin the carboxylate efflux, mainly citrate and malate was by a factor of 10–100 higher than in ryegrass. This can lead to a high rate of complexation of Cd by citrate (~ 85%) in the rhizosphere soil solution of lupin. These results seem to support the hypothesis that the Cd uptake of the root is restricted by the complexation of Cd by citrate.

**Key words:** Cd-acquisition / Cd inflow / Cd soil solution concentration / lupinus species / root-shoot ratio / ryegrass

## Das Cadmiumaneignungsvermögen von *Lupinus albus* L., *Lupinus angustifolius* L. und *Lolium multiflorum* Lam.

Mehrere Pflanzenarten mobilisieren Bodenphosphate (P) und Kationen wie Fe und Al durch die Exsudation organischer Anionen und Protonen. Deshalb untersuchten wir das Cd-Aneignungsvermögen von P-, Fe-, Al-mobilisierenden Arten (*Lupinus albus* L., *Lupinus angustifolius* L.) im Vergleich zu einer nicht mobilisierenden Pflanzenart (*Lolium multiflorum* Lam.). Die Pflanzen wuchsen in zwei stark unterschiedlichen Böden (saurer Humuspodsol, karbonathaltiger Lössunterboden). Die Cd-Aufnahme in die Sprosse war bei Weidelgras 5 bis 10 mal höher als bei Blauen bzw. Weißen Lupinen. Dieses Ergebnis hat mehrere Ursachen: 1. Das Wurzellängen/Sprossmasseverhältnis des Weidelgrases ist 2–3 mal größer als das der Lupinenpflanzen. 2. Bei Weidelgras wird ein größerer Teil des aufgenommenen Cd in die Sprosse verlagert. 3. Die Cd-Aufnahme bei Lupinen ist im sauren Boden (Podsol) und bei P-Mangel auch im Kalkboden niedriger als bei Weidelgras.

Während im Podsol die Cd-Konzentration der Bodenlösung unter Lupine geringer war als in der Kontrolle (Gefäße ohne Pflanzen), war sie im Kalkboden höher. Bei den Lupinen war der Efflux organischer Säureanionen, vor allem Citrat und Malat, um den Faktor 10–100 höher als bei Weidelgras. Diese Exsudation kann zu einer hohen Cd-Komplexierung, insbesondere durch Citrat, in der Rhizosphärenbodenlösung führen (~ 85%). Diese Ergebenisse deuten darauf hin, dass das komplexierte Cd von den Wurzeln schlechter aufgenommen wird als das freie Cd.

## 1 Introduction

*Lupinus albus* L. is generally adapted to grow well in soils of low available P (P stressed conditions). Under such conditions, lupin plants form proteoid cluster roots, which release mainly citrate, malate and protons (*Dinkelaker* et al., 1989; *Neumann* et al., 1999). Previous studies have shown that citrate enhanced P mobilization of P in P-fixing soils (*Gerke*, 1992). Consequently, white lupin has a greater ability to acquire P than other plant species (*Römer* and *Gerke*, 1998).

Not only is P mobilized in the proteoid rhizosphere soil but also metal cations such as Al or Fe (*Gerke* et al., 1994) or micronutrient cations e.g. Zn or Cu (*Dinkelaker* et al., 1989; *Meyer* et al., 1995). The effect on the acquisition of toxic heavy metals by root excreted mobilizing agents is not well known. *Mench* and *Martin* (1991) concluded that the higher Cd-solubility in the rhizosphere of *Nicotiana tabacum* L. is responsible for the higher Cd uptake of this plant species, compared with other plant species. The complexation of Al(III) by citrate may be an adaptive mechanism to alleviate Al toxicity in soil (*Bartlett* and *Riego*, 1972; *Gerke*, 1997). The selection of genotypes with a high citrate efflux is therefore beneficial and is a focus in plant breeding (*Fuente* et al., 1997). In the cases of Cd, however, considerable variations of mobilization in soil were documented among organic acid anions and the soil pH (*Naidu* and *Harter*, 1998; *Herms* and *Brümmer*, 1984). To evaluate the effect of mobilization processes in the rhizosphere of white lupin on the acquisition of Cd, we used lupin grown on a humic podzol and a calcareous loess subsoil at different P supply. For comparison we used *Lupinus angustifolius* L., which does not form proteoid root clusters (*Egle* et al., 1999) and *Lolium multiflorum* Lam. showing, even at low P supply, only a small carboxylate efflux (*Gerke*, 1995).

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## 2 Materials and methods

### 2.1 Comparative uptake of Cd by *L. albus, L. angustifolius* and *L. multiflorum* on a humic podzol (experiment 1 and 2)

The plants were cultivated under ambient conditions outdoor for 35 days (exp. 1) or in a climate chamber for 44 days (exp. 2).

Soil characteristics of the humic podzol, with low available P are presented in Table 1. Fourteen days before cultivation, 2 mg Cd/kg soil as $Cd(NO_3)_2$*$4H_2O$ were added to all treatments.

The following cultivars of lupin plants and grass were used:

| No | Cultivar | Country |
|---|---|---|
| *L. albus* L. | | |
| 1 | Amiga | Chile |
| 2 | Ares | France |
| 3 | Borki | Germany |
| 4 | Lublanc | France |
| 5 | Lutop | France |
| 6 | Minori | Germany |
| 7 | Nelly | Hungary |
| 8 | Vladimir | Ukraine |
| 9 | Feli | Germany |
| 10 | Weibit | Germany |
| *L. angustifolius* L. | | |
| 11 | Bordako | Germany |
| 12 | Borweta | Germany |
| *Lolium multiflorum* Lam. | | |
| 13 | Lirasand | Germany |

The PVC pots were filled with 0.7 kg soil and planted with lupin seeds (4 seedlings per pot) and inoculated with Bradyrhizobium lupinum (Jost Ltd., Iserlohn, Germany). The pots sown with grass were fertilized with 210 mg N $kg^{-1}$, as $Ca(NO_3)_2$. In experiment 2, all treatments were additionally provided with 60 mg P*$kg^{-1}$ soil, as $CaHPO_4$. The moisture content of the soil was adjusted to 70% of the maximum water holding capacity and monitored daily. In experiment 2, the following conditions were chosen: day/night regime: 14/10 h, temperature: 21/16°C, relative humidity: 70%, PAR: 240 µE $m^{-2}s^{-1}$. At harvest, shoots and roots were separated and dried at 105°C. Dry matter yield was determined by gravimetry. A proportion of the shoots and roots was digested in $HNO_3$ (65%) at 175°C under pressure. In the solutions resulting from the digestion, Cd concentrations were determined by FAAS. Soil solutions of planted soil and control (without plants) were recovered by column displacement, according to *Adams* (1974). In the soil solution, the pH was determined by potentiometry, Cd concentrations were determined by GFAAS.

### 2.2 Comparative Cd uptake by *Lupinus albus* L. (cv. Vladimir), *Lupinus angustifolius* L. (cv. Bordako) and *Lolium multiflorum* Lam. (cv. Lirasand) at different P supply on a calcareous loess subsoil (experiment 3)

For this experiment we used a soil extremely low in available P and soil organic matter (Table 1). The plants were cultivated in 3 L PVC pots filled with a mixture of 2.5 kg soil and 0.5 kg quartz sand to improve physical parameters. Per kg substrate, 1.5 mg Cd were added as $Cd(NO_3)_2$ and 60 mg K as $K_2SO_4$. Phosphate was added at rates of 33 mg P ($P_0$) or 333 mg P ($P_1$) per kg substrate as $CaHPO_4$. Twelve lupin seedlings or 200 mg grass seed were sown per 3 kg substrate. The plants were placed in a growth chamber under the same conditions, as described in experiment 2.

**Table 1:** Characteristics of the humic podzol (Hodenhagen) and the calcareous loess subsoil (Elliehausen).

**Tabelle 1:** Charakteristika des humosen Podsol (Hodenhagen) und des karbonathaltigen Lössunterbodens (Elliehausen).

| Site | Clay % | Silt % | Sand % | $CaCO_3$ % | pH $CaCl_2$ | Corg % | $P_2O_5$-CAL* mg $kg^{-1}$ | Total-Cd mg $kg^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Hodenhagen | 3 | 6 | 91 | 0 | 5.2 | 3.2 | 8.0 | 0.40 |
| Elliehausen | 16 | 75 | 9 | 9 | 7.7 | 0.3 | 1.4 | 0.01 |

* CAL = Calcium-Acetate-Lactate-Extraction

The plants were harvested 13 days (2 replicates) and 25 days (4 replicates) after sowing. Shoot and root dry matter, Cd content of the shoots and roots were determined, as described for experiment 1 and 2. The root length was determined by the method of *Newman* (1966). The Cd inflow (net uptake rate per unit root length (acc. to *Tinker* and *Nye*, 2000)) was calculated after *Williams* (1948) in two ways: the "total inflow" based on the Cd amounts of roots and shoots and the "shoot inflow" based on the Cd amounts of shoots only, considering the time period between the first and second harvest. A linear root growth was assumed for the period.

### 2.3 Excretion of organic acid anions by *L. albus* L. (cv. Minori and Nelly), *L. angustifolius* L. (cv. Bordako, Borweta) and *Lolium multiflorum* Lam. (cv. Limella) at different P supply

The plants were precultivated in PVC pots (3 L) filled with 4 kg quartz sand. The total substrate was fertilized with 600 mg N as $Ca(NO_3)_2$, 1000 mg K as $K_2SO_4$, 120 mg Mg as $MgSO_4$*$7H_2O$, micronutrients according to Hoagland, as modified by *Schilling* et al. (2000). Per 4 kg substrate 0.1 mg Fe was given as Fe-EDDHA. Additionally, one half of the lupin pots received 12 mg P $kg^{-1}$ as $NaH_2PO_4$ ($P_1$), the other half received no P ($P_0$). In the grass pots 6 mg P $kg^{-1}$ ($P_0$) or 36 mg P $kg^{-1}$ ($P_1$) were added. The difference in the P treatments between lupin and grass is due to the relatively high quantities of P in the lupin seeds. At harvest, sand was removed from the roots by washing. The intact plant roots were dipped into a washing solution of 50 µM $CaCl_2$ remove organic anions that came from root injuries the washing procedure. The washing solution was discharged and the roots were then dipped for further 4 hours into 50 µM $CaCl_2$ solution. These solutions were purified and preconcentrated by solid phase extraction as described by *Gerke* et al. (1994). Organic acids in the eluates were determined by HPLC (Waters Corporation, Milford USA) as described by *Keller* and *Römer* (1998) at the following conditions: column: Merck Polyspher OA KC (length: 300 mm, diameter: 7.8 mm); eluent: 0.015 M $H_2SO_4$; flow rate: 0.3 ml $min^{-1}$, temperature: 52°C, detection: at 210 nm). Root samples were taken to determine the root length, according to *Newman* (1966).

## 3 Results

### 3.1 Dry matter yield, Cd concentration of shoots and roots

The effect of phosphate application and altered experimental conditions on dry matter yield and Cadmium concentration in the shoots and roots are presented in Table 2.

Phosphate application increased shoot dry matter yield in ryegrass by a factor of 2.3 (Table 2). For the lupin cultivars this increase was about a factor of 1.6–1.8 (Table 2).

**Table 2:** Shoot dry matter, Cd concentration of shoots and roots and the proportion of total Cd in shoots of white lupin (10 cv.), of blue lupin (2 cv.) and of ryegrass (1 cv.) at two P levels (–P treatment under open air conditions, +P treatment in climate chamber) on a humic podzol (Hodenhagen).

**Tabelle 2:** Sprosstrockenmasse, Cd-Konzentration in der Spross- und Wurzeltrockenmasse, relativer Anteil der Gesamt-Cd-Menge im Spross von 10 Sorten Weißen Lupinen, 2 Sorten Blauen Lupinen und Weidelgras bei 2 P-Niveaus (–P-Versuch im Freien, +P-Versuch in der Klimakammer) auf einem humosen Podsol (Hodenhagen).

| | Shoot dm g per pot | | Cd-concentration in shoot, mg $kg^{-1}$ | | Cd-concentration in root, mg $kg^{-1}$ | | Cd-proportion of shoot in % | |
|---|---|---|---|---|---|---|---|---|
| | –P | +P | –P | +P | –P | +P | –P | +P |
| *L. albus* | | | | | | | | |
| 1 | 3.0 abc | 4.5 bc | 0.61 a | 0.61 ab | 22 ab | 21 ab | 7 | 6 |
| 2 | 2.9 bc | 5.2 abc | 0.45 bcd | 0.54 abc | 20 ab | 24 a | 6 | 5 |
| 3 | 3.0 abc | 4.5 bc | 0.46 bcd | 0.53 abc | 22 ab | 21 ab | 5 | 5 |
| 4 | 3.0 abc | 4.4 c | 0.48 bcd | 0.52 abc | 21 ab | 20 b | 6 | 6 |
| 5 | 3.2 ab | 4.7 bc | 0.38 d | 0.38 cd | 18 bc | 19 b | 6 | 5 |
| 6 | 2.9 bc | 4.8 abc | 0.53 abc | 0.63 a | 15 c | 19 b | 9 | 8 |
| 7 | 3.5 a | 5.6 ab | 0.40 d | 0.32 d | 18 bc | 18 b | 6 | 4 |
| 8 | 3.1 abc | 5.1 abc | 0.55 ab | 0.61 ab | 19 bc | 22 ab | 7 | 7 |
| 9 | 2.6 c | 4.3 c | 0.42 cd | 0.48 bcd | 26 a | 24 a | 4 | 4 |
| 10 | 3.3 ab | 5.8 a | 0.40 d | 0.45 bcd | 20 bc | 20 b | 5 | 6 |
| *L. angustifolius* | | | | | | | | |
| 11 | 1.8 | 3.0 | 2.15 | 3.54 | 10 | 10 | 29 | 35 |
| 12 | 1.5 | 3.2 | 2.37 | 4.07 | 8 | 12 | 31 | 36 |
| *Lolium multiflorum* | | | | | | | | |
| 13 | 0.8 | 1.8 | 5.62 | 4.42 | 29 | 22 | 24 | 28 |

Different letter combination show significant differences between cultivars at p = 0,05

Irrespective of P supply and other climatic conditions, the Cd concentration in the shoots was approximately 9–12 fold higher in ryegrass compared with white lupin, and approximately 1.2–2.5 times higher as compared with blue lupin. In all 3 plant species, the Cd concentrations in the root were much higher than in the shoot. In white lupin, only 6% of the Cd was apparently translocated to the shoot, whereas in the case of blue lupin it was 29–36% and in the case of ryegrass it was 24–28%. Among the cultivars of white lupin, we found a high genotypic variation.

Table 3 shows the results, with respect to dry matter production and Cd acquisition at two P levels and the same climatic conditions in the calcareous loess subsoil. A higher P availability in soil significantly increased shoot dry matter in ryegrass but not in the lupin cultivars. Also in the calcareous loess subsoil, Cd concentration in the shoot of the lupin cultivars were relatively low (0.14–1.04 mg $kg^{-1}$), compared with ryegrass (3.4–5.2 mg $kg^{-1}$).

## 3.2 Root length/shoot weight ratios (root/shoot-ratio), Cd inflow

The root/shoot-ratio, a measure of the root surface, which provides the shoot with inorganic nutrients, is by a factor of two or more higher in ryegrass compared with the lupin cultivars (Table 4).

**Table 3:** Shoot dry matter, Cd concentration and Cd removal of shoots and roots, as well as relative Cd portion of the total Cd amount of plants in shoots of one cv. of each species at two P levels on a calcareous loess subsoil (Elliehausen).

**Tabelle 3:** Sprosstrockenmasse, Cd-Gehalte der Spross- und Wurzeltrockenmasse sowie die Cd-Entzüge und der relative Cd-Anteil der Sprosse am Gesamt-Cd-Entzug bei je einer Sorte der drei Spezies bei variiertem P-Angebot auf einem Lössunterboden (Elliehausen).

| | Treatment | Shoot dm g | Cd-conc. in shoot mg $kg^{-1}$ | Cd-amount in shoot µg per pot | Cd-conc. in root mg $kg^{-1}$ | Cd-amount in root µg per pot | Cd-proport. of shoot in % |
|---|---|---|---|---|---|---|---|
| First harvest (13 days after sowing) | | | | | | | |
| *L. albus* | –P | 2.9 | 0.11 | 0.32 | 12.0 | 8.6 | 4 |
| | +P | 2.9 | 0.09 | 0.26 | 9.0 | 8.8 | 3 |
| *L. ang.* | –P | 1.4 | 0.71 | 0.96 | 11.6 | 7.8 | 11 |
| | +P | 1.4 | 0.66 | 0.95 | 9.5 | 6.8 | 12 |
| *Lolium m.* | –P | 0.8 | 6.86 | 5.28 | 21.4 | 5.1 | 51 |
| | +P | 0.7 | 4.23 | 3.00 | 12.7 | 4.3 | 41 |
| Second harvest (25 days after sowing) | | | | | | | |
| *L. albus* | –P | 6.7 a | 0.17 a | 1.14 a | 14.0 a | 36 a | 3 |
| | +P | 7.0 a | 0.14 a | 0.98 a | 13.6 a | 36 a | 3 |
| *L. ang.* | –P | 2.6 a | 1.04 a | 2.67 a | 11.6 a | 27 a | 9 |
| | +P | 2.8 a | 0.91 b | 2.51 a | 11.1 a | 27 a | 8 |
| *Lolium m.* | –P | 4.2 a | 5.19 a | 21.60 a | 21.1 aa | 38 a | 36 |
| | +P | 5.6 b | 3.56 b | 19.80 a | 14.5 b | 34 a | 37 |

a > b at P < 0,05; different for each P level within each plant species.

Additionally, the Cd inflow (Table 5) was quite different among plant species. Considering the "total inflow", white lupin showed the lowest inflow, but the differences between the plant species being relatively small. If the Cd taken up by roots and translocated into the shoot is only considered ("shoot inflow"), the differences are much more striking. The Cd inflow of ryegrass was 10 times higher than that of white lupin and by about 5 times higher than that of blue lupin. Phosphate application decreased the Cd inflow in the lupin cultivars, as well as in ryegrass.

## 3.3 pH and Cd concentration of soil solutions

In both soils, the plants affected the pH and Cd concentration in the soil solution (Tables 6 and 7). The pH was always higher in soil with plants than in the control. The Cd solubility was by a factor of 10 or higher in the humic podzol as compared with the calcareous loess subsoil. In the podzol, Cd solubility was higher in the control treatment than the planted soil. In the calcareous loess subsoil, Cd solubility was higher under lupins, compared with the control.

The pH of the soil solution of the humic podzol vs. the Cd soil sulution concentration resulted in an exponential relationship between these two parameters (Figure 1). The variability of this relation suggests that beside the pH effects, other soil parameters of the rhizosphere are involved in the modification of the Cd solubility.

**Table 4:** Root length and root length/shoot weight ratios at the 2nd harvest depending on the P supply (loess subsoil).
**Tabelle 4:** Wurzellängen und Wurzellängen/Sprossgewicht – Verhältnisse zur 2. Ernte in Abhängigkeit vom P-Angebot (Lössunterboden).

| | Treatment | Root length cm per pot | rel. | Root/shoot-ratio cm $mg^{-1}$ |
|---|---|---|---|---|
| *L. albus* | –P | 23720 | 100 a | 3.6 a |
| | +P | 33108 | 140 b | 4.8 b |
| *L. ang.* | –P | 14468 | 100 a | 5.6 a |
| | +P | 16025 | 110 a | 5.8 a |
| *Lolium m.* | –P | 42897 | 100 a | 10.3 a |
| | +P | 80048 | 187 b | 14.4 b |

a > b at $p < 0,05$; different for each P level and for each plant species.

**Table 5:** Cd inflow of roots between 13th and 25th day based on the quantity of Cd taken up by root and shoot ("total inflow") or based on the quantity of Cd taken up by root and translocated into the shoot ("shoot inflow").
**Tabelle 5:** Cd-Nettoaufnahmeraten der Wurzeln im Zeitraum zwischen dem 13. und dem 25. Tag auf Basis der Cd-Mengen in der Gesamtpflanze („Gesamtaufnahmerate") oder auf Basis der Cd-Mengen, die von der Wurzel aufgenommen und in den Spross verlagert wurden („Sprossaufnahmerate).

| | Treatment | "total inflow" (A) | "shoot inflow" (B) |
|---|---|---|---|
| | | $10^{-18}$ mol $cm^{-1}$ $s^{-1}$ | |
| *L. albus* | –P | 18.8 b | 0.55 d |
| | +P | 13.6 c | 0.35 a |
| *L. ang.* | –P | 20.8 b | 1.68 c |
| | +P | 19.3 b | 1.38 c |
| *Lolium m.* | –P | 24.1 a | 7.97 a |
| | +P | 14.3 c | 5.12 b |

a > b at $p < 0,05$

## 3.4 Organic acid exudation of roots

The efflux of organic acid anions by cultivars of the three plant species as affected by the P status is shown in Table 8. In general, the efflux of organic acid anions by lupin cultivars was much higher by a factor of 10–100 fold than that of ryegrass. In the three cultivars Minori, Nelly and Bordako, the citrate efflux was higher in the –P treatment than in the +P treatment.

# 4 Discussion

From the ecological point of view, the uptake of Cd in plant parts, which are harvested, is of central importance. Regardless to the P supply, the three plant species showed wide variations in shoot Cd content and its uptake in both soils. The Cd content of ryegrass was 28 and 5, respectively, times higher than the corresponding Cd concentration of white and blue lupin. In agreement with this result, *Gerke* et al. (1999) found that the graminaceous species *Triticum aestivum* L. showed a much higher Cd concentration in shoots compared with white lupin (cv. Jet Neuf). The concentration of mineral nutrients in the shoots is influenced by several factors of the nutrient acquisition process such as root/shoot-ratio, inflow and the duration of uptake (*Claassen* and *Jungk*, 1984). The experimental data show that the root/shoot-ratio was about a factor of 2.3 times higher in ryegrass than in the lupin species which, however, does not explain the differences in Cd shoot concentrations (Tab. 5). This could be attributed to differences between ryegrass and lupin in Cd translocation from the root to the shoot. On the basis of nutrient uptake data into shoot only, the calculated values of Cd inflow ("shoot inflow", Table 5) were much higher in ryegrass compared with lupin. Therefore we believe that the "total Cd inflow" (Table 5) based on the Cd uptake into the roots **and** shoots is of minor relevance to explain the variations of Cd accumulation of shoots. Apart from that, the

**Table 6:** pH value and Cd concentration of soil solutions from soil without plants (control) and from soil with lupin or ryegrass on a humic podzol, 44 days after sowing.
**Tabelle 6:** pH-Wert und Cd-Konzentration in den Bodenlösungen 44 Tage nach der Saat in den Gefäßen ohne Pflanzen (Kontrolle) und im Boden unter Lupinen und Weidelgras (humoser Sandboden).

| | N° | pH | µg Cd $l^{-1}$ |
|---|---|---|---|
| control | | 4.75 f | 34 b |
| *L. albus* | 1 | 5.15 e | 20 d |
| | 2 | 5.18 e | 43 a |
| | 3 | 5.41 cd | 27 c |
| | 4 | 5.14 e | 26 c |
| | 5 | 5.30 de | 21 d |
| | 6 | 5.23 e | 16 d |
| | 7 | 5.71 b | 5 e |
| | 8 | 5.18 e | 15 d |
| | 9 | 5.49 c | 10 e |
| | 10 | 5.43 cd | 17 d |
| *L. ang.* | 11 | 5.86 a | 3.4 f |
| | 12 | 5.90 a | 2.0 f |
| *Lolium m.* | | 5.64 | n.d.* |

a > b at $p < 0,05$
* n.d.: not determined

**Table 7:** pH value and Cd concentration of soil solutions from soil without plants (control) and from soil with lupin or ryegrass on a calcareous loess subsoil, 25 days after sowing.
**Tabelle 7:** pH-Wert und Cd-Konzentration in den Bodenlösungen 25 Tage nach der Saat in den Gefäßen ohne Pflanzen (Kontrolle) und im Boden unter Lupinen und Weidelgras im Lössunterboden.

| | Treatment | pH | µg Cd $l^{-1}$ |
|---|---|---|---|
| control | –P | 7.8 b | 1.68 b |
| | +P | 7.8 b | 1.18 b |
| *L. albus* | –P | 8.1 a | 3.4 a |
| | +P | 8.1 a | 3.5 a |
| *L. ang.* | –P | 8.1 a | 3.1 a |
| | +P | 8.1 a | 3.2 a |
| *Lolium m.* | –P | 8.1 a | 1.3 b |
| | +P | 8.2 a | 1.3 b |

a > b at $p < 0,05$

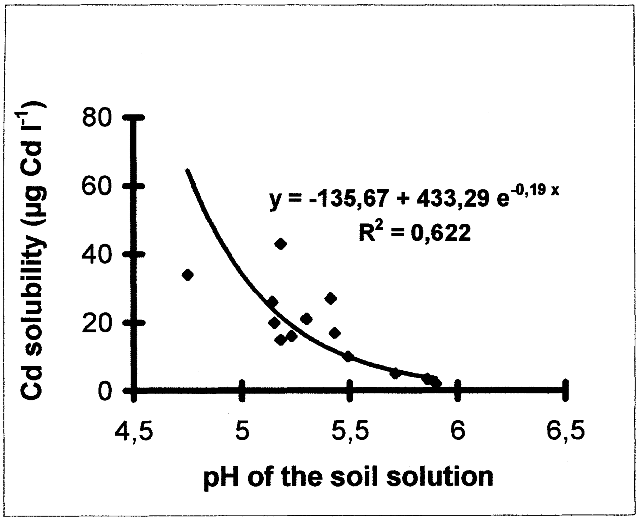


**Figure 1:** Relationship between pH of the soil solution and the Cd solubility in a humic podzol, 44 days after sowing.
**Abbildung 1:** Beziehung zwischen ph-Wert und Cd-Konzentration in den Bodenlösungen, 44 Tage nach der Aussaat (humoser Podsol).

**Table 8:** Organic acid exudation of roots by *L. albus*, *L. angustifolius*, and *Lolium multifl.*, 21 days after sowing with P (+P) or without P (–P) supply (substrate: quartz sand).
**Tabelle 8:** Wurzelexsudation organischer Säuren von *L. albus*, *L. angustifolius* und *Lolium multifl.*, 21 Tage nach der Saat bei variiertem P-Angebot (Substrat Quarzsand).

| | *L. albus* | | | | *L. angustifolius* | | | | *Lolium multifl.* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | cv. Minori | | cv. Nelly | | cv. Borweta[1] | | cv. Bordako | | cv. Lirasand | |
| | –P | +P | –P | +P | –P | +P | –P | +P | –P | +P |
| | nmol $cm^{-1}$ $h^{-1}$ | | | | | | | | | |
| Citrate | 0.32 a | 0.15 b | 0.45 a | 0.26 b | 4.27 a | 5.23 a | 6.80 a | 3.64 b | 0.024 | 0.037 |
| Malate | 0.43 | 0.87 | 1.18 | 1.65 | 1.75 | 7.02 | 7.19 | 5.23 | 0.009 | 0.043 |
| Succinate | 0.27 | 0.23 | 0.26 | 0.28 | 0.71 | 0.57 | 0.73 | 0.40 | 0.008 | 0.013 |
| Formiate | 0.67 | 0.59 | 0.67 | 0.63 | 1.66 | 1.70 | 2.31 | 1.11 | n.b. | n.b. |
| Acetate | 0.61 | 0.68 | 0.64 | 0.97 | 1.08 | 1.53 | 1.83 | 1.14 | 0.060 | 0.059 |

a > b at $p < 0{,}05$; for the comparison between –P and +P for citrate
The standard deviation was between 20 and 30%
1) Some plants showed antracnose (Colletotrichum ssp.) symptoms
n.b. not dedected

determination of heavy metals in the roots is still questionable. Because of methodological problems, it is, for instance, difficult to distinguish between Fe adsorbed at the root surface, Fe located in the apparent free space or Fe taken up into the cortex cells. According to *Bienfait* et al. (1985) and *Strasser* and *Römheld* (1998), most of the Fe in the root is located in an extracellular fraction and may contribute to Cd adsorption (*Qi* et al., 1998). Recently *Yu* and *Tang* (2000) showed that L. *angustifolius* plants have a greater proportion of tightly bound apoplastic Cd than *Pisum sativum* L. plants. If Cd replaces Ca in the cell walls of the cortex cells, the concentration of Cd, which is truly taken up by the root symplast, will be overestimated. In general, the CEC of roots of dicotyledonous species is higher than that of monocotyledonous plant species. Previous studies by *Grauer* and *Horst* (1992) have shown that the CEC of *Lupinus luteus* L. was about 50% higher than that of *Secale cereale* L.. Consequently the apoplastic Cd fraction may be greater in lupin than in grass, explaining the high apparent retention of Cd in lupin roots. It is possible that a great but unknown proportion of Cd in the lupin roots has not passed the Casparian strip but may be adsorbed in the root apoplast. If this explanation is valid, it may not be the translocation from the root into the shoot, which differs between the lupin cultivars and ryegrass, but it would be primarily the uptake of Cd into the root cells of white and blue lupin cultivars which is restricted.

In which way do mobilization processes affect the acquistion of Cd by ryegrass and lupin cultivars? Lupin plants were inoculated by rhizobia and without N-fertilizer. From $N_2$-fixation a reduced pH in the rhizosphere soil was expected. But in both soils the rhizosphere pH was higher in the presence of lupin plants than the control treatment (without plants). This discrepancy is difficult to explain and is also in contradiction to *Dinkelaker* et al. (1989), who found that in the proteoid rhizosphere the pH was reduced. It may be that the loess soil contained sufficient reactive $CaCO_3$, which neutralized the released protons. Or the protons may be neutralized during the percolation process collecting soil solution after the method of *Adams* (1974). Regarding the humic podzol *Gerke* (1995) found also in some cases an increase in pH as a result of organic acid excretion and discussed the exchange of $OH^-$ with organic anions like citrate. He used the same method as *Adams* (1974). The question emerges how selective this method works. It is not clear how thick the soil layer around the root was. It may be that it was partially thicker than 1 mm and that soil not influenced by exudates was included (*Li* et al., 1997). But for an assessment of Cd solubility in the collected soil solution it has to be emphasized that with increasing pH, the decrease of Cd solubility in soil can be expected (*Brümmer* et al., 1986). The results obtained on calcareous soil revealed that Cd solubility in the presence of lupin was higher than in soil without plants (Tab. 7). This result can be interpreted on the basis of the higher solubility of Cd-citrate complexes as compared with free Cd in the calcareous soil. The concentration of citrate in the proteoid rhizosphere soil solution of white lupin can exceed 1 mM (*Gerke* et al. 1999). Under these conditions, more than 85% of the Cd is complexed by citrate if the stability constants given by *Martell* and *Smith* (1989) are used.

In the humic podzol, however the Cd concentration of soil solution was lower in soil planted with lupin as compared with the control. This result is in agreement with *Gerke* et al. (1999). *Naidu* and *Harter* (1998) also found that the direction and extent of the effect of organic acid anions strongly depends on the soil used. In acid soils, such as the humic podzol, carboxylates may be bound to Fe(Al)-surfaces and may increase the negative surface charge as well as Cd sorption. In calcareous soils, where the Cd sorption capacity is high, the Cd complexation by citrate may increase the Cd solubility.

## Acknowledgments

The project was partially funded by the *Deutsche Forschungsgemeinschaft.*

## 5 References

*Adams, F.* (1974): Soil solution. In Carson, E. W.: The plant root and its environment. University Press of Virginia, USA.

*Bartlett, R.* and *Riego, R.* (1972): Effect of chelation on the toxicity of aluminium. Plant and Soil 37, 419–423.

*Bienfait, H. F., v. d. Briel, W.,* and *Mesland-Mul, N. T.* (1985): Free Space Iron Pools in Roots: generation and mobilization. Plant Physiol. 78, 596–600.

*Brümmer, G., Gerth, J.,* and *Herms, U.* (1986): Heavy metal species, mobility and availability in soils. Z. Pflanzenernähr. Bodenkd. 149, 382–398.

*Claassen, N.* and *Jungk, A.* (1984): Bedeutung der Kaliumaufnahmerate, Wurzelwachstum und Wurzelhaaren für das Kaliumaneignungsvermögen verschiedener Pflanzenarten. Z. Pflanzenernähr. Bodenkd. 147, 267–289.

*Dinkelaker, B., Römheld, V.,* and *Marschner, H.* (1989): Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*L. albus* L.). Plant Cell Environ. 12, 285–292.

*Egle, K., Römer, W., Gerke, J.,* and *Keller, H.* (1999): The influence of phosphorus nutrition on the organic acids exudation of the roots of three lupin species. 9th Intern. Lupin Conference 20–24 June 1999, Klink/Müritz, Germany, in press.

*Fuente, J. M. de la, Ramirez-Rodriguez, V., Cabrera-Ponce, J. L.,* and *Herrera-Estrella, L.* (1997): Aluminum Tolerance in Transgenic Plants by Alteration of Citrate Synthesis. Science 276, 1566–1568.

*Gerke, J.* (1992): Phosphate, aluminum and iron in the soil solution of three different soils in relation to varying concentration of citric acid. Z. Pflanzenernähr. Bodenkd. 155, 339–343.

*Gerke, J.* (1995): Chemische Prozesse der Nährstoffmobilisierung in der Rhizosphäre und ihre Bedeutung für den Übergang vom Boden in die Pflanze. Habilitationsschrift, Cuvillier Verlag, Göttingen.

*Gerke, J.* (1997): Aluminum and iron(III) species in the soil solution including organic complexes with citrate and humic substances. Z. Pflanzenernähr. Bodenkd. 160, 427–432.

*Gerke, J., Römer, W.,* and *Jungk, A.* (1994): The excretion of citric and malic acid by proteoid roots of *Lupinus albus* L.: effects on soil solution concentration of phosphate, iron and aluminium in the proteoid rhizosphere in samples of an oxisol and a luvisol. Z. Pflanzenernähr. Bodenkd. 157, 289–294.

*Gerke, J., Wessel, E., Egle, K.,* and *Römer, W.* (1999): Heavy metal acquisition by white lupin and yellow lupin. 9th International Lupin Conference, 20–24 June 1999, Klink/Müritz, Germany, in press.

*Grauer, U. E.* and *Horst, W. J.* (1992): Modelling cation amelioration of aluminum phytotoxicity. Soil Sci. Soc. Am. J. 56, 166–172.

*Herms, U.* and *Brümmer, G.* (1984): Einflussgrößen der Schwermetalllöslichkeit und -bindung in Böden. Z. Pflanzenernähr. Bodenkd. 147, 400–414.

*Keller, H.* and *Römer, W.* (1998): Ausscheidung organischer Säuren bei Spinat in Abhängigkeit von der P-Ernährung und deren Einfluss auf die Löslichkeit von Cu, Zn und Cd im Boden, 187–195. In Merbach, W. (Ed.): 8. Borkheider Seminar zur Ökophysiologie des Wurzelraumes. B. G. Teubner Verlagsgesellschaft, Stuttgart, Leipzig.

*Li, M., Shinano, T.*, and *Tadano, T.* (1997): Distribution of exudates of lupin roots in the rhizosphere under phosphorus deficient conditions, Soil. Sci. Plant Nutr. 43, 237–245.

*Martell, A. E.* and *Smith, R. M.* (1989): Critical stability constants. Plenum Press, New York.

*Mench, M.* and *Martin, E.* (1991): Mobilization of cadmium and other metals from two soils by root exudates of *Zea mays* L., *Nicotiana tabacum* L and *Nicotiana rustica* L. Plant and Soil 132, 187–196.

*Meyer, U., Gerke, J.,* and *Römer, W.* (1995): Einfluss von Citrat auf die Mobilisierung und Aufnahme von Zn und Cu bei Weidelgras und Weißer Lupine. In Merbach, W. (Ed.): 5. Borkheider Seminar zur Ökophysiologie des Wurzelraumes. B. G. Teubner Verlagsgesellschaft, Stuttgart, Leipzig, 137–143.

*Naidu, R.* and Harter, R. (1998): Effect of different organic ligands on cadmium sorption by and extractability from soil. Soil Sci. Am. J. 62, 644–650.

*Neumann, G., Massonneau, A., Martinoia, E.,* and *Römheld, V.* (1999): Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. Planta 208, 273–382.

*Newman, E. J.* (1966): A method of estimating the total length of root in a sample. J. Appl. Ecol. 3, 139–145.

*Qi, L, Chun-rong, Z., Huai-man, C.,* and *Ying-xu, C.* (1998): Transformation of cadmium species in rhizosphere. Acta Pedologica Sinica, Vol. 35, No. 4, 461–467.

*Römer, W.* and *Gerke, J.* (1998): Phosphat- und Eisenmobilisierung durch Wurzelexsudate bei *Lupinus albus* L.. In Wink M. (Ed.): Lupinen in Forschung und Praxis, 15–25.

*Schilling, G., Kerschberger, M., Kummer, K. F.,* and *Peschke, H.* (2000): Pflanzenernährung und Düngung. Verlag Eugen Ulmer, Stuttgart.

*Strasser, O.* and *Römheld, V.* (1998): Bedeutung des apoplastischen Eisens in den Wurzeln von Tomate und Gerste in Bodenkulturen für die Ernährung der Pflanze. In Merbach, W. (Ed.): 8. Borkheider Seminar zur Ökophysiologie des Wurzelraumes. B. G. Teubner Verlagsgesellschaft, Stuttgart, Leipzig, 80–86.

*Tinker, P. B.* and *Nye, P. H.* (2000): Solute Movement in the Rhizosphere. Oxford Univ. Press, New York, Oxford.

*Williams, R. F.* (1948): The effects of phosphorus supply on the rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in gramineous plants. Aust. J. Sci. Res. (B) 1, 333–361.

*Yu, Q.* and *Tang, C.* (2000): Lupin and pea differ in root cell wall buffering capacity and fractionation of apoplastic Calcium. J. Plant Nutr. 23, 529–539.

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