Federal Agricultural Research Centre

Institute of Plant Nutrition and Soil Science

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Factors affecting the availability of uranium in soils

Published as: Landbauforschung Völkenrode Sonderheft 278

Braunschweig
Federal Agricultural Research Centre (FAL)
2005

Sonderheft 278
Special Issue

FAL
Bundesforschungsanstalt für Landwirtschaft

Landbauforschung
Völkenrode
FAL Agricultural Research

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Bibliographic information published by Die Deutsche Bibliothek
Die Deutsche Bibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data is available in the Internet at http://dnb.ddb.de .

2005

Landbauforschung Völkenrode - FAL Agricultural Research
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Preis / Price: 8 €

ISSN 0376-0723
ISBN 3-86576-002-3

In memory of my mother.

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1 Introduction

International attention has been paid to the danger represented by the hundreds of tons of depleted uranium (DU) discharged into the environment via ammunition during the wars in Iraq in 1990 and in Kosovo in 1998. During the combats inhalation and wounds were the main pathways of direct body contamination. A direct relation between uranium (U) and the so called "Desert War Syndrome" of Gulf War is contested, but many of the symptoms associated to chemical and radiological U toxicity have been described for American soldiers and native people of Iraq (Anon. V, 1999; Anon. VI, 2000; Anon. IX, 2000). The creeping endangerment by permanent contamination via the food chain during the period after strike operations could be considerably higher. Penetrating projectiles will be destroyed after the collision in small pieces and dust. These finest particles content a high amount of DU and they will deposit on the vegetation, on soil surface and in water-bodies. But lots of projectiles do not hit their targets and they are missed. They are posing the second threat: after penetration of the soil surface they are resting in different soil horizons, corroding over longer periods and the ingredients being included in the nutrient cycle bit by bit (Anon. V, 1999; Anon. VI, 2000; Erikson et al., 1990; Stoetzel et al., 1983; UNEP II, 2001).

Areas contaminated with U exist in all the countries where its technology has been developed. High U levels in the environment like for other heavy metals and radionuclides can become a hazard for the organisms life. U can effect as a chemical toxin directly or by detrimental irradiation. As a toxic substance it can produce structural and functional defects mainly in kidneys. U can be accumulated in lungs and bones from where it irradiates to internal organs. Ionizing radiation can produce alterations in the deoxyribonucleic acid increasing the risk of cancer or changes in reproductive cells (Anon. XII, 2000; Durakoviae, 1999; Ribera et al., 1996; WHO, 2001; Zajiv, 2000).

Uranium has been mined during 200 years to be used as the yellow color in pottery and jewellery, as a catalyst in chemical processes, as a steel alloying constituent, and also for medical applications (Anon. II, 2000; Anon. XIII, 2000). It was during the World War II when the energy arisen from the fission of the nucleus of ^{235}U was recognized as a powerful energy source to prepare the atomic bombs. Nowadays, more than 16 % of the world electric energy is produced by nuclear power plants requiring U, and it is still considered the longest term energy source available (Anon. VII, 2000; Anon. XIII, 2000). U leaves the mine as the oxide UO_2. Natural U is mainly formed by the ^{238}U and ^{235}U isotopes, accounting for 99.3 and 0.7 % respectively. Differences in mass between

both isotopes let them be separated and makes it possible to increase or enrich the proportion of ^{235}U, to 3 - 4 % for fuel or more than 90 % for bomb-grade U. During the enrichment process a by-product reduced to 0.2 - 0.3 % in ^{235}U, called DU is produced. At first facilities tried to include DU in consumers products, mixed with other metals, in fertilizers or counterweights for planes because of its high density (19.05 g cm^{-3}). But people denied to use them because of the public concern on the hazard of their radioactivity. So thousands of tons of the produced by-product DU are storing as a waste in special repositories in the USA and Europe, becoming a great environmental problem (Anon. III, 2000; Anon. IV, 2000; Anon. X, 1999; Anon. XIII, 2000; IAEA, 2001).

DU can be converted to the metallic form, some of its properties as its high density were found useful for the production of weaponry. Projectiles made of U metal are able to withstand the high firing velocities of modern weapons. U based shells fired from tanks and aircrafts are able to penetrate and destroy heavily armed tanks at greater ranges than other types of anti-tank shells. Other U metal advantage is its pyrophoricity, this makes that finely divided particles present spontaneous ignition and ammunitions can burn in the air after hitting a target. Officially in the seventies the Department of Energy of the United States started the investigation to use DU metal for military purposes. It was also considered the best strategic replacement for tungsten in ammunitions, most of which was imported from China (Erikson et al., 1990; NRL, 2000).

As it was mentioned above two situations defined appear when these ammunitions are used, and they have different consequences in the final quantity and quality of DU in soils after impacts. Projectiles that hit against a hard target, normally will burn. A powder arises during this process whose oxidation depends on the temperatures reached and the size/frequency distribution of the dispersed particles. At high temperature they are ceramized making them less soluble, and can be transported in the air. When the penetrators impact against a soft target they can be broken, or stay relatively intact and shallow buried in the surface. Erikson et al. (1990) reviewed the factors that affect the oxidation of DU metal in the ground. They mentioned that the oxidation rates of U metal were more rapid in contact with water vapor at 25 ?C (0.024 mg cm^{-2} U) than in dry air (0.000026 mg cm^{-2} U), CO_2 (0.000001 mg cm^{-2} U) and O_2 and water vapor (0.00041 mg cm^{-2} U). The reaction rates depend on the temperature and titanium base alloy of the ammunitions reduces them till 16 times (Erikson et al., 1990; Hanson, 1974; Stoetzel et al., 1983).

Much information about U behavior can be obtained from studies of models in laboratory, but it is very difficult to predict what will happen with the contaminated soils in the field. For that reason,

Fernald at Ohio (USA), a processing center of U metal and UF_4 between 1951 and 1989, was chosen by the Department of Energy of the United States to evaluate different technologies for large scale remediation of U contaminated soils. Widespread U was estimated at 2 - 4 millions m^3 of soil with above regulatory standards of 52 mg kg^{-1} (Elless et al., 1997a; Elless and Lee, 1998). U concentration at firing sites could exceed 10,000 mg kg^{-1} and U contamination of the underlying groundwater was also found. During the investigation many different sources of U were identified at this place. They found that 80 % of the U was in the more oxidized form (VI) (Mason et al., 1997). Also coarse fractions of intact and fragmented ammunition projectiles were found in soils and catch sand boxes. Many of them showed evidence of the oxidation of their surfaces (yellow or green coatings), dehydrated and the hydrated schoepite were identified by X ray diffraction analysis. Schoepite ($UO_2(OH)_2$-nH_2O) is a form commonly found at DU contaminated sites, it is produced by weathering and considered to have a slow solubility (Buck et al., 1996; Morris et al., 1996).

Many cleaning techniques for remediation were tested. Acid or alkaline solutions as used in mines were the most successful to reduce total and available U forms in soils (Duff et al., 1998; Francis and Dodge, 1998). Among the organic substances citric acid increased U solubility in soils, and the U uptake by plants up to 1,000 times (Ebbs et al., 1998b; Huang et al., 1998). Mobilization of U during the clean up of soils creates the problem of leaching of U to the water bodies. A radical procedure after contamination accidents would be to remove the layer of contaminated soil completely as it was done in Holland in 1992, after the crash of a plane containing a counterweight made of DU (Anon. V, 1999; Layton and Armstrong, 1994). It could then be transported to be cleaned or stored as radioactive waste in controlled sites. This technique is expensive, so it can not be used for large areas. Different amendments have also been tested to immobilize heavy metals in situ (Anon. VIII, 2001; Basta et al., 2001). Phosphorus was found to be successful to stop quickly their availability (Bolan et al., 2003), but normally large amounts are required making it also an expensive practice. Such immobilization procedures may not be recommended for highly radioactive elements because they would not resolve the problem of continuous radiation from the soil.

Great concern about DU contamination appeared immediately after the finish of the Kosovo war. In 2001 a mission of the United Nations Environmental Programme (UNEP) was sent to take samples of soil, water, vegetation and air in the areas identified as more affected. UNEP report

minimized the hurtful effect of dispersed U (UNEP I, 2001; UNEP II, 2001) but recommended to start investigations on the ammunitions left in the soils. After cleaning operations only few projectiles had been picked up from the ground surface, most of them stayed buried in soils at different depths. Erikson et al. (1990) calculated for an intact ammunition several milligrams of UO_2 would be oxidized in a few hours, and then they would be quickly oxidized to UO_3 in the form of uranyl ion (UO_2^{2+}) which is very soluble and easily leached by percolation water. The selection of a decontamination procedure, will depend on the source term and the concentration of the contaminant, the soil characteristics and also the future use of the land.

It is known that U can impact on soil properties and the potential yield of plants. Meyer et al. (1998b) found in a substrate contaminated with schoepite that respiration, the most sensible parameter, was affected at 500 mg kg^{-1}, but wood decomposition was decreased only at 25,000 mg kg^{-1} U. Meyer and McLendon (1997) found a decrease in plant biomass, fecundity and long term survivability at the highest level (25,000 mg kg^{-1}). In the same experiment growing stimulation was described for one of the three species tested at the lower levels of contamination (50 and 500 mg kg^{-1} DU) (Meyer et al., 1998a).

This work is proposed to get understanding in the behavior of uranium in the environment, and specifically to generate knowledge about soil factors that affect its availability and could be managed in a contaminated soil to produce food without health risk.

Objectives of the research:

- Review of the recent knowledge on uranium in the environment with special view to soil protection and food security.
- Investigation of the effects of chemical soil factors on the plant availability of U in contaminated soils.
- Discussion and evaluation of agronomic measures to reduce the transfer of U from contaminated soil via growing plants into the food chain.

2 Review on uranium in the environment

The release of U to the environment represents a potential risk of chemical and radiological toxicity to human health (Schnug et al., 2003). Therefore, in mines and processing nuclear industries much precaution is required for the workers to reduce exposure to U and in general the civil population had been maintained locally isolated from those activities. With the U liberation by virtue of DU contamination of large areas due to ammunition tests and acts of war (e.g. in Iraq and Kosovo) a new general behavior of U in the environment is presented, emphasis is devoted to contaminated soils, as a possible path of U to water and plants, increasing the risk of entering to the food chain.

2.1 Physical and chemical properties of uranium

Uranium is the heaviest metal in nature. It has 14 natural isotopes, being the most abundant: ^{238}U (99.28 %) and ^{235}U (0.71 %). All of them are radioactive, this means that their nuclei emit radiation particles to achieve a stable configuration. Uranium is member of two decay series, the uranium and the actinium series, being a stable isotope of lead the last element of them. The energy released during the disintegration of these nuclei warms the interior of the earth and determines the process that moves the continents and the elements cycles in the earth crust (Anon. XIII, 2000; Cowart and Burnett, 1994; Harmsen and Haan, 1980; Luckey, 1991). These decay sequences are given in table 2.1. Radioactivity produced by U mainly consists of alpha particles formed by two protons and two neutrons, a helium nucleus with a net charge $^{2+}$, they have a high energy but they do not penetrate more than 1 mm of tissues normally (Luckey, 1991; Ribera et al., 1996). Besides the ^{235}U isotope is fissile, this means its nucleus can be split spontaneously releasing an enormous amount of energy in chain reactions (Anon. XIII, 2000).

The radioactivity is a function of the number of atoms and their probabilities of decay in a time unit, the specific activity of each isotope is expressed in the International System Units, as Bq g^{-1} or MBq kg^{-1}, where 1 Bq (becquerel) = 1dps (decay per second). The activity of ^{235}U (78,400 Bq g^{-1}) is higher than the ^{238}U activity (12,445 Bq g^{-1}), this determines that activity of enriched U > natural U > depleted U (Anon. XI, 2000; Luckey, 1991).

Tab. 2.1: Uranium and actinium decay series (modified from Eisenbud, 1987; cited by Luckey, 1991)

Atom	Uranium family					Actinium family				
			Rays					Rays		
	Isotope	Half-life	Alpha	Beta	Gamma	Isotope	Half-life	Alpha	Beta	Gamma
U	238	4.5×10^9 y	*	-	*	235	7×10^8 y	*	-	*
	234	2.5×10^5 y	*	-	*					
Th	234	24 d	-	*	*	231	25.6 h	-	*	*
	230	8×10^4 y	*	-	*	227	18.2 d	*	-	*
Pa	234	1.2 m	-	*	*	231	3.3×10^4 y	*	-	*
Ac						227	21.6 y	*	*	-
Ra	226	1622 y	*	-	*	223	11.4 d	*	-	*
Fr						223	22 m	-	*	*
Rn	222	3.8 d	*	-	-	219	4.0 s	*	-	*
At	218	2 s	*	-	-	215	10^{-4} s	*	-	-
Po	218	3 m	*	-	-	215	10^{-3} s	*	-	-
	214	10^{-4} s	*	-	-	211	0.5 s	*	-	-
	210	138 d	*	-	-					
Bi	214	19.7 m	-	*	-	211	2.2 m	*	-	*
	210	5 d	-	*	-					
Tl	210	1.3 m	-	*	*	207	4.8 m	-	*	-
	206	4.2 m	-	*	-					
Pb	214	268 m	-	*	*	211	36 m	-	*	*
	210	22 y	-	*	*					
	206	Stable	-			207	Stable			

Half life (t ½) is the time in which the half of the atomic nucleus of a radioactive element is disintegrated.
Isotopes are elements with the same number of protons and electrons, but with different number of neutrons, ^{234}U and ^{238}U are isotopes. Nuclides have different number of electrons and protons, so they have different properties, ^{210}Po and ^{210}Pb are nuclides.

The ^{235}U and ^{238}U isotopes have similar chemical properties. The metal U is chemically very active and can react with most elements except the rare gases. It forms oxides with air, producing either UO_2 or U_3O_8. At room temperature, humidity is the principal cause of oxidation. In fragmented state (chips, powder or turnings) the metal U becomes pyrophoric (Erikson et al., 1990; Ribera et al., 1996).

Uranium IV and VI are the oxidation states typically observed in the environment. Under strongly reducing conditions U occurs in the tetravalent oxidation state: U(IV). It hydrolyzes in solution to form monomolecular hydroxo-complexes, such as $U(OH)_n^{4-n}$. In reduced ground waters U(IV) is complex bounded by sulphate, chloride and phosphate, by fluoride at pH values less than 4 and also by organic humic and fulvic acids. Still under low oxygen contents the U is quickly oxidized to the hexavalent state (VI) and the uranyl ion (UO_2^{+2}) is formed in aqueous solution. U forms mono- and poly-nuclear hydrolysis products. At pH 5 and higher the major species in solution is $(UO_2)_3(OH)_5^+$. Aqueous uranyl forms complexes with halogens and with most oxo-anions, such as NO_3^-, SO_4^{-2}, ClO_4^-, PO_4^{3-}, HPO_4^{-2} and CO_3^{2-}, and carboxylic acids as well. The formation of uranyl carbonate complexes is favored by high pH, high uranyl ion activities and high partial CO_2 pressures. Uranyl phosphates may be important in systems with pH from 6 to 9, when the ratio of P/C is larger than 10. Sulphates, F^- and possibly Cl^- complexes are important when their concentrations are high. Organic acids, which contains several functional groups per molecule also form strong complexes with U (Erikson et al., 1990; Fellows et al., 1998; Harmsen and Haan, 1980; Mortvedt, 1994).

The high affinity of U to oxo-anion groups, to the silanol group and carboxylic acid groups, causes that U is strongly adsorbed in soils and that U(IV) is stronger retained than U(VI). The sorption of U has been shown to be rapid (90 % occurs in a few hours), it is strongest in the low concentration range and is favored by a high pH (Willet and Bond, 1995). Adsorption of U on goethite in the range of pH 4.5 to 6.5 grows while $(UO_2)_3(OH)_5^+$ increases and then it decreases because of formation of carbonates. Similar tendency occurs with hematite, ferric amorphous oxy-hydroxide and other Fe oxides, and also to smectite by edge co-ordination reactions with increasing pH. Uranium is normally accumulated in organic horizons of mineral soils, but the role of humic substances is not clear (Barnett et al., 2000; Duff and Amrhein, 1996; Esteves da Silva et al., 1996; McKinley et al., 1995; Tipping, 1996; Willet and Bond, 1995; Zachara and McKinley, 1993; Zhang et al., 1997).

If the activity of UO_2^{2+} in solution was risen above the equilibrium value it would precipitate providing control over its solubility. Both HPO_4^{2-} and PO_4^{3-} may form precipitates with UO_2^{2+}, however concentration of total U in soil solution may be higher than calculated activities, due to hydrolysis and the presence of organic and inorganic ligands which would avoid the precipitation or co-precipitation in solid phase of U. Water with high carbonate content may significantly increase the solubility of U in natural waters, in particular at pH 6 to 7 or higher. Complexing of uranyl by fulvic acids has been suggested to facilitate the transport of U in the groundwater, although the competence of $CaCO_3$ can diminish its effectiveness (Duff and Amrhein, 1996; Fellows et al., 1998; Harmsen and Haan, 1980; Elless and Lee, 1998; Mortvedt, 1994).

Under reducing conductions, uranyl is reduced to the U(IV) form, which tends to precipitate in clay layers, calcite or phosphates. U(IV) oxides are less soluble than U(VI).

U(VI) can also be reduced by micro-organisms. U enrichment in very rich organic soils, is believed to be the result of UO_2^{2+} transport, adsorption, or complexation by humic materials, and reduction of dissolved U(VI) to U(IV), followed by the formation of uraninite (UO_2) (Casas et al., 1998; Fellows et al., 1998; Harmsen and Haan, 1980).

2.2 Geochemistry of uranium

U is widely distributed in nature. U occurs in most rocks in concentrations of 2-4 mg kg^{-1} and is as common in the earth's crust as tin, tungsten and molybdenum. Geologically the main natural sources of U are hydrothermal veins, sedimentary rocks, and pyritic conglomerate beds of Precambrian age. Many rocks have higher values than the average. Felsic rocks, such as granite, usually contain more U than mafic rocks, such as basalt and dunite. It also is found in phosphate rock, lignite, and monazite sands at levels that can be commercially recovered. Uranium can also precipitate as organic and inorganic carbonates. The main geographical locations of minerals containing U are Australia, Canada, Russia, South Africa and the USA. U is an important constituent of about 155 minerals. The most common U minerals in ore deposits include uraninite (UO_2) and coffinite ($USiO_4$) in reduced environments, and carnotite [$K_2(UO_2)_2(VO_4)_2 \cdot 3H_2O$)], tyuyamunite [$Ca(UO_2)_2(VO_4)_2 \cdot 2.5\text{-}8H_2O$], autunite [$Ca(UO_2)_2(PO_4)_2 \cdot 10\text{-}12H_2O$)], uranophane [$Ca(UO_2)_2Si_2O_7 \cdot 6H_2O$] in oxidized zones (Alloway, 1995; Cowart and Burnett, 1994; Erikson et al., 1990; Kabata Pendias and Pendias, 1984; Pais and Jones, 1997; Ribera et al., 1996).

Water is the main transport vector for U. In confined aquifers, U occurs in the tetravalent state, whereas in unconfined or surface water its state is hexavalent. U content in natural water generally is in the range 0.1-10 ?g L^{-1}. The soluble products of rock weathering are transported into the groundwater and eventually into the rivers and then the ocean. The actual content of sea water is specified at a level of 3 ?g L^{-1} U (Cowart and Burnett, 1994; Pais and Jones, 1997). Reductive precipitation of U(IV) in anoxic marine sediments is globally the most significant sink for dissolved U. This is the reason because higher aquatic plants generally contain greater U concentration than terrestrial plants (Fellows et al., 1998). U content of surface waters varies rather within narrow limits, possibly as a result of adsorption to bottom sediments or due to precipitation. The high capacity of sediments for fixing U is used to remove dissolved U from mine waste waters in treatment ponds. Contaminated sediments persisting in closed environments (pool, pond, lake) can be re-suspended by water movements and mobilization (Amrhein et al., 1993; Batson et al., 1996; Ribera et al., 1996; Fellows et al., 1998).

2.3 Uranium in soils

Uranium total values from 0.1 to 11 mg kg^{-1} are considered to be the typical soil background (Alloway, 1995; Harmsen and Haan, 1980; Kabata Pendias and Pendias, 1984; Pais and Jones, 1997). In the table 2.2 reference data from surveys of soils performed in USA and China are presented:

Tab. 2.2: Uranium concentration in samples of the upper layer of soils in the United States, Alaska and China (Xu et al., 1993)

Country	Geometric mean	Geometric deviation	Arithmetic mean	Range
			[mg kg^{-1}]	
United States	2.30	1.73	2.70	0.29 - 11
Alaska	2.30	1.86	2.80	< 0.22 - 45
China	2.79	1.50	3.03	0.42 - 21.1

The average value of the earth crust is 1.7 mg kg^{-1} (Xu et al., 1993). During the weathering process, U can be leached or accumulated in some horizons of the soil profile. Jones (1991) did not find significant differences in U content between grouping of soils (Alfisols vs Mollisols), moisture regime or degree of weathering for surface soils in South Illinois. Evans et al. (1997) suggested that acid leaching could be important in the mobility and distribution of U and other radionuclides in the

profile of 12 typic Haplorthods (Spodosols). In tropical environments, U is retained in the red soils, because it has a great affinity for iron minerals (Evans et al., 1997; Yoshida et al., 1998; Xu et al., 1993). U is mainly accumulated in the A horizons of soils. High U contents have been described in very rich organic matter soils coming from the sequestration and reducing of U (Fellows et al., 1998; Tipping, 1996).

Anthropogenic U contamination of soils is caused by the different steps of mining, uses and disposal of U containing products or by-products. Although U is decreased in tailings of mines, their U content can be 10 to 100 folds higher than in natural soils (Dressen and Marple, 1979; Dressen and Williams, 1982). In the San Joaquin Valley, USA, the irrigation with U containing water increased the U levels in the upper centimeters of agricultural soils (Amrhein et al., 1993). The use of natural P fertilizers in agriculture is also a possible source of U enrichment in soils. Average concentrations of U in such fertilizers are by a factor of 100 higher than that of soils, mainly depending on the origin of the rock phosphates (Heiland, 1986; Karhunen and Vermeulen, 2000; Lehr, 1980; Schnug et al., 1996).

The use of DU metal in ammunitions, both to replace tungsten and to reduce existing stockpiles of UF_6, brought a new type of contamination, whereby oxidized U particles or metal pieces are released to the environment. Several hundreds tons of U in ammunitions were released in the Iraq and Kosovo wars. Although the UNEP mission in 2001 did not detect U contamination all over the soil surface, values till 400 mg kg^{-1} have been mentioned 30 cm around the metal projectiles buried in the ground (UNEP II, 2001).

The concentration values considered hazardous depend on the source term of the pollutant and the future use of the contaminated soil. Conditions that increase the rate of formation of soluble complexes and decrease the rate of sorption of labile U in soil are expected to enhance its mobility. Water in contaminated soils plays a key role because U can be mobilized and transported both in vertical direction (groundwater) and at the surface (Layton and Armstrong, 1994).

2.4 Uranium in plants

There exists a wide variation among plant species in the ability to uptake U from soils via roots or the translocation within the plant (Dressen and Williams, 1982; Gulati et al., 1980; Lakshmanan and Venkateswarlu, 1988; Morishima et al., 1976; Morishima et al., 1977; Schnug et al., 1996;

Singh, 1997; Van Netten and Morley, 1981; Van Netten and Morley, 1982; Van Netten, 1983; Whicker et al., 1999).

Very few is known about the responsible mechanisms. It has been proposed that the U distribution in plants followed a pattern similar to Ca, which is accumulated more in older than in younger leaves (Mortvedt, 1994). As a typical value for U in plant 0.04 mg kg^{-1}, with a maximum of 0.4 mg kg^{-1} is reported by Vidal Perez et al. (1998) and Dressen and Marple (1979); or 0.01 mg kg^{-1} reported by Pais and Jones (1997). There are also great differences among organs in the same plant. In general the following ranking for concentrations seems to occur: roots > leaves > fruits/grains (Gulati et al., 1980, Lakshmanan and Venkateswarlu, 1988; Morishima et al., 1976; Morishima et al., 1977; Singh, 1997). In all the cases the U content in plant increases with higher values of U in soils or water (Lakshmanan and Venkateswarlu, 1988; Morishima et al., 1976; Morishima et al., 1977; Singh, 1997). Some species have developed the ability to tolerate high concentration of heavy metals. Such plants can be used as bio-indicators in geo-chemical explorations or, more practical, to extract those contaminants from polluted soils. Much information was generated for phytoremediation of soil contaminated with Cs, after the Chernobyl explosion in 1986, but for U the knowledge is comparatively scarce. Many members of the *Brassica* species are classified as hyper-accumulators, and hydroponically grown sunflowers (*Helianthus annuus*) have been reported as a means to remove U from contaminated waters (Chaney et al., 1997; Salt et al., 1995).

In view of the known health risk provoked by U, its accumulation in edible parts of plants and the possible entering into the food chain must be prevented certainly. The sampling and analysis of representative diets in New York City showed that in particular two categories of plant origin foodstuffs could contribute to U uptake via ingestion: foods consisting of vegetables, potatoes or beans, and bakery products (as well as cereals, rice and grain). These products accounted for 50 % of total U intakes (Linsalata, 1994). A Japanese work, directly relating U content in diet and soil, pointed out that leafy vegetables generally contain higher concentration of radionuclides than fruits and grains, so some foods are potentially more dangerous than others (Morishima et al., 1977). In an epidemiological study, Yukawa et al. (1999) estimated the annual effective dose of internal radiation from food obtained in a high natural radiation area to be 30 times higher for high background U levels (6 to 28 mg kg^{-1} U) than controls (0.8 to 2.7 mg kg^{-1} U).

The concentration ratio (CR), defined as concentration of total U in plant tissue/concentration of total U in soil, determines the transfer probability from soil to human diet. It has been used as a measure to estimate the health risk. The CR data base for natural series of radionuclides is relatively limited compared with data obtained for fallout produced radionuclides. Substantial variability of the CR was reported for U, the overall geometric means of CR for U was 0.0045 (Mortvedt, 1994; Sheppard and Evenden, 1988) depending on soil properties and crop species.

CR data are not linearly related to the radionuclides concentration of soils, generally they decrease with increases in substrate at low-level concentrations, above this range the CR values asymptotically decrease till a value that does not change with further increases in soil concentration (Sheppard and Evenden, 1988). This threshold was reported to be at 20 mg kg^{-1} of total U for soils derived from the Precambrian shield (Sheppard and Sheppard, 1985), and at 8 mg kg^{-1} for a marsh soil (Martinez Aguirre et al., 1997).

Sheppard and Evenden (1992b) and Sheppard (1998) determined that the amounts of soil adhering to plants could also be a source of contamination, as they could range from 0.03–4 g dry soil per kg dry plant for washed material, up to as high as 450 g dry soil per kg dry plant for short annual crops and forage crops.

2.5 Hazardous effects of uranium

There are two different health hazards produced by U. The first is the short-time chemical toxicity of soluble compounds like UO_2^{2+}, by influencing directly the function of internal organs, especially of the kidneys. The second is in the long time influence, because of the effect of the short distance alpha radiation of the U staying in the body, which could cause the development of cancer and genetic defects by deformation of chromosomes (Schott, 2003).

Uranium penetrates into the organism by different paths: pulmonary (inhalation), ingestion (gastro-intestinal system) or trans-cutaneous (skin and wounds) (Anon. XII, 2000; Ribera et al., 1996; WHO, 2001). Its fate is then determined by the U compound solubility and U valence.

Only a minor part (10 %) of the uranium that enters the animal or human organisms by the respiratory route, will be retained in the bronchial tree, the highest proportion (till 65 %) will attain to the gastro-intestinal tract and the rest will be exhaled. Insoluble compounds that remain in the lungs, will affect the alveolar tissue by irradiating and soluble compounds will then be transferred to the extra-cellular fluids and entail the diffusion of U throughout the organism.

The rate of ingested radionuclide absorption across the mammalian gastro-intestinal tract into the blood depends on a number of typical biological characteristics: age, feeding state, the presence or absence of complexing agents, oxidants or reductants respectively on chemical form and oxidation state of the contaminant.

Only 12 % of all ingested U (soluble and insoluble) will be absorbed by the intestine and transport into the blood over 6 days. The remainder will be excreted in faeces (WHO, 2001; Durakoviae, 1999; Zajiv, 2000).

In the organism, blood is the principal carrier of U to target organs. From the U absorbed in blood, approximately 67 % is filtered by kidneys and excreted in urine during the next 24 hours (WHO, 2001).

Insoluble U oxides do not seem to be a significant toxic risk when applied through the skin (WHO, 2001).

Long term ingestion of U by humans leads to progressive or irreversible kidney injury. Both structural and functional kidney's damages are known. UO_2^{2+} ions depress glomerular function, tubular secretion of organic anions and re-absorption of filtered glucose in the proximal tubule. In addition damaging U effects on liver and the whole nervous system were described. U can cross the blood-brain barrier, placenta, foetus and can also be in milk (Durakoviae, 1999; Ribera et al., 1996).

Bone is the principal storage site for U in the body. Uranyl ions are complexed by phosphate ions on the surface of bone crystals, releasing Ca ions. U deposited in bones and other organs will be subsequently released back to the blood stream. Ionizing radiation affects the molecules directly or indirectly through the formation of free radicals, it engenders a cascade of reactions that can affect cells throughout the organisms. U damage produced at molecular level determines modifications in cellular functions such as permeability, mobility, protein synthesis, and mitotic cycles. Macromolecules like deoxyribonucleic acid, proteins and polypeptides are particularly affected. These damages take place either in the cytoplasm or in the nucleus. Their effects are then of somatic nature (affecting the non reproductive part of the cell) or of genetic nature (affecting its reproductive part). The radiation doses to osteo-progenitors cells (stem cells), living bone surfaces and the bone marrow are usually considered to be of greater biological significance than doses absorbed by other tissues, due to the fact that they can produce bone sarkomas and leukaemias. In the case of genetic cellular damage it may result in cell death or higher mutation rates. These mutations contribute to the cellular transformation phenomena, precursory to cancerous colonies.

Anomalies and malformations can be produced by radiation on the embryo or foetus during pregnancy. Mutagenesis results mostly from high doses (Durakoviae, 1999; Ribera et al., 1996).

Critical values for U have been established by the World Health Organization (WHO). It is accepted a daily intake of 0.5 µg kg^{-1} body weight for soluble U or 5 µg kg^{-1} body weight for insoluble forms. The radiation effective dose for general population is < 1 mSv $year^{-1}$, and for workers < 20 mSv $year^{-1}$ during 5 years, or 50 mSv in one year. The U.S.EPA proposed a drinking water standard for U of 20 µg L^{-1} based on kidney damage (Layton and Armstrong, 1994). Limit for U in water has been established at 2 µg L^{-1} by WHO (2001).

2.6 Hormesis caused by uranium

The word hormesis came from the same root as hormone. Like homeopathy recognizes, every agent may be stimulatory in small amounts and inhibitory in large amounts. A hormetic doses is any dose which produces bio-positive effects (Anon I, 2001; Brucer, 2002; Calabrese and Baldwin, 1998; Freney et al., 1965; Javad Mortazavi, 2002; Luckey, 1991; Luckey, 1998; Reijnders, 2002; Sheppard et al., 1987). Biological examples of hormesis include the stimulation produced at low doses by dietary antibiotics, drugs, toxic metals as Hg, Pb or Se, hormones, vitamins, essential trace minerals or UV light action upon skin.

Large doses of ionizing radiation (acute or chronic) are harmful for life. However, it was not always believed that radiation could be a great danger. At the beginning of the last century it was very common to get a dose of radiation for better health, through going to a spa or drinking radioactive water (Javad Mortazavi, 2002). Later, damages by excessive use of these products, accidents with X-ray and the consequences of the atomic bombs directed the attention to the dangerous aspects of nuclear energy and radiation (Luckey, 1998; Javad Mortazavi, 2002; Parsons, 2001).

Luckey (1991) reviewed the results of experiments following large and small whole-dose body exposures to ionizing radiation. He found that results were diametrically opposite and postulated it would be due to a hormetic effect. Natural environmental or background radiation varies locally, and all the regional population is exposed in equal measure. Few is known about the effect of sub-environmental radiation or the application of low levels of ionizing radiation. Hormetic doses vary from ambient to 1,000 times background levels (Luckey, 1991). Experimental results with protozoans indicate that both external and internal sources of ionizing radiation increased the

growth rates of these organisms and they were decreased in a direct proportion to the reduction in radiation (Luckey, 1991). But information about the benefits of radiation coming from non vertebrates or lower vertebrates can not be extrapolated to humans, because organisms rising in the evolutionary scale decrease their radio-resistance (Fellows et al., 1998; Ribera et al.,1996).

After the World War II, agricultural chemists experimented with antibiotics, Luckey (1991) and colleagues added them to the livestock diet, expecting they would suppress the intestinal flora, decreasing the animals growth. Contrarily they caused growth. Since then, the addition of antibiotics to animal food is a common practice. Intensive reviews were performed in the following years, founding that very often toxins have a positive effect on the health of organisms at low doses, producing the so called chemical hormesis (Anon. I, 2001; Calabrese and Baldwin, 1998; Calabrese, 2004; Luckey, 1998). Calabrese and Baldwin (1998) found that the chemical hormetic dose-response range is usually within a 10–fold range. However, stimulatory effects had been reported over dose ranges of two or more order of magnitude, depending on the agent, endpoint, and model assessed. Majority of low dose stimulation are 30 to 60 % greater than the controls.

Hormetic responses are observed in numerous species from a broad range of taxonomic groups including microbes, plants and animals. And for a broad range of biological end points that involve growth, survival, longevity, reproduction, and also numerous metabolic and physiological response, e.g. metallothionein synthesis, DNA synthesis, RNA synthesis, mitosis, oxygen consumption, altered hepatic loci, photosynthesis rate, tissue regeneration, immune response, stress protein synthesis, germination of seeds (Calabrese and Baldwin, 1998).

In the figure 2.1 a dose-response or ß-curve for chemical or ionizing radiation hormesis is presented. The range of stimulatory doses is between background and the no observed adverse effect level (NOAEL), at the transition between bio-positive to bio-negative effects.

Several decades ago, it was also thought that U could stimulate the growth of plants. Most of the research was done in agricultural plants, adding U salts to fertilizers and nutrient solutions. The results were very variable, and the same concentration that stimulated the growth of a specie could cause death to others. However, they got the general accord that low U salt concentration had a positive effect on the growth of plants and it could be necessary as a nutrient, but overcoming a certain concentration, it had detrimental effect on the superior plants. Recently, Meyer et al. (1998a) also found hormesis in grass plants cultivated in pots contaminated with DU.

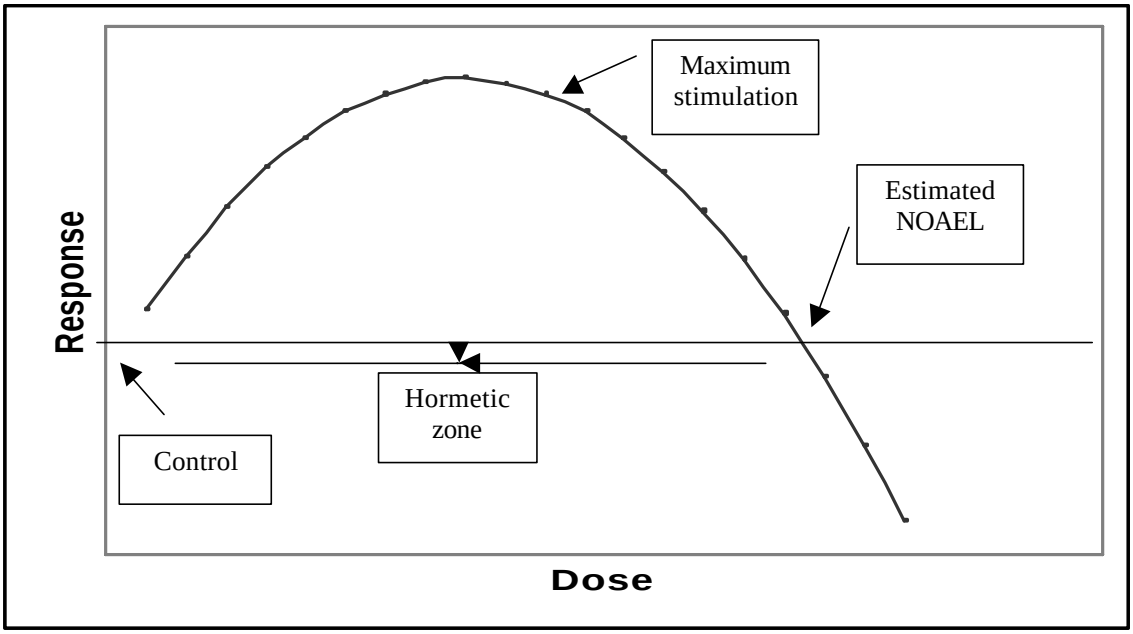


Fig. 2.1: Dose- response curve (or ß-curve) depicting characteristics of the chemical and ionizing radiation hormetic zone (following http://accesstoenergy.com, 2004)
NOAEL: no adverse effect level

3 Materials and methods

3.1 Preparation of the contamination material

For the experimental tests it was necessary to synthesize an artificial form of contamination material. Metallic form of U is pyrophoric; it could easily burn in contact with the air during its milling or manipulation during the laboratory procedures. Because of this, its use is not allowed in routine works. For this reason the contamination of the substrates in the experiments was carried out with the green modification of finely pulverized U_3O_8. It was prepared from $UO_2(NO_3)_2 \cdot 6H_2O$, by precipitation with ammonia to $(NH_4)_2U_2O_7$, then by incineration at 450?C to UO_3 and finally at 720?C to U_3O_8 following Fleckenstein (1972). This form was expected to have a more similar behavior to the environmentally oxidized DU metal than the uranyl-nitrate, a very toxic form, which is commonly used in ecological experiments.

3.2 Greenhouse experiment

A greenhouse experiment to evaluate how selected soil factors will affect U uptake by plants from contaminated soils was installed. The test substrates derived from the same site with two different managed soils: a grassland soil and a forest soil. To simulate different levels of soil fertility substrate from topsoil and subsoil were used.

The experimental design is shown in table 3.1. Uranium treatments were the following soil contamination levels: 0 (control), 250, 500 and 1,000 mg kg^{-1} U. Two different fertilization treatments were tested in the course of the experiment. First, when the experiment started (part I), $CaHPO_4$ was given to half of the pots (control and P-fertilization) to simulate deficient and sufficient P nutrition of plants.

At the beginning of the 2nd experimental year in each treatment two pots were fertilized with lime in the form of $CaCO_3$ additional (part II). To achieve several cuttings during the experiment, a perennial grass (*Lolium perenne*) was chosen as test plant.

Tab. 3.1: Experimental design of the pot experiment (Braunschweig, from Sept. 2000 to Oct. 2001)

Soil substrates	U contamination [mg kg^{-1}]	Fertilizer application (1)	
		Part I	Part II
		$CaHPO_4$ 4 replications	$CaCO_3$ 2 replications
Grassland, topsoil…… GT	0	0	0
			+
		+	0
			+
Grassland, subsoil…..…. GS	250	0	0
			+
		+	0
			+
Forest, topsoil…… FT	500	0	0
			+
		+	0
			+
Forest, subsoil…… FS	1,000	0	0
			+
		+	0
			+

(1) Fertilizers addition: 6.8 g $CaHPO_4$ per kg of soil (0.15 P %), 5 g $CaCO_3$ per kg of soil

Soil characteristics

Four different soil substrates were used in the experiment. They were taken from the experimental site of the FAL. Plants and roots or litter were removed before taking the soil material. The grassland soil was classified as Podzolic Brownearth[1] in the German Classification, the topsoil was the upper layer: 0-25 cm and the subsoil was the subsurface layer: 25-50 cm. The forest soil was classified as Podzol[2]. The extraction occurred from the same depths as in the grassland site. The soil samples collected in the field were maintained in a plastic container to avoid excessive loss of humidity till the preparation of the pots. The characteristics of the used soil substrates are presented in the table 3.2.

[1] FAO Classification: Dystric Cambisol/Orthic Luvisol
[2] FAO Classification: Leptic Podzol

Tab. 3.2: Soil characteristics before treatment at the start of the experiment

Sample site	Soil type	German Classification	Sample depth [cm]		pH	C_t [%]	N_t [mg g^{-1}]	P_{CAL} [mg kg^{-1}]	K_{CAL} [mg kg^{-1}]
Grassland	silty-loamy-sand	Podzolic Brownearth	0-25	(GT)	5.9	1.2	1.0	108	261
			25-50	(GS)	4.8	0.5	0.4	20	246
Forest	sandy	Podzol	0-25	(FT)	3.5	2.0	1.1	48	25
			25-50	(FS)	3.8	0.6	0.4	20	5

pH: determined in a soil:solution ratio 1: 2.5 0.01 M $CaCl_2$
C_t: total carbon determined by a LECO carbon analyzer
N_t: total nitrogen determined by Kjeldahl
P, K: extractable phosphorus and potassium by calcium acetic lactate (according to Schüller, 1969)

Uranium contamination treatments

In the following table the amounts of U added to each pot to reach the decided contamination levels are presented. The amounts were calculated for 1,500 g of dry soil per pot.

Tab. 3.3: Calculations of U treatment per pot

Contamination level	U [g]	U_3O_8 [g]
uncontaminated (control)	0	0
250 g kg^{-1}	0.375	0.442
500 g kg^{-1}	0.750	0.884
1,000 g kg^{-1}	1.500	1.768

Fertilization treatments

$CaHPO_4$ addition to increase P levels in the substrates was calculated according to Seaman et al. (2001b) with the aim to reduce U leaching in columns. The estimated amount for 1.5 kg of dry soil was 10.2 g $CaHPO_4$. Calculations for liming to regulate and increase the pH value of the soil substrates were made on the basis of published recommendations to preserve the soil fertility (referred as Ca amounts), following Kerschberger and Franke (2001). Based on the described calculation that 6 tons of $CaCO_3$ per hectare are required to increase the pH values from 4 to 6 in soils like FS, the calculated amount for 1.5 kg of dry soil was 3 g of Ca (7.5 g $CaCO_3$).

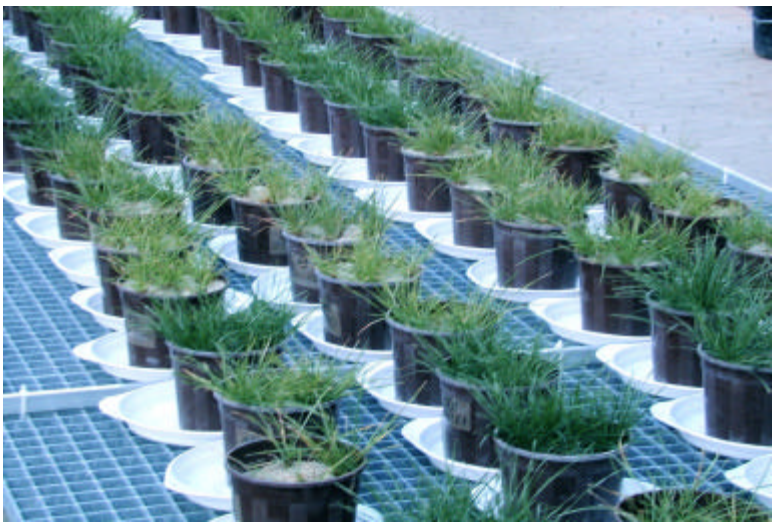
Testing plant

The selected testing plant was *Lolium perenne*, a perennial ryegrass, variety “Lisuna”. In agricultural production it is used in mixtures under maize. It builds up a thick sward with the leaves base near the ground. Remarkable features of this variety are its persistence, and good health, the

very good mildew and rust resistance, which were important under the climatic conditions in the greenhouse over the winter. Because of its relatively low growth rate this variety has been selected in order to minimize the production of contaminated plant material.

Experimental procedure

Plastic pots of about 2 L capacity with compatible collecting pans were used (pic. 3.1). A round filter paper and over it a plastic lid with cuts to let water go through, were placed at the bottom of the pot. 1,500 g of soil (DW basis) was filled into each pot. Maximum moisture holding capacity (MHC_{max}) and soil moisture content were determined for each soil substrate. Soil moisture was adjusted at 70 % MHC_{max} by wetting with deionized water. For Part I of the experiment, the P fertilized pots were prepared by mixing the weighted portions of $CaHPO_4$ with the soil by hand. For the artificial U contamination, the prepared soil material for each separate pot was filled in a plastic bag, the corresponding U oxide powder portion was added, the pouch was closed and then carefully mixed by shaking it. After filling the pot, five holes (1 cm depth) at regular distance one from each other were made in the top of the soil. Five grass seeds were sown in each hole and then covered with soil. Finally, 200 g of washed quartz pieces were added on top to avoid direct contact of the growing grass leaves with the contaminated soil and to protect the soil surface against silting. The prepared pots were weighted for control and placed on plastic dishes from where the irrigation water could be taken up by growing plants. For the part II, quartz pieces were removed from each



Pic. 3.1: General view of the pot experiment

pot and weighted portions of $CaCO_3$ were added to the top of the pots. Then quartz pieces were placed again.

Conditions during the experiment

At the beginning of the experiment deionized water was added to the dishes to provide as much water as plants required. After some weeks the pots were found to be continuously humid, then the water was given on the top to avoid an oxygen deficiency of the roots. Periodical determinations of the changes in pot weights considering the contribution of growing roots and shoots were carried out, and the consumed water was supplemented. All pots were placed outdoor to get natural air and sun light during the day, every time the weather conditions allowed it. During the winter season, when danger of frost appeared (from December 2000 to April 2001), pots were transferred to a heated greenhouse, with temperature control (13-15 ?C) and artificial light additional (from 7 to 9 a.m. and 4 to 6 p.m.). Temperatures and humidity of the pots adjacency were registered daily. For the sufficient plant nutrition a commercial liquid fertilizer[3] was periodically applied. By the time enough plant dry matter had been developed to make the chemical determinations, plants were harvested. The grass plants were cut at 1-1.5 cm above the quartz surface in the pots.

The experiment lasted 407 days, the chronology of sampling dates and the fertilizer applications are presented in table 3.4.

Sampling

Plant samples were taken for the determination of total dry weight and total content of U, P and Ca in all cuts. At the end of the experiment soil pH was directly measured in pots by a direct soil probe[4], and soil samples were taken for determining pH in the laboratory too, as well as to quantify the extractable U and P. Roots were visually estimated at the end of the experiment.

[3] "Fischers SUPER", Ingredients: 1.6 % nitrate-N, 1.6% ammonium-N, 2.8 % carbamide-N, 6 % P_2O_5 water soluble P, 6 % K_2O water soluble and microelements: B, Cu, Fe, Mo and Zn.
[4] pH-meter 351i with a single-rod measuring cell "Sen Tix SP", WTW GmbH & Co. KG; http://www.WTW.com

Tab. 3.4: Chronology of cuts and fertilizer applications performed during the pot experiment

	Date	Duration of the experiment [S days]	Fertilizer applications*
		Part I	
Starting	14-09-00	0	0
Cuts			
			1
1st	07-11-00	56	
			2
2nd	01-02-01	140	
			1
3rd	29-03-01	196	
			2
4th	21-06-01	280	
			3
		Part II	
5th	02-08-01	322	
			2
6th	30-08-01	350	
			2
7th	26-10-01	407	

*number of fertilizations between each 2 cuts

3.3 Analytical methods

3.3.1 Soil analysis

Soil samples from the pot experiment were air dried and sieved (mesh size 2 mm).

All chemicals used were of “pro analysis” grade.

Solution extractions and procedures

?? DTPA-extraction solution (Lindsay and Norvell, 1978):

0.005 M diethylene-triamine pentacetic acid (DTPA)

0.01 M calcium chloride ($CaCl_2$)

0.1 M triethanolamine (TEA)

The pH was adjusted at 7.3 with hydrochloric acid (HCl). For a soil:solution ratio 1:2, 10 g of dry soil and 20 ml of the extracting solution were shaken for 2 hours at 27 rpm. The suspensions were filtered through Schleicher & Schuell N 593 ½ filter paper.

?? AAAcEDTA-extraction solution (Lakanen and Erviö, 1971):

0.5 M ammonium acetate (CH_3COONH_4)

0.5 M acetic acid (CH_3COOH)

0.02 M sodium ethylene-diamine-tetracetic acid (Na_2EDTA)

The pH was adjusted to 4.65 with CH_3COOH or CH_3COONH_4. For a soil:solution ratio 1:10, 5 g of dry soil and 50 ml of the extracting solution were shaken in polyethylene bottles for 1 hour at 27 rpm. The suspensions were filtered through Schleicher & Schuell N 593 ½ filter paper.

The high concentration of organic compounds in the extracts resulted in instabilities of the calibration of the ICP-QMS (Inductively Coupled Plasma-Quadrupole Mass Spectrometry) system. To avoid this, the following treatment was applied to the filtrated solutions: 10 ml of the AAAcEDTA filtrate and the whole extracted solution of the DTPA (6-15 ml) extraction were transferred to a ceramic crucible and evaporated on a sand bed at 200 ?C to dryness and then ashed in a muffle furnace at 550 ?C over night. After cooling, 0.2 ml of concentrated HNO_3 were added to each crucible and evaporated, ashes were then dissolved with 10 ml of 2.5 % HNO_3 for ICP-QMS determination.

For the determination of P in soil two methods were used: CAL (Schüller, 1969) in the characterization of the control substrates, and water soluble P (Van der Paauw, 1971) at the end of the experiment. P was determined by photometry using a Perkin-Elmer 550SE UV/VIS.

3.3.2 Plant analysis

Plant material was prepared for yield determination (DW) by drying at 80 °C for 22 h in a drying cabinet. For the following chemical analyses the plant material was dried at 105 °C for other 5 hours and afterwards milled, to get homogeneous samples. 200 mg of the prepared plant material were digested with 4 ml 65 % HNO_3 + 1 ml 30 % H_2O_2 in closed vessels under pressure in a microwave furnace. The digested material was diluted to 10 ml with bi-distilled water.

ICP-QMS analysis

For the ICP-QMS measurements a further dilution with 2 % HNO_3, containing 1 µg L^{-1} of Rh as an internal standard was carried out. All dilutions were controlled by means of a balance. The mass calibration of the ICP-QMS was performed by standard solutions in the range of 0 to 40 µg L^{-1} of ^{238}U. The detection limit for U in plant and soil extraction digests were determined at 10 ng L^{-1} U. No enrichment steps were required for the concentration range in question. U in soil and U, P, and

Ca in plant were analyzed directly by means of ICP-QMS employing a VG Elemental Plasma Quad 3. Six standards and two samples for quality control were placed at the beginning, in the middle and the end of the daily measurements.

3.4 Statistical methods

Results were statistically analyzed by linear regression analysis, standard error of the mean, analysis of variance, Tukey and LSD tests, using the statistical software-package COHORT.

3.5 Safety measures

1. Uranium and its compounds are hazardous materials, both from the viewpoint of radioactivity and chemical toxicity. Although the total amount of uranium employed in the experiments was below the threshold values from which on German law requests permission by government authorities, a number of safety measures were employed.
2. For incorporation of U the soil had to be dry in order to reach homogeneity of the mixture within shortest time possible. Dry soil and U_3O_8 powder were mixed in bags of extra strong polyethylene, sealed and stored until set up of the experiment.
3. Immediately after opening the bags the soil was wetted with deionized water in order to avoid any dust development.
4. Staff always wore disposable dust protection masks, overalls and latex gloves when attending the experiment or during sampling and sample preparation.
5. All plant material harvested was spent in analysis.
6. The soil surfaces of the vegetation pots were covered with a layer of quartz sand to avoid dust development from dry surfaces.
7. Filtration residues from soil extractions were collected and disposed according to the regulations for low radioactive wastes.
8. After the experiment the soil of each individual pot was transferred to a polyethylene bag, sealed after air-drying and stored in a refrigerator for further experiments.

4 Results

4.1 Effect of uranium application on growth of Lolium perenne

High U levels in soil can be toxic for plants, but some authors have also mentioned the possibility of stimulatory effects on plant growth if U is added at low doses, called “hormesis”. This research work aimed to investigate the effect of U oxide application to soil at doses between those found by Meyer et al. (2004) as stimulatory and toxic, and similar to those found in contaminated soils with depleted uranium (UNEP, 2001).

Effect of soil substrate qualities on yield of Lolium perenne

To investigate to what extent the soil substrate quality determined the grass growth during the experiment, the cumulative yields of aboveground mass for the non contaminated substrates (controls) were calculated based on the dry matter yield per cut and thus compared (fig. 4.1).

As expected grassland topsoil (higher pH, organic matter and initial fertility) showed the highest, and forest subsoil (lower pH, organic matter and fertility) the lowest dry matter production during the experiment.

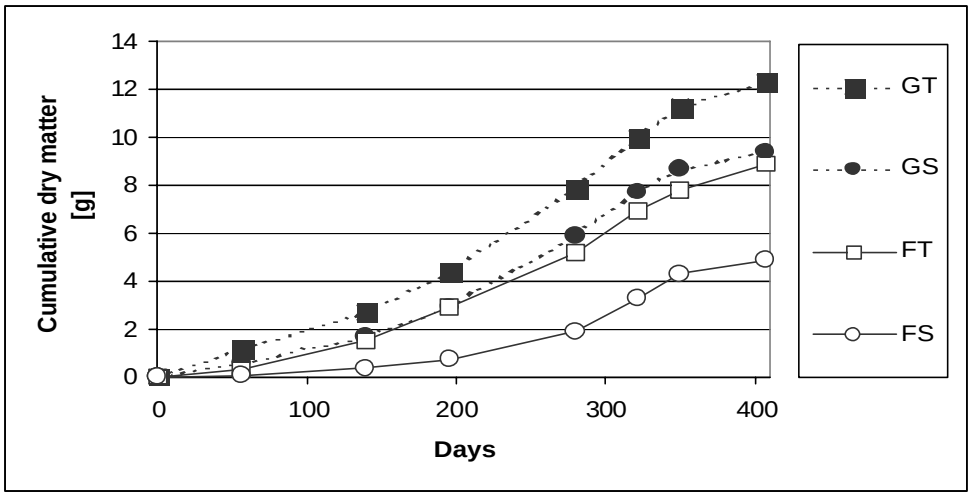


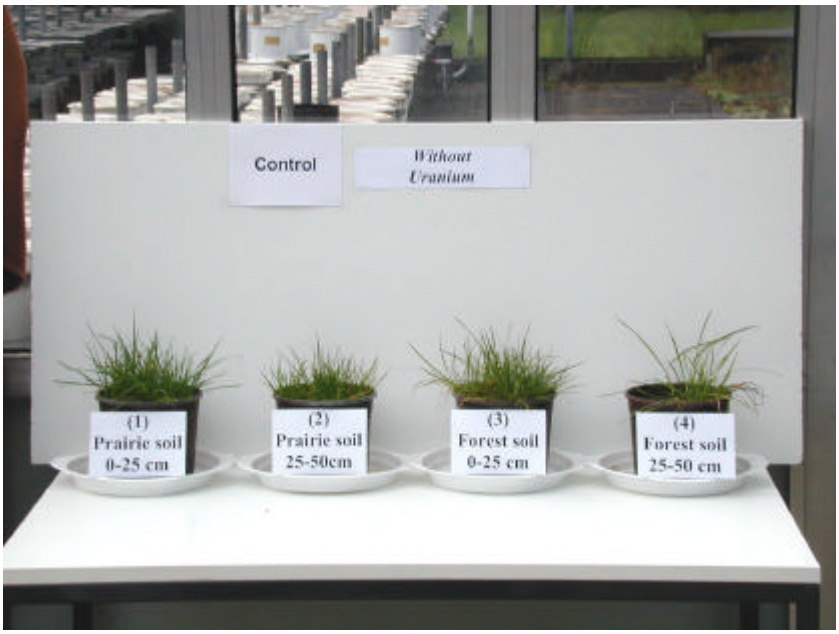
Fig. 4.1: Cumulative dry matter of *Lolium perenne* in non contaminated, unfertilized soils during the experiment term
GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil

There were no significant differences in yield of *Lolium perenne* in the grassland subsoil and the forest topsoil (fig. 4.1). Statistical differences for aboveground mass production were found

comparing the different substrates during the cuts 1 to 4, but not from cuts 5 to 7 after the longest growth period of spring (cut 3 to 4) (appendix, tab. A.1).

Analyzing the nutrient concentration in plant is a useful tool to access the nutrient uptake efficiency and thus indirectly the level of soil fertility. For that reason, mean contents of P and Ca in plant grown on non contaminated unfertilized substrate were calculated during the entire experimental time (tab. A.2) and compared with bibliographic references (tab. A.3). Low levels of Ca in forest substrate were determined, which could have limited the growth in these substrates.

Picture 4.1 shows the differences in plant growth of *Lolium perenne* in the control treatments of all soil substrates (cut 7).



Pic. 4.1: Growth of *Lolium perenne* in control substrates (cut 7)

4.1.1 Effect of uranium application on yield of Lolium perenne

The effect of soil contamination with U on grass growth was tested by using the least significant difference (LSD). The effect of the U contamination levels on the grass yields depending on the soil substrate are shown separately in the figures 4.2, 4.3, 4.4 and 4.5, but only for such cuts with significant differences (for all the substrates and cuts see in appendix, tab. A.4).

In the grassland topsoil (fig. 4.2), the yields were significantly increased after 8 weeks (cut 1) only with the 250 mg U kg^{-1} soil dose, and with all the U doses after 28 weeks (cut 3). After that either the grass yields decreased with increased U doses in the substrates (cuts 4 and 5) or no significant effects were observed. A similar tendency to increase yields till cut 3 and to decrease them later was found for grass yields in the grassland subsoil (fig. 4.3).

The addition of U to the forest topsoil (fig. 4.4) showed statistically significant effects on yields only in two cuts: after 8 weeks (cut 1) when the yields were decreased following all the U doses, and after 28 weeks (cut 3), when the effect was completely changed and the yields increased following higher U doses.

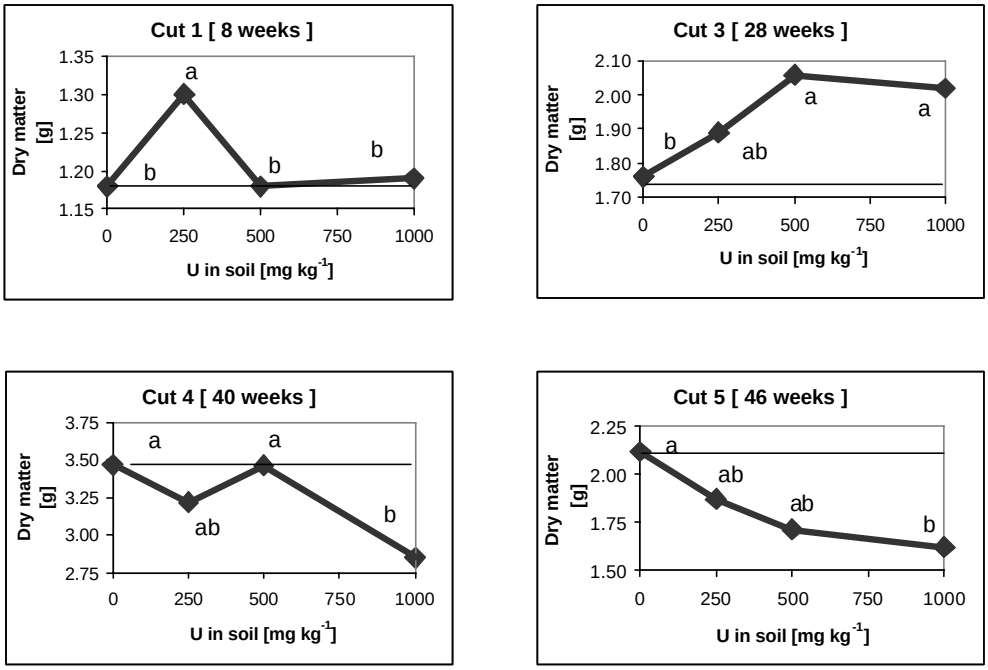


Fig 4.2: Dry matter of *Lolium perenne* following U contamination (grassland topsoil)
Significant differences are declared by different letters (LSD, at 5 % level)

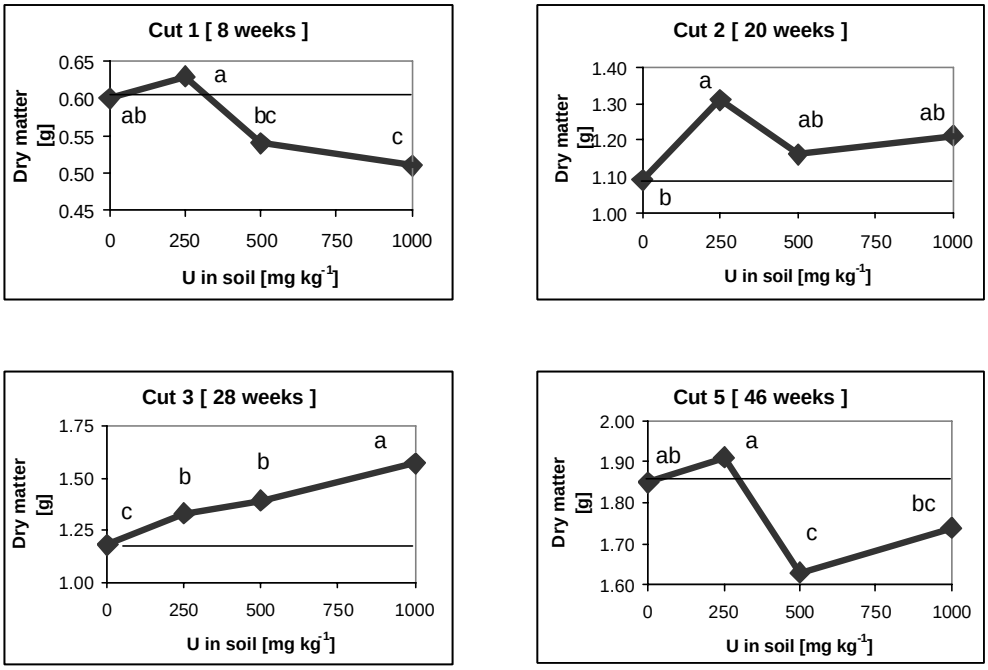


Fig. 4.3: Dry matter of *Lolium perenne* following U contamination (grassland subsoil)
Significant differences are declared by different letters (LSD, at 5 % level)

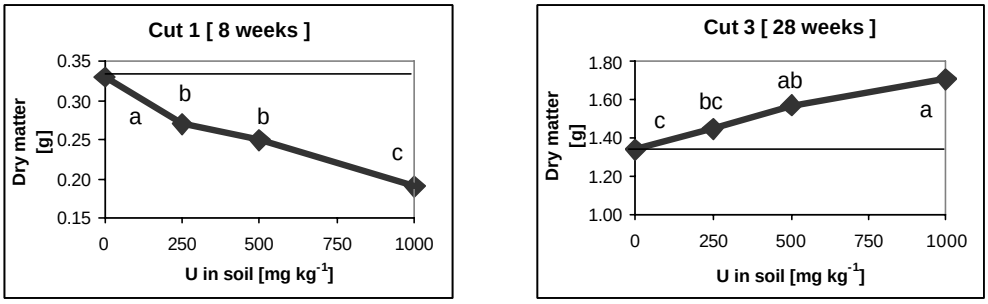


Fig. 4.4: Dry matter of *Lolium perenne* following U contamination (forest topsoil)
Significant differences are declared by different letters (LSD, at 5 % level)

Yields were significant decreased in forest subsoil (fig. 4.5) following U doses till week 28 (cut 3), after that a positive effect of U on yields was observed in the course of time (cut 4), being statistically significant at the U doses of 250 and 500 mg kg^{-1} U at the end of the experiment (58 weeks, cut 7). This was the only case where a complete type dose-response curve (cp. fig. 2.1) was found, although with only two points of U doses in the range of a stimulatory or "hormetic effect".

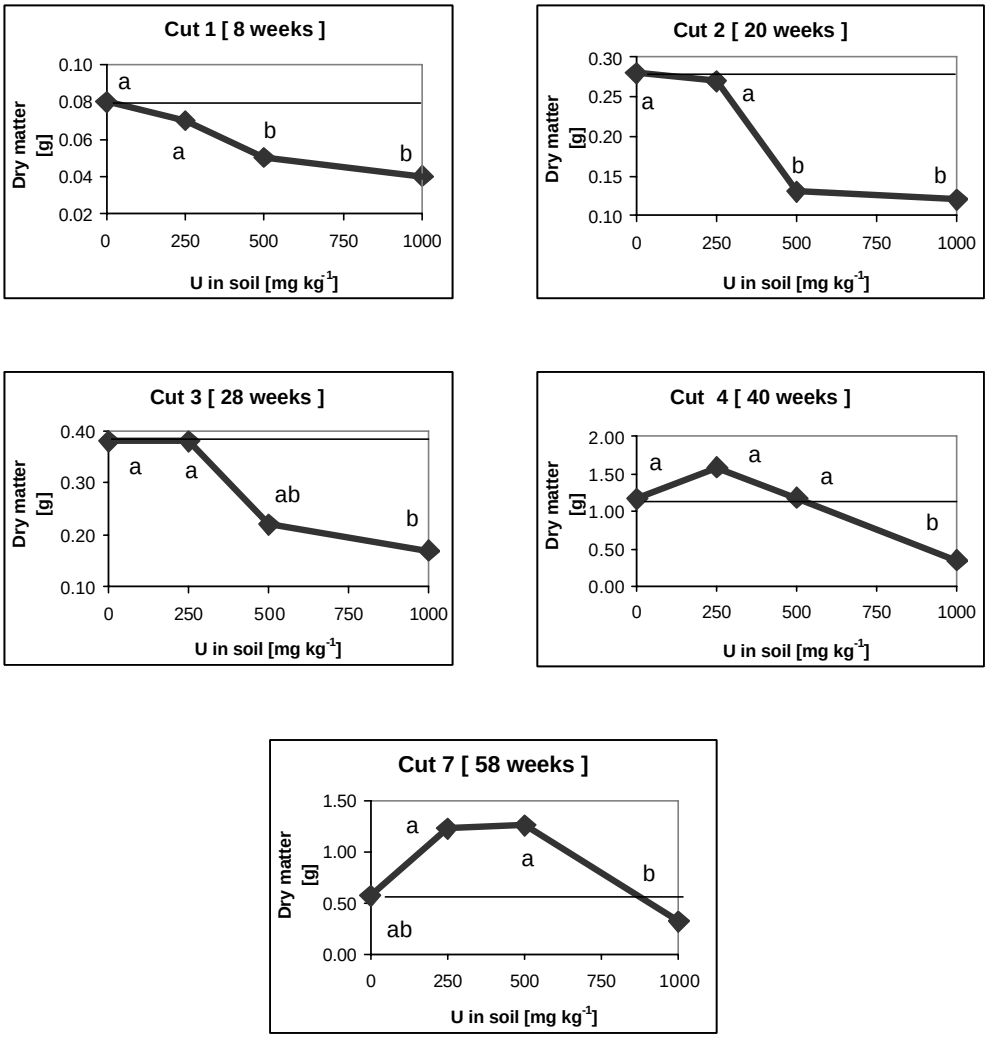
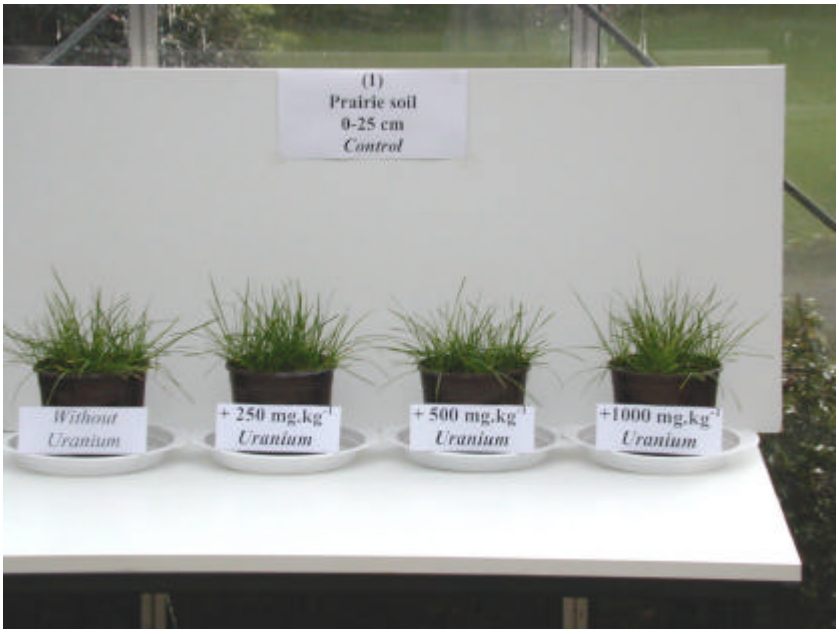
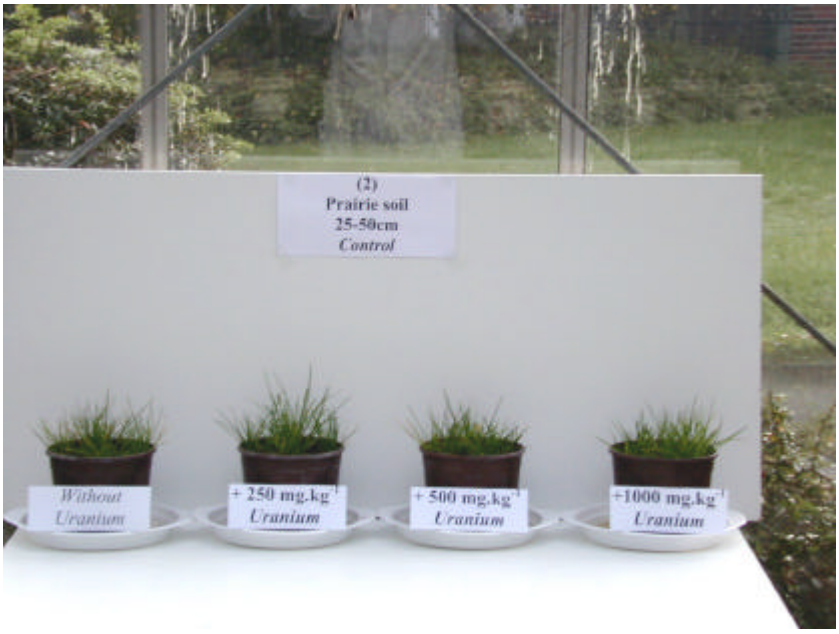


Fig. 4.5: Dry matter of *Lolium perenne* following U contamination (forest subsoil)
Significant differences are declared by different letters (LSD, at 5 % level)

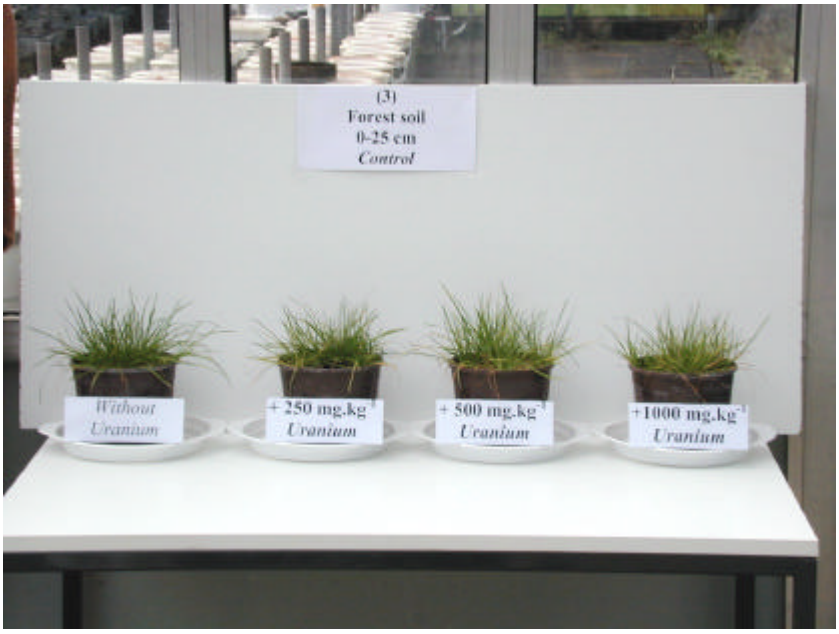
The growth of *Lolium perenne* at cut 7 is shown for all soil substrates and levels of U contamination in pictures 4.2 to 4.5. For this cut, little differences were observed between the contaminated and non contaminated treatments in GT, GS and FT substrates. But the grass growth was clearly stimulated in U contaminated FS substrate at the 250 and 500 mg kg^{-1} doses (tab. A.4).



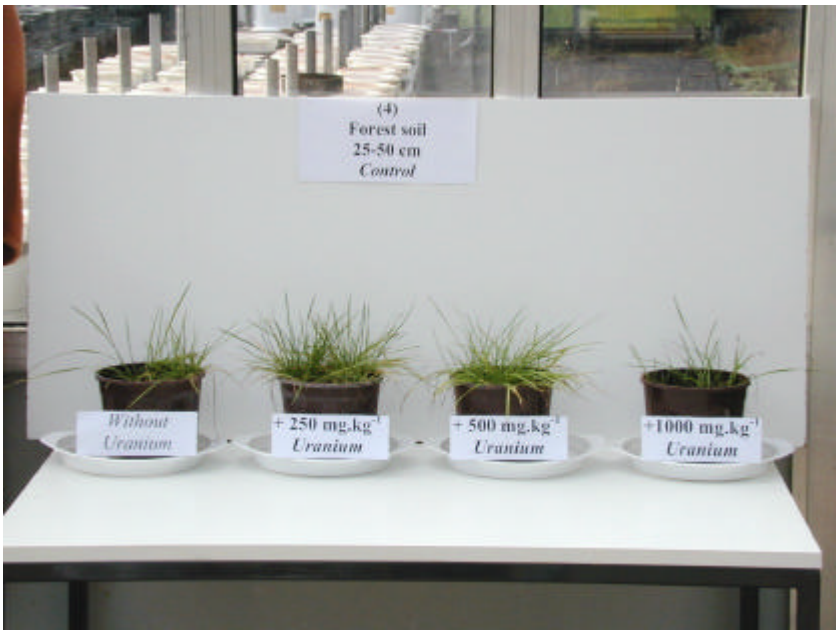
Pic. 4.2: Growth of *Lolium perenne* in grassland topsoil (GT) substrate contaminated with U (cut 7)



Pic. 4.3: Growth of *Lolium perenne* in grassland subsoil (GS) substrate contaminated with U (cut 7)



Pic. 4.4: Growth of *Lolium perenne* in forest topsoil (FT) substrate contaminated with U (cut 7)



Pic. 4.5: Growth of *Lolium perenne* in forest subsoil (FS) substrate contaminated with U (cut 7)

Comparison of the total dry matter of Lolium perenne growing on contaminated substrates

Because of the changing effect of U on yields in the time, the total cumulative dry matter for each substrate/U contamination combination during the experiment was calculated as an indicator of the complete process. The ratios of cumulative dry matter between non treated or control soil substrates, and between U contaminated and control for each soil substrate are presented in table 4.1.

Tab. 4.1: Total dry matter[1] of *Lolium perenne* affected by increasing U contamination relative to control

Soil substrate	Among control substrates	0	250	500	1,000
GT	100	100	100	99	93
GS	76	100	102	96	97
FT	73	100	97	99	99
FS	39	100	**130**	93	46

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
0, 250, 500 and 1,000 are U doses in mg kg^{-1} U
[1] sum of the replications and cuts for each substrate/U contamination combination

At the end of the experiment similar levels of total cumulative dry matter were found in GT, GS and FT, between each control and the U contaminated in the range of the levels tested. A clear increase of dry matter yield was only observed in FS when 250 mg kg^{-1}U had been added, and a detrimental effect with the addition of 1,000 mg kg^{-1} U.

Comparison of total nutrient uptake by Lolium perenne growing on contaminated soil substrates

In order to know if the pattern of cumulative dry matter was related to nutrient content in plant, the total cumulative elements uptake for each soil substrate/U contamination combination were determined and calculated as contaminated/control ratios (tab. 4.2).

Tab. 4.2: Total macronutrients uptake[1] by *Lolium perenne* affected by increasing U contamination relative to control

Soil substrate	Among control substrates	0	250	500	1,000
			P uptake ratios		
GT	100	100	99	92	104
GS	64	100	94	94	100
FT	72	100	80	65	82
FS	32	100	**133**	92	58
			Ca uptake ratios		
GT	100	100	104	100	87
GS	56	100	92	85	75
FT	33	100	87	92	79
FS	17	100	**125**	42	25

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
0, 250, 500 and 1,000 are U doses in mg kg^{-1} U
[1] sum of the replications and cuts for each substrate/U treatment combination

The uptake of P by the grass in grassland substrates was little affected by U. In FT it decreased in U contaminated pots, whereas in FS it increased at the U dose 250 mg kg^{-1} and decreased at highest levels of contamination. When considering all the substrates (tab. A.5, A.6) the effect on P was not statistically significant. Ca uptake was also increased in FS at the U dose 250 mg kg^{-1}, and except for GT, higher levels of U contamination were associated with lower total Ca uptake by grass.

The doses of U applied to soils showed to be alternatively inhibitory and stimulatory for the growth of *Lolium perenne* under the conditions of this experiment. The stimulatory effect appeared to be delayed in the substrate with the lowest fertility (FS) compared to the other soil substrates. At the end of the experiment at least for GT, GS and FT in the range of the U levels tested, cumulative dry matter depended on soil quality. An increment of cumulative dry matter and P and Ca elements uptake was associated with the 250 mg kg^{-1} U dose in FS.

4.1.2 Effect of uranium application on root growth of Lolium perenne

Most metal ions enter plant cells by an energy dependent process which showed a relative lack of selectivity. However, transport within endodermis, or the high cation exchange capacity in xylem cell walls could limit the uptake and movement to shoots, respectively. To resist toxic effect, plants can detoxify the heavy metals by different ways as compartmentalization in vacuoles, precipitation, etc. But roots can also secrete chelating substances or acidify their environment, which could enhance U uptake as other metals. Also rhizosphere microorganisms (mycorrhizal fungi, root colonizing bacteria) could increase or diminish its bio-availability (Hinsinger, 1998; Salt et al., 1995).

U redistribution within the plant indicates that the roots are an effective barrier to U, and only a small fraction of the soil U is taken up and distributed within the plant (Van Netten, 1983; Van Netten and Morley, 1982). U may be precipitated on the outer root membrane rather than accumulated in the interior of the root. Plant U uptake mechanisms have not been well studied. As it is competitively depressed by Ca^{2+} (Mortvedt, 1994), it was proposed that both could present a similar physiology in plant, being translocated via xylem sap with the transpiration stream and showing low transport in phloem. This would agree with the U content and distribution in plant described by several authors: root > leaves > grain or fruits (Gulati et al., 1980; Lakshmanan & Venkateswarlu, 1988; Morishima et al., 1976; Morishima et al, 1977; Singh, 1997).

At the end of the experiment, pots were turned over and soil was separated from the plastic recipients to observe the roots distribution. Differences among the color of roots, compaction of the soil root mass or destruction of paper in the base of the pot appeared as significant for the comparison.

A qualitative comparison of root growth between the control pots and those with the highest U dose contamination was performed. In this experiment no apparent difference between root development at 0, 250 or 500 mg kg^{-1} U was found for each soil substrate at the end of the experiment, but very strong impairment of root mass was observed at 1,000 mg kg^{-1} U, specially for FS.

A view of the roots in the best quality substrate (GT) is given in picture 4.6. A big part of the roots built up a root mat around the peripheral zone of the root ball, the roots were clear and formed an open mat, and the paper was integrated or partially decomposed. The results were completely different for roots in FS (pic. 4.7): lesser amounts of roots grew than in the control, they were

darker, and the soil was compressed. The filter paper at the bottom was not integrated to the soil-root ball. At the 1,000 mg kg^{-1} U dose soil was broken in pieces without any formation of roots, showing they had serious difficulties to grow.

Pic. 4.6: Root distribution in the base of the control pot of the grassland topsoil (GT) substrate

Pic. 4.7: Root distribution in control and U contaminated (1,000 mg kg^{-1}) FS substrate

4.2 Soil chemical factors affecting the plant availability of uranium

The effect of DU in soil is more based on its properties as a toxic heavy metal than on its radioactivity. However, few and contradictory information exists about the U behavior in the soil-plant system (Gulati et al., 1980; Lakshmanan and Venkateswarlu, 1988, Meyer and McLendon, 1997; Meyer et al., 1998a; Meyer et al., 2004; Morishima et al., 1976; Morishima et al., 1977; Singh, 1997; Van Netten and Morley, 1981; Van Netten and Morley, 1982).

If an element will be adsorbed on the particles, precipitated or mobilized in the ground water and taken up by plants, besides its chemical forms, it will depend on the soil characteristics (pH, content of organic matter, oxides and clay) and the concentration of other elements in the soil solution. This means that the risk of U entering the food chain could be altered by modifying selected soil and management factors.

In this investigation the effects of some soil factors on the U availability were studied in soil and plant variables in a pot experiment. The factors tested will be presented in the following order: soil substrate, P supply, pH changes caused by liming. The U availability, water soluble P and pH in soil were determined only at cut 7. In plants, yield and U concentration per cut were determined, and U uptake by *Lolium perenne* was calculated during the entire experiment.

4.2.1 Effect of phosphorus fertilization and liming on different soil factors

4.2.1.1 Phosphorus fertilization

In the pot experiment an ANOVA (tab. A.7) showed that mobile and available P in soil had been significantly affected by soil substrate, fertilizers and their interactions.

Tab. 4.3: Water soluble P[1] in soil [mg kg^{-1}] depending on substrate and fertilization treatment (cut 7)

Treatment	GT	GS	FT	FS
Unfertilized	15 ± 2.1	12 ± 4.7	15 ± 7.6	8 ± 4.1
P fertilization	40 ± 6.4	97 ± 52.7	291 ± 12.3	122 ± 66.3
Liming	15 ± 2.3	9 ± 1.5	13 ± 7.3	19 ± 23.6
P & liming	30 ± 1.7	69 ± 52.7	59 ± 19.4	76 ± 56.1

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
[1] Mean values and standard deviation

The addition of $CaHPO_4$, alone or with lime increased the water soluble P compared to the control soils (tab. 4.3). Also the addition of lime slightly increased P in FS.

For all soil substrates the water soluble P increment was lower when both fertilizers had been added, probably because of a lower solubilization of P at higher pH or re-precipitation of calcium phosphates. To avoid interferences on the result interpretation, no data from limed soils was used to evaluate P fertilization effect on the studied variables.

When $CaHPO_4$ was applied alone, the highest mean level (291 mg kg^{-1}) of P was determined in the FT substrate, and the lowest was in the GT (40 mg kg^{-1}) (tab.4.3). Levels as the last one can be found in heavily P fertilized soils (Indiati and Singh, 2001)

4.2.1.2 Lime addition

$CaCO_3$ is generally added to increase pH and Ca levels in soil. Ca has been suggested to be an antagonist to heavy metals (Mortvedt, 1994), so both parameters were considered.

Soil pH determination and calcium content in plants after liming

In an ANOVA (appendix, tab. A.7) the soil pH was found to be significantly affected by soil substrates, fertilizer application and their interactions. The addition of lime as $CaCO_3$, the P fertilization in form of $CaHPO_4$ and the combination of both amendments, increased the pH of the soils in the pot experiment (tab. 4.4) in the following order: P fertilization & liming > liming > P fertilization > unfertilized.

The average of the pH values determined in $CaCl_2$ (pH 5.8) was 0.2 units higher than directly determined in soil (pH 5.6). This difference was probably due to dilution in the laboratory measurements. As a good relationship was found for both determinations of the soil pH ($y = 1.0312 + 0.7998\ x$, R^2: 79 %, $P < 0.001$), and $CaCl_2$ is the most widely used in commercial and research laboratories, it will be used for the analysis onwards.

To avoid interferences of P addition in the results of soil pH and Ca in plant, the substrates fertilized with P were not considered when liming effect was evaluated. The mean pH of the control soil substrates was in the range of 3.7–6.4, after the addition of $CaCO_3$ in the range of 5.9–6.9. Although the grassland substrates reached at the end of the experiment higher pH values than the substrates derived from forest, the increment was highest for forest substrates: almost 2 units, against 0.5 and 1.5 for GT and GS, respectively. This could be attributed to the higher solubility of $CaCO_3$ at the low pH of the forest soils.

Tab. 4.4: Soil pH[1] depending on substrate and fertilization treatment (cut 7)

Treatment	GT	GS	FT	FS
	Soil pH determined in dried soil [0.01 M $CaCl_2$]			
Unfertilized	6.4 ± 0.13	5.2 ± 0.32	3.7 ± 0.18	4.2 ± 0.23
P fertilization	6.5 ± 0.09	5.7 ± 0.18	4.4 ± 0.08	4.9 ± 0.10
Liming	6.9 ± 0.11	6.7 ± 0.07	5.9 ± 0.33	6.1 ± 0.32
P & liming	6.8 ± 0.11	6.6 ± 0.11	6.1 ± 0.07	6.2 ± 0.08
	Soil pH determined directly in fresh soil			
Unfertilized	6.5 ± 0.41	5.4 ± 0.32	3.9 ± 0.14	4.3 ± 0.36
P fertilization	6.3 ± 0.35	5.7 ± 0.13	4.5 ± 0.28	5.2 ± 0.27
Liming	6.6 ± 0.17	6.3 ± 0.50	4.9 ± 0.25	5.7 ± 0.46
P & liming	6.9 ± 0.32	6.4 ± 0.25	5.6 ± 0.21	5.9 ± 0.17

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
[1] Mean values and standard deviation

Calcium levels in plants

After fertilization it was found that the Ca content in plant was affected by the soil substrate, the fertilization and their interactions (ANOVA, tab. A.7). Mean values and coefficients of variation of Ca in *Lolium perenne* are presented in table 4.5. The Ca levels in plant were in the following order: P fertilization = P fertilization & liming > liming > unfertilized.

Tab. 4.5: Ca content[1] in *Lolium perenne* [%] depending on substrate and fertilizer treatment (cut 7)

Treatment	GT	GS	FT	FS
Unfertilized	0.62 ± 0.07	0.41 ± 0.02	0.29 ± 0.05	0.23 ± 0.07
P fertilization	0.75 ± 0.06	0.86 ± 0.07	1.12 ± 0.08	1.10 ± 0.15
Liming	0.77 ± 0.06	0.80 ± 0.05	1.01 ± 0.10	1.00 ± 0.14
P & Liming	0.82 ± 0.04	0.99 ± 0.08	1.08 ± 0.12	1.12 ± 0.14

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
[1] Mean and standard deviation

Liming increased 0.25-fold the level of Ca in leaves in the GT, 2-fold in GS, and 4-fold in the forest substrates compared to unfertilized. All the values (0.75-1.12 %) were in the range of normal levels for Ca in plants (tab. A.3).

As fertilizing with $CaHPO_4$ increased Ca in plants (tab. 4.5), and P will be shown below to have a strong effect in reducing U levels in plant, only substrates with the addition of $CaCO_3$ were considered to study the Ca and U relationship in plant.

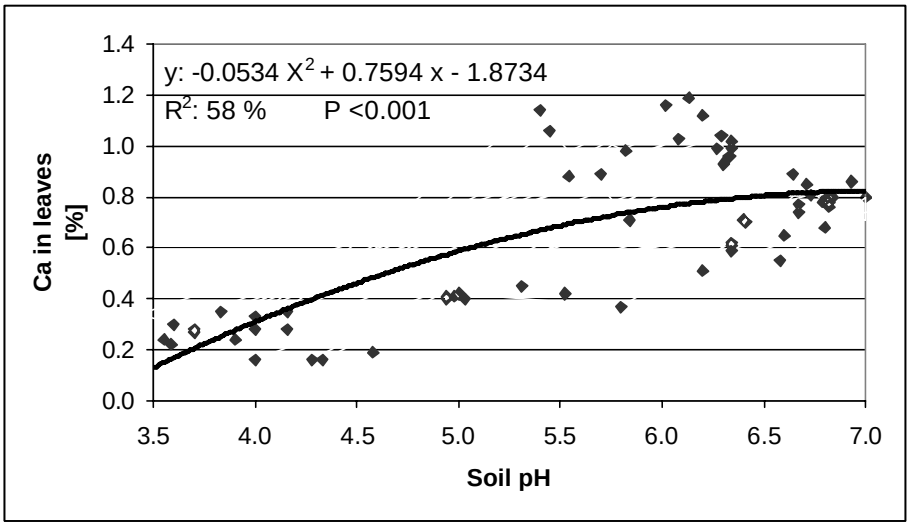


Fig. 4.6: Relationship between soil pH and Ca content in *Lolium perenne* (cut 7)

In this study the relationship soil pH/Ca in plant (fig. 4.6) shows that they both increased till pH 6.5 approximately, but higher pH levels were associated to lower Ca content in leaves. This is because after liming the pH of forest substrates (6.0) were still lower than grassland ones (6.8), but the Ca content in forest had been increased 20 % over grassland substrates (tab. 4.5).

4.2.1.3 Extracting solutions

The soil extractions were done by using two extracting solutions: DTPA (Lindsay and Norvell, 1978) and a stronger extraction agent: AAAcEDTA (Lakanen and Erviö, 1971). Their performance to determine the U availability in soil was compared. As it was expected both extracting solutions extracted different amounts of U from the tested soils.

The DTPA extracting solution appeared to be more sensitive to detect differences in the characteristics of the non fertilized soils. U extracted by it showed the highest correlation with U content in plant (n: 32) (tab. 4.6). U extracted by AAAcEDTA showed a better correlation with U content in plant tissues and plant uptake when all the data after the fertilization was included (n: 128). U extracted by DTPA rather represents the U labile, or immediately available for plants and the U extracted by AAAcEDTA, the mobilized, which is in equilibrium with labile fraction.

Although they extracted different fractions of U from soils, similar tendencies for the results were found for both extracting solutions. Although sometimes information could appear as

duplicated, the results of U in soil extracted by both extracting solution will be presented in the next sections.

Tab. 4.6: Correlation coefficients between U in soil and plant variables in unfertilized and fertilized substrates (cut 7)

U extractable in soil [mg kg^{-1}]	Yields [g]	U in plant [mg kg^{-1} DW]	U uptake [µg]
	Non fertilized substrates (n: 32)		
DTPA	Ns	0.843 ***	0.542 ***
AAAcEDTA	Ns	0.769 ***	0.579 ***
	Fertilized substrates (n: 128)		
DTPA	0.224*	0.576 ***	0.642 ***
AAAcEDTA	Ns	0.715 ***	0.714 ***

*** : P < 0.001, *: P < 0.05, Ns: not statistically significant

4.2.2 Effect of soil substrate

This part of results refers only to the unfertilized substrates of the pot experiment. The selected substrates presented a wide range of soil characteristics. One extreme was the grassland topsoil, with a high organic matter content, slightly acid, with reasonable fertility for crop growth. In contrast the forest subsoil, with low organic matter content and low pH, was poor in terms of fertility.

An ANOVA for the effect of soil substrate and U treatment on the soil and plant variables studied is shown in appendix, table A.8. The results will be presented in tables 4.7 to 4.13 as the effect of all soil substrate/U treatment combinations. The linear regression equations for the studied variables following U in the soil will also be presented when they were significant (tab. A.10-A.14).

Effect of the soil substrate fertility on the extractable uranium in contaminated treatments

The U extracted by DTPA increased from the best to the worst soil substrate (GT to FS) and followed U doses applied. For the 1,000 mg kg^{-1} dose, the extracted U from FS (45 mg kg^{-1}) was almost 30-fold higher than the U amount from GT (1.6 mg kg^{-1}) (tab. 4.7).

The U extracted by AAAcEDTA showed similar levels for both forest substrates and the GS at each U dose, and they were higher than for GT. For the 1,000 mg kg^{-1} dose the extracted U from the

substrate with the lowest fertility (317 mg kg^{-1}) was only 3-fold higher than that from the best fertility (117 mg kg^{-1}) (tab. 4.7).

Although both extracting solutions extracted increasing amounts of U, following the U doses applied to the soil substrates, the DTPA showed to be more sensitive to differences in the soil substrate than the AAAcEDTA, as the last extracted similar amounts from GS, FT and FS.

In all cases the extracted U in soil showed significant linear regressions following total U levels in soil (tab. 4.7 and A.10). Mean amount of U extracted by AAAcEDTA in contaminated soils (150 mg kg^{-1}) were 13-fold higher than that of DTPA (11.64 mg kg^{-1}).

Tab. 4.7: Effect of soil substrate and U contamination on U extracted by DTPA and AAAcEDTA in unfertilized substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
			U extracted by DTPA [mg kg^{-1}]		
0	<LLD	<LLD	<LLD	0.01	<LLD
250	0.49	3.67	3.30	7.25	3.68
500	0.77	5.48	9.74	18.29	8.57
1,000	1.64	18.95	24.66	45.43	22.67
Mean for soil substrate	0.72	7.02	9.42	17.75	$LSD_{0.05}$ 3.78
b	0.002	0.019	0.025	0.046	
R^2	94 %	85 %	97 %	93 %	
P	***	**	***	***	
			U extracted by AAAcEDTA [mg kg^{-1}]		
0	0.02	0.03	0.04	0.04	0.03
250	25	79	78	86	67
500	54	130	160	148	123
1,000	117	309	297	317	260
Mean for soil substrate	49	129	134	138	$LSD_{0.05}$ 32.3
b	0.118	0.305	0.297	0.314	
R^2	98 %	89 %	98 %	99%	
P	***	***	***	***	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : $P < 0.001$ ** : $P < 0.05$ *: $P < 0.01$ Ns: no significant
LLD: lower than level of detection

Effect of the soil substrate fertility on the yield of Lolium perenne in contaminated treatments

In this section both, cumulative aboveground dry matter and U uptake during the experiment term and yield and uptake determined only at cut 7 were considered (tab. 4.8 to 4.13).

Statistical differences for mean values of the cumulative dry matter (ANOVA, tab. A.9) following the quality of the substrates were determined. No differences were found for GS and FT (tab. 4.8). Mean dry matter yield was statistically increased by the U dose 250 mg kg^{-1}, but as it has been shown in chapter 4.1 it probably mainly depended on the bad fertility of the FS substrate (tab. A.11). When only cut 7 is considered (tab. 4.9), interactions existed between substrates and U treatment (ANOVA, tab. A.8). The yield decreased at 1,000 mg kg^{-1} U compared to control. The dry matter production was similar for both topsoil substrates and higher than for the substrate derived from both subsoils. The U level of 250 mg kg^{-1} increased the yields in the GT substrate (20 %), and the U levels of 250 and 500 mg kg^{-1} increased them 3-fold over control in the lowest fertile substrates. For this last substrate the highest U level showed an inhibitory effect as it has been shown in chapter 4.1.

Tab. 4.8: Effect of soil substrate and U contamination on the cumulative yield per pot of *Lolium perenne* [g] in unfertilized substrates

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	12.32	9.36	8.96	4.74	8.85 ab
250	12.26	9.53	8.71	6.18	9.17 a
500	12.21	8.96	8.79	3.79	8.44 ab
1,000	11.46	9.06	8.87	2.16	7.89 b
Mean for soil substrate	12.06 a	9.23 b	8.83 b	4.22 c	$LSD_{0.05}$ 0.74
b	$-9.0 \cdot 10^{-4}$	$-4.2 \cdot 10^{-4}$	$-2.3 \cdot 10^{-5}$	-0.0033	
R^2	63 %	21 %	< 1 %	48 %	
P	*	Ns	Ns	Ns	

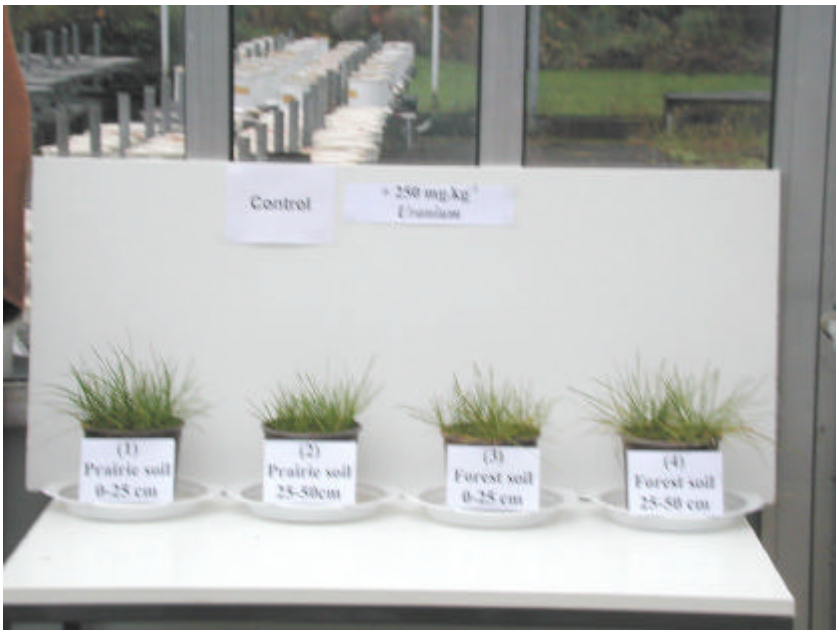
GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

Tab. 4.9: Effect of soil substrate and U contamination on yield per pot of *Lolium perenne* [g] in unfertilized substrates (cut 7)

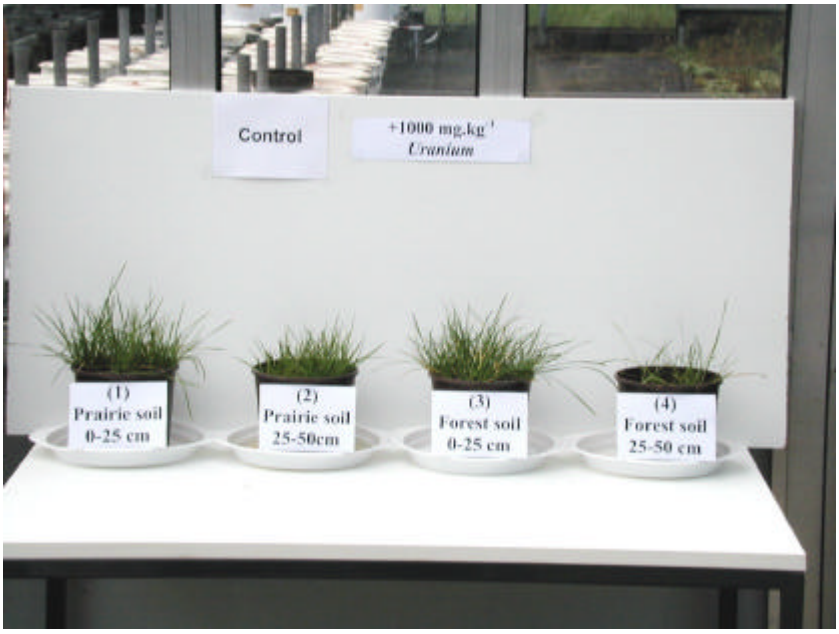
U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	1.00	0.69	1.13	0.41	0.81
250	1.20	0.70	1.10	1.23	1.06
500	1.00	0.75	0.89	1.26	0.93
1,000	1.01	0.75	0.97	0.32	0.76
Mean for soil substrate	1.05	0.72	1.02	0.74	$LSD_{0.05}$ 0.16
b	$-6.0 \cdot 10^{-5}$	$6.0 \cdot 10^{-5}$	$-1.7 \cdot 10^{-4}$	$-3.1 \cdot 10^{-4}$	
R^2	4 %	6 %	22 %	7 %	
P	Ns	Ns	Ns	Ns	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

In pictures 4.8 and 4.9 the growth of *Lolium perenne* in the four substrates at 250 and 1,000 mg kg^{-1} U dose are presented, respectively.



Pic. 4.8: Growth of *Lolium perenne* in different substrates contaminated with 250 mg kg^{-1} U (cut 7)



Pic. 4.9: Growth of *Lolium perenne* in different substrates contaminated with 1,000 mg kg^{-1} U (cut 7)

Effect of soil substrate fertility on the uranium content of Lolium perenne in contaminated treatments

When U concentration during the entire experiment term is considered (tab. 4.10), a progressive increment of mean values following U levels and decreasing soil fertility shows their straight dependency of these both factors (ANOVA, tab. A.8). Mean values of U content for the contaminated substrates were doubled as U doses did. If GT is not considered mean U content for contaminated substrates was also doubled following decreasing soil fertility.

Most of the mean values for U content in plant were far above the range 0.04-0.40 mg kg^{-1} DW, considered as “normal” range for herbaceous plants in non contaminated soils (in Dressen and Marple, 1979; Pais and Jones, 1997).

Tab. 4.10: Effect of soil substrate and U contamination on U content in *Lolium perenne* [mg kg^{-1}] in unfertilized substrates considering the entire experiment

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	0.06	0.03	0.02	0.07	0.05
250	0.33	1.01	1.63	3.16	1.53
500	0.31	1.48	4.49	6.68	3.24
1,000	0.62	2.63	4.93	14.20	5.59
Mean for soil substrate	0.33	1.29	2.77	6.03	$LSD_{0.05}$ 1.44
b	$5.1 \cdot 10^{-4}$	0.0025	0.0050	0.0142	
R^2	24 %	56 %	27 %	37 %	
P	***	***	***	***	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

Differences for U content in plant at cut 7 (tab. 4.11) were statistically significant for substrate and U treatment (tab. A.8). The mean U content in leaves increased following the U contamination levels, but different situations appeared depending on soil substrate. Little differences of U content among the three levels of contamination were found in both top soil substrates, whereas in both subsoil substrates the U content in leaves showed an almost linear relationship following U in soil (tab. A.10). In the forest subsoil the plant U contents were similar for the 250 and 500 mg kg^{-1} U.

Tab. 4.11: Effect of soil substrate and U contamination on U content in *Lolium perenne* [mg kg^{-1}] in unfertilized substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	<LLD	0.04	0.03	<LLD	0.02 b
250	0.75	1.20	3.07	4.11	2.28 a
500	0.55	2.47	4.32	4.79	3.03 a
1,000	0.81	4.01	3.86	8.39	4.27 a
Mean for soil substrate	0.53 c	1.93 bc	2.82 ab	4.32 a	$LSD_{0.05}$ 1.63
b	$6.3 \cdot 10^{-4}$	0.004	0.003	0.008	
R^2	40 %	80 %	31 %	77 %	
P	Ns	**	Ns	**	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

Effect of soil substrate fertility on uranium uptake by Lolium perenne in contaminated treatments

The cumulative U uptake by grass was obtained by multiplication of yield by U concentration in plant for each sample (tab. 4.12). Interactions between substrates and U treatment were determined for cumulative U uptake (ANOVA, tab. A.9). Forest substrates showed a higher uptake of U than grassland substrates. The highest U uptake was determined for the FT, which had showed nor the highest yields neither the highest U levels in plant. This probably occurred because the highest U levels reached in FS reduced the yields with the concomitant diminish of the U uptake. At 1,000 mg kg^{-1} U dose, the cumulative U uptake by *Lolium perenne* in FT was more than 100 times higher than that of the control in this substrate.

In the last cut the U uptake by the *Lolium perenne* was significantly higher in forest than in grassland substrates (tab. 4.13). As for U concentration in the cut 7 (tab. 4.11), no significant difference for U uptake was found for mean values among U contamination levels. When only the last cut was considered the lowest fertile substrate (FS) showed the highest U uptake values at 250 and 500 mg kg^{-1} doses associated with the highest yields obtained (tab. 4.9).

Tab. 4.12: Effect of soil substrate and U contamination on cumulative U uptake per pot by *Lolium perenne* [µg] in unfertilized substrates

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	0.65	0.30	0.22	0.21	0.35
250	3.93	9.06	15.5	18.2	11.68
500	3.41	12.3	39.7	24.9	20.07
1,000	8.06	23.5	50.4	28.7	27.66
Mean for soil substrate	4.01	11.3	26.4	18.0	$LSD_{0.05}$ 6.63
b	0.0068	0.0222	0.0509	0.0260	
R^2	78 %	94 %	81 %	63 %	
P	**	***	**	*	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

Tab. 4.13: Effect of soil substrate and U contamination on the U uptake per pot by *Lolium perenne* [µg] in unfertilized substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	<LLD	0.02	0.03	<LLD	0.02 b
250	0.89	0.84	3.45	4.77	2.49 a
500	0.53	1.73	3.43	6.20	2.51 a
1,000	0.81	3.05	3.81	2.83	2.62 a
Mean for soil substrate	0.56 b	1.41 ab	2.68 a	3.06 a	$LSD_{0.05}$ 1.37
b	$5.8 \cdot 10^{-4}$	0.003	0.003	0.002	
R^2	31 %	79 %	34 %	9 %	
P	Ns	Ns	Ns	Ns	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant
LLD: lower than level of detection

4.2.3 Summary of the relative effect of the fertilizer additions on the studied variables

As many factors and variables have been studied in this experiment, a comparison of the relative effect of fertilization at each U level of contamination, independently of the soil substrate, will contribute to the understanding of the results (tab. 4.14).

The addition of $CaHPO_4$ alone or in combination with $CaCO_3$ notably reduced the U contents in the soil substrates and in *Lolium perenne* and consequently the U uptake. The simulation of liming by incorporation of $CaCO_3$ within the upper layer of the substrates increased the U extracted by DTPA, but had little effect on the U extracted by AAAcEDTA. It resulted in better growth of the grass, and diminution of the U content in plant, as well as the U uptake by *Lolium perenne* at the 250 and 500 mg kg^{-1} U dose. At the 1,000 mg kg^{-1} U dose, yields were almost increased 2-fold over the control, concomitant the mean U content was declined to approximately half, showing that at least in this case the effect of liming could be due to dilution of U in plant tissues.

Below in this section the effect of each fertilization treatment on the studied variables will be referred separately. First the general relationship between the tested fertilizers and studied variables will be presented in figures. Then, for each fertilizer treatment, the effect of substrates and U treatments on each soil and plant variables will be presented in tables, and the data will be compared with the corresponding unfertilized substrate (chapter 4.2.2).

Tab. 4.14: Comparison of the fertilized/unfertilized ratios[1] for the mean values of all studied soil and plant variables at different levels of U contamination (cut 7)

Soil and plant variables	Unfertilized	P fertilization	Liming	P fertilization & liming
Level of U contamination: 250 mg kg^{-1}				
U by DTPA	100	4	158	4
U by AAAcEDTA	100	21	106	23
Yield	100	107	141	131
U in leaves	100	6	43	5
U uptake	100	6	59	6
Level of contamination: 500 mg kg^{-1}				
U by DTPA	100	6	201	5
U by AAAcEDTA	100	27	93	21
Yield	100	104	136	117
U in leaves	100	4	32	5
U uptake	100	4	40	5
Level of contamination: 1,000 mg kg^{-1}				
U by DTPA	100	3	170	3
U by AAAcEDTA	100	26	93	22
Yield	100	110	190	150
U in leaves	100	7	47	7
U uptake	100	12	104	12

[1]Mean values of the 4 substrates

4.2.4 Effect of phosphorus fertilization

P has been mentioned to affect the behavior of heavy metals in soil. High applied doses has been used to immobilize them (Alloway, 1995; Bolan et al., 2003; Hettiarachchi et al., 2001; Ruby et al., 1994; Seaman et al., 2001a).

Effect of phosphorus fertilization on the uranium availability in soils

Increased levels of water soluble P in soil decreased the plant available U extracted by DTPA and AAAcEDTA extracting solution (fig. 4.7 and 4.8). The curve of U extracted by DTPA reached down to a minimum of U at P level of 150 mg kg^{-1}. However, for both extracting solutions little diminution of U appeared when the water soluble P levels in soil exceeded 25 - 30 mg kg^{-1} (fig. 4.8).

In the substrates fertilized with $CaHPO_4$ an ANOVA (tab. A.8) determined that the mean U extracted by DTPA was significantly affected by the soil substrate, the U treatment and their interactions, whereas mean U extracted by AAAcEDTA was only affected by U contamination.

Mean levels of U extracted by DTPA in the contaminated soils after $CaHPO_4$ addition (tab. 4.15) ranged from 0.21 to 0.76 mg kg^{-1} U and by AAAcEDTA from 18 to 68 mg kg^{-1} U. These values are similar or lower than those of the (unfertilized) most fertile soil substrate (GT) at the same levels of U contamination (tab. 4.7).

After $CaHPO_4$ addition the extracted U amounts were similar for the different substrates (tab. 4.15). Although the levels of extractable U in soils were strongly reduced, they still showed a positive linear relationship following the U contamination for all soil substrates (tab. A.12). U extracted by DTPA was relatively more reduced than that extracted by AAAcEDTA.

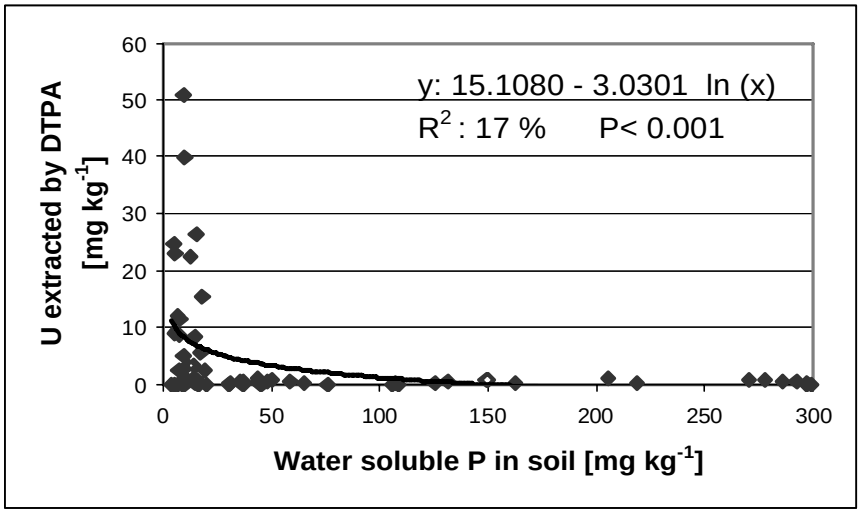


Fig. 4.7: Relationship between water soluble P in soil and U extracted by DTPA (cut 7)

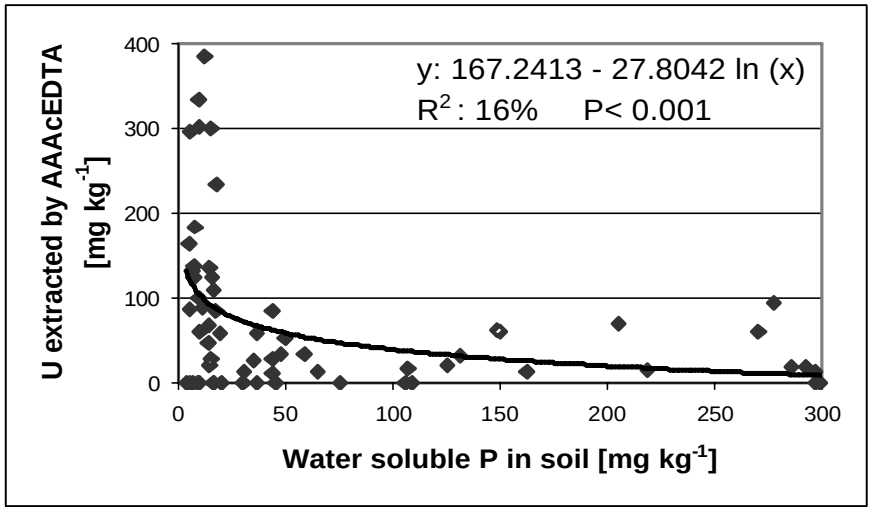


Fig. 4.8: Relationship between water soluble P in soil and U extracted by AAAcEDTA (cut 7)

The effect was important for the highest U level in FS: U extracted by DTPA at 1,000 mg kg^{-1} U (0.74 mg kg^{-1}) was reduced 60-fold compared to that without fertilizer (45.4 mg kg^{-1}, tab. 4.7). Similarly, U extracted by AAAcEDTA was reduced 5-fold (317 down to 61 mg kg^{-1}, tab. 4.7). Mean U extracted by AAAcEDTA was 85-fold of that extracted by DTPA, which was comparatively higher than that ratio of non fertilized substrates (13-fold, tab. 4.7).

Tab. 4.15: Effect of soil substrate and U contamination on U extracted by DTPA and AAAcEDTA in P fertilized substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
			U extracted by DTPA [mg kg^{-1}]		
0	<LLD	0.01	0.01	0.01	0.01
250	0.12	0.21	0.32	0.21	0.21
500	0.29	0.36	0.34	0.38	0.34
1,000	0.61	0.99	0.71	0.74	0.76
Mean for soil substrate	0.25	0.39	0.34	0.34	LSD $_{0.05}$ 0.05
b	$6.2 \cdot 10^{-4}$	$9.8 \cdot 10^{-4}$	$6.5 \cdot 10^{-4}$	$7.2 \cdot 10^{-4}$	
R^2	99 %	96 %	93 %	98 %	
P	***	***	***	***	
			U extracted by AAAcEDTA [mg kg^{-1}]		
0	0.01	0.01	0.02	0.03	0.02 c
250	12	15	29	13	18 b
500	27	27	18	33	26 b
1,000	55	77	78	61	68 a
Mean for soil substrate	24 a	30 a	31 a	27 a	LSD $_{0.05}$ 9.7
b	0.056	0.077	0.072	0.062	
R^2	99 %	95 %	73 %	99 %	
P	***	***	**	***	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA. Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

Effect of phosphorus fertilization on the yields of Lolium perenne

In this experiment an increment of water soluble P level in soil till 50 mg kg^{-1} increased the plant yields (fig 4.9), higher amounts of P in soil produced little changes.

In P fertilized substrates significant differences between the mean yields for the soil substrate effect were determined, but not for the effects caused by U treatments (tab. A.8). After the $CaHPO_4$ addition the grass yield in GT was statistically higher than in the other substrates (tab. 4.16). When yields were compared to non fertilized, U contaminated substrates (tab. 4.9), the yield of *Lolium*

perenne growing in GT and GS showed an increment of 25 %. At this cut the yields of *Lolium perenne* in substrates derived from forest were not increased over unfertilized treatment.

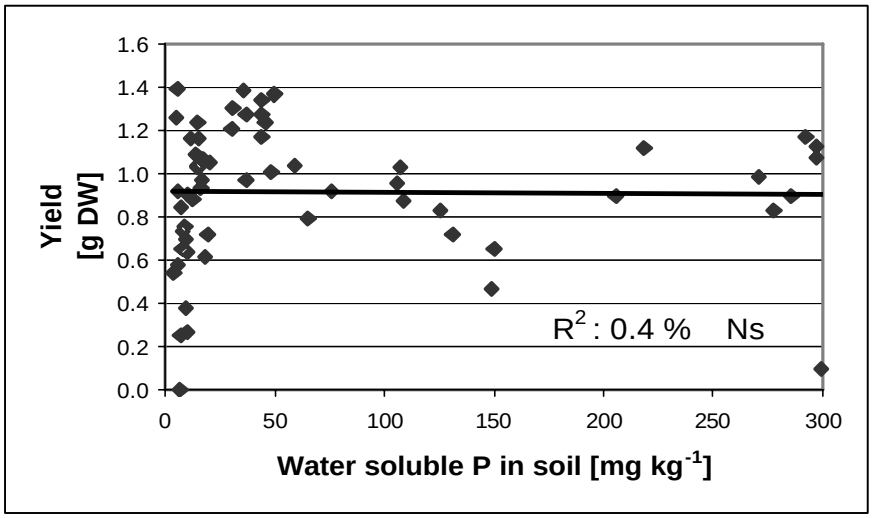


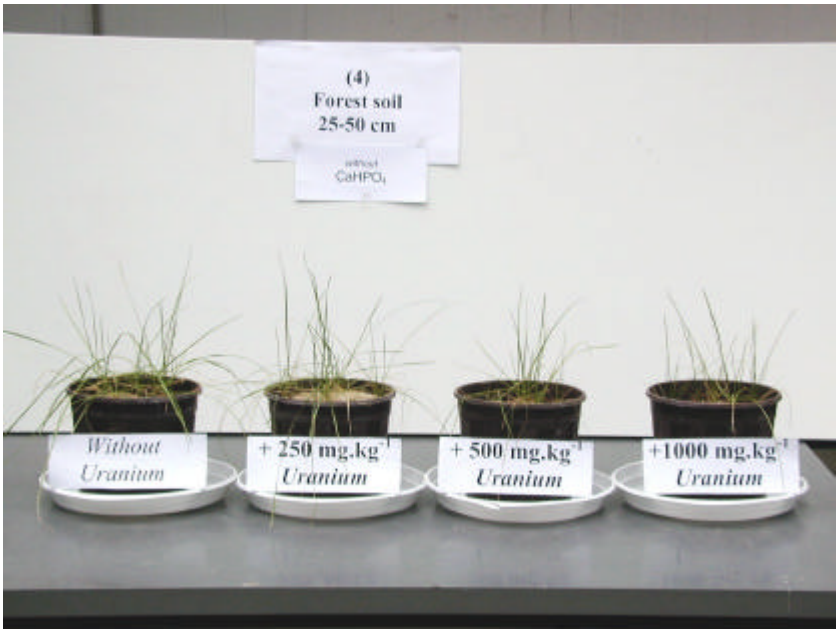
Fig. 4.9: Relationship between water soluble P in soil and yield of *Lolium perenne* (cut 7)

The growth of *Lolium perenne* in contaminated FS, non fertilized and fertilized with $CaHPO_4$, are presented in pictures 4.10 and 4.11. The pictures belong to the cut 3. Although $CaHPO_4$ increased yields over control and no differences for U treatments could be observed, plants were not vigorous, showing the fertility problems of the substrate.

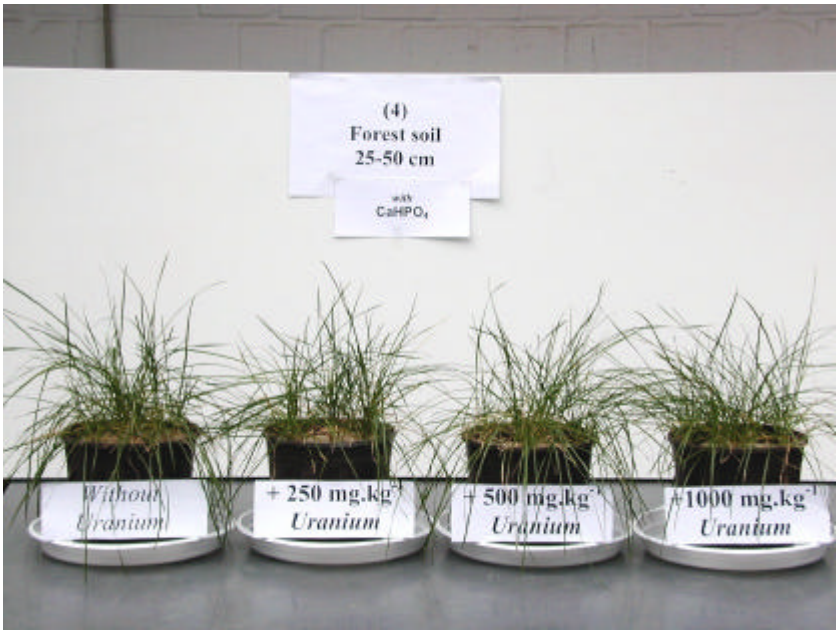
Tab. 4.16: Effect of soil substrate and U contamination on yield per pot of *Lolium perenne* [g] in P fertilized substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	1.26	0.89	1.01	0.75	0.98 a
250	1.33	0.91	1.05	1.12	1.10 a
500	1.33	0.92	1.03	0.88	1.04 a
1,000	1.17	1.03	0.91	0.56	0.92 a
Mean for soil substrate	1.27 a	0.94 b	1.00 b	0.78 b	$LSD_{0.05}$ 0.19
b	$-1.0 \cdot 10^{-4}$	$1.4 \cdot 10^{-4}$	$1.2 \cdot 10^{-4}$	$-2.6 \cdot 10^{-4}$	
R^2	10 %	20 %	16 %	18 %	
P	Ns	Ns	Ns	Ns	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant



Pic. 4.10: Growth of *Lolium perenne* in unfertilized FS substrates contaminated with U (cut 3)



Pic. 4.11: Growth of *Lolium perenne* in P fertilized FS substrates contaminated with U (cut 3)

Effect of phosphorus fertilization on the uranium content of Lolium perenne

As in soil, the U concentration in plant was reduced after the $CaHPO_4$ addition, showing a similar tendency (fig. 4.10).

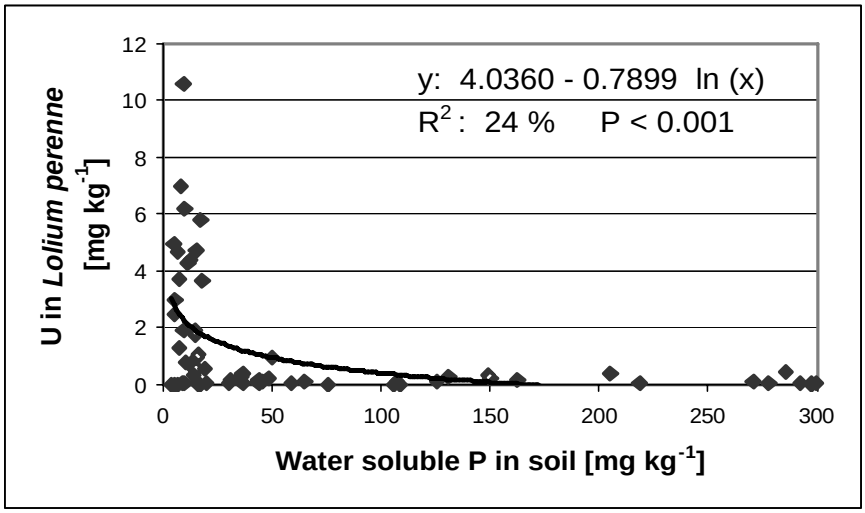


Fig. 4.10: Relationship between water soluble P in soil and U content in *Lolium perenne* (cut 7)

In the substrates fertilized with P, an ANOVA (tab. A.8) determined that the mean U content in *Lolium perenne* at cut 7 was significantly affected by the U treatment but not by the soil substrates. Mean U concentration of all substrates at 1,000 mg kg^{-1} U dose (0.33 mg kg^{-1}, tab. 4.17) was reduced at averaged 13-fold when compared to non fertilized treatments (4.3 mg kg^{-1}, tab. 4.11). As in soil the best performance of $CaHPO_4$ to reduce the U content of *Lolium perenne* was obtained in the low fertile soil substrate (FS) and at the highest U contamination level (1,000 mg kg^{-1}): U content in leaves was diminished 30-fold compared to non fertilized treatment (tab. 4.11).

U content in plant after $CaHPO_4$ addition was still increased following U soil dose (tab. 4.17). But the tendency for each soil substrate was not so straight as for U extracted in soil, as linear regression were less significant (tab. A.12). GT at 1,000 mg kg^{-1} U dose was the only case which was over the typical levels for non contaminated soil (< 0.40 mg kg^{-1}, following Dressen and Marple, 1997; Pais and Jones, 1997).

Tab. 4.17: Effect of soil substrate and U contamination on U content in *Lolium perenne* [mg kg^{-1}] in P fertilized substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	0.05	<LLD	0.02	0.01	0.02 b
250	0.12	0.10	0.04	0.10	0.09 b
500	0.18	0.17	0.23	0.16	0.18 ab
1,000	0.68	0.28	0.08	0.27	0.33 a
Mean for soil substrate	0.26 a	0.14 a	0.09 a	0.14 a	$LSD_{0.05}$ 0.14
b	$6.4 \cdot 10^{-4}$	$2.8 \cdot 10^{-4}$	$7.7 \cdot 10^{-5}$	$2.6 \cdot 10^{-4}$	
R^2	69 %	74 %	5 %	66 %	
P	*	**	Ns	*	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA. Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

Effect of phosphorus fertilization on the uranium uptake by Lolium perenne

U uptake was strongly associated to the U content of *Lolium perenne* and showed the same tendency following the $CaHPO_4$ addition (fig. 4.11).

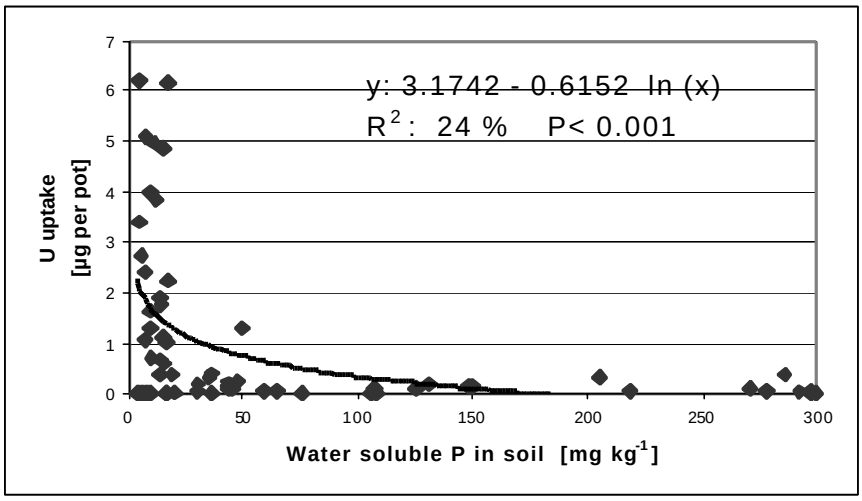


Fig. 4.11: Relationship between water soluble P in soil and U uptake by *Lolium perenne* (cut 7)

In the substrates fertilized with P an ANOVA (tab. 4.8) determined that means of U uptake were statistically different among U treatments but not significant among soil substrates. The U uptake for each soil substrate followed linearly the increased contamination levels except for FT (tab. 4.18 and A.12). Mean U uptake at 1,000 mg kg^{-1} U dose (0.34 µg per pot) was almost 8-fold lower than the corresponding non fertilized treatment (2.62 µg per pot, tab. 4.13).

GT with P fertilization showed the highest U uptake (0.33 µg per pot) because of its higher yields (tab. 4.16) and U content (tab. 4.17) compared with the other substrates, but it was still lower than for the same unfertilized soil substrate (0.56 µg per pot, tab. 4.13).

Tab. 4.18: Effect of soil substrate and U contamination on U uptake per pot by *Lolium perenne* [µg] in P fertilized substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*	
0	0.06	<LLD	0.02	0.01	0.02	b
250	0.16	0.10	0.05	0.05	0.09	ab
500	0.24	0.16	0.21	0.12	0.18	ab
1,000	0.86	0.28	0.07	0.15	0.34	a
Mean for soil substrate	0.33 a	0.13 a	0.09 a	0.09 a	$LSD_{0.05}$	0.21
b	$8.0 \cdot 10^{-4}$	$2.7 \cdot 10^{-4}$	$6.9 \cdot 10^{-5}$	$1.4 \cdot 10^{-4}$		
R^2	59 %	79 %	5 %	64 %		
P	*	**	Ns	*		

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant
LLD: lower than level of detection

4.2.5 Effect of liming: influence of soil pH and calcium content in Lolium perenne

The highest U solubility of uranyl cation occurs at pH 4-5. Uranyl is the chemical species which can be taken up by plants (Ebbs et al., 1998a). At higher soil pH it increases the concentrations of $(UO_2)_3(OH)_5^+$ which is easily adsorbed by soil reducing the U availability. The addition of lime increases the soil pH, but carbonates can form very soluble complexes with U (Ebbs et al., 1998a; Mortvedt, 1994; Duff and Amrhein, 1996; Erikson et al., 1990; Fellows et al., 1998).

Influence of pH on the extractable uranium in soils

No clear tendency could be observed for the U extracted by both of the extracting solutions following soil pH (fig. 4.12 and 4.13), as high and low U values were determined along the entire pH range studied. U amounts up to 300 mg kg^{-1} U extracted by AAAcEDTA (higher than the mean maximum level in non limed soil, tab. 4.7) were determined in the range of pH 3.5-6.5.
In the limed soil substrates an ANOVA (tab. A.8) showed that the U extracted by both extracting solutions had been affected by the soil substrate, the U contamination level and their interactions. Extractable U levels by both solutions were higher in subsoils than in topsoils, and followed the U doses in soil (tab. 4.19). Mean U levels extracted by DTPA after liming were almost twice as much than those in similar non fertilized treatments (tab. 4.7). This was higher for subsoil substrates than for topsoil substrates, probably because of the lower buffer capacity of these substrates. Contrarily, in some samples (GT, GS) in limed substrates the U amount extracted by the AAAcEDTA was diminished compared to non limed treatments (tab. 4.7). Mean amounts of U extracted by AAAcEDTA (145 mg kg^{-1}) in limed soil were 7-fold higher than those extracted by DTPA (20.3 mg kg^{-1}) in contaminated substrates.

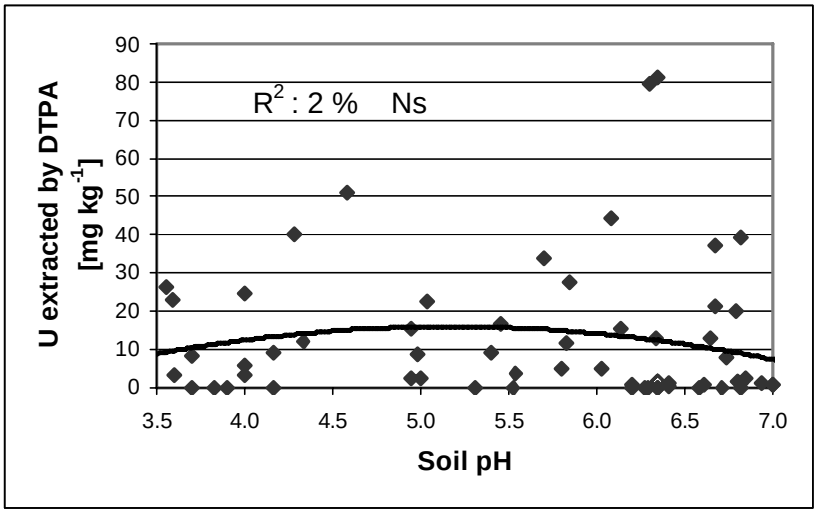


Fig. 4.12: Relationship between soil pH and U extracted by DTPA (cut 7)

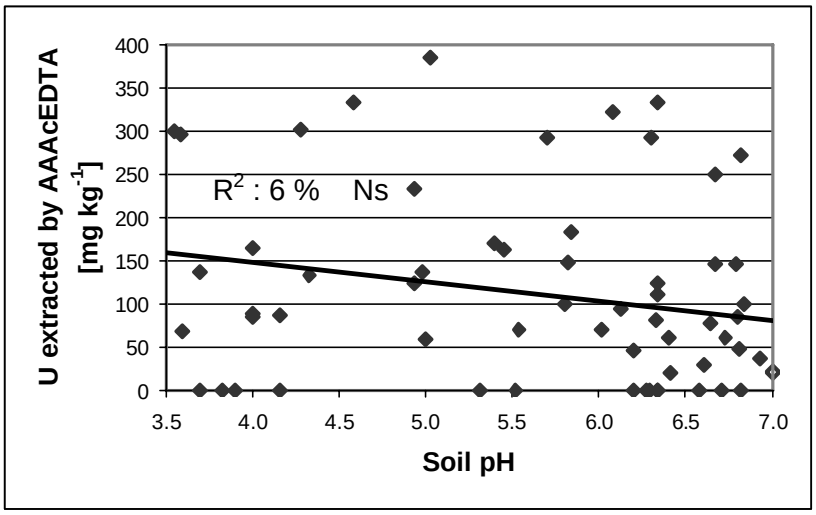


Fig. 4.13: Relationship between soil pH and U extracted by AAAcEDTA (cut 7)

Tab. 4.19: Effect of soil substrate and U contamination on U extracted by DTPA and AAAcEDTA in limed substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
			U extracted by DTPA [mg kg^{-1}]		
0	<LLD	0.01	<LLD	0.01	<LLD
250	0.72	10.45	4.29	14.10	7.39
500	1.02	20.55	10.51	22.11	13.55
1,000	1.93	38.03	39.10	80.27	39.83
Mean for soil substrate	0.92	17.26	13.47	29.12	$LSD_{0.05}$ 3.12
b	0.002	0.038	0.040	0.080	
R^2	90 %	99 %	91 %	93 %	
P	***	***	***	***	
			U extracted by AAAcEDTA [mg kg^{-1}]		
0	0.01	0.03	0.02	0.03	0.03
250	21	69	70	87	62
500	42	145	159	172	130
1,000	92	260	307	313	243
Mean for soil substrate	39	119	134	143	$LSD_{0.05}$ 13.04
b	0.092	0.260	0.310	0.311	
R^2	98 %	99 %	99 %	98 %	
P	***	***	***	***	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : $P < 0.001$ ** : $P < 0.05$ *: $P < 0.01$ Ns: no significant
LLD: lower than level of detection

Influence of pH on the yield of Lolium perenne

The yields of *Lolium perenne* were increased as soil pH was increased by the addition of $CaCO_3$. The better performance (1.5–2.5 g) was determined in the range of pH 5.3–6.3, and then decreased at higher pH levels (fig. 4.14).

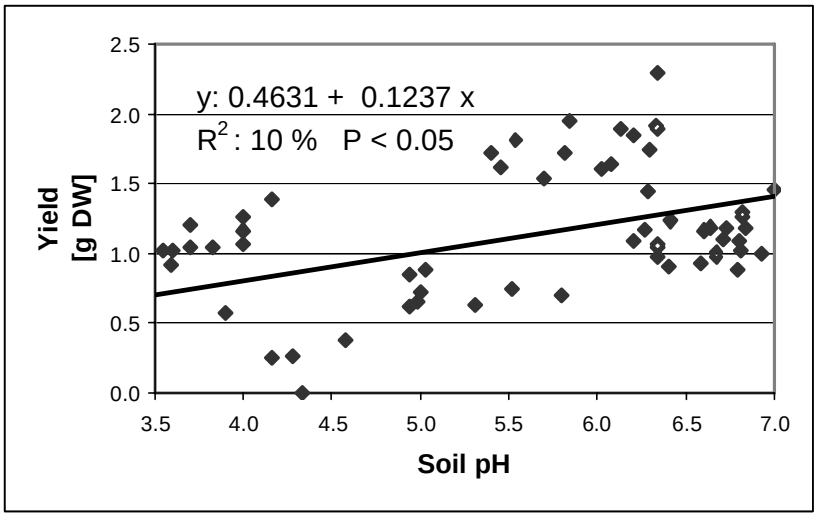


Fig. 4.14: Relationship between soil pH and yield of *Lolium perenne* (cut 7)

In limed substrates, an ANOVA (tab. A.8) determined that no significant effect on mean yields was due to U treatments, but they were significantly different for soil substrates.

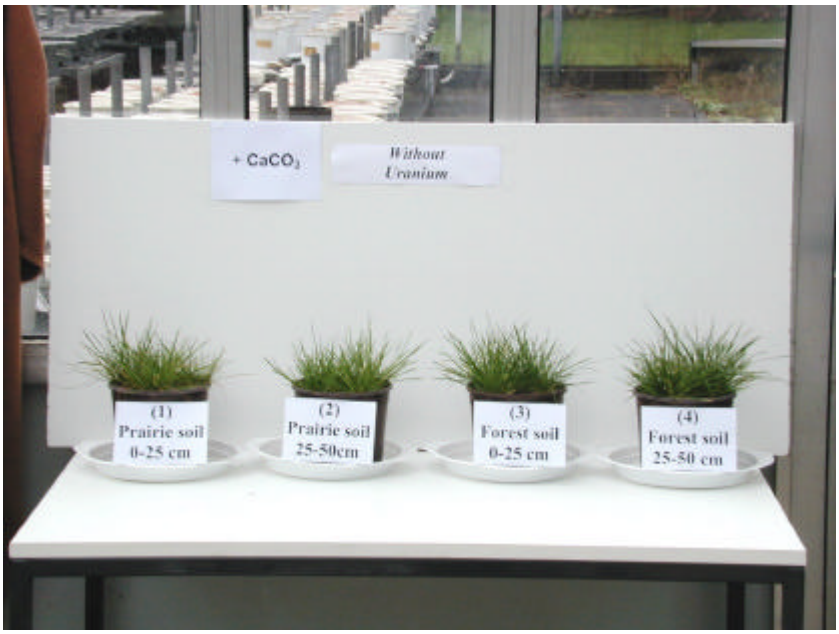
The addition of $CaCO_3$ resulted in higher growth in forest soil than in grassland soil substrates, both in contaminated and in non contaminated treatments (tab. 4.20). This difference was clearer when the poorest soil substrate was contaminated with the highest U amount (1,000 mg kg^{-1}): plant growth in limed FS (2.02 g) was increased 6-fold in comparison with the non fertilized FS substrate (0.32 g, tab. 4.9).

The different influence of liming on the growth of *Lolium perenne* is shown in pictures 4.12 and 4.13 depending on the soil substrate and the U contamination of the soil.

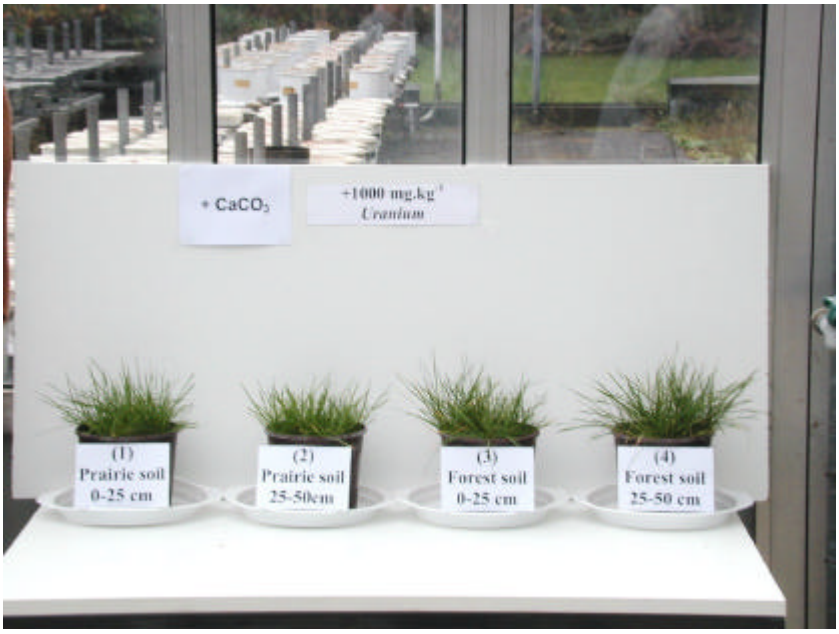
Tab. 4.20: Effect of soil substrate and U contamination on yield per pot of *Lolium perenne* [g] in limed substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	0.94	1.19	1.31	1.87	1.33 a
250	1.46	1.18	1.71	1.90	1.58 a
500	1.01	0.95	1.72	1.78	1.37 a
1,000	1.14	1.13	1.59	2.02	1.47 a
Mean for soil substrate	1.09 c	1.11 c	1.58 b	1.90 a	$LSD_{0.05}$ 0.20
b	$9.5 \cdot 10^{-5}$	$-7.9 \cdot 10^{-5}$	$2.0 \cdot 10^{-4}$	$1.3 \cdot 10^{-4}$	
R^2	3 %	5 %	16 %	7 %	
P	Ns	Ns	Ns	Ns	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant



Pic. 4.12: Growth of *Lolium perenne* in different non contaminated soil substrates after liming (cut 7)



Pic. 4.13: Growth of *Lolium perenne* in different U contaminated soil substrates after liming (cut 7)

Influence of pH on the uranium content in Lolium perenne

The addition of $CaCO_3$ increased the soil pH and the Ca content in leaves as showed in tab. 4.4 and 4.5. The U content in plant showed a decreasing tendency following the increased soil pH and Ca content in leaves (fig. 4.15 and 4.16). The lowest U contents were observed in the range of pH 5.3 - 6.2, that could be attributed to the dilution effects corresponding with the higher growth because of liming (fig. 4.14). The highest level of Ca in leaves also coincided with this pH (fig. 4.6 and 4.15, tab. 4.5), and could be caused by changes in speciation of U (e.g. the soluble uranyl form).

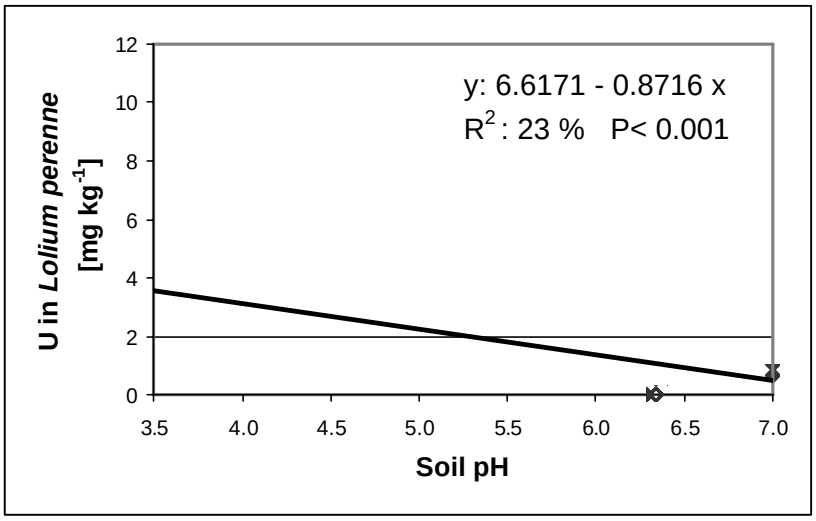


Fig. 4.15: Relationship between soil pH and U content in *Lolium perenne* (cut 7)

In limed substrates an ANOVA (tab.a.8) determined that the mean U content in leaves of *Lolium perenne* were statistically different for U contamination and soil substrates.

Although, they both presented very low determination coefficients (R^2), the Ca/U relationship in plant (fig. 4.16) showed a better fit than the pH/U relationship (fig. 4.15). Mean U concentration in plant followed the U contamination in soil substrates, showing linear regression for each substrate (tab. 4.21).

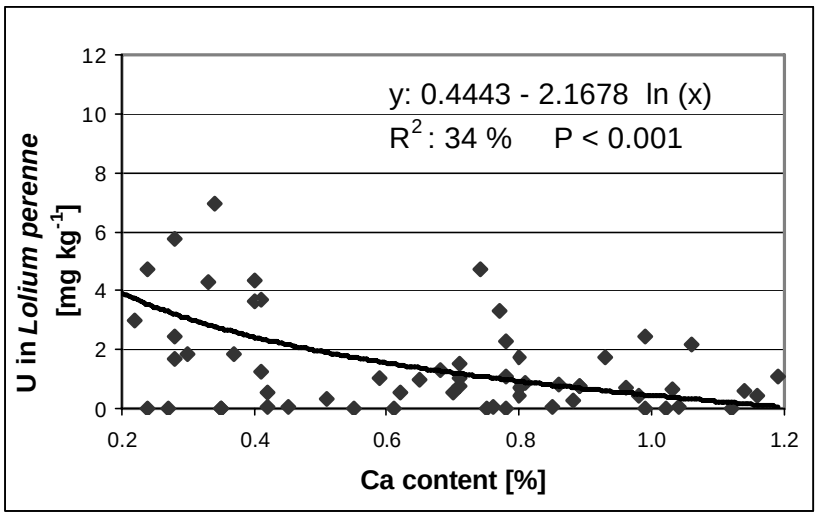


Fig. 4.16: Relationship between Ca and U content in *Lolium perenne* (cut 7)

Tab. 4.21: Effect of soil substrate and U contamination on U content in *Lolium perenne* [mg kg^{-1}] in limed substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	0.02	0.04	0.02	LLD	0.02 c
250	0.88	0.81	0.34	0.89	0.73 b
500	0.63	2.20	0.52	1.86	1.30 ab
1,000	1.55	3.50	0.68	2.11	1.96 a
Mean for soil substrate	0.77 bc	1.64 a	0.39 c	1.21 ab	$LSD_{0.05}$ 0.66
b	0.001	0.003	$6.2 \cdot 10^{-4}$	0.002	
R^2	76 %	70 %	82 %	76 %	
P	**	**	**	**	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant

When $CaCO_3$ was added (tab. 4.21) the U concentration in plant was decreased in both forest substrates and subsoil of grassland substrates but increased in the GT compared to non fertilized soil substrates (tab. 4.11). The mean U content of *Lolium perenne* growing on U contaminated GT was increased almost 45 % after $CaCO_3$ addition compared to non limed (tab. 4.11). Except for

forest topsoil at 500 mg kg^{-1} U, all other U contents in *Lolium perenne* were higher than the 0.4 mg kg^{-1} U given as typical for plants in non contaminated soils by Dressen and Marple (1979).

Influence of pH on uranium uptake by Lolium perenne

It was found a tendency of U uptake diminution at higher pH of soil. But as for all the other variables investigated in this chapter, both high and low values were obtained along the calculated regression curve (fig. 4.17).

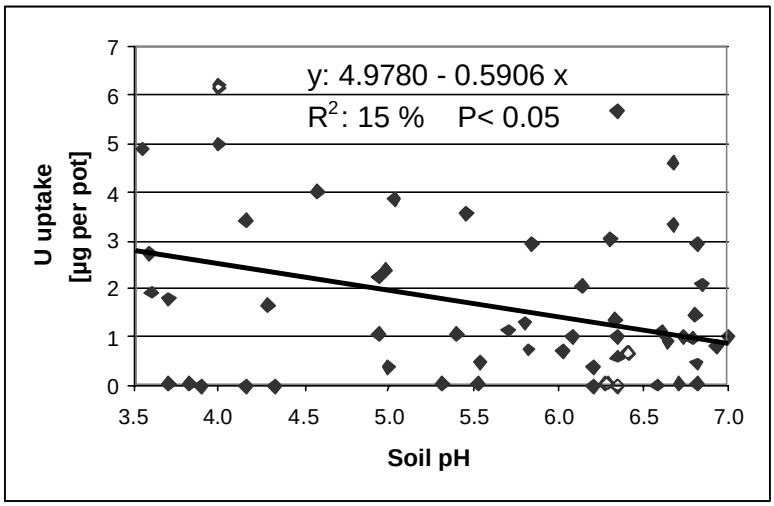


Fig. 4.17: Relationship between soil pH and U uptake by *Lolium perenne* (cut 7)

In the limed substrates an ANOVA showed that the mean U uptake at cut 7 had been significantly affected by the U treatment and the soil substrate (tab. A.8)

As for U concentration in plant, mean U uptake showed a linear association to U doses in all substrates (tab. 4.22). Plants growing in subsoils showed a higher U uptake than those in topsoils. With the addition of $CaCO_3$ the forest topsoil showed the lowest U uptake. The forest subsoil showed the highest U uptake by *Lolium perenne*. The mean U uptake by plants growing on contaminated grassland substrates was increased almost 55 % after liming (1.14 mg kg^{-1}) compared to non fertilized (0.74 mg kg^{-1}, tab. 4.13), probably because of its buffer capacity was reduced. U uptake was decreased in forest substrates. Mean U uptake in contaminated samples (1.85 mg kg^{-1}, tab. 4.22) was decreased almost 40 % compared to unfertilized (2.54 mg kg^{-1}, tab. 4.13).

Tab. 4.22: Effect of soil substrate and U contamination on U uptake per pot by *Lolium perenne* [µg] in limed substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
0	0.01	0.05	0.03	<LLD	0.02 c
250	1.02	0.96	0.58	1.70	1.07 bc
500	0.63	2.16	0.90	3.25	1.74 ab
1,000	1.77	3.77	1.09	4.36	2.75 a
Mean for soil substrate	0.84 bc	1.73 ab	0.65 c	2.33 a	$LSD_{0.05}$ 0.85
b	0.002	0.004	$9.9 \cdot 10^{-4}$	0.004	
R^2	80 %	78 %	79 %	78 %	
P	**	**	**	**	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant
LLD: lower than level of detection

U availability in limed soil substrates was not dependent on soil pH, as interactions with the extracting solution existed. At the highest U dose (1,000 mg kg^{-1}) yields after liming (tab. 4.20) were increased only 10 % in GT against 6-fold in forest subsoil compared to non limed substrate (tab. 4.9). Mean U content (1.96 mg kg^{-1}) at that dose in limed soils (tab. 4.21) was half of that of non limed (4.27 mg kg^{-1}, tab. 4.11) as it was shown in table 4.14. At this U dose U content was reduced to 25 % in FS, but it was doubled for GT in limed compared to non limed substrates. U content in *Lolium perenne* grown in limed soils were over standard level (> 0.4 mg kg^{-1}, following Dressen and Marple, 1979). It cannot be discarded that U also could have produced a stimulatory effect on yields of *Lolium perenne* after liming. Mean U uptake (tab. 4.22) was increased in grassland but decreased in forest compared to unfertilized substrates (tab. 4.13), but at the highest U contamination level liming also increased the U uptake by *Lolium perenne* growing in the low fertile soil substrate (FS).

4.2.6 Effect of phosphorus fertilization and liming on the studied soil and plant variables

No special figures are presented to show the effect of the addition of the both fertilizers on the studied variables. For the fertilization treatments an ANOVA (tab. A.8) determined that the U extracted by DTPA had been affected by fertilization, soil substrate and their interactions. U extracted by AAAcEDTA had been only significantly affected by U treatment. The yields of *Lolium perenne* were significantly affected only by the soil substrate. The U content and uptake were influenced by both factors.

Tab. 4.23: Effect of soil substrate and U contamination on U extracted by DTPA and AAAcEDTA in P fertilized and limed substrates (cut 7)

U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
			U extracted by DTPA [mg kg^{-1}]		
0	<LLD	0.01	0.01	0.04	0.01
250	0.14	0.26	0.18	0.22	0.20
500	0.33	0.51	0.24	0.30	0.35
1,000	0.70	1.36	0.52	0.68	0.82
Mean for soil substrate	0.29	0.54	0.24	0.31	$LSD_{0.05}$ 0.13
b	$7.1 \cdot 10^{-4}$	0.001	$5.0 \cdot 10^{-4}$	$6.3 \cdot 10^{-4}$	
R^2	83 %	94 %	96 %	92 %	
P	***	***	***	***	
			U extracted by AAAcEDTA [mg kg^{-1}]		
0	0.01	0.06	0.06	0.03	0.04 d
250	14	15	12	15	14 c
500	25	31	31	27	28 b
1,000	59	63	47	64	58 a
Mean for soil substrate	24 a	27 a	23 a	26 a	$LSD_{0.05}$ 5.4
b	0.059	0.063	0.048	0.063	
R^2	98 %	98 %	88 %	87 %	
P	***	***	***	***	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA. Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant
LLD: lower than detection level

Tab. 4.24: Effect of soil substrate and U contamination on yield, U content and U uptake by *Lolium perenne* in P fertilized and limed substrates (cut 7)

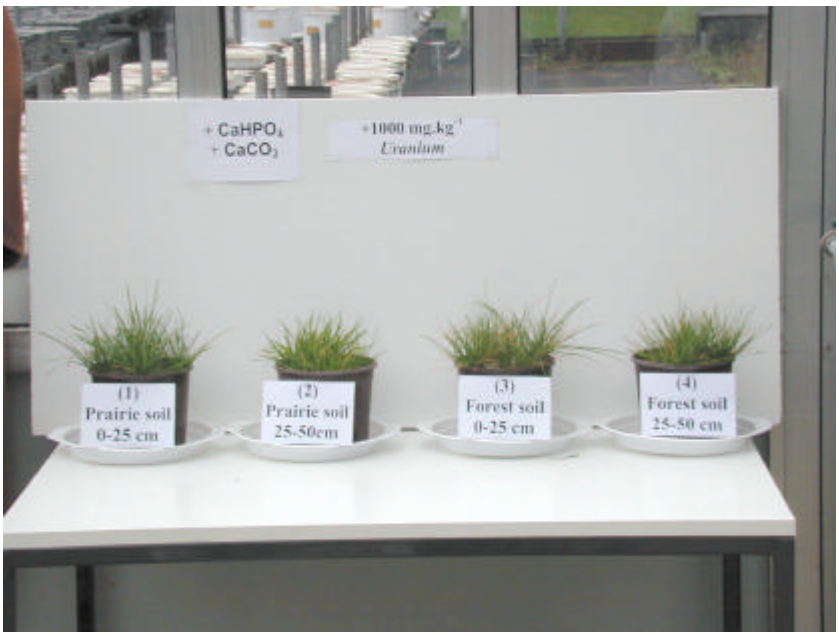
U contamination [mg kg^{-1}]	GT	GS	FT	FS	*Mean for U treatment*
			Yield [g per pot]		
0	1.20	0.97	1.25	1.24	1.17 a
250	1.32	0.84	1.44	1.37	1.24 a
500	1.20	1.00	1.56	1.31	1.27 a
1,000	1.07	0.85	1.57	1.19	1.17 a
Mean for soil substrate	1.20 b	0.91 c	1.46 a	1.28 ab	$LSD_{0.05}$ 0.16
b	$-1.8 \cdot 10^{-4}$	$-7.5 \cdot 10^{-5}$	$3.0 \cdot 10^{-4}$	$-8.8 \cdot 10^{-5}$	
R^2	30 %	3 %	34 %	9 %	
P	Ns	Ns	Ns	Ns	
			U content in *Lolium perenne* [mg kg^{-1}]		
0	0.02	<LLD	0.04	0.01	0.02 b
250	0.13	0.23	0.07	0.04	0.12 ab
500	0.29	0.24	<LLD	0.07	0.15 ab
1,000	0.37	0.82	0.05	0.14	0.35 a
Mean for soil substrate	0.20 ab	0.32 a	0.04 b	0.06 b	$LSD_{0.05}$: 0.19
b	$3.5 \cdot 10^{-4}$	$8.0 \cdot 10^{-4}$	$2.9 \cdot 10^{-7}$	$1.3 \cdot 10^{-4}$	
R^2	52 %	61 %	<1 %	72 %	
P	*	*	Ns	**	
			U uptake by *Lolium perenne* [µg per pot]		
0	0.02	<LLD	0.05	0.01	0.02 b
250	0.18	0.19	0.11	0.06	0.13 b
500	0.35	0.35	<LLD	0.09	0.17 ab
1,000	0.37	0.37	0.08	0.16	0.30 a
Mean for soil substrate	0.23 ab	0.25 a	0.06 c	0.08 c	$LSD_{0.05}$ 0.11
b	$3.4 \cdot 10^{-4}$	$5.5 \cdot 10^{-4}$	$1.1 \cdot 10^{-5}$	$1.5 \cdot 10^{-4}$	
R^2	50 %	85 %	<1 %	76 %	
P	Ns	**	Ns	**	

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Means of substrate and U treatment without letters are presented when interactions between these factors were determined by an ANOVA. Means with the same letters were not significantly different at P = 0.05 determined by the Tukey test.
b, R^2 and P: coefficients and level of significance of linear regression equations between soil and plant variables and U dose. *** : P < 0.001 ** : P < 0.05 *: P < 0.01 Ns: no significant
LLD: lower than detection level

By addition of both fertilizers, as for P alone, the available U was diminished in all the substrates but it still showed a linear regression following the levels of U contamination (tab. 4.23). Only

when lime had been added the yields were higher in forest than in grassland soil substrates (tab. 4.24). Although all the values were very low, the substrates derived from grassland showed higher U content and U uptake than the substrates derived from forest. As for P alone the U concentration in *Lolium perenne* in the grassland substrates were higher than in the forest substrates. Except grassland subsoil, at 1,000 mg kg^{-1} the U levels were all under the typical values for plants in non contaminated soils (< 0.4 mg kg^{-1} DW; Dressen and Marple, 1979).

Picture 4.14 shows the growth of *Lolium perenne* in different substrates contaminated with 1,000 mg kg^{-1} U, and fertilized with P and lime, at cut 7.



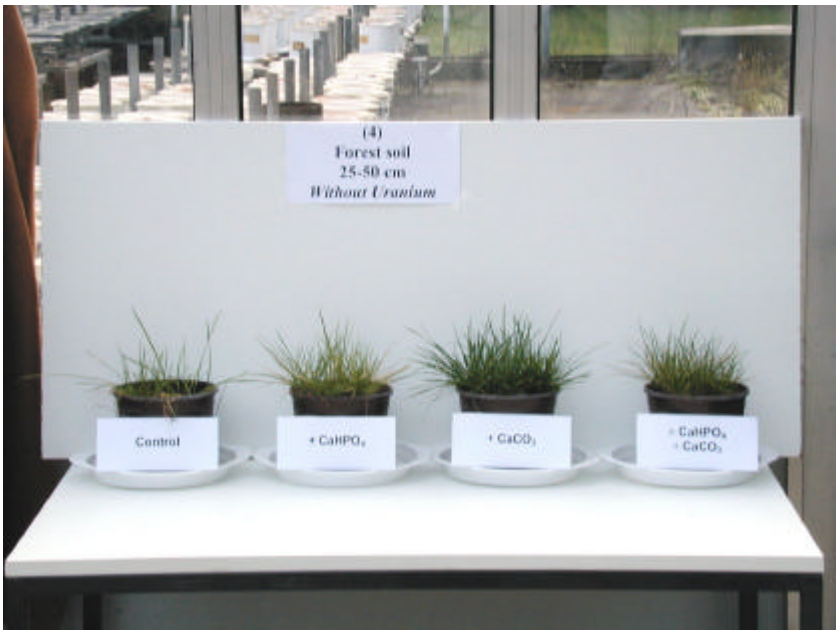
Pic. 4.14: Growth of *Lolium perenne* in different substrates contaminated with U (1,000 mg kg^{-1}), fertilized with P and lime (cut 7)

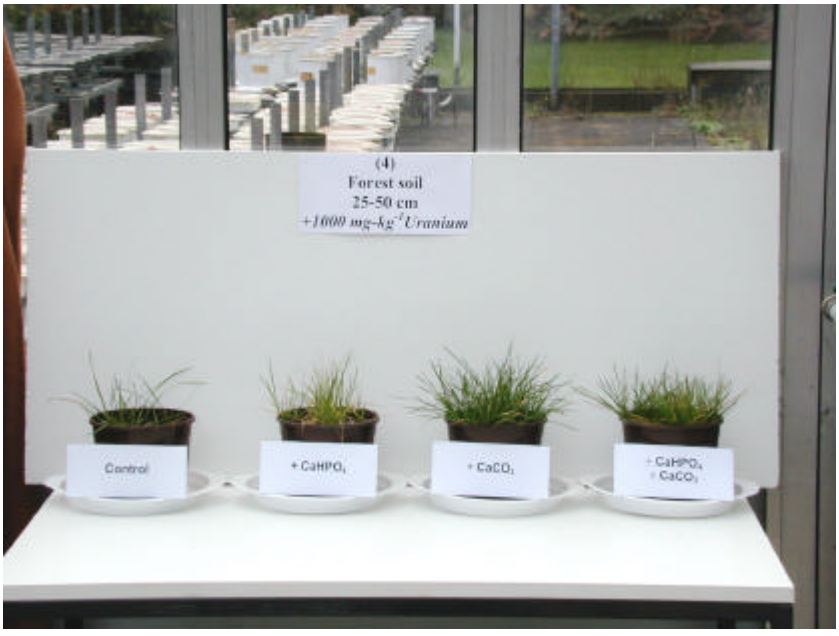
The following pictures (pic. 4.15-4.18) demonstrate the influence of different fertilizer management on *Lolium perenne* plants growing in GT, GS, FT and FS with and without U contamination (0 and 1,000 mg kg^{-1} U) before cut 7.

Pic. 4.15: Influence of different fertilizer treatments on the growth of *Lolium perenne* in non contaminated and high U contaminated GT substrates (cut 7)

Pic. 4.16: Influence of different fertilizer treatments on the growth of *Lolium perenne* in non contaminated and high U contaminated GS substrates (cut 7)

Pic. 4.17: Influence of different fertilizer treatments on the growth of *Lolium perenne* in non contaminated and high U contaminated FT substrates (cut 7)

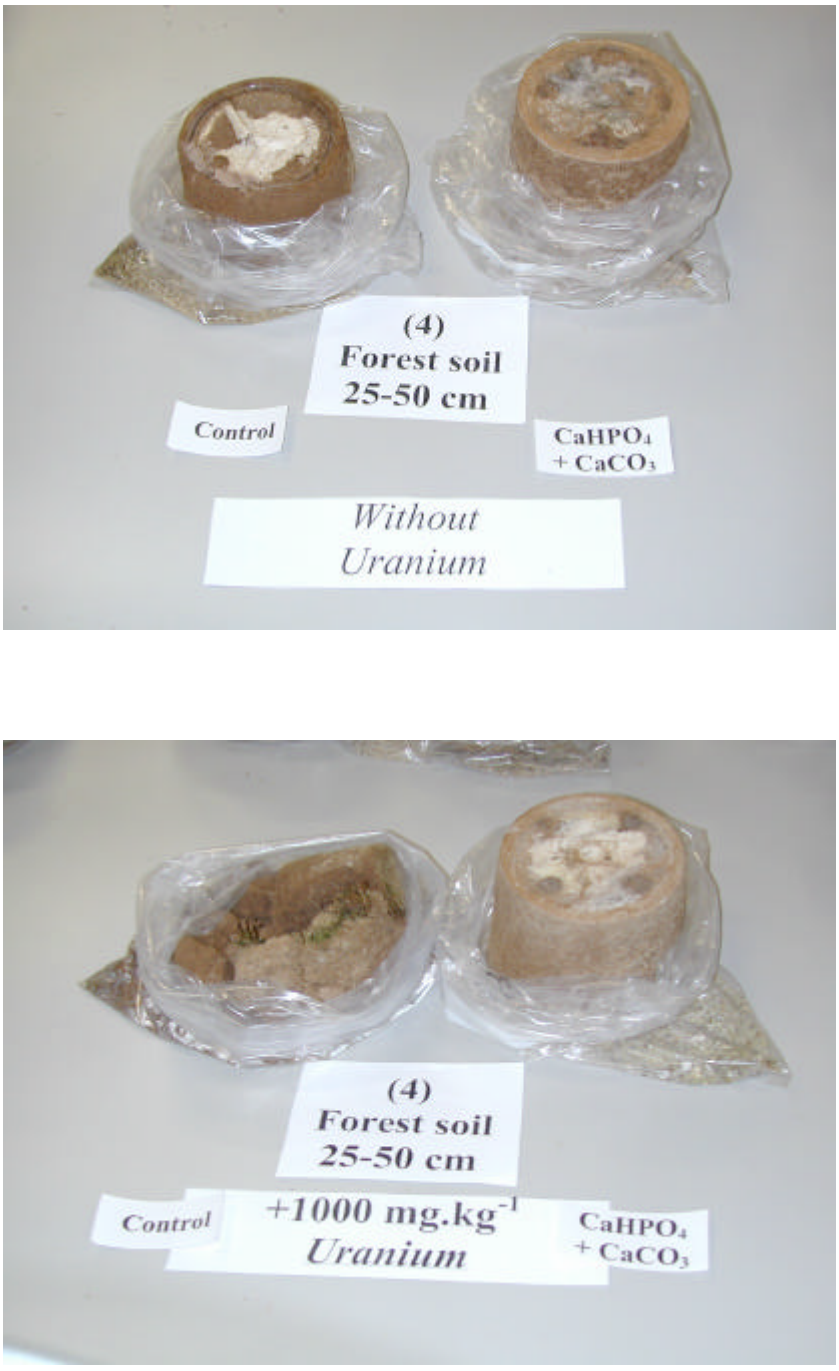




Pic. 4.18 Influence of different fertilizer treatments on the growth of *Lolium perenne* in non contaminated and high U contaminated FS substrates (cut 7)

The root distribution in GT and FS at control and 1,000 mg kg^{-1} U is shown in pictures 4.19 and 4.20. It can be observed that the root balls were formed by forest substrate (pic. 4.20), and less changes were produced in GT (pic. 4.19).

Pic. 4.19: Root distribution in high U contaminated GT substrates, unfertilized and fertilized with P and lime



Pic. 4.20: Root distribution in non contaminated and high U contaminated FS substrates, unfertilized and fertilized with P and lime

Comparison of the fertilizer effects on reduction of uranium content in Lolium perenne

A comparison of all fertilizer treatments tested to reduce U in each soil substrate are shown in the fig. 4.18-4.21. The LSD test, at a level of 5 %, was used for statistical analysis.

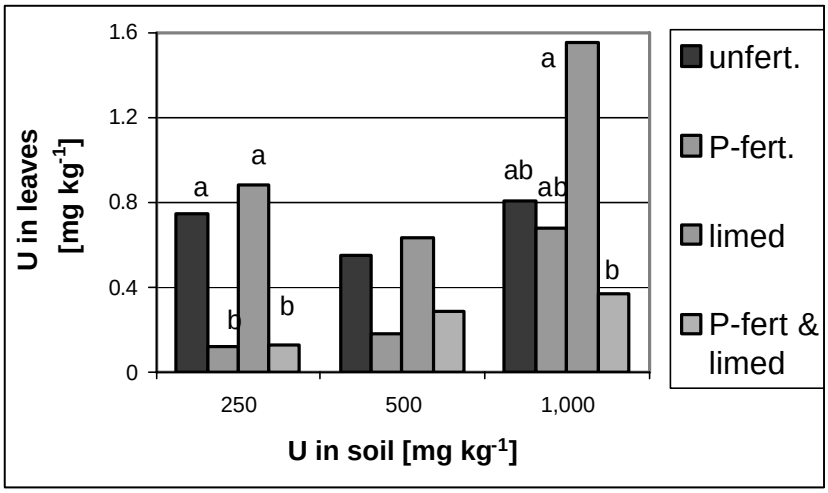


Fig. 4.18: Effect of fertilizer addition on U content in *Lolium perenne*, GT (cut 7)
Significant differences are declared by different letters (LSD, at 5 % level)

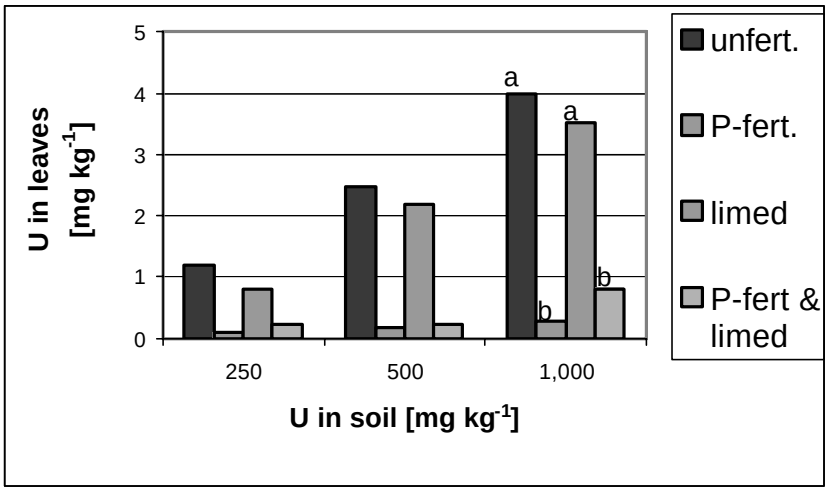


Fig. 4.19: Effect of fertilizer addition on U content in *Lolium perenne*, GS (cut 7)
Significant differences are declared by different letters (LSD, at 5 % level)

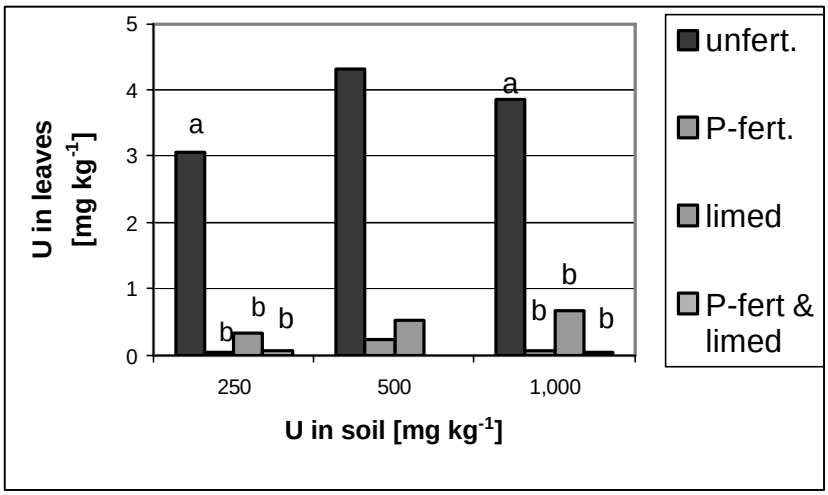


Fig. 4.20: Effect of fertilizer addition on U content in *Lolium perenne*, FT (cut 7)
Significant differences are declared by different letters (LSD, at 5 % level)

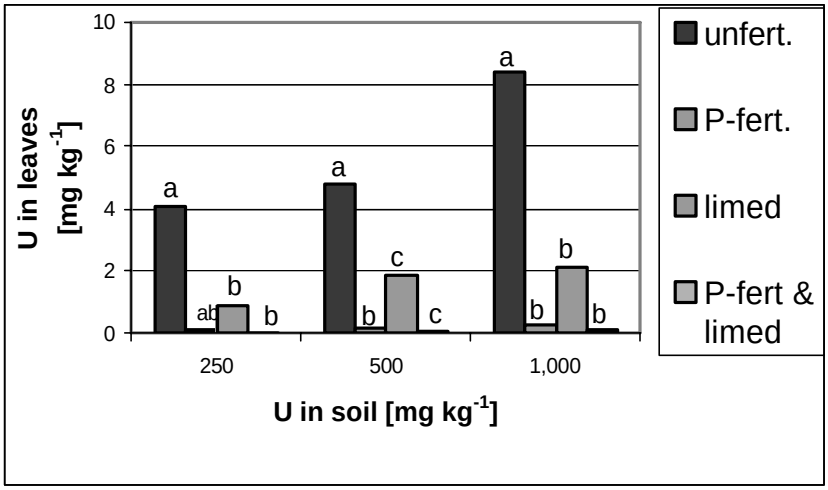


Fig. 4.21: Effect of fertilizer addition on U content in *Lolium perenne*, FS (cut 7)
Significant differences are declared by different letters (LSD, at 5 % level)

It can be observed that plants growing in the substrate derived from grassland were able to maintain low U concentrations in leaves except when $CaCO_3$ had been added (fig. 4.18-4.21). Levels of U decreased by addition of $CaCO_3$ probably occurred by dilution effect. $CaHPO_4$ alone or with lime showed the most pronounced effect to reduce U availability for plants.

In the experiments of this research work even at contamination levels of 1,000 mg kg^{-1} U, the U contents in plants were reduced to nearly normal values (< 0.4 mg kg^{-1}; following Dressen and

Marple, 1979) by the P addition. Figures 4.18 - 4.21 show that although not in all cases it was statistically significant, P alone or in combination with lime decreased the U content in *Lolium perenne*. The effect of liming was dependent on the properties of soil substrates.

The levels of P and Ca in leaves were affected by soil substrate and fertilization treatment (tab. A.16 and A.17). Except for GT the content of P in leaves were high in P fertilized substrates.

Complete data set in tables A.18-A.22

5 Discussion

5.1 Critical assessment of the used experimental methods

The experiments of this work were designed to quantify the effects of U on plant growth and to evaluate the relative effects of some selected soil factors on the U availability. The range of U doses (0 up to 1,000 mg kg^{-1} U) used for soil contamination within the pot experiment were chosen to represent the levels of U contamination that have been frequently found in areas where ammunitions with DU were used, as Kosovo (UNEP II, 2001) or nuclear and testing weapon sites (Elles et al., 1997b; Elles and Lee, 1998; Hanson, 1974; Mason et al., 1997; Meyer and McLendon, 1997; UNEP II, 2001). The soils represented a broad range in fertility parameters from low (forest subsoil) until optimal (grassland topsoil) fertility. The treatments were selected to produce changes of the levels of P, Ca and the pH of soils, which were expected to influence the U behavior.

Uranium source (preparation and chemical nature)

When DU ammunitions are used, metal U is oxidized in contact with the air. Schoepite ($UO_2(OH)_2$-nH_2O) seems to be one of the more common forms of U metal after weathering in the earth crust, and has been determined in the surface of the ammunitions and in all the soils where DU was released (Erikson et al., 1990; Meyer and McLendon, 1997; Meyer et al., 1998b; Meyer at al., 2004; Morris et al., 1996; UNEP II, 2001).

The green modification (Fleckenstein, 1972) prepared for the experiments of this research work consists of a mixture of U oxides. The first question to solve was, if this artificial U source would have the same behavior in the environment as DU coming from ammunitions. An answer to this question can be made by a comparison of the U availability for plants, between the results of this work and those obtained by Meyer and McLendon (1997) and Meyer et al. (2004) using schoepite which came directly from a weapon testing site (fig. 5.1).

Some variables of the work of Meyer et al. (2004) had to be recalculated from the original data and the comparison below was made under the assumptions that results from both studies derived from different grass plants growing in pots where the substrates had been contaminated with similar amounts of U.

The most important differences between both experiments were:

a) The experiment of Meyer et al. (2004) lasted 82 days with only one cut. For the comparison the average of the data of the 1st and 2nd cuts of this work (at 56 and 140 days, respectively) were used.

b) The mean value of the U concentration in aboveground mass obtained for the three native grasses tested by Meyer et al. (2004) was used. The experiments of this work were conducted with *Lolium perenne*.

c) Data for the highest water regime from Meyer et al. (2004) were used which seemed most similar to the conditions of the experiment of this work.

d) In the experiment reported here the U_3O_8 was mixed with the whole soil substrate of the pot. Meyer et al. (2004) applied milled schoepite to the upper 6 cm of a sand substrate and then they calculated the U concentration, as an average for the whole pot.

e) Meyer et al. (2004) calculated the plant U content on the basis of plant ashes, whereas the U content of grass leaves in the greenhouse experiment was calculated on the effective grown dry matter per cut.

f) The U contamination levels were not exactly the same in both experiments, but their range is comparable. So, the U concentration in the plant material of Meyer´s experiment was calculated corresponding to the given doses here (fig. 5.1).

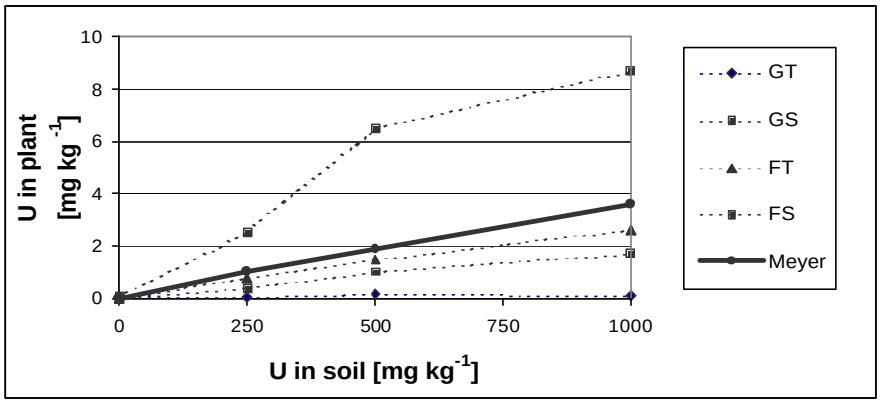


Fig. 5.1: Comparison of results on U content in plants depending on U contamination of soils, with results from Meyer et al. (2004)
GT, GS, FT and FS: native soil substrates, contamination with uranium oxides, greenhouse experiment, Braunschweig, 2001
Meyer: sand substrate, contamination with schoepite

The results showed that both U sources in soil yielded U plant concentration in the same range (fig. 5.1), indicating that the U_3O_8 manufactured especially for this experimental investigations was a suitable source to study the behavior of DU in soils instead of substrates directly contaminated by ammunitions.

Greenhouse experiment

Many types of amendments have been studied in the last years to change the physicochemical properties of soils and to reduce the solubility of contaminants, and consequently their mobility and bio-availability (Boisson et al., 1999; Ebbs et al., 1998b; Mason et al., 1997; Seaman et al., 2001b). Because of the formation of insoluble phosphates, different sources of P proved to be cost-effective to immobilize Cd, Zn or Pb in situ (Anon. VIII, 2001; Basta et al., 2001; Bolan et al., 2003; Hettiarachchi et al., 2001; Ma et al., 1993; Ruby et al., 1994; Seaman et al., 2001a). Apatite is the most commonly used P form, but also commercial fertilizers could be used for this purpose. Hydroxy-apatite has been shown to be successful to immobilize U in batch and leaching columns at an application of 0.5 % w/w, but it was affected by the addition of Ca, illite and zeolite (Seaman et al., 2001b). The objective of this research work was particularly to investigate the effect of common P fertilizers on the U uptake on agricultural soils. In this work $CaHPO_4$ was applied at 0.15 P % w/w as a source of P and Ca, this is higher than usually used in P fertilization but still lower than used for the remediation of contaminated soils (Seaman et al., 2001a; Hettiarachchi et al., 2001).

Acidic soils require liming to increase their fertility, to sustain crop production and to improve the activity of microorganisms in soils. Liming has been effective to reduce Cd, Zn or Sr uptake from contaminated soils (Haneklaus and Schnug, 2001). Because of the increased solubility of U in the uranyl-CO_3 form, many works consider it to have a negative effect on U mobility, however, most are referred to very high amounts of lime added (Buck et al., 1996; Duff et al., 1998; Mason et al., 1997) and not to practical applications in crop fields.

Analytical methods

Soil extraction

When U in soil is increased by contamination, besides the total U content the levels of available U needs to be determined. Sheppard and Evenden (1992a) and Sheppard et al. (1992) found that $NaHCO_3$ as a extracting solution was strong and reflected total soil U rather than its bio-availability. But with the CH_3COONH_4 lower amounts of U were extracted which correlated well to U in plants.

The extracted amounts of U are probably more sensitive to the pH of the extracting solutions than other heavy metals. The importance of the pH for the U extraction was clearly demonstrated by several authors who tested different organic and inorganic substances to increase U availability within the scope of phytoremediation techniques (Ebbs et al., 1998b; Huang et al., 1998). Neither acids nor chelating agents or citrate alone extracted high amounts of U, but citric acid reduced the pH and notably enhanced the extraction of U. Rare earth which are often studied together with U in soils, showed better correlation between levels in soils and plants using an acid inorganic extracting solution (Li et al., 2001).

Plant analysis by ICP determination

Although U is not a new contaminant in the environment, other radionuclides, like Pu, Cs and Sr, have received more attention, especially after the Chernobyl accident (Fellows et al., 1998). One of the reasons could be the difficulties of its measurement, alpha radiation from U is not directly measurable and nuclear methodologies are not easily available, so in the past U determination was very laborious and only conducted by few laboratories at high costs and skills. Due to this, only few samples could be processed, which is especially a problem for ecological studies which have to rely on a broader statistical basis. New possibilities for environmental research with U arise with the availability of Inductively Coupled Plasma-Quadrupole Mass Spectrometry (ICP-QMS), because it can measure at very low concentration (ppb) an appreciable quantity of samples. For the determination of U with radiochemical methods, it is necessary to gather the radioactivity of all relevant U nuclides, whereas with the ICP-QMS only needs to capture ^{238}U because it accounts to 99.3% of the weight of natural uranium. The most important feature of the ICP-QMS compared with radiochemical or optical emission spectroscopy (ICP-OES) is, that no enrichment steps are required for the concentration range. The digestion processes for plant material and the determination of ^{238}U by ICP-QMS in soils, plants and waters was described by Lamas et al. (2002).

A comparison of U^{238} measured by ICP-QMS with radiochemical methods, conducted by Sparovek et al. (2001), showed a good agreement of both methods.

5.2 Effect of uranium application on growth and yield of Lolium perenne

It has been suggested that low U dose in soil could be beneficial for the plant growth because of the "so called hormetic effect". But even if this would be true, U is still a toxic element, which could enter by plants in the food chain.

For the conditions of the greenhouse pot experiment it was found, that the same U dose in soil produced stimulatory and inhibitory effects on the grass growth in different moments (fig. 4.2-4.5). But, except for forest subsoil (FS), if the accumulated yields of *Lolium perenne* were considered, they did not result in net diminution or increment and the growth was predominantly determined by the quality of the soil fertility (tab. 4.1).

As in grassland substrates the dry matter was increased at the first cuts by all U doses, and further growth was reduced. Contrarily, in forest topsoil (FT) at first growth was decreased by U contamination and later increased by the same levels. The process could be described for the tested substrates and U contamination levels as a stimulation-detrimental alternative effect on growth.

In the soil substrate with the lowest fertility (FS) the process was delayed. The stimulation of growth was statistically significant at 250 mg kg^{-1} U dose and started in the cut 4, evidently associated to the longer time between cuts 3–4, following the highest growth of spring time, which provided the grass with a higher mineral element uptake and DW production. At 500 mg kg^{-1} U dose this effect just appeared significant in the cut 7, and could be reasonably expected to occur at 1,000 mg kg^{-1} U dose in a longer time, as happened in the other soils.

The hormetic effect on the growth of crops is typically represented by a beta (ß-) curve dose response (fig. 2.1). A contribution from toxicology have to be considered. The ß-curve divided in two parts: the first, the ascendent, where the stimulation occurs (from the control up to the maximum positive effect), and the second or the descendent part, where inhibitory and stimulating effects coexist, till down to the non observed adverse effect level (NOAEL), from which on only inhibitory effects are to observe. Many half curves with opposite sense appeared during the experiment and a complete ß-curve for grass growth/dose response was observed only in the forest subsoil at cut 7 (fig. 4.5). The results above mentioned could be included within the hormetic effect.

The controversy about the existence of hormesis is old (Anon. I, 1998; Brucer, 2002; Calabrese and Baldwin, 1998; Calabrese, 2004; Luckey, 1991; Luckey, 1998), as some authors found it in fertile, others in poor soils, it appeared and disappeared, it could be observed in one specie but not in another one (Meyer et al., 1998a). This experiment shows that all these results could be part of the same process. The experimental design and its duration were fundamental to reach this conclusion which would have been completely different if it had consisted of only one soil or 1–2 cuts.

Another way to interpret the results is following the behavior of each U dose in the time, and to compare their relative effect on yields. They could be represented as waves of different amplitude, at 250 mg kg^{-1} U the range of highest-lowest yields and U in plant is narrower than at 500 and 1,000 mg kg^{-1} U. In this context, the effect of a low U dose could pass unnoticed in many experiments, and for the highest dose could only be observed the positive or the negative part of the wave. A plausible explanation of these results is that *Lolium perenne* could have displayed a reply to the stress produced by U in the range of doses applied to soil. The whole process could be influenced by the U concentration reached in plant tissues in each stage. These changing effects of U during the time put doubts about the concept of hormesis as was referred till now (see review, section 2.6).

Waves of stimulating-inhibitory effect of toxic elements in plant could be more general as it was supposed before, but being a short time effect, they are not frequently described. A homeostatic regulation mechanism for excessive uptake of rare earths (REs) in plants was suggested to regulate their concentration in maize (Wuang et al., 2001). It could be passed by translocation from roots to leaves or vice versa, as happened when REs are applied to soil or leaves, and was interpreted as a detoxification mechanism. The addition of N, P, K separated or as NPK fertilizer to soils with low U dose, could change the relative growth and U concentration of root and aboveground mass (Morishima et al., 1977). But it has also been proposed that U could increase the root permeability and decrease the content of chlorophyll (Jain and Aery, 1997). An increment in the permeability of root tissues by U, could increase the uptake of nutrient elements low or deficient, increasing yields.

Data of table 4.2 also showed that although the total growth was not affected in the GT, GS and FT substrates, a lower total accumulated nutrient uptake following U contamination levels was found at the end of the experiment. At a first view this could be attributed to a more efficient use of nutrients, but it could also be a progressive damage due to plant toxicity.

Meyer and McLendon (1997) found detrimental effects on the growth of *Schizachyrium scoparium* at 25,000 mg kg^{-1} DU in soil applied as schoepite. Sheppard and Evenden (1992a) found no biological effects with U levels lower than 300 mg kg^{-1} U applied as uranyl-nitrate.

The U doses tested in this experiment appeared to be in the range where *Lolium perenne* was able to display a stress reply in the time. This specie had also shown adaptive reply to Pb and other elements before (Alloway, 1995). It is not possible to generalize these results, perennial grasses have more opportunities of adaptation than annual crops because of the longer period of growth. Besides in the field, soil quality, seasonal demand, water regime, time of growth, practical management, and fertilization could affect the U availability and the same dose could be toxic for other species.

The hormesis concept is associated to the discredit of the homeopathy, probably because they share the same promise of better health or growth, which not always could be demonstrated. Scientists investigated for a long time the use of stimulating doses of toxins to increase the yields (Appleby, 2001; Baniecki, 2003; Freney, 1965; Sheppard et al., 1987). Yearly tons of REs are applied to soil and plant in vast areas of China for this purpose (Hu et al., 2003). Often toxic heavy metals (including U) are contained in fertilizers, especially phosphates (Heiland, 1986; Karhunen and Vermeulen, 2000; Lehr, 1980; Schnug et al., 1996), which could have in some extent contributed to increase yield in the field. Expectations of hormetic effects are often invoked in network toxicological publications to reduce the fear of increasing contaminants in the environment.

The most important contribution of this part of the work is to provide evidence of the U effects on plant growth in terms of successive stimulation-inhibition stages, taking part of a bigger process probably of stress reply, but reducing the stigma of hormesis. More studies will be necessary to know the reply of other species and if different threshold of U toxicity exists for each one.

5.3 Soil chemical factors affecting the plant availability of uranium

Although U is the source of many other radionuclides in the environment, its behavior is less known. This could be due to the assumption that uranium is less dangerous than other elements, or rather that many investigators desisted to study it, because of their contradictory results and the many factors that govern its availability.

In this work extractable U followed the U doses applied and all the results were modulated by the soil substrate The effect appeared like a mosaic of combinations of organic matter and pH in table 4.7.

The free-uranyl cation, a very soluble form of U, was expected to predominate in the pH range of the forest soils (pH 3.4-4.6). In contrast the U-hydroxides, which are highly adsorbed to soil surfaces and thus are less available for plants, predominate at the pH of the grassland soils (pH 6-6.5). The strong relation between U and organic matter has been attributed to its great affinity for the oxygen of the carboxylic and phenol groups. The organic matter effect is still not completely understood as U tends to be accumulated in organic horizons, but it can be mobilized when it is adsorbed to fractions of low molecular weight. In this study in both top soils lower availability of U was found than in the corresponding subsoils obviously because of adsorption by organic matter. The magnitude of the "soil quality effect" can be quantified by comparing the mean U concentrations extracted by DTPA in contaminated substrates at cut 7: 1 mg kg^{-1} U in the GT with 10–24 mg kg^{-1} U in the other substrates (tab. 4.7)

However, at cut 7, the addition of U increased the yields of *Lolium perenne* in the poorest soil (FS) at 250 and 500 mg kg^{-1} U dose by more than 200 % (fig. 4.5, tab. A.4), diluting the U content in plant. For the evaluation of the effects of the soil substrate on the U content in plant it is necessary to consider the whole process in the soil plant relationship to avoid contradictory conclusions.

It was postulated above (chapter 5.2), that waves of stimulatory and inhibitory effects on growth could occur as a reply to stress induced by U, in the range of U doses tested in these soils. Little differences for mean U contents or U uptake in FT in the contaminated substrates at cut 7 would be in agree with this postulation (tab. 4.11 and 4.13).

The grassland topsoil showed the highest capacity to reduce the U availability for plants. U content in *Lolium perenne* grown in this soil was lower than 2 mg kg^{-1} U during all the entire experiment (tab. A.15 and the data set in tab. A.19). For the other soil substrates, although a very

wide range of U values were determined specially for forest soils (tab. A.15), the U concentration in plant tended to be lower at cut 7 (tab. 4.11). For all U doses in this experiment values lower than 5 mg kg^{-1} U in tissue did not show detrimental effect on plant growth. This fact may support that a threshold could exist to the adaptation reply (tab. 4.11).

These results suggest that on soils with higher fertility the transfer of U from soil to plant and into the food chain is smaller. As a first consequence of it a broader group of plants could grow in fertile compared to poor soils, contaminated with U.

Addition of high amounts of P compounds have been demonstrated to be effective to reduce heavy metals in soils (Basta et al., 2001; Boisson et al., 1999; Bolan et al., 2003; Hettiarachchi et al., 2001; Ma et al., 1993; McGowen et al., 2001; Ruby et al., 1994; Seaman et al., 2001a) During the dissolution of the $CaHPO_4$ (and other P compounds) the soil pH around the fertilizer grain is lowered down to 2, the acidification causes the dissolution of metal compounds resulting in its increase in the soil solution which are then precipitated by P (Bolan et al., 2003).

In this work although the U extracted from soils still showed dependency on the U doses applied and the soil types (tab. 4.15), the addition of $CaHPO_4$ reduced the mean extractable U in the investigated substrates below 1 mg kg^{-1} (extracted by DTPA). This suggested that U could have been precipitated by P in soil. The levels of U in *Lolium perenne* were reduced down a level close to the typical values for herbaceous plants in non contaminated soils (< 0.4 mg kg^{-1} U, given by Dressen and Marple, 1979).

Natural levels of P in the soil substrates extracted by water were in the range of 8-15 mg kg^{-1} P (tab. 4.3), after the $CaHPO_4$ addition, they raised to 40 and 97 mg kg^{-1} P in GT and GS, respectively, which are values found in intensively P fertilized agricultural soils (Indiati and Singh, 2001). The levels of water extractable P in substrates derived from forest soils were extremely high (122-291 mg kg^{-1} P), probably because of the higher dissolution of $CaHPO_4$ at the acid pH of these soils, they represented 15 and 19-folds over the control for the forest topsoil and subsoil, respectively. As it happened with U, the substrate from grassland topsoil also buffered the effect of the addition of $CaHPO_4$, as no increase in pH was found, and the levels of Ca in plants were only increased by 20 %, compared to non fertilized treatments (tab. 4.4 and 4.5).

It is well known that in pot experiments very often the levels of factors are exaggerated. The level of P tested in this experiment has been frequently used to immobilize U and other metals in laboratory studies (Bolan et al., 2003; Seaman et al., 2001a; Seaman et al., 2001b).

Although levels so high are frequently added to highly weathered tropical soils to increase their fertility and up to 32,000 mg kg^{-1} P as triple superphosphate were necessary to reduce Pb uptake by tall fescue (*Festuca arundinacea*) (Hettiarachchi et al, 2001), it is important to recognize that large scale use of P compounds can contaminate surface and ground water, becoming a problem of P itself.

The soil-plant system seems to be able to incorporate high levels of P without showing visible effects, but long term immobilization of U in soils, probably will not be recommended for all the cases and lower amounts of P fertilizer could be still more effective to reduce U availability and to increase the yields, if they were applied in combination with other practices, as liming or addition of organic matter. As precipitation of P-metal compounds occurs quickly (Bolan et al., 2003; Seaman et al, 2001b), the levels of P in water for different sources of P, could be estimated through rapid tests after a short stabilization.

In this experiment tentatively it could be said that P levels in soil, higher than 80 mg kg^{-1} P did not produce additional reduction of U in soil extractants or in plants (fig. 4.7, 4.8, 4.10, 4.11), and lower than 40 mg kg^{-1} P (tab. 4.3) were not able to reduce at all the levels of contamination the content of U in plants below typical values (< 0.4 mg kg^{-1} DW, Dressen and Marple, 1979) in the substrate from grassland topsoil (tab. 4.17). These both values could be indicators for next trials.

Few elements have such an affinity for carbonates, as the uranyl ion (Harmsen and Haan, 1980; Fellows et al., 1998). These anions can complex U from the other components in the soil, making it very mobile in groundwater. Carbonates have been used to release U in mines and contaminated sites and can dissolve instable precipitated meta-autunita (Casas et al., 1998; Harmsen and Haan, 1980; Mason et al., 1997).

In spite of all these disadvantages, liming is still the best option to increase yields on acid soils. In this study the addition of $CaCO_3$ increased the pH, the levels of Ca and slightly the levels of P in forest soil, and also increased the yields (fig. 4.14) with simultaneous diminution of the U concentration in plants in the very acidic forest soils (fig. 4.15, tab. 4.21).

Ebbs et al. (1998a) found the greatest U concentration in shoots of peas at pH 5.0, suggesting that the free cationic uranyl ion could be the specie most readily taken up and transported in plants. But the U uptake at pH 6.0 was less than 20 % of that. In the experiment of this work the highest U concentration in plants was found at pH 3.5–4.5, and the lowest at pH 5.5–6.5, coinciding with the

highest yields (tab. 4.14). The lower U content in leaves could partly been due to a dilution effect. Consequently, the U uptake did not show a clear tendency (fig. 4.17).

The $CaCO_3$ addition produced contrasting results in the uptake of U, in grassland and forest soil, still producing similar tendencies on U concentration and yields. When both, P fertilizer and lime, were applied, pH, Ca in plant and P in soil showed intermediate effect for the studied parameters (tab. 4.24). The U extractable content in the substrates and in plants were lowered down to the level of the fertile, non treated soil (tab. 4.24).

In summary, the results of this work revealed a highly variable behavior of U. The accumulation of U in plants appeared to be the price of the adaptive reply of *Lolium perenne* when the U load is increased in the soil. All soil factors tested in this study affected the uranium availability for plants (tab. 4.14). Fertile soils showed a lower U transfer to plants after U contamination. Only when $CaHPO_4$ had been added, extractable U in soil and concentration in plants were reduced near to natural background levels. $CaCO_3$ with its effect on pH and nutrient availability, showed the best effect increasing the yields in acid soils. But the $CaCO_3$ addition failed to reduce the U concentration in grass leaves down to background levels. In fact, it increased the extractable U levels in soils. The addition of both amendments appears to be a realistic option to reduce the U transfer in the soil plant cycle.

6 Conclusions

Evaluation of agronomic measures to reduce the transfer of uranium into the food chain

Complete ammunitions or pieces of them staying shallow buried in the soil after combat are converted in dirty bombs, from where U can be oxidized and mobilized to the groundwater and taken up by plants, entering the food chain. U levels up to 500 mg U kg^{-1} soil were found by UNEP near the buried ammunitions 1.5 years after the war (UNEP II, 2001).

Based on the present study the following results should be considered at the time to decide the management of these soils to produce food:

- *Lolium perenne*, a perennial grass could tolerate the U soil concentrations in this range, probably displaying an adaptation process during their growing period. These soils could be used for grass, risk of U entering the food chain is low as there is a low coefficient for transfer from plant to animal (Linsalata, 1994). It could be previously necessary to select species for grazing, with a mechanism of adaptation or tolerance to U but not being high accumulators. The addition of $CaCO_3$ to acid soils, could increase grass yields, and reduce the U concentration in plant.

- In the experiment of this work the most fertile soils controlled the U availability in the range tested (250-1,000 mg kg^{-1} U), maintaining the U concentration in plant lower than 2 mg kg^{-1}, which is more than 5-fold the plant U content which is actually considered as a “safe” limit. Fertile soil could be used for grain crops and intensive crops of high yields, if the soil pH were maintained in the range of the lower U availability. Grain crops appear to have low risk of U entering the food, because grains do not accumulate U. The plants could still benefit of the slow release of U.

- If U contaminated soil needs to be used for vegetable production, specially for leafy or root crops which have a great capacity to take up U from soils, this could be a great problem, especially if most of the food is produced in the same contaminated areas (see chapter 2.4).

In vegetable farms, many different types of amendments are used to maintain high levels of fertility. As U availability can be modified by organic matter content of soil substrates, and probably by many other environmental factors, it is difficult to estimate how other combinations of factors could change the U availability for plants. More studies would be necessary for food production under such conditions.

- It has been observed that trees can accumulate U (Kabata-Pendias and Pendias, 1984), probably by xylem transport. Forest soils in Europe were limed to alleviate the negative effects of acid rain.

Addition of $CaCO_3$ to acid forest soils to reach pH levels of low solubility of U could reduce the mobility and U uptake in the long time, but it could increase the releasing of U by biological mineralization in short time.

- The pH could be a useful indicator of the U availability in soil and plant uptake. A lower U concentration in plant occurred from pH 5.5 to 6.5. Maintaining soil pH at those values could be a first step to reduce plant availability of U in arable soils.

- The $CaCO_3$ contrarily to the divalent Ca or Sr (Haneklaus and Schnug, 2001) can enhance U levels in soil and still dissolve certain forms of precipitated U increasing its availability. In the context of reducing U transfer it is necessary to avoid over-liming, specially in low organic matter soils.

- The results showed that P amounts lesser than 0.15 % P as $CaHPO_4$ could effectively reduce U availability to plants.

- Maintaining a sufficient organic matter content in arable soils and a suitable P supply at a near neutral pH value, would be effective measures to reduce the U uptake by plants.

7 Summary

International attention has been paid to the danger represented by the hundreds of tons of depleted uranium (DU) discharged into the environment during the wars of Iraq (1990, 2002) and Kosovo (1998). Projectiles containing DU are destroyed after the collision in small pieces and dust, the finest particles are deposit on the vegetation, on soil surface and enter in water-bodies. But lots of projectiles did not hit their targets and are missing. They are normally very shallow in the ground, corroding there over a long period. DU concentrations of up to 500 mg kg^{-1} U have been determined by UNEP in Kosovo near the ammunitions found only 1.5 years after the war. The DU is less radioactive than the natural U, it is still a dangerous a-emitter and bears significant chemical toxicity to men and animals.

This work was conducted to contribute to the understanding of the behavior of DU in the environment and specifically to generate knowledge about the soil factors that affect the U availability for plants and which could be managed in contaminated soils to produce food with lower health risk.

Contamination of substrates has been carried out with finely pulverized green modification of U_3O_8. This form was identified to have a similar behavior like the environmentally oxidized DU metal from ammunitions. In a greenhouse experiment different levels of soil fertility were simulated through substrates derived from a side by side grassland and forest soil. Substrates were taken from the topsoil (0 - 25 cm) and the subsoil (25 - 50 cm). Uranium was applied to the substrates at 0 (control), 250, 500 and 1,000 mg kg^{-1} U. In the first part of the experiment $CaHPO_4$ was incorporated in half of the pots, in a second part of the experiment the half of the replications were treated with $CaCO_3$ in such amounts which increased the pH of the substrates from 4 to 6. The experiment was conducted with *Lolium perenne* and lasted 58 weeks. Dry matter and U, P and Ca concentration were determined for the seven cuts of the experiment. In the last cut pH and extractability of U and P in the substrates were also determined.

The research work yielded the following main results:

The type of extracting solution (DTPA and AAAcEDTA) used to analyze the U mobility in the soil, had a great influence on the results, probably associated to their pH. On an average AAAcEDTA extracted larger amounts of U from the substrates: 13-fold, 7-fold and 85-fold, in non fertilized, limed and P fertilized substrates, respectively. The U extracted by DTPA showed the best

correlation with U in plants for non fertilized soils (0.843***) and U extracted by AAAcEDTA showed the best correlation (0.714***) with U content in plant tissues when all the data after fertilization was used. The addition of U produced a stimulatory - inhibitory effect on the growth of *Lolium perenne* at each U dose tested in this work. In the lowest fertile soil substrates the stimulation appeared after the longest time between the cuts, which seems to be related to a diminish of U concentration in plant, associated with higher dry matter production and the uptake of nutrients.

Fertile soils buffered U levels in soil and plant. The maximal levels found in grass growing on this soil substrates were around 2 mg kg^{-1} U during the whole of the experiment. Highest values of U occurred in plants grown on substrates with low fertility. *Lolium perenne* was able to maintain U levels in plants down to 5 mg kg^{-1} DW.

Different fertilization strategies were tested for their ability to reduce U levels in soils and plants. U in soil and plant were lowest at pH 5.5 – 6.5. The addition of $CaCO_3$ was effective on substrates from the forest soil, increasing yields and reducing the U concentrations in plants, but not on the substrates derived from the grassland soil. Only $CaHPO_4$ was effective to reduce U in all substrates.

For this source term of U, in the range tested, the different systems studied were able to incorporate the U without visible effects or diminution of plant growth, which makes it more dangerous as it could easily enter into the food chain.

Zusammenfassung

In der jüngsten Vergangenheit tritt eine neue, nicht zu unterschätzende Gefährdung des Ökosystems Boden auf: seit mehr als zehn Jahren wird bei militärischen Auseinandersetzungen Munition eingesetzt, deren Penetratoren unter Verwendung von abgereicherten Uranabfällen aus der Kernspaltung, sogenanntem „Depleted Uranium“ (DU), hergestellt werden. Berichten des UNEP zufolge wurden während der Gefechte im Kosovo mehr als 30.000 Geschosse abgefeuert. Beim Aufprall auf harte Ziele werden die Geschosse zerstört, feinste uranhaltige Staubpartikel lagern sich auf Pflanzen, der Bodenoberfläche und Gewässern ab. Der weitaus größere Teil der verschossenen Munition (bis zu 90 %) verfehlt nachweislich sein Ziel und dringt in den Boden ein. Die Projektile liegen nahezu unauffindbar, umgeben von Pflanzenwurzeln, Bodenwasser und Bodentieren in unterschiedlichen Bodenschichten. Infolge fortschreitender Korrosion werden toxische Uranverbindungen freigesetzt. In unmittelbarer Umgebung der wenigen wiedergefundenen Geschosse wurden im Rahmen der UNEP-Untersuchungen Bodengehalte von bis zu 500 mg kg^{-1} U festgestellt, eine permanente Quelle für unkontrollierbare Schwermetallkontamination. Pflanzenwurzeln können diese giftigen Stoffe aufnehmen. Im Verlauf des Pflanzenwachstums erfolgt die teilweise Einlagerung des aufgenommenen Urans in die oberirdischen Organe der Pflanzen. Gelangen belastete Pflanzen oder Pflanzenteile in die Nahrungskette, geht von ihnen neben der Schwermetalltoxizität auch eine radiologische Gefahr durch ? -Strahlung aus.

In einem dreifaktoriellen Gefäßversuch mit *Lolium perenne*, von dem insgesamt 7 Schnitte geerntet wurden, wurden Faktoren evaluiert, die den Boden-Pflanze-Transfer von Uranverbindungen beeinflussen. Zur Kontamination des Versuchsbodens mit Uran wurde eine aus Uranylsalzen gewonnene grüne Modifikation von U_3O_8 verwendet. Der Einfluss von Bodenfruchtbarkeit und gesteigerter U-Kontamination auf Wachstum, Ertrag, U-, P- und Ca-Gehalt des Weidelgrases wurden untersucht. Die Applikation meliorativer Mengen an Phosphat und Karbonat als Maßnahmen zur Beeinflussung des U-Transfers Boden-Pflanze wurde geprüft.

Zwei Methoden zur Extraktion mobilen Urans aus den Bodensubstraten wurden verglichen. Mit Ammoniumacetat-EDTA (AAAcEDTA)-Lösungen konnten mehr als 10fach größere Mengen an U aus Böden extrahiert werden, als mit DTPA-Lösungen. Dabei erklärte die Variabilität der mit DTPA extrahierten U-Konzentrationen der Substrate deutlich höhere Anteile der U-Gehalte der Pflanzen (71%) als die mit AAAcEDTA extrahierten (50%).

Beim Einfluss der U-Kontaminationen auf die Biomasseproduktion der Pflanzen zeigten sich sowohl hemmende als auch stimulierende Effekte. Letztere sind jedoch zu unsicher, als das hieraus sicher auf eine hormetische Wirkung von geringen U-Kontaminationen geschlossen werden könnte. Eine deutliche Beziehung zwischen der potentiellen Fruchtbarkeit der Bodensubstrate und der U-Aufnahme durch die Pflanzen wurde für alle U-Kontaminationsstufen nachgewiesen. In den Blättern des Weidelgrases fanden sich auf den produktiveren Substraten im Mittel nur 40% der U-Gehalte, die das Gras der unfruchtbareren Varianten aufwies.

Die geringste U-Aufnahme der Pflanzen wurde im Bereich von Boden-pH-Werten zwischen 5,5 – 6,5 festgestellt. Die Applikation von Ca-Phosphat reduzierte den U-Transfer aus dem Bodensubstrat in die oberirdische Pflanzensubstanz in allen Substrat- und Kontaminationsvarianten signifikant.

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Tab. A.1: Yield of *Lolium perenne* [g], non contaminated soil substrates (control)

Soil substrate	Cut number						
	1	2	3	4	5	6	7
GT	1.18 a	1.48 a	1.76 a	3.47 a	2.12 a	1.30 a	1.00 a
GS	0.60 b	1.09 c	1.18 b	3.01 ab	1.85 a	0.95 a	0.75 a
FT	0.33 c	1.21 b	1.34 b	2.32 b	1.68 a	0.94 a	1.13 a
FS	0.08 d	0.28 d	0.38 c	1.17 c	1.43 a	1.00 a	0.57 a
ANOVA	***	***	***	***	Ns	Ns	Ns

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
Cut 1-4, n:4, cuts 5-7: n:2
Means with different letters in columns are significantly different at the 5 % levels determined by the least significant difference (LSD)
****: P < 0.001, **: P < 0.01, *: P < 0.05, Ns: no significant*

Tab. A.2: Content of P and Ca in *Lolium perenne*[1] [%], non contaminated soil substrates (control)

Element	GT	GS	FT	FS
			[%]	
P	0.69 ± 0.19	0.59 ± 0.21	0.57 ± 0.25	0.57 ± 0.25
Ca	0.64 ± 0.14	0.51 ± 0.16	0.32 ± 0.14	0.34 ± 0.21

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
[1] Mean and standard deviation (entire experiment)

Tab. A.3: Interpretative values for *Lolium perenne*, taken from Plant Analysis Handbook, 1991, USA

Element	Low	Sufficient	High
		[%]	
P	0.30 - 0.34	0.35 - 0.40	> 0.40
Ca	0.20 - 0.24	0.25 - 0.30	> 0.30

Tab. A.4: Effect of U contamination levels on yields of *Lolium perenne*

	Grassland topsoil		Grassland subsoil		Forest topsoil		Forest subsoil	
	U in soil mg kg^{-1}	Dry weight g	U in soil mg kg^{-1}	Dry weight g	U in soil mg kg^{-1}	Dry weight g	U in soil mg kg^{-1}	Dry weight g
Cut 1 n: 4	LSD: 0.106		LSD: 0.066		LSD:0.043		LSD: 0.015	
	250 a	1.299	**250a**	0.627	**0 a**	0.335	**0 a**	0.076
	1,000 b	1.188	**0 ab**	0.598	**250 b**	0.275	**250 a**	0.068
	500 b	1.185	**500 bc**	0.536	**500 b**	0.247	**500 b**	0.052
	0 b	1.176	**1,000 c**	0.509	**1,000 c**	0.191	**1,000 b**	0.038
Cut 2 n: 4	LSD: 0.122		LSD: 0.157		LSD: 0.124		LSD: 0.065	
	1,000 a	1.594	**250 a**	1.309	500 a	1.250	**0 a**	0.280
	500 a	1.584	**1,000 ab**	1.214	250 a	1.243	**250 a**	0.272
	250 a	1.493	**500 ab**	1.160	0 a	1.212	**500 b**	0.132
	0 a	1.485	**0 b**	1.089	1,000 a	1.151	**1,000 b**	0.116
Cut 3 n: 4	LSD: 0.197		LSD: 0.146		LSD: 0.161		LSD: 0.203	
	500 a	2.059	**1,000 a**	1.567	**1,000 a**	1.715	**0 a**	0.380
	1,000 a	2.024	**500 b**	1.393	**500 ab**	1.572	**250 a**	0.379
	250 ab	1.886	**250 b**	1.331	**250 bc**	1.446	**500 ab**	0.222
	0 b	1.760	**0 c**	1.176	**0 c**	1.340	**1,000 b**	0.172
Cut 4 n: 4	LSD: 0.391		LSD: 0.715		LSD: 0.472		LSD: 0.813	
	0 a	3.474	0 a	3.006	500 a	2.351	**250 a**	1.590
	500 a	3.461	250 a	2.786	0 a	2.316	**500 a**	1.182
	250 ab	3.222	500 a	2.549	250 a	2.257	**0 a**	1.167
	1,000 b	2.853	1,000 a	2.489	1,000 a	2.104	**1,000 b**	0.346
Cut 5 n: 2	LSD: 0.424		LSD: 0.141		LSD: 0.452		LSD: 1.210	
	0 a	2.116	**250 a**	1.914	1,000 a	1.760	250 a	1.604
	250 ab	1.866	**0 ab**	1.846	0 a	1.682	0 a	1.430
	500 ab	1.706	**1,000 bc**	1.743	500 a	1.653	500 a	0.949
	1,000 b	1.620	**500 c**	1.631	250 a	1.524	1,000 a	0.566
Cut 6 n: 2	LSD: 0.256		LSD: 0.256		LSD: 0.359		LSD: 0.597	
	0 a	1.298	0 a	0.948	1,000 a	0.973	250 a	1.035
	250 a	1.289	500 a	0.940	0 a	0.944	0 a	0.999
	500 a	1.209	250 a	0.863	250 a	0.873	500 a	0.627
	1,000 a	1.134	1,000 a	0.778	500 a	0.857	1,000 a	0.608
Cut 7 n: 2	LSD: 0.247		LSD: 0.609		LSD: 0.432		LSD: 1.045	
	250 a	1.200	0a	0.751	0 a	1.128	**500 a**	1.262
	1,000 a	1.009	500 a	0.750	250 a	1.096	**250 a**	1.228
	0 a	1.001	250 a	0.748	500 a	1.040	**0 ab**	0.573
	500 a	0.997	1,000 a	0.704	1,000 a	0.974	**1,000 b**	0.322

Means with different letters in columns are significantly different at the 5 % levels determined by the least significant difference (LSD)

Tab. A.5: ANOVA for the effect of cut number, soil substrate and U contamination on P and Ca uptake by *Lolium perenne*

Source of variation	***P***	***Ca***
Cut (C)	***	***
Substrate (S)	***	***
U contamination (U)	Ns	***
C x S	***	***
C x U	Ns	***
S x U	Ns	Ns
C x S x U	Ns	***

***: P < 0.001, **: P < 0.01, *: P < 0.05, Ns: no significant

Tab. A.6: Content of P and Ca in *Lolium perenne,* in U contaminated, unfertilized substrates during the entire experiment

Level of U contamination [mg kg^{-1}]	GT	GS	FT	FS
		P		
0	0.69 (27)	0.59 (36)	0.57 (44)	0.57 (44)
250	0.65 (26)	0.54 (33)	0.48 (35)	0.56 (43)
500	0.62 (26)	0.56 (36)	0.42 (26)	0.55 (45)
1,000	0.74 (22)	0.56 (30)	0.49 (45)	0.52 (48)
		Ca		
0	0.64 (22)	0.51 (31)	0.32 (44)	0.34 (62)
250	0.64 (20)	0.47 (45)	0.30 (43)	0.29 (55)
500	0.62 (19)	0.46 (43)	0.30 (40)	0.15 (47)
1,000	0.61 (24)	0.42 (48)	0.25 (44)	0.13 (38)

Mean and CV% (between brackets), over the entire experimental term

Tab. A.7: ANOVA for pH determined in $CaCl_2$ and humid soil directly, water soluble P in soil and Ca in plant (cut 7)

Source of variation	pH in $CaCl_2$	pH in humid soil	Water soluble P in soil	Ca content in plant
Soil substrate (S)	***	***	***	***
Fertilization (F)	***	***	***	***
S x F	***	***	***	***

***: P < 0.001, **: P < 0.01, *: P < 0.05, Ns: no significant

Tab. A.8: ANOVA for the effect of substrate and U contamination on the studied soil and plant variables (cut 7)

Source of variation	U by DTPA	U by AAAcEDTA	Yield	U content in leaves	U uptake by leaves
			Non fertilized substrates		
Substrate (S)	***	***	***	**	***
U contam. (U)	***	***	**	***	***
S x U	***	*	**	Ns	Ns
			After P fertilization		
Substrate (S)	***	Ns	***	Ns	Ns
U contam. (U)	***	***	Ns	**	*
S x U	**	Ns	Ns	Ns	Ns
			After liming		
Substrate (S)	***	***	***	**	**
U contam. (U)	***	***	Ns	***	***
S x U	***	***	Ns	Ns	Ns
			After P fertilization and liming		
Substrate (S)	**	Ns	***	*	**
U contam. (U)	***	***	Ns	*	***
S x U	*	Ns	Ns	Ns	Ns

***: P < 0.001, **: P < 0.01, *: P < 0.05, Ns: no significant

Tab. A.9: ANOVA for the effect of substrate and U contamination on the cumulative dry matter and U uptake by *Lolium perenne* during the entire experiment

Source of variation	Cumulative dry matter	Cumulative U uptake
Soil substrate (S)	***	***
U contamination (U)	*	***
Interactions S x U	Ns	**

***: P < 0.001, **: P < 0.01, *: P < 0.05, Ns: no significant

Tab. A.10: Soil and plant variables following U contamination levels in unfertilized soil substrates (control), (cut 7) (n: 8)

Soil substrate	Regression equations	R^2	P
	U extracted by DTPA [mg kg^{-1}]		
GT	y: 0.0204 + 0.0016 x	94	***
GS	y: - 1.2243 + 0.0189 x	85	**
FT	y: - 1.6637 + 0.0253 x	97	***
FS	y: - 2.5225 + 0.0463 x	93	***
	U extracted by AAAcEDTA [mg kg^{-1}]		
GT	y: - 2.6403 + 0.1177 x	98	***
GS	y: - 4.2137 + 0.3055 x	89	***
FT	y: 3.8665 + 0.2970 x	98	***
FS	y: 0.5314 + 0.3138 x	99	***
	Yields [g]		
GT	y: 1.0784 - $6.0 \cdot 10^{-5}$ x	4	Ns
GS	y: 0.6974 + $6.0 \cdot 10^{-5}$ x	6	Ns
FT	y: 1.0979 - $1.7 \cdot 10^{-4}$ x	22	Ns
FS	y: 0.8729 - $3.1 \cdot 10^{-4}$ x	7	Ns
	U content in Lolium perenne [mg kg^{-1}]		
GT	y: 0.2515 + $6.3 \cdot 10^{-4}$ x	40	Ns
GS	y: 0.1935 + 0.0039 x	80	**
FT	y: 1.3373 + 0.0034 x	31	Ns
FS	y: 0.9228 + 0.0078 x	77	**
	U uptake by Lolium perenne [µg per pot]		
GT	y: 0.3046 + $5.8 \cdot 10^{-4}$ x	31	Ns
GS	y: 0.0875 + 0.0030 x	79	**
FT	y: 1.3217 + 0.0031 x	34	Ns
FS	y: 2.2837 + 0.0018 x	9	Ns

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, Ns: no significant

Tab. A.11: Plant variables following U contamination levels in unfertilized soil substrates considering the data of all the experiment

Soil substrate	Regression equations	R^2	P
	Cumulative yield [g] (n: 8)		
GT	$y: 12.4422 - 9.0 \cdot 10^{-4} x$	63	*
GS	$y: 9.4101 - 4.2 \cdot 10^{-4} x$	21	Ns
FT	$y: 8.8501 - 2.3 \cdot 10^{-5} x$	< 1	Ns
FS	$y: 5.6447 - 0.0033 x$	48	Ns
	U content in Lolium perenne [mg kg^{-1}] (n: 56)		
GT	$y: 0.1066 + 5.1 \cdot 10^{-4} x$	24	***
GS	$y: 0.1926 + 0.0025 x$	56	***
FT	$y: 0.5816 + 0.0050 x$	27	***
FS	$y: -0.1934 + 0.0142 x$	37	***
	Cumulative U uptake by Lolium perenne [µg per pot] (n: 8)		
GT	$y: 1.0412 + 0.0068 x$	78	**
GS	$y: 1.5687 + 0.0222 x$	94	***
FT	$y: 4.1898 + 0.0509 x$	81	**
FS	$y: 6.6300 + 0.0260 x$	63	*

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, Ns: no significant

Tab. A.12: Soil and plant variables following U contamination levels in P fertilized soil substrates (cut 7) (n: 8)

Soil substrate	Regression equations	R^2	P
	U extracted by DTPA[mg kg^{-1}]		
GT	y: - 0.0159 + 6.2 · 10^{-4} x	99	***
GS	y: - 0.0375 + 9.8 · 10^{-4} x	96	***
FT	y: 0.0611 + 6.5 · 10^{-4} x	93	***
FS	y: 0.0220 + 7.2 · 10^{-4} x	98	***
	U extracted by AAAcEDTA [mg kg^{-1}]		
GT	y: - 0.5695 + 0.0557 x	99	***
GS	y: - 3.8778 + 0.0767 x	95	***
FT	y: - 0.2073 + 0.0719 x	73	**
FS	y: - 0.2507 + 0.0619 x	99	***
	Yields [g]		
GT	y: 1.3167 - 1.0 · 10^{-4} x	10	Ns
GS	y: 0.8779 + 1.4 · 10^{-4} x	20	Ns
FT	y: 1.0537 - 1.2 · 10^{-4} x	16	Ns
FS	y: 0.9046 - 2.6 · 10^{-4} x	18	Ns
	U content in Lolium perenne [mg kg^{-1}]		
GT	y: - 0.0228 + 6.4 · 10^{-4} x	69	*
GS	y: 0.0183 + 2.8 · 10^{-4} x	74	**
FT	y: 0.0598 + 7.7 · 10^{-5} x	5	Ns
FS	y: 0.0218 + 2.6 · 10^{-4} x	66	*
	U uptake by Lolium perenne [µg per pot]		
GT	y: - 0.0217 + 8.0 · 10^{-4} x	59	*
GS	y: 0.0147 + 2.7 · 10^{-4} x	79	**
FT	y: 0.0577 + 6.9 · 10^{-5} x	5	Ns
FS	y: 0.0183 + 1.4 · 10^{-4} x	64	*

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, Ns: no significant

Tab. A.13: Soil and plant variables following U contamination levels in limed soil substrates (cut 7) (n: 8)

Soil substrate	Regression equations	R^2	P
	U extracted by DTPA [mg kg^{-1}]		
GT	y: 0.1058 + 0.0019 x	90	***
GS	y: 0.6883 + 0.0379 x	99	***
FT	y: - 4.0012 + 0.0399 x	91	***
FS	y: - 5.9883 + 0.0803 x	93	***
	U extracted by AAAcEDTA[mg kg^{-1}]		
GT	y: - 1.6017 + 0.0920 x	98	***
GS	y: 4.7297 + 0.2603 x	99	***
FT	y: - 1.6592 + 0.3100 x	99	***
FS	y: 6.7118 + 0.3115 x	98	***
	Yields [g]		
GT	y: 1.0475 + $9.5 \cdot 10^{-5}$ x	3	Ns
GS	y: 1.1485 - $7.9 \cdot 10^{-5}$ x	5	Ns
FT	y: 1.4955 + $2.0 \cdot 10^{-4}$ x	16	Ns
FS	y: 1.8395 + $1.3 \cdot 10^{-4}$ x	7	Ns
	U content in Lolium perenne [mg kg^{-1}]		
GT	y: 0.1768 + 0.0014 x	76	**
GS	y: 0.0880 + 0.0035 x	70	**
FT	y: 0.1208 + $6.2 \cdot 10^{-4}$ x	82	**
FS	y: 0.3065 + 0.0021 x	76	**
	U uptake by Lolium perenne [µg per pot]		
GT	y: 0.0827 + 0.0016 x	80	**
GS	y: 0.0894 + 0.0033 x	78	**
FT	y: 0.2159 + $9.9 \cdot 10^{-4}$ x	79	**
FS	y: 0.4572 + 0.0043 x	78	**

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
***: P < 0.001, **: P < 0.01, *: P < 0.05, Ns: no significant

Tab. A.14: Soil and plant variables following U contamination levels in P fertilized and limed soil substrates (cut 7) (n: 8)

Soil substrate	Regression equations	R^2	P
	U extracted by DTPA[mg kg^{-1}]		
GT	y: - 0.0149 + $7.1 \cdot 10^{-4}$ x	83	**
GS	y: - 0.0578 + 0.0014 x	94	***
FT	y: 0.0218 + $5.0 \cdot 10^{-4}$ x	96	***
FS	y: 0.0309 + $6.3 \cdot 10^{-4}$ x	92	***
	U extracted by AAAcEDTA [mg kg^{-1}]		
GT	y: - 1.2339 + 0.0588 x	98	***
GS	y: - 0.4839 + 0.0629 x	99	***
FT	y: 1.4894 + 0.0482 x	88	***
FS	y: - 1.2361 + 0.0634 x	97	***
	Yields [g]		
GT	y: 1.2746 - $1.8 \cdot 10^{-4}$ x	30	Ns
GS	y: 0.9471 - $7.5 \cdot 10^{-5}$ x	3	Ns
FT	y: 1.3244 + $3.0 \cdot 10^{-4}$ x	34	Ns
FS	y: 1.3172 - $8.8 \cdot 10^{-5}$ x	9	Ns
	U content in Lolium perenne [mg kg^{-1}]		
GT	y: 0.0480 + $3.5 \cdot 10^{-4}$ x	52	*
GS	y: - 0.0258 + $8.0 \cdot 10^{-4}$ x	61	*
FT	y: 0.0405 + $2.9 \cdot 10^{-7}$ x	<1	Ns
FS	y: 0.0061 + $1.3 \cdot 10^{-4}$ x	72	**
	U uptake by Lolium perenne [µg per pot]		
GT	y: 0.0773 + $3.4 \cdot 10^{-4}$ x	50	Ns
GS	y: 0.0102 + $5.5 \cdot 10^{-4}$ x	85	**
FT	y: 0.0565 + $1.1 \cdot 10^{-5}$ x	<1	Ns
FS	y: 0.0141 + $1.5 \cdot 10^{-4}$ x	76	**

GT: grassland topsoil, GS: grassland subsoil, FT: forest topsoil, FS: forest subsoil
***: $P < 0.001$, **: $P < 0.01$, *: $P < 0.05$, Ns: no significant

Tab. A.15: Effect of U contamination level on U content in *Lolium perenne* (both in mg kg^{-1})

	Grassland topsoil		Grassland subsoil		Forest topsoil		Forest subsoil	
	U in soil	U in plant	U in soil	U in plant	U in soil	U in plant	U in soil	U in plant
Cut1 n: 4	LSD: 0.060		LSD: 0.294		LSD: 0.214		LSD: 1.543	
	1,000 a	0.113	**1,000 a**	1.782	**1,000 a**	2.637	**1,000 a**	10.085
	0 a	0.112	**500 b**	1.062	**500 b**	1.429	**500 b**	5.708
	500 ab	0.088	**250 c**	0.379	**250 c**	0.754	**250 c**	2.317
	250 b	0.044	**0 d**	0.000	**0 d**	0.000	**0 d**	0.043
Cut 2 n: 4	LSD: 0.170		LSD: 0.182		LSD: 0.481		LSD: 5.024	
	500 a	0.273	**1,000 a**	1.666	**1,000 a**	2.659	**500 a**	7.343
	1,000 ab	0.147	**500 b**	1.004	**500 b**	1.495	**1,000 a**	7.008
	0 ab	0.116	**250 c**	0.455	**250 c**	0.867	**250 ab**	2.762
	250 a	0.100	**0 d**	0.041	**0 d**	0.044	**0 b**	0.029
Cut 3 n: 4	LSD: 0.123		LSD: 3.302		LSD:1.060		LSD: 2.181	
	250 a	0.236	**250 a**	3.402	**1,000 a**	3.961	**1,000 a**	11.570
	1,000 ab	0.175	**1,000 ab**	2.970	**500 b**	1.936	**500 b**	8.100
	500 bc	0.087	**500 ab**	1.668	**250 bc**	0.982	**250 c**	5.303
	0 c	0.055	**0 b**	0.079	**0 c**	0.037	**0 d**	0.370
Cut 4 n: 4	LSD: 0.273		LSD: 0.564		LSD: 2.722		LSD: 26.326	
	1,000 a	1.299	**1,000 a**	2.706	**1,000 a**	7.314	**1,000 a**	44.300
	250 b	0.358	**500 b**	1.245	**500 b**	4.528	**500 b**	9.060
	500 bc	0.232	**250 c**	0.523	**250 bc**	2.314	**250 b**	3.026
	0 c	0.048	**0 c**	0.021	**0 c**	0.008	**0 b**	0.026
Cut 5 n: 2	LSD: 1.151		LSD: 0.442		LSD: 9.901		LSD: 8.005	
	1,000 a	1.358	**1,000 a**	2.176	**1,000 a**	9.976	**1,000 a**	11.964
	500 ab	0.480	**500 b**	1.169	**500 ab**	5.070	**500 ab**	6.874
	250 ab	0.232	**250 c**	0.482	**250 ab**	2.040	**250 b**	2.282
	0 b	0.020	**0 d**	0.027	**0 b**	0.017	**0 b**	0.034
Cut 6 n: 2	LSD: 0.819		LSD: 0.906		LSD: 14.847		LSD: 6.488	
	250 a	0.614	**1,000 a**	3.081	500 a	12.680	1,000 a	6.084
	1,000 a	0.456	**500 b**	1.717	1,000 a	4.100	500 a	4.890
	500 a	0.441	**250 c**	0.603	250 a	1.415	250 a	2.328
	0 a	0.053	**0 c**	0.031	0 a	0.040	0 a	0.028
Cut 7 n: 2	LSD: 0.747		LSD: 2.801		LSD: 5.915		LSD: 5.423	
	1,000 a	0.809	**1,000 a**	4.014	500 a	4.325	**1,000 a**	8.389
	250 ab	0.750	**500 ab**	2.467	1,000 a	3.865	**500 ab**	4.790
	500 ab	0.552	**250 b**	1.203	250 a	3.069	**250 ab**	4.106
	0 c	0.004	**0 b**	0.036	0 a	0.029	**0 b**	0.000

Means with different letters in columns are significantly different at the 5 % levels determined by the least significant difference (LSD)

Tab. A.16: ANOVA for the effect of soil type, U contamination and fertilization on P and Ca content in plant (cut 7)

Source of variation	*Content in plant*	
	P	***Ca***
Soil substrate (S)	***	***
U contamination (U)	Ns	Ns
Fertilization (F)	***	***
S x U	Ns	Ns
S x F	***	***
U x F	*	Ns
S x U x F	Ns	Ns

***: P < 0.001, **: P < 0.01, *: P < 0.05, Ns: no significant

Tab. A.17: Effect of fertilizer treatments on the macronutrients content of *Lolium perenne* (cut 7)

Elements	GT	GS	FT	FS
		P		
Unfertilized	0.8 c	0.9 c	0.5 d	0.8 c
P fertilization	1.2 a	2.0 a	3.0 a	2.0 a
Liming	0.8 c	0.8 c	0.7 c	0.6 d
P & liming	1.0 b	1.5 b	2.1 b	1.6 b
		Ca		
Unfertilized	0.6 b	0.4 c	0.3 c	0.2 b
P fertilization	0.7 a	0.9 b	1.1 a	1.1 a
Liming	0.8 a	0.8 b	1.0 b	1.0 a
P & liming	0.8 a	1.0 a	1.1 ab	1.1 a

Means with different letters in columns are significantly different at the 5 % levels determined by the least significant difference (LSD)

Tab. A.18: Dry weight of *Lolium perenne*, grassland topsoil (GT) (in grams)
(*) Caterpillar attacked samples

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	1.178 1.273 1.116 1.136	1.435 1.514 1.538 1.453	1.753 1.809 1.755 1.723	3.270 3.470 3.866 3.289	+	2.218 2.257	1.298 1.186	0.704* 1.186
						-	2.275 1.958	1.396 1.201	1.067 0.936
	250	1.390 1.193 1.284 1.328	1.581 1.360 1.518 1.513	1.933 1.812 1.736 2.065	3.289 3.170 3.426 3.006	+	1.967 1.909	1.181 1.201	1.455 0.503*
						-	1.821 1.912	1.203 1.375	1.236 1.164
	500	1.163 1.132 1.299 1.146	1.518 1.527 1.683 1.609	2.258 1.901 2.044 2.033	3.245 3.563 3.869 3.169	+	2.055 1.708	1.341 1.204	0.999 1.025
						-	1.700 1.713	1.217 1.201	0.903 1.091
	1,000	1.182 1.143 1.220 1.208	1.659 1.651 1.600 1.468	1.997 1.869 2.005 2.225	3.060 3.018 2.637 2.698	+	1.815 1.891	1.176 1.223	1.093 1.178
						-	1.481 1.759	1.141 1.128	1.047 0.972
+	0	1.293 1.094 1.296 1.208	1.536 1.534 1.514 1.548	1.906 1.891 2.094 1.872	3.372 3.426 3.904 3.541	+	2.411 2.265	1.206 1.190	1.172 1.236
						-	2.202 1.925	1.276 1.281	1.278 1.237
	250	1.101 1.288 1.026 1.244	1.506 1.662 1.550 1.433	1.841 1.928 1.913 1.980	3.784 2.848 3.868 2.926	+	2.215 1.979	1.183 1.121	1.328 1.306
						-	2.178 2.222	1.230 1.172	1.307 1.344
	500	1.356 1.297 1.168 1.247	1.560 1.580 1.668 1.616	1.935 2.115 1.929 2.118	3.246 3.872 3.099 3.666	+	1.972 2.283	1.369 1.206	1.237 1.158
						-	1.961 1.972	1.163 1.221	1.388 1.273
	1,000	1.334 1.306 1.188 1.298	1.709 1.666 1.578 1.810	2.162 2.248 2.126 2.622	3.646 3.760 3.863 5.163	+	2.128 2.129	1.118 1.130	1.227 0.914
						-	2.054 2.297	1.180 1.132	1.369 0.972*

Tab. A.18: Dry weight of *Lolium perenne*, grassland subsoil (GS) (in grams)
(*) Caterpillar attacked samples

$CaHPO_3$ addition	U contamination mg kg^{-1}	Cut 1 09-11.00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06.01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.585 0.616 0.606 0.585	1.086 1.095 1.168 1.009	1.177 1.272 1.217 1.038	2.880 3.446 2.915 2.783	+	1.748 2.086	1.408 1.243	1.107 1.266
						-	1.913 1.779	0.928 0.968	0.633* 0.751
	250	0.616 0.720 0.617 0.556	1.302 1.292 1.280 1.364	1.275 1.316 1.278 1.457	3.080 2.132 2.747 3.186	+	2.063 1.766	1.242 1.022	1.187 1.180
						-	1.889 1.940	0.921 0.806	0.716 0.693
	500	0.497 0.520 0.600 0.528	1.244 0.930 1.276 1.189	1.285 1.337 1.444 1.506	3.268 2.980 2.146 1.804	+	1.854 1.910	1.193 1.305	1.012 0.889
						-	1.634 1.629	1.043 0.837	0.848 0.652
	1,000	0.506 0.533 0.513 0.486	1.335 1.212 1.231 1.079	1.676 1.454 1.581 1.557	2.518 2.587 2.742 2.110	+	2.017 2.015	0.983 1.175	0.972 1.297
						-	1.750 1.737	0.830 0.727	0.615 0.881
+	0	0.643 0.755 0.712 0.652	1.294 1.139 1.273 1.245	1.522 1.572 1.415 1.563	3.218 3.111 3.198 3.417	+	2.347 2.189	1.239 0.923	0.986 0.948
						-	2.392 2.533	0.940 1.132	0.870 0.917
	250	0.678 0.758 0.683 0.643	1.260 1.301 1.235 1.145	1.238 1.556 1.754 1.287	2.761 2.960 3.987 2.707	+	2.218 2.084	1.159 0.998	0.806 0.883
						-	2.023 1.928	0.978 0.908	1.031 0.793
	500	0.751 0.694 0.623 0.624	1.181 1.182 1.347 1.261	1.541 1.231 1.469 1.440	2.911 3.016 2.991 3.107	+	2.092 2.295	1.112 1.195	0.965 1.026
						-	2.186 2.289	1.014 1.101	1.008 0.829
	1,000	0.686 0.689 0.736 0.756	1.209 1.230 1.335 1.272	1.423 1.493 1.423 1.358	3.350 3.204 3.064 3.182	+	2.101 2.026	1.047 1.269	0.565 1.135
						-	2.048 1.952	1.161 1.011	1.172 0.895

Tab. A.18: Dry weight of *Lolium perenne*, forest topsoil (FT) (in grams)
(*) Caterpillar attacked samples

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.353 0.370 0.304 0.312	1.315 1.152 1.201 1.180	1.235 1.362 1.497 1.268	2.826 2.332 2.070 2.035	+	1.937 2.057	1.249 1.364	1.172 1.444
						-	1.608 1.756	0.833 1.056	1.049 1.208
	250	0.234 0.290 0.274 0.303	1.125 1.252 1.340 1.255	1.388 1.530 1.305 1.561	2.570 2.334 2.098 2.028	+	1.784 2.136	1.352 1.361	1.605 1.816
						-	1.654 1.394	0.970 0.777	1.028 1.164
	500	0.270 0.272 0.233 0.215	1.273 1.293 1.216 1.217	1.473 1.481 1.608 1.727	2.563 2.469 1.765 2.606	+	2.069 1.978	1.381 1.496	1.726 1.724
						-	1.795 1.511	0.956 0.758	0.733* 1.040
	1,000	0.167 0.184 0.198 0.217	1.106 1.033 1.279 1.188	1.673 1.726 1.738 1.724	1.902 2.106 2.296 2.112	+	1.798 1.955	1.385 1.268	1.642 1.543
						-	1.863 1.658	1.018 0.929	0.921 1.028
+	0	0.995 0.976 0.987 1.011	1.363 1.365 1.364 1.352	1.593 1.706 1.855 1.656	2.881 3.080 2.890 2.987	+	2.328 2.485	1.327 1.140	1.477 1.031
						-	2.210 2.140	0.913 0.949	0.952 1.078
	250	0.983 0.870 1.005 0.793	1.350 1.369 1.465 1.374	1.673 1.771 1.689 1.827	2.706 3.006 2.454 2.783	+	1.917 1.951	1.355 1.184	1.598 1.273
						-	1.388 1.699	0.882 0.903	0.973 1.126
	500	0.940 0.941 1.048 0.894	1.461 1.475 1.383 1.398	1.917 1.781 1.692 1.891	3.191 3.457 3.270 3.288	+	2.010 2.038	1.289 1.240	1.593 1.528
						-	2.054 1.258	1.012 0.678	1.172 0.898
	1,000	0.943 1.149 0.972 1.005	1.468 1.569 1.561 1.501	1.906 1.857 1.766 1.895	3.554 2.969 3.110 3.421	+	2.288 1.832	1.128 1.205	1.546 1.597
						-	1.731 1.582	0.813 0.809	0.833 0.988

Tab. A.18: Dry weight of *Lolium perenne*, forest subsoil (FS) (in grams)
(*) Caterpillar attacked samples

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.079 0.087 0.077 0.063	0.271 0.261 0.370 0.220	0.426 0.235 0.685 0.175	1.486 0.741 2.133 0.308	+	2.687 2.368	1.712 1.711	1.853 1.897
						-	1.931 0.929	0.859 1.140	0.253* 0.573
	250	0.068 0.072 0.074 0.060	0.247 0.312 0.288 0.241	0.265 0.383 0.498 0.371	1.972 1.148 1.812 1.430	+	2.258 1.800	1.572 1.822	1.897 1.913
						-	1.509 1.700	1.206 0.864	1.391 1.066
	500	0.052 0.039 0.070 0.046	0.116 0.123 0.133 0.156	0.279 0.170 0.253 0.188	1.591 1.571 1.156 0.410	+	2.241 2.203	1.704 1.645	1.620 1.949
						-	1.260 0.638	0.832 0.423	1.262 nd
	1,000	0.050 0.039 0.036 0.027	0.156 0.100 0.068 0.139	0.193 0.172 0.076 0.248	0.431 0.330 0.154 0.471	+	2.088 1.802	1.754 1.804	1.742 2.302
						-	0.415 0.717	0.568 0.648	0.267 0.378
+	0	0.371 0.454 0.402 0.405	1.159 1.273 1.222 1.168	1.531 1.620 1.636 1.656	3.892 3.760 3.617 3.600	+	1.834 2.025	1.196 1.223	1.338 1.146
						-	2.199 1.969	0.844 1.047	0.540 0.958
	250	0.439 0.436 0.408 0.450	1.242 1.237 1.282 1.217	1.540 1.607 1.583 1.679	3.446 3.718 3.892 3.047	+	1.758 1.917	1.157 1.201	1.300 1.442
						-	1.988 1.985	1.226 1.073	1.117 0.136*
	500	0.463 0.412 0.435 0.321	1.140 1.296 1.230 1.123	1.554 1.510 1.641 1.731	3.725 3.706 4.098 4.049	+	1.677 1.963	1.207 1.404	1.308 1.311
						-	2.133 1.760	1.169 0.766	1.041 0.717
	1,000	0.497 0.424 0.469 0.392	1.304 1.123 1.304 1.188	1.682 1.504 1.514 1.619	3.614 2.977 3.790 2.947	+	1.604 1.632	1.191 1.176	1.075 1.308
						-	1.936 1.703	1.083 0.694	0.655 0.465

Tab. A.19: U content in *Lolium perenne*, grassland topsoil (GT) (in mg kg^{-1} dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.200 0.069 0.110 0.071	0.128 0.097 0.134 0.104	0.043 0.047 0.074 nd	0.057 0.050 0.042 0.044	+	0.029 0.020	0.081 0.050	0.031 0.004
						-	0.021 0.020	0.054 0.053	0.005 0.004
	250	0.060 0.031 0.036 0.049	0.115 0.074 0.105 0.106	0.139 0.243 0.306 0.257	0.194 0.217 0.278 0.744	+	0.343 0.562	0.272 0.338	0.700 1.052
						-	0.182 0.282	0.202 1.026	0.549 0.952
	500	0.113 0.078 0.078 0.084	0.124 0.218 0.197 0.553	0.043 0.049 0.077 0.178	0.245 0.185 0.290 0.207	+	0.232 0.189	0.264 0.244	0.812 0.444
						-	0.480 0.481	0.472 0.410	0.764 0.341
	1,000	0.175 0.107 0.084 0.087	0.289 0.175 0.066 0.059	0.094 0.107 0.213 0.285	1.133 1.426 nd 1.338	+	0.641 0.550	0.658 0.556	1.338 1.759
						-	0.774 1.942	0.398 0.514	0.566 1.053
+	0	0.034 0.025 0.037 0.021	0.038 0.124 0.021 0.317	0.037 0.053 0.130 0.096	0.028 0.029 0.023 0.030	+	0.051 0.030	0.044 0.042	0.017 0.014
						-	0.029 0.033	0.057 0.041	0.016 0.083
	250	0.116 0.036 0.049 0.047	0.085 0.040 0.075 0.051	0.068 0.046 0.048 0.030	0.045 0.094 0.104 0.053	+	0.065 0.103	0.073 0.087	0.099 0.170
						-	0.066 0.103	0.095 0.068	0.164 0.078
	500	0.126 0.073 0.038 0.179	0.062 0.058 0.106 0.362	0.061 0.046 0.127 0.045	0.100 0.103 0.127 0.088	+	0.087 0.112	0.181 0.138	0.139 0.448
						-	0.213 0.084	0.166 0.242	0.245 0.116
	1,000	0.072 0.069 0.076 0.054	0.258 1.941 0.083 0.182	0.181 0.188 0.085 0.102	0.805 0.233 0.129 0.128	+	0.217 0.153	0.263 0.520	0.195 0.543
						-	0.145 0.175	0.354 0.169	0.953 0.417

Tab. A.19: U content in *Lolium perenne*, grassland subsoil (GS) (in mg kg^{-1} dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	us us us us	0.056 0.030 0.042 0.036	0.121 0.075 0.069 0.053	0.020 0.020 0.017 0.028	+	0.017 0.012	0.024 0.025	0.040 0.037
						-	0.025 0.029	0.030 0.032	0.036 0.036
	250	0.270 0.483 0.249 0.514	0.406 0.463 0.371 0.580	0.610 9.602 2.576 0.820	0.506 0.497 0.387 0.704	+	0.487 0.555	0.546 0.531	0.776 0.849
						-	0.399 0.565	0.446 0.760	0.542 1.864
	500	1.105 0.870 0.818 1.455	1.214 0.890 0.871 1.041	2.076 1.125 1.553 1.918	0.999 0.991 1.061 1.928	+	1.174 0.779	1.631 1.460	3.291 1.103
						-	1.304 1.035	1.531 1.904	1.253 3.682
	1,000	1.838 2.037 1.558 1.694	1.804 1.683 1.717 1.458	2.808 2.437 2.844 3.790	2.774 2.095 2.528 3.429	+	1.871 2.081	2.377 1.631	4.729 2.266
						-	2.016 2.336	3.473 2.689	3.663 4.365
+	0	nd us us us	0.031 0.021 0.015 0.021	0.081 0.057 0.058 0.058	0.024 0.019 0.017 0.016	+	0.016 0.028	0.064 0.406	us us
						-	0.017 0.028	0.040 0.071	us us
	250	0.074 0.042 0.152 0.050	0.053 0.058 0.030 0.068	0.028 0.013 0.011 0.021	0.037 0.042 0.068 0.056	+	0.091 0.068	0.186 0.201	0.160 0.291
						-	0.181 0.070	0.148 0.168	0.121 0.085
	500	0.008 0.013 0.026 0.095	0.060 0.043 0.039 0.023	0.042 0.080 0.046 0.037	0.101 0.127 0.091 0.060	+	0.090 0.100	0.227 0.198	0.301 0.185
						-	0.133 0.103	0.151 0.126	0.234 0.105
	1,000	0.111 0.086 0.055 0.267	0.206 0.049 0.171 0.087	0.088 0.077 0.061 0.064	0.171 0.105 0.131 0.144	+	0.067 0.196	0.316 0.187	1.259 0.387
						-	0.081 0.169	0.173 0.254	0.186 0.382

Tab. A.19: U content in *Lolium perenne*, forest topsoil (FT) (in mg kg^{-1} dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	us us us us	0.037 0.052 0.055 0.031	0.044 0.036 0.045 0.022	us 0.007 0.012 0.012	+	0.006 0.016	0.074 0.047	0.015 0.035
						-	0.017 0.017	0.054 0.026	0.028 0.031
	250	1.084 0.565 0.681 0.685	0.799 0.719 0.763 1.189	0.765 1.377 0.795 0.993	0.859 0.883 1.808 5.705	+	0.437 0.483	0.516 0.253	0.437 0.251
						-	2.442 1.639	1.669 1.161	1.865 4.273
	500	1.437 1.621 1.260 1.397	1.537 1.695 1.323 1.426	1.695 1.730 2.593 1.724	3.187 4.775 3.270 6.879	+	2.371 1.027	0.517 0.908	0.614 0.435
						-	5.634 4.507	20.132 5.228	6.942 1.708
	1,000	2.698 2.609 2.572 2.671	3.065 3.201 2.039 2.331	3.671 5.735 2.705 3.735	8.479 5.256 5.947 9.573	+	1.822 1.846	1.036 1.350	0.627 0.740
						-	4.980 14.971	2.837 5.364	2.982 4.748
+	0	us us us 0.033	0.059 0.086 0.010 0.079	0.031 0.003 0.023 0.218	0.008 us 0.019 0.008	+	0.010 0.010	0.030 0.043	0.040 0.041
						-	0.010 0.033	0.035 0.037	0.042 us
	250	0.018 us us us	0.026 0.044 0.077 0.031	0.014 0.072 0.430 0.013	0.060 0.071 0.089 0.043	+	0.065 0.051	0.120 0.087	0.134 us
						-	0.354 0.142	0.654 0.083	0.043 0.047
	500	0.000 0.006 0.023 0.027	0.034 0.047 0.135 0.114	nd 0.282 0.083 0.048	0.073 0.080 0.045 0.112	+	0.114 0.071	0.099 0.209	0.001 0.001
						-	0.089 0.244	0.119 0.219	0.034 0.420
	1,000	0.106 0.079 0.029 0.018	0.091 0.036 0.031 0.036	0.065 0.081 0.627 0.073	nd 0.232 0.286 0.366	+	0.168 0.160	0.120 0.102	0.046 0.062
						-	0.196 0.563	0.195 0.237	0.068 0.094

Tab. A.19: U content in *Lolium perenne*, forest subsoil (FS) (in mg kg^{-1} dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.004 0.064 0.035 0.035	0.072 us 0.009 0.035	0.102 0.209 0.048 1.123	0.009 0.020 0.037 0.036	+	0.029 0.029	0.035 0.025	us us
						-	0.028 0.041	0.027 0.030	us 0.001
	250	2.135 2.371 2.381 2.381	2.129 2.365 3.023 3.530	6.556 4.381 5.962 4.315	1.415 5.122 2.312 3.256	+	0.913 0.921	0.482 1.087	1.072 0.711
						-	2.102 2.463	2.689 1.968	2.448 5.764
	500	6.662 6.925 4.622 4.622	4.102 4.088 4.128 17.053	10.096 9.685 6.992 5.627	5.962 5.038 6.405 18.834	+	5.188 2.806	1.569 1.334	2.207 1.504
						-	9.829 3.919	4.656 5.124	4.916 4.664
	1,000	9.285 12.416 9.320 9.320	6.595 6.595 7.421 7.421	12.754 12.754 10.387 10.387	19.262 39.690 92.890 25.358	+	3.518 3.082	2.898 2.721	1.745 2.467
						-	9.160 14.768	9.361 2.808	6.184 10.595
+	0	us 0.021 0.009 us	0.184 0.074 0.002 0.100	0.005 0.005 0.016 0.006	0.060 0.071 0.047 0.028	+	us us	0.066 0.040	0.012 0.001
						-	us us	0.027 0.051	0.001 0.011
	250	0.004 0.009 0.056 us	us 0.053 0.043 0.260	0.025 0.006 0.057 0.036	0.073 0.142 0.065 0.257	+	0.084 0.078	0.040 0.076	0.023 0.057
						-	0.062 0.114	0.086 0.041	0.044 0.166
	500	0.009 0.006 us 0.116	us us us 0.037	0.060 0.017 0.018 0.037	0.260 0.083 0.107 0.199	+	0.122 0.165	0.072 0.153	0.061 0.079
						-	0.144 0.308	0.058 0.170	0.046 0.265
	1,000	0.099 0.025 0.103 0.391	0.033 0.036 0.014 nd	0.072 0.054 0.084 0.097	0.174 0.225 0.245 1.714	+	0.232 0.256	0.287 0.122	0.197 0.081
						-	0.435 0.737	0.137 0.199	0.221 0.328

Tab. A.20: P content in *Lolium perenne*, grassland topsoil (GT) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.68 0.74 0.67 0.63	0.79 0.82 0.73 0.79	0.82 0.74 0.66 nd	0.37 0.37 0.38 0.38	+	0.56 0.54	1.04 0.95	0.81 0.72
						-	0.55 0.63	0.97 1.01	0.80 0.86
	250	0.59 0.70 0.67 0.54	0.82 0.73 0.74 0.88	0.68 0.67 0.66 0.80	0.38 0.32 0.36 0.42	+	0.49 0.46	0.89 0.92	0.76 0.72
						-	0.47 0.60	0.81 0.85	0.74 0.78
	500	0.67 0.70 0.62 0.70	0.73 0.76 0.68 0.76	0.62 0.64 0.63 0.67	0.42 0.34 0.27 0.29	+	0.48 0.59	0.90 1.09	0.87 0.97
						-	0.53 0.54	0.7 0.93	0.76 0.73
	1,000	0.67 0.66 0.63 0.65	0.81 0.76 0.75 0.82	0.68 0.71 0.69 0.63	0.32 0.95 nd 1.05	+	0.48 0.45	0.97 0.83	0.77 0.68
						-	0.65 0.64	0.90 0.93	0.80 0.76
+	0	0.66 0.67 0.61 0.61	0.97 1.04 1.03 0.92	1.08 0.95 0.94 0.75	0.67 0.58 0.60 0.54	+	0.74 0.77	1.18 1.16	1.05 0.98
						-	1.06 1.09	1.44 1.27	1.47 1.14
	250	0.62 0.61 0.72 0.63	0.93 0.80 0.84 0.91	0.76 0.76 0.87 0.84	0.49 0.45 0.49 0.54	+	0.76 0.69	1.05 0.95	1.00 0.90
						-	0.94 0.91	1.24 1.28	1.10 1.17
	500	0.74 0.66 0.71 0.74	0.86 1.01 1.08 0.94	0.92 0.86 0.96 0.84	0.55 0.57 0.53 0.44	+	0.65 0.73	0.94 1.13	1.05 0.99
						-	0.89 0.95	1.20 1.26	1.29 1.17
	1,000	0.68 0.67 0.68 0.66	0.83 1.10 1.01 0.81	1.46 0.79 0.95 0.69	1.43 1.52 1.43 1.20	+	0.67 0.62	1.05 0.94	1.07 1.07
						-	0.84 0.82	1.06 1.25	1.25 1.35

Tab. A.20: P content in *Lolium perenne*, grassland subsoil (GS) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.48 0.55 0.54 0.56	0.73 0.70 0.64 0.69	0.62 0.58 0.56 0.60	0.32 0.25 0.28 0.29	+	0.44 0.37	0.83 0.85	0.75 0.68
						-	0.38 0.51	0.82 0.93	0.84 1.03
	250	0.52 0.62 0.53 0.52	0.70 0.59 0.64 0.62	0.67 0.45 0.54 0.42	0.29 0.30 0.26 0.22	+	0.49 0.47	0.90 0.94	0.89 0.83
						-	0.44 0.41	0.82 0.71	0.88 0.79
	500	0.49 0.56 0.58 0.57	0.73 0.80 0.68 0.70	0.45 0.54 0.49 0.54	0.22 0.30 0.22 0.30	+	0.43 0.44	0.82 0.79	0.83 0.88
						-	0.44 0.44	0.76 0.81	0.79 0.97
	1,000	0.48 0.48 0.50 0.45	0.68 0.67 0.70 0.64	0.49 0.44 0.51 0.50	0.26 0.78 0.31 0.34	+	0.37 0.42	0.74 0.74	0.82 0.74
						-	0.44 0.46	0.74 0.79	0.92 0.76
+	0	nd 0.74 0.72 0.81	0.96 1.08 0.95 1.07	0.89 1.03 1.00 0.90	1.35 1.95 1.73 1.68	+	0.85 0.96	1.48 1.53	1.81 1.78
						-	1.11 0.99	1.89 1.77	2.09 2.25
	250	0.78 0.63 0.82 0.71	0.83 1.03 0.92 0.97	0.91 0.84 0.73 0.89	1.52 1.44 0.43 1.83	+	0.75 0.72	1.34 1.26	1.58 1.37
						-	1.06 0.93	1.78 1.84	2.12 2.28
	500	0.66 0.71 0.71 0.77	0.77 1.01 0.91 0.86	0.76 0.85 0.71 0.83	1.43 1.54 1.30 0.54	+	1.05 0.61	1.35 1.28	1.59 1.35
						-	0.76 0.90	1.66 1.76	1.77 2.12
	1,000	0.66 0.63 0.77 0.77	0.97 0.84 0.87 0.95	0.82 0.69 0.57 0.82	1.67 1.43 1.70 1.87	+	0.90 0.91	1.26 1.35	1.52 1.39
						-	0.63 1.26	1.43 1.75	1.88 1.87

Tab. A.20: P content in *Lolium perenne*, forest topsoil (FT) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.35 0.27 0.28 0.25	0.67 0.60 0.67 0.65	0.68 0.57 0.57 0.61	1.22 0.38 0.90 1.13	+	0.55 0.44	0.80 0.76	0.72 0.68
						-	0.40 0.36	0.51 0.51	0.48 0.54
	250	0.37 0.23 0.28 0.32	0.69 0.61 0.52 0.57	0.56 0.51 0.47 0.47	1.06 0.36 0.39 0.34	+	0.47 0.40	0.86 0.90	0.76 0.72
						-	0.41 0.39	0.55 0.48	0.54 0.49
	500	0.29 0.33 0.33 0.32	0.60 0.56 0.59 0.57	0.38 0.38 0.45 0.39	0.27 0.31 0.31 0.38	+	0.41 0.43	1.07 0.97	0.86 0.69
						-	0.31 0.35	0.57 0.56	0.50 0.48
	1,000	0.34 0.34 0.30 0.31	0.50 0.56 0.62 0.65	0.36 0.33 0.40 0.33	0.31 0.28 0.99 1.05	+	0.43 0.42	1.00 1.10	0.77 0.78
						-	0.34 0.39	0.74 0.59	0.53 0.47
+	0	1.32 1.23 1.31 1.39	2.66 2.75 2.66 2.71	2.10 2.19 2.16 2.23	2.84 2.69 2.87 2.26	+	1.82 1.69	2.50 2.59	2.45 2.27
						-	1.99 1.86	2.98 2.62	2.99 3.23
	250	1.20 1.26 1.25 1.54	2.80 2.81 2.60 2.67	2.09 2.12 2.10 2.16	3.00 2.81 2.79 3.24	+	1.91 1.74	2.42 2.23	2.19 2.43
						-	2.37 2.17	3.14 2.87	2.79 3.04
	500	1.41 1.49 1.32 1.61	2.50 2.66 2.53 2.52	nd 2.32 2.02 1.90	2.29 2.55 2.77 2.67	+	1.43 1.50	2.18 2.39	1.95 2.11
						-	1.39 2.23	2.76 3.19	2.78 3.00
	1,000	1.21 1.34 1.16 1.03	2.17 2.66 2.08 1.77	1.77 1.98 1.96 2.26	2.57 3.31 3.06 2.90	+	1.42 1.39	2.15 2.25	1.59 2.07
						-	2.00 2.03	2.83 2.74	2.97 3.32

Tab. A.20: P content in *Lolium perenne*, forest subsoil (FS) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.18 0.15 0.40 0.40	1.02 1.03 0.89 0.77	0.60 0.65 0.65 0.64	0.37 0.50 0.35 0.42	+	0.32 0.33	0.67 2.96	0.62 0.57
						-	0.39 0.38	0.67 0.49	0.73 0.91
	250	0.11 0.16 0.34 0.34	1.01 0.83 0.88 0.81	0.94 0.73 0.60 0.55	0.45 0.49 0.47 0.46	+	0.37 0.32	0.73 0.69	0.72 0.56
						-	0.28 0.48	0.50 0.66	0.68 0.59
	500	us us 0.35 0.35	0.96 0.57 0.76 0.64	0.90 0.51 0.70 0.49	0.48 0.46 0.48 0.41	+	0.38 0.41	0.71 0.69	0.64 0.49
						-	0.53 0.53	0.65 0.69	0.86 0.87
	1,000	us us 0.38 0.38	0.77 0.77 0.45 0.45	0.46 0.46 0.47 0.47	0.46 0.39 0.32 0.57	+	0.46 0.49	0.75 0.71	0.58 0.58
						-	0.53 0.56	0.77 0.74	0.98 0.99
+	0	0.85 0.93 0.98 0.93	1.41 1.28 1.22 1.22	1.81 1.72 1.52 1.64	1.41 1.26 1.25 1.34	+	1.15 1.01	1.69 1.38	1.70 1.64
						-	1.27 1.27	1.30 1.76	1.63 2.49
	250	1.04 1.01 0.92 1.00	1.37 1.46 1.52 1.54	1.44 2.16 1.48 2.00	1.53 1.55 1.31 1.62	+	1.10 1.15	1.52 1.62	1.44 1.51
						-	1.05 1.10	1.52 1.32	1.90 1.71
	500	1.01 1.04 1.17 1.33	1.30 1.39 1.53 1.36	1.42 1.65 1.93 1.88	2.21 1.62 1.42 1.48	+	1.20 1.16	1.52 1.54	1.49 1.53
						-	1.08 1.20	1.41 1.31	1.75 1.60
	1,000	1.08 0.96 0.98 1.03	1.28 1.31 1.41 nd	1.73 1.61 1.83 1.68	1.65 1.44 1.73 1.62	+	1.26 1.01	1.76 1.35	1.92 1.30
						-	1.11 1.17	1.74 1.78	2.62 2.14

Tab. A.21: Ca content in *Lolium perenne*, grassland topsoil (GT) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.63 0.76 0.79 0.65	0.71 0.66 0.70 0.65	0.88 0.76 0.71 nd	0.68 0.63 0.53 0.65	+	0.39 0.37	0.93 0.79	0.78 0.75
						-	0.28 0.31	0.67 0.66	0.61 0.55
	250	0.66 0.74 0.68 0.63	0.69 0.71 0.74 0.74	0.69 0.77 0.70 0.75	0.50 0.60 0.65 0.65	+	0.35 0.37	0.75 0.77	0.80 0.71
						-	0.28 0.31	0.60 0.64	0.70 0.65
	500	0.69 0.73 0.65 0.71	0.64 0.67 0.65 0.76	0.70 0.69 0.68 0.74	0.63 0.53 0.49 0.54	+	0.37 0.40	0.77 0.85	0.86 0.80
						-	0.32 0.31	0.65 0.58	0.71 0.51
	1,000	0.66 0.67 0.71 0.65	0.77 0.75 0.74 0.71	0.68 0.75 0.69 0.64	0.26 0.68 nd 0.31	+	0.36 0.40	0.81 0.74	0.68 0.80
						-	0.37 0.36	0.63 0.60	0.62 0.59
+	0	0.73 0.80 0.74 0.80	0.86 0.78 0.72 0.71	0.96 0.88 0.87 0.74	0.72 0.73 0.55 0,65	+	0.40 0.39	0.84 0.90	0.88 0.85
						-	0.32 0.36	0.69 0.72	0.80 0.69
	250	0.70 0.73 0.87 0.75	0.67 0.65 0.65 0.69	0.79 0.75 0.89 0.78	0,57 0,68 0,58 0.66	+	0.37 0.40	0.78 0.86	0.81 0.85
						-	0.36 0.34	0.68 0.73	0.74 0.74
	500	0.73 0.82 0.86 0.75	0.66 0.71 0.78 0.65	0.82 0.79 0.88 0.82	0.62 0.59 0.69 0.49	+	0.35 0.38	0.78 0.82	0.74 0.78
						-	0.36 0.33	0.78 0.74	0.81 0.64
	1,000	0.85 0.93 0.88 0.79	0.63 0.75 0.66 0.62	1.55 0.78 0.91 0.73	0.27 0.69 0.76 0.25	+	0.42 0.35	0.86 0.75	0.82 0.82
						-	0.31 0.34	0.66 0.69	0.76 0.84

Tab. A.21: Ca content in *Lolium perenne*, grassland subsoil (GS) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.66 0.79 0.78 0.84	0.48 0.60 0.58 0.53	0.59 0.62 0.53 0.58	0.40 0.36 0.34 0.37	+	0.47 0.41	0.80 0.81	0.85 0.76
						-	0.27 0.29	0.40 0.38	0.45 0.42
	250	0.79 0.90 0.86 0.79	0.46 0.50 0.55 0.48	0.58 0.43 0.50 0.36	0.38 0.37 0.22 0.18	+	0.54 0.61	0.85 0.91	0.89 0.81
						-	0.34 0.27	0.39 0.30	0.42 0.37
	500	0.75 0.77 0.85 0.90	0.45 0.45 0.53 0.50	0.39 0.55 0.45 0.50	0.18 0.23 0.18 0.21	+	0.48 0.66	0.66 0.80	0.77 0.78
						-	0.40 0.33	0.38 0.34	0.41 0.41
	1,000	0.81 0.78 0.76 0.75	0.40 0.45 0.54 0.38	0.36 0.39 0.43 0.39	0.16 0.17 0.18 0.17	+	0.61 0.73	0.73 0.78	0.74 0.78
						-	0.33 0.29	0.33 0.32	0.40 0.40
+	0	nd 1.02 1.10 1.03	0.70 0.76 0.62 0.68	0.82 0.82 0.81 0.68	0.73 0.34 0.33 0.30	+	0.79 0.82	0.96 0.93	0.91 1.03
						-	0.70 0.64	0.86 0.81	1.00 0.87
	250	0.79 0.82 0.81 0.89	0.60 0.78 0.62 0.63	0.78 0.74 0.65 0.78	0.33 0.31 0.27 0.39	+	0.83 0.89	1.04 1.00	1.03 1.00
						-	0.67 0.55	0.74 0.78	0.90 0.86
	500	0.92 0.99 0.96 0.96	0.68 0.67 0.63 0.60	0.72 0.63 0.59 0.71	0.33 0.34 0.29 0.35	+	0.67 0.71	0.94 0.95	0.92 0.92
						-	0.86 0.56	0.70 0.74	0.85 0.78
	1,000	0.91 0.92 0.77 0.78	0.59 0.57 0.66 0.55	0.63 0.57 0.44 0.64	0.72 0.22 0.80 0.82	+	0.59 0.83	0.82 0.90	0.95 1.16
						-	0.63 0.87	0.76 0.69	0.87 0.79

Tab. A.21: Ca content in *Lolium perenne*, forest topsoil (FT) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-02-01	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.57 0.56 0.51 0.61	0.31 0.30 0.45 0.33	0.32 0.30 0.34 0.26	0.16 0.14 0.15 0.14	+	0.94 1.31	0.84 0.97	0.99 1.04
						-	0.29 0.26	0.24 0.17	0.35 0.27
	250	0.49 0.49 0.57 0.59	0.40 0.33 0.27 0.32	0.39 0.25 0.24 0.29	0.14 0.15 0.16 0.15	+	1.34 1.03	1.10 0.94	1.16 0.88
						-	0.22 0.21	0.19 0.23	0.30 0.33
	500	0.47 0.45 0.63 0.53	0.31 0.32 0.34 0.34	0.27 0.30 0.29 0.27	0.13 0.15 0.15 0.16	+	1.01 1.05	1.01 0.91	1.14 0.98
						-	0.22 0.23	0.28 0.23	0.34 0.28
	1,000	0.47 0.40 0.27 0.24	0.31 0.29 0.40 0.38	0.37 0.30 0.29 0.26	0.13 0.09 0.12 0.10	+	1.14 0.82	0.94 0.93	1.03 0.89
						-	0.19 0.18	0.17 0.16	0.22 0.24
+	0	1.33 1.28 1.27 1.49	1.37 1.58 1.61 1.44	1.48 1.46 1.53 1.51	1.23 1.29 1.31 1.39	+	1.30 1.38	1.03 1.03	1.06 1.15
						-	1.40 1.44	0.89 1.01	1.01 1.05
	250	1.23 1.16 1.25 1.50	1.54 1.69 1.47 1.33	1.42 1.46 1.46 1.33	1.35 1.37 0.49 1.33	+	1.42 1.39	1.09 1.08	1.14 1.23
						-	1.11 1.36	1.27 0.96	1.18 1.14
	500	1.42 1.63 1.32 1.64	1.44 1.25 1.42 1.38	nd 1.42 1.40 1.24	0.49 0.45 1.07 1.18	+	1.19 1.34	1.05 1.09	1.14 1.06
						-	0.92 1.00	1.03 1.01	1.17 1.21
	1,000	1.21 1.30 1.20 0.93	1.20 1.30 1.25 1.15	1.31 1.38 1.32 1.49	nd 1.30 1.18 1.40	+	1.11 1.15	0.97 0.97	0.83 1.07
						-	1.05 1.12	0.82 0.89	1.04 1.20

Tab. A.21: Ca content in *Lolium perenne*, forest subsoil (FS) (in % of dry weight)
Us: under standard, nd: no data

$CaHPO_4$ addition	U contamination mg kg^{-1}	Cut 1 09-11-00	Cut 2 01-11-00	Cut 3 29-03-01	Cut 4 21-06-01	$CaCO_3$ addition	Cut 5 02-08-01	Cut 6 30-08-01	Cut 7 26-10-01
-	0	0.36 0.31 0.34 0.34	0.23 0.22 0.21 0.20	0.73 0.54 0.67 0.95	0.18 0.19 0.16 0.23	+	0.85 0.92	1.06 0.88	1.12 1.02
						-	0.29 0.22	0.26 0.17	0.35 0.24
	250	0.19 0.46 0.29 0.29	0.19 0.18 0.18 0.16	0.55 0.53 0.60 0.58	0.14 0.17 0.16 0.15	+	1.33 1.14	1.28 1.06	1.19 0.96
						-	0.38 0.19	0.22 0.14	0.28 0.28
	500	us us 0.24 0.24	0.12 0.12 0.13 0.12	0.27 0.23 0.26 0.17	0.13 0.12 0.12 0.13	+	0.91 0.69	0.96 0.84	1.06 0.71
						-	0.17 0.09	0.14 0.08	0.16 0.16
	1,000	us us 0.13 0.13	0.14 0.14 0.12 0.12	0.19 0.19 0.20 0.20	0.15 0.11 0.16 0.13	+	0.70 0.65	0.96 0.91	0.93 0.99
						-	0.11 0.13	0.11 0.12	0.16 0.19
+	0	0.93 0.82 0.86 0.86	1.33 1.47 1.25 1.30	1.51 1.78 1.72 1.66	1.16 1.12 1.22 1.20	+	1.31 1.30	1.04 0.99	1.04 1.04
						-	1.17 1.27	0.70 0.93	1.08 1.11
	250	0.90 1.00 0.87 0.94	1.50 1.45 1.61 1.36	1.63 1.89 1.70 1.57	1.38 1.30 1.12 1.24	+	1.41 1.35	1.06 1.04	1.11 1.04
						-	1.05 0.92	0.88 0.66	1.07 0.92
	500	0.86 0.89 0.99 0.95	1.41 1.37 1.37 1.28	1.41 1.53 1.70 1.69	0.58 0.45 0.48 0.58	+	1.36 1.28	1.02 0.97	1.06 1.05
						-	1.15 1.16	0.81 0.88	0.99 1.42
	1,000	0.93 0.77 0.92 0.82	1.30 1.27 1.40 nd	1.53 1.48 1.52 1.52	0.56 0.51 0.56 1.54	+	1.54 1.25	1.25 1.06	1.45 1.19
						-	1.04 0.91	0.76 0.81	1.07 1.18

Tab. A.22: Availability of U and P, and pH of grassland topsoil (GT), at cut 7

$CaHPO_4$ addition	U treatment mg kg^{-1}	$CaCO_3$ addition	Soil determination				
			U mg kg^{-1}		P in water mg kg^{-1}	pH	
			DTPA	AAAcEDTA		$CaCl_2$	Wet Soil
-	0	+	0.000 0.000	0.015 0.015	13.8 16.4	7.06 7.04	6.62 6.79
		-	0.000 0.000	0.010 0.036	16.6 16.3	6.34 6.58	7.40 6.42
	250	+	0.596 0.841	19.176 22.170	19.2 15.3	7.00 7.00	6.57 6.79
		-	0.280 0.694	20.977 28.616	14.5 15.3	6.41 6.60	6.35 5.95
	500	+	1.097 0.950	36.733 47.764	12.9 12.7	6.93 6.81	6.77 6.73
		-	0.949 0.587	61.071 46.746	10.3 14.0	6.40 6.20	6.48 6.26
	1,000	+	1.536 2.327	83.598 99.788	12.4 14.5	6.80 6.84	6.75 6.57
		-	1.721 1.559	123.845 109.699	15.9 16.4	6.34 6.34	6.41 6.45
+	0	+	0.004 0.002	0.009 0.019	31.8 32.6	6.72 6.80	6.89 7.28
		-	0.002 0.002	0.010 0.010	36.6 45.3	6.55 6.40	5.77 6.98
	250	+	0.100 0.189	14.397 12.828	31.1 27.1	6.77 6.82	6.85 6.90
		-	0.120 0.120	13.770 11.230	30.8 44.0	6.40 6.35	6.43 6.52
	500	+	0.303 0.355	23.419 27.496	29.6 29.6	6.77 6.76	6.19 6.78
		-	0.288 0.283	27.270 27.585	35.4 44.0	6.60 6.42	6.25 6.31
	1,000	+	0.468 0.935	53.251 64.537	30.6 29.4	6.70 7.06	6.97 7.16
		-	0.647 0.575	52.670 57.940	49.9 36.6	6.50 6.36	6.07 6.37

Tab. A.22: Availability of U and P, and pH of grassland subsoil (GS), at cut 7

$CaHPO_4$ addition	U treatment mg kg^{-1}	$CaCO_3$ addition	Soil determination				
			U mg kg^{-1}		P in water mg kg^{-1}	pH	
			DTPA	AAAcEDTA		$CaCl_2$	Wet Soil
-	0	+	0.008 0.004	0.026 0.036	11.5 10.1	6.71 6.82	6.82 7.00
		-	0.004 0.004	0.004 0.049	9.8 8.8	5.31 5.52	6.06 5.65
	250	+	12.935 7.963	77.867 60.295	8.9 8.7	6.64 6.73	6.07 6.30
		-	2.463 4.870	58.588 99.158	19.3 9.4	5.00 5.80	5.37 5.34
	500	+	21.093 20.012	145.685 145.018	8.8 9.7	6.67 6.79	5.72 5.99
		-	2.418 8.540	122.993 136.994	7.3 7.4	4.94 4.98	5.15 5.08
	1,000	+	36.940 39.114	248.854 271.132	6.2 8.1	6.67 6.82	6.72 5.75
		-	15.448 22.443	233.502 384.273	17.9 12.4	4.94 5.03	5.32 5.19
+	0	+	0.012 0.011	0.082 0.029	53.4 52.5	6.50 6.64	6.47 6.72
		-	0.002 0.019	0.010 0.020	108.7 75.7	5.76 5.71	5.86 5.96
	250	+	0.279 0.244	14.228 14.801	46.1 47.7	6.42 6.68	6.55 6.64
		-	0.196 0.216	16.453 13.761	107.2 65.0	5.70 5.86	5.65 5.66
	500	+	0.468 0.558	29.535 32.388	41.5 174.8	6.48 6.68	6.23 6.11
		-	0.445 0.276	32.783 21.183	48.1 125.4	5.60 5.56	5.58 5.70
	1,000	+	1.153 1.566	62.488 62.665	122.7 13.4	6.52 6.71	6.31 6.04
		-	1.041 0.942	84.428 68.834	43.9 205.3	6.10 5.54	5.59 5.67

Tab. A.22: Availability of U and P, and pH of forest topsoil (FT), at cut 7

$CaHPO_4$ addition	U treatment mg kg^{-1}	$CaCO_3$ addition	Soil determination				
			U mg kg^{-1}		P in water mg kg^{-1}	pH	
			DTPA	AAAcEDTA		$CaCl_2$	Wet Soil
-	0	+	0.001 0.002	0.036 0.015	9.9 10.1	6.27 6.29	4.98 4.63
		-	0.002 0.002	0.041 0.039	19.9 30.1	3.83 3.70	3.82 4.12
	250	+	5.067 3.512	69.297 70.057	8.8 9.7	6.02 5.54	5.27 5.03
		-	3.190 3.403	67.747 88.799	14.4 11.5	3.60 4.00	3.87 3.88
	500	+	9.304 11.711	169.942 148.406	29.6 10.0	5.40 5.82	4.89 4.55
		-	11.279 8.198	183.571 136.043	7.9 14.7	3.43 3.70	3.98 3.76
	1,000	+	44.389 33.805	321.680 292.121	17.4 7.9	6.08 5.70	5.16 4.82
		-	23.050 26.262	294.878 299.403	5.7 15.1	3.59 3.55	4.12 4.02
+	0	+	0.011 0.004	0.084 0.031	63.9 71.5	6.00 6.01	5.43 5.69
		-	0.004 0.018	0.024 0.019	299.3 297.1	4.35 4.33	3.97 4.93
	250	+	0.154 0.214	10.015 13.809	66.6 63.2	5.96 6.06	5.80 5.60
		-	0.359 0.281	45.257 12.765	307.9 297.1	4.40 4.36	4.62 4.44
	500	+	0.201 0.284	42.117 19.747	11.7 62.5	6.08 6.10	5.67 5.26
		-	0.310 0.370	18.554 18.252	292.3 285.6	4.35 4.55	4.66 4.36
	1,000	+	0.488 0.560	47.446 47.517	65.2 67.8	6.10 6.18	5.28 5.72
		-	0.701 0.714	94.888 60.164	277.7 270.6	4.42 4.30	4.70 4.56

Tab. A.22: Availability of U and P, and pH of forest subsoil (FS), at cut 7

$CaHPO_4$ addition	U treatment mg kg^{-1}	$CaCO_3$ addition	Soil determination				
			U mg kg^{-1}		P in water mg kg^{-1}	pH	
			DTPA	AAAcEDTA		$CaCl_2$	Wet Soil
-	0	+	0.008 0.007	0.033 0.040	12.1 14.7	6.20 6.34	5.22 6.21
		-	0.009 0.006	0.043 0.040	6.8 5.6	4.16 3.90	3.89 4.31
	250	+	15.328 12.872	93.725 79.928	11.2 7.3	6.13 6.33	5.40 6.07
		-	9.009 5.499	86.660 84.876	5.3 17.4	4.16 4.00	4.25 3.94
	500	+	16.690 27.527	162.930 181.914	77.3 6.8	5.45 5.84	5.06 5.57
		-	24.645 11.934	163.858 132.527	5.0 6.4	4.00 4.33	4.35 4.45
	1,000	+	79.512 81.033	291.678 333.573	15.6 8.2	6.30 6.34	5.73 6.27
		-	39.954 50.911	301.766 332.626	9.8 9.4	4.28 4.58	4.98 4.68
+	0	+	0.040 0.032	0.035 0.030	58.5 1156.2	6.32 6.35	5.86 5.94
		-	0.006 0.024	0.033 0.030	3.7 105.7	4.80 5.13	5.10 5.42
	250	+	0.206 0.225	14.353 16.639	6.1 52.2	6.14 6.24	6.20 5.87
		-	0.221 0.204	14.055 12.787	218.5 162.5	4.86 4.83	5.23 5.38
	500	+	0.314 0.283	29.409 23.733	137.3 57.8	6.25 6.31	5.78 5.71
		-	0.379 0.385	33.158 32.458	58.7 131.1	4.88 4.84	5.41 5.13
	1,000	+	0.555 0.811	58.454 69.227	168.6 54.3	6.14 6.28	6.07 6.09
		-	0.659 0.825	60.005 61.991	150.1 148.8	4.88 4.92	5.29 4.59

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