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Comparative studies on the solubility of uranium and phosphorus in phosphate-fertilisers and their uranium transfer to plants

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Comparative studies on the solubility of uranium and phosphorus in phosphate-fertilisers and their uranium transfer to plants

Von der Fakultät für Lebenswissenschaften

der Technischen Universität Carolo-Wilhelmina

zu Braunschweig

zur Erlangung des Grades eines

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Dedication

I dedicate this work to my great mother, my dear wife, and my sweet daughters, Mahsima and Mahshid.

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I would like to appreciate all persons that their help and support gave me the possibility to complete this thesis.

First of all, many thanks to my supervisor Prof. Dr. Dr. Dr. h.c. Ewald Schnug who gave me this opportunity to work under his supervision in *the Institute for Crop and Soil Science* of *the Federal Research Centre for Cultivated Plants* (*Julius Kuehn Institute, JKI)*. I would like to thank the other members of my committee, Prof. Dr. Robert Kreuzig and Prof. Dr. Dirk Selmar.

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Finally, I would like to express my deepest appreciation to my mother, my wife, my daughters, and all my family for their support, encouragement, patience and endless love.

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List of acronyms and abbreviations

AA	Ascorbic acid
AAAc-EDTA	Acid ammonium acetate EDTA (pH=4.65)
AAC	Alkaline ammonium citrate
AAc	Acetic acid
Ac	Acetate
Am	Americium
ANOVA	Analysis of variance
AR	*Aqua regia*
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical
BCR	Community Bureau of Reference
Bq	Becquerel (a unit of radioactivity)
C	Carbon
Ca	Calcium
CA	Citric acid
CAL	Calcium-acetat-lactate
CEC	Cation exchangeable capacity
CF	Compound fertilisers
Cl	Chloride
Cm	Curium
cv.	Cultivar
CR	Concentration ratio
DAP	Diammonium phpsphate
DCB	Dithionite-citrate-bicarbonate (sodium citrate-sodium ditionite buffer)
DCP	Dicalcium phosphate
DM	Dry matter
DTPA	Diethylenetetraaminepentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency (of USA)
EU	European Union
FA	Formic acid
FAO	Food and Agriculture Organization (of United Nations)
Fe	Iron
GLM	General linear model
HOx	Oxalic acid
I	Ionic strength
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
ICP-QMS	Inductively coupled plasma quadruple mass spectroscopy

JKI	Julius Kühn-Institut
K	Potassium
K_d	Distribution coefficient
M	mol l^{-1}
MAP	Ammonium phosphate
MCP	Monocalcium phosphate
MD	Mineral dünger (mineral fertiliser)
Mg	Magnesium
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
Na	Sodium
NAC	Neutral ammonium citrate
O	Oxygen
OM	Organic matter
OMF	Organo-mineral fertiliser
P	Phosphorous
PAPR	Partially acidulated phosphate rock
PR	Phosphate rock
Pu	Plutonium
R, r	Correlation coefficient
S	Sulphur
SP	Superphosphate
SPF	Straight phosphorus-fertilisers
SPSS	Statistical Package for the Social Sciences
SSP	Single superphosphate
TF	Transfer factor
TSP	Triple superphosphate
U	Uranium
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten (Association of Agricultural Investigation and Research Institutes of Germany)
W	Water
WHO	World Health Organization
Zn	Zinc

1 Introduction

Agriculture was developed at least 10,000 years ago, and it has made evident developments since the time of the earliest cultivation. Agricultural practices such as domestication, fertilisation, irrigation, and disease controlling were developed over a long time but great strides were made in the last century. Following industrial and health care developments, the world population increased rapidly and the necessity of food security opened another view on crop production. The first aim of agricultural practices is to provide human food and health. To this aim, the researches concentrated on fertilisers, irrigation systems, pesticides and plant breeding investigations in the last century. After progress in breeding techniques and genetic engineering methods, the modern commercial varieties of grains such as wheat, corn, and barley were produced. New hybrids, improved disease resistance and dryness tolerance increased the quantity of agricultural production in the most strategic crops, vegetables, and fruits enormously (Ruttan, 1999; Cassman, 1998; Berg and Singer 2003; FAO Statistics Division, 2008). Food safety and quality is as important as food security and quantity in human and animal feeding. Concerns over the food safety and quality have increased worldwide in last two decades. Attention to these concerns as priority issues is similar for farmers, consumers, industry, and traders alike. Foodborne disease and hazards are significant in all parts of the world, and the reported incidences of disease have increased over last two decades (FAO & WHO, 2002).

The entering of heavy metals and radionuclides into human bodies is a food-borne hazard coming from farming activities. Uranium, the heaviest naturally occurring radionuclide in the environment, is one of these elements. Uranium and uranium series radionuclides can be present in the environment anywhere, and their natural levels can also reach levels of concern. Uranium has chemical and radiological effects on human health. The chemical toxic effect of uranium as a heavy element mainly supersedes its radiological toxicity (Sheppeherd *et al*., 2005; Caddia and Iversen, 1998).

Uranium and other heavy metals and radionuclides contents are increased in soils in different ways (additionally to some natural amount that can be found in all soils).

Uranium can be increased or loaded to soil via natural pedogenesis processes with uranium being released from parent materials to soil and soil solution, and via human activities. Mining phosphate ores can carry uranium to the soil and river water resources surrounding the mines. Phosphate rocks may contain considerable amounts of uranium (Romero Guzman *et al*., 1995; Kratz and Schnug 2006; Rothbaum *et al*., 1979; Makweba and Holm 1993; Takeda *et al*., 2006; Sattouf, 2007; Pantelica, *et al*., 1997; Uyanik *et al*., 1999). Uranium contents of phosphate rocks vary according to geographical origin from less than 10 up to more than 200 $mg\ kg^{-1}$ (Kratz *et al*., 2007). In general, the uranium content in sedimentary phosphate rocks is higher than in igneous phosphate rocks. The uranium content in mineral phosphate fertilisers is also considerable, and it is related to phosphate content and origin of phosphate rock as initial phosphorus source. The uranium contents of ground phosphate rock, triple super phosphate, and simple super phosphate of Minjingu, an area in Tanzania, are as high as 377, 571, and 315 $mg\ kg^{-1}$, respectively (Makweba and Holm 1993). Based on a world wide literature survey, Kratz and Schnug (2006) reported mean uranium concentrations of 6 to 146 $mg\ kg^{-1}$ in different types of phosphorus-containing mineral fertilisers and less than 2.2 $mg\ kg^{-1}$ in mineral fertilisers without phosphorus.

Anyway, different amounts of uranium and other heavy metals and radionuclides are transferred to phosphorus-fertilisers via the chemical production processes. Then, phosphate rocks, as a direct application or original source for producing the phosphate fertilisers, can load various amounts of uranium onto soil depending on the original mines from which they are extracted, and the type of initial minerals. Kratz and Schnug (2006) reported that a mean value of uranium from 7.0 to 23 $g\ ha^{-1}\ yr^{-1}$ can be loaded onto soil using of 22 kg P $ha^{-1}\ yr^{-1}$ (50 kg P_2O_5 ha^{-1}) from various phosphorus-containing fertilisers.

An increase of uranium concentration in topsoil of fields with long applied fertilisers has been reported by several authors (Rogasik *et al*., 2007; Takeda *et al*., 2006; Makweba and Holm, 1993; Rothbaum *et al*., 1979), while some others found different results. Jones (1992) reported no changes in uranium concentration in the field soil over 82 years with applied phosphate rock and phosphorus-fertilisers. It may be assumed that the fertiliser-derived uranium was leached from the topsoil, lost via surface soil erosion or taken up by plants. Also, Mortvedt (1994) reported no differences between uranium concentrations of nonfertilised and fertilised soil with the triple superphosphate (TSP) made from Florida phosphate rock, which, according to data collected by Kratz and Schnug (2006), has a uranium concentration in the range of 65-141 $mg\ kg^{-1}$.

With increasing uranium content and concentration in soil, uranium concentration may increase in plant tissues, animal bodies, and human bodies in the long run. Uranium can cause damage to the health of humans and animals. The toxic effects of uranium are mainly based on its chemical toxicity as heavy element rather than the radiation dose (Caddia and Iversen, 1998; Sheperd *et al*., 2005).

Total amount of uranium and uranium-to-phosphorus ratio vary in different types of phosphorus-containing mineral fertilisers (Kratz and Schnug, 2006). Also, solubility and transferability of uranium in different P-fertilisers is not the same. Assessing uranium solubility and transferability in P-fertilisers is necessary for fertiliser recommendation with regard to food safety and controlling uranium entering the food chain. Finding an extraction method for assessing solubility and transferability of uranium and phosphorus in each type of P-fertiliser at the same time is very important. To this end, comparing uranium and phosphorus solubility in standard extractions can be useful. Transferability of phosphorus and uranium need a bio response test to follow them from fertilisers to plant tissues. More investigations are necessary to find out how much uranium can transfer from phosphorus sources to plants, and differences between uranium uptakes by plants from various types of phosphorus sources.

The following chapter gives an overview of the state of knowledge on the chemistry and geochemistry of uranium, its environmental occurrence, behaviour of uranium in soil, and parameters affecting the transferability of uranium to plants. From this, key questions of this thesis are derived.

2 Literature review

2.1 Geochemistry of uranium and its occurrence in the environment

- Chemical and physical properties of uranium: Uranium is the 92nd element of periodic table, and its relative molecular mass is 238.0289. Uranium is the heaviest naturally occurring radionuclide in the environment. It has 15 isotopes of which three are present in nature, ^{238}U, ^{235}U and ^{234}U. Abundances of these three primordial isotopes are 99.2745%, 0.7200% and 0.0055%, respectively (Caddia and Iversen, 1998). Uranium has a high melting point about 1132.3 °C, boiling point of 3818 °C, and specific gravity equal to 18.95 g/cm^3. Oxidation states of uranium are +3, +4, +5, and +6 (Environment Canada, 2002). However, under natural condition tetravalent and hexavalent oxidation states of uranium are dominant.

- Uranium minerals: Uranium exists in the environment as uraninite (UO_2) or pitchblende (U_3O_8) or as secondary minerals (Some Chemistry of Uranium). Uranium (+IV) minerals include uraninite (UO_2 through $UO_{2.25}$) and coffinite ($USiO_4$). Uranium (+VI) as the uranyl cation (UO_2^{2+}) is a basic structural constituent of nearly 200 minerals including primary oxides, carbonates, silicates, phosphates, and vanadates (*Table 2.1*).

- Uranium content in the earth crust: Uranium is found in the Earth’s crust as different types of minerals. During the pedogenic processes, uranium is released to the soil, ground water, and rivers. The mean uranium content in the Earth’s crust is 2-4 $mg\ kg^{-1}$ and it varies in different minerals (Lamas, 2005). Uranium content in major rock types varies from 0.003 to 6 $mg\ kg^{-1}$ (*Table 2.2*). Uranium can be distributed in nature in different forms and ways. It can be found in plant tissues, animal bodies, sea water, some foods, and the human body in rare quantities.

Table 2.1 Uranyl minerals that may form in porous media (adapted from Giammar, 2001 and Lamas, 2005).

Mineral	Composition
Oxides and Hydroxides:	
Schoepite	$(UO_2)_8O_8(OH)_{12}\cdot 12H_2O$
Meta-schoepite	$(UO_2)_8O_8(OH)_{12}\cdot 10H_2O$
Dehydrated schoepite	$UO_3\cdot(2\text{-}x)H_2O$
Becquerelite	$Ca(UO_2)_6O_4(OH)_6\cdot 8H_2O$
Clarkeite	$Na[(UO_2)O(OH)]\cdot H_2O$
Compreignacite	$K_2U_6O_{19}\cdot 11H_2O$
Carbonates:	
Rutherfordine	UO_2CO_3
Liebigite	$Ca_2UO_2(CO_3)_3\cdot 11H_2O$
Silicates:	
Soddyite	$(UO_2)_2SiO_4\cdot 2H_2O$
Uranophane	$Ca(H_3O)_2(UO_2SiO_4)_2\cdot 3H_2O$
β−uranophane	$Ca(UO_2)SiO_3(OH)_2\cdot 5H_2O$
Weeksite	$K_2(UO_2)_2Si_6O_{15}\cdot 4H_2O$
Coffinite	$USiO_4$
Phosphates:	
Autunite	$Ca(UO_2)_2(PO_4)_2\cdot 10H_2O$
Meta-autunite	$Ca(UO_2)_2(PO_4)_2\cdot(2\text{-}6)H_2O$
Uranyl orthophosphate	$(UO_2)_3(PO_4)_2\cdot 4H_2O$
Sodium meta-autunite	$Na_2(UO_2)_2(PO_4)_2\cdot 8H_2O$
Meta-ankoleite	$K_2(UO_2)_2(PO_4)_2\cdot 6H_2O$
Phosphuranylite	$Ca(UO_2)_3(PO_4)_2(OH)_2\cdot 6H_2O$
Saleeite	$Mg(UO_2)_2(PO_4)_2\cdot 10H_2O$
Vanadates:	
Carnotite	$K_2(UO_2)_2(VO_4)_2.3H_2O$
Tyuyamunite	$Ca(UO_2)_2(VO_4)_2.(2.5\text{-}8)H_2O$

- Uranium content in soil: Natural uranium content in soil varies from 0.1 to 11.2 *mg kg*$^{-1}$ in different areas. Uranium concentration in soil increases by some human activities like mining uranium ores, uranium enrichment processes, storing the by-products or use of the by-products and depleted uranium, and agricultural activity for fertilizing the soil with phosphorus sources. In contaminated soils, uranium content can be reached 10 to 100-fold higher than in natural soils (Lamas, 2005). Uranium content of surface soils of several countries is shown in *Table 2.3*.

Table 2.2 Uranium content in major rock types (Kabata-Pendias and Pendias, 1984; Lamas, 2005)

Rock type		Uranium content (*mg kg*$^{-1}$)
	Magmatic Rocks	
Ultramafic rocks:		
	Dunites, Peridotites, Pyroxenites	0.003-0.010
Mafic rocks:		
	Basalts, Gabbros	0.3-1.0
Intermediate rocks:		
	Diorites, Syenites	1.4-3.0
Acid rocks:		
	Granites, Gneisses	2.5-6.0
Acid rocks (volcanic):		
	Rhyolites, Trachytes, Dacites	5
	Sedimentary Rocks	
	Argillaceous sediments	3-4
	Shales	3.0-4.1
	Sandstones	0.45-0.59
	Limestones, Dolomites	2.2-2.5

- Uranium content in plants: Range of uranium concentration in plants on uncontaminated soils is 0.01 to 0.4 *mg kg*$^{-1}$ of dried matter (DM) with a mean of 0.04 *mg kg*$^{-1}$ (Lamas, 2005). Of course uranium concentration is higher in plants growing on contaminated soils than in plants on natural soils. On a loamy sand texture soil, uranium concentration of *Lolium perenne* reached 45 *mg kg*$^{-1}$ of dried matter (DM) after using 1000 *mg* uranium per kilogram of soil (Schroetter *et al.*, 2003).

Table 2.3 Uranium contents of surface soils of different countries (Kabata-Pendias and Pendias, 1984; Lamas, 2005)

Country	**Range (*mg kg^{-1}*)**	**Mean (*mg kg^{-1}*)**
Canada	0.72-2.05	1.22
Great Britain	-	2.60
Germany	0.42-11.02	-
India	-	11.00
Italy	1.5-8.0	3.17
Poland	0.10-2.33	0.79
U.S.	0.30-10.70	3.70
U.S. (Alaska)	0.22-45	2.3
Russia	-	3.8
China	0.42-21.1	2.79

- no result

Uranium concentration in plants varies in different tissues. In most of plants investigated, the highest uranium concentration was in roots, and the lowest in fruits or grains (Lamas, 2005; Laroche *et al.,* 2005; Netten and Morley, 1983; Chen *et al.*, 2005; Ribera *et al.,* 1996; Morishima *et al.*, 1977). Sometimes, differences between uranium concentration of roots and other tissues are very large, for instance, uranium concentration of roots in *Phaseolus vulgaris* in a hydroponics culture was about 2400 times more than uranium concentration of leaves at seedlings stage, and at least 156 times more than in stems so, more than 99% of uranium was accumulated in the roots (Laroche *et al.,* 2005).

There are some other contradictory results about the ratio of uranium concentration in shoots and roots, for instance, Singh's report (1997) implies that the highest concentration of uranium in different tissues (root, straw, and grain/fruit or nut) of wheat, rice, chick-pea, lentil, spinach, carrot, radish, brinjal, and beet plants was found in leaves; also Lakshmanan and Venkateswarlu (1988) reported some concentration factor of uranium in fresh tissues of radish, according to that report, uranium concentration of fresh radish leaves is about 1.8, 3.0, and 5.6 times more than uranium concentration of fresh radish roots growing on a farm soil under well water irrigation, on a contaminated soil under well water irrigation, and on a contaminated soil under contaminated water irrigation, respectively. In contrast to the last two reports, Morishima *et al.* (1977) reported almost equal concentrations of uranium 0.049 and 0.066 μg uranium g^{-1} ash) in leaf and root of radish, respectively.

Total amount of uranium in plants varies greatly, and is related to the kind and species of plants, and is directly affected by concentration and speciation of uranium in soil solution, situation of mineral nutrients, microbial activities in soil, existence and situation of organic components in soil solution, and environmental conditions; also it is indirectly affected by some chemical and physical properties of soil such as redox potential, pH, CEC, iron oxides and hydroxides, the kind and quantity of clay minerals, texture, aeration, and drainage (see *Sections 2.3* and *2.4*).

2.2 Behaviour of uranium in soil

Uranium is found in different forms and situations in soil. Plants remove nutrients and unessential metals from the readily exchangeable and soluble fractions of the soil. Negligible amounts of uranium remain in the soluble and exchangeable forms over any significant period of time (Hossner *et al.,* 1998). Five different forms of uranium there are in soil:

- soluble uranium as hydrated ions and uranil complexes,
- exchangeable uranium that can be retained as cation forms by soil colloids (mineral and organic forms),
- precipitated uranium,
- uranium minerals, and
- accumulated uranium in tissues of soil microorganisms.

-Uranium in aqueous system:

Uranium can exist in oxidation states of +III, +IV, +V, and +VI in aqueous solution; however, under most natural aqueous environments the tetravalent and hexavalent states dominate. Dissolved uranium (III) easily oxidizes to uranium (+IV) under reducing conditions found in nature. The uranium (V) aqueous species (UO_2^+)

readily changes to uranium (+IV) and uranium (+VI). This reaction can be described as follows:

$$2UO_2^{+} + 4H_3O^{+} \longleftrightarrow UO_2^{2+} + U^{4+}$$

Consequently, uranium will exist in the +6 oxidation state under oxidizing conditions, and in the +4 oxidation state under reducing conditions. Both uranium species, UO_2^{2+} and U^{4+}, hydrolyze readily. The U^{4+} ion is more readily hydrolyzed than UO_2^{2+} as would be expected from its higher ionic charge.

In acidic and strongly reducing conditions, uranium is reduced to tetravalent state and it is complex-bounded by hydroxyl, sulphate, chloride, phosphate, and fluoride at pH values of less than four, and also by organic humic and fulvic acids (EPA, 1999; Lamas, 2005). Langmuir (1978) calculated uranium (+IV) speciation in a system containing typical natural water containing chloride, fluoride, phosphate, and sulphate in concentrations of 10, 0.2, 0.1, and 100 $mg\ l^{-1}$, respectively. The speciation of dissolved uranium (+IV) at pH values of greater than 3 is dominated by hydrolytic species, and complexes with chloride, phosphate, fluoride, and sulphate are not important above pH 3 (EPA, 1999; Szecsody *et al.*, 1998).

Uranium in the VI oxidation state is relatively soluble and can be detected in almost any natural water. Seawater is the largest reservoir of dissolved uranium, and contains uranium at a highly uniform value of 3.3 $\mu g\ l^{-1}$. Uranium is present in seawater as a stable uranium (+VI) carbonate complex. A small fraction of the total oceanic uranium is associated with particulate organic carbon (McManus *et al.*, 2005).

In groundwater, the weathering of uranium-bearing rocks and minerals is the source of dissolved uranium. The concentration of uranium in groundwater is usually in the range 0.1-50 $\mu g\ l^{-1}$ (Rivas, 2005; Giammar, 2001), and in contaminated groundwater plumes, concentration of uranium is much more, i.e., uranium concentration of groundwater in some uranium mill tailing in United States varied from 0.07 – 3.05 $mg\ l^{-1}$ (Baumgartner *et al.*, 2000).

Uranium solubility in aqueous systems is predominantly controlled by three factors: oxidation-reduction potential, pH, and dissolved carbonate (Giammar, 2001; EPA, 1999; Ervanne, 2004). Uranium (+VI) is considerably more soluble than uranium (+IV). Under reducing conditions, uranium (+IV) complexes with hydroxide or fluoride are the only dissolved species (Giammar, 2001). Uranyl has different hydrated degrees in pure water at different pHs (*Figure 2.1*). At pH values of less than 4, near to 100% of uranyl species is free uranyl ions (UO_2^{2+}), while the most abundant of uranyl species at a pH of more than eight, are uranyl hydroxides with a negative charge after hydrolysis (*Figure 2.1*). Anyhow, in an open system, equilibrated with air CO_2, abundant uranyl species at pH values of more than 7.5, are uranyl carbonates (*Figure 2.2*).

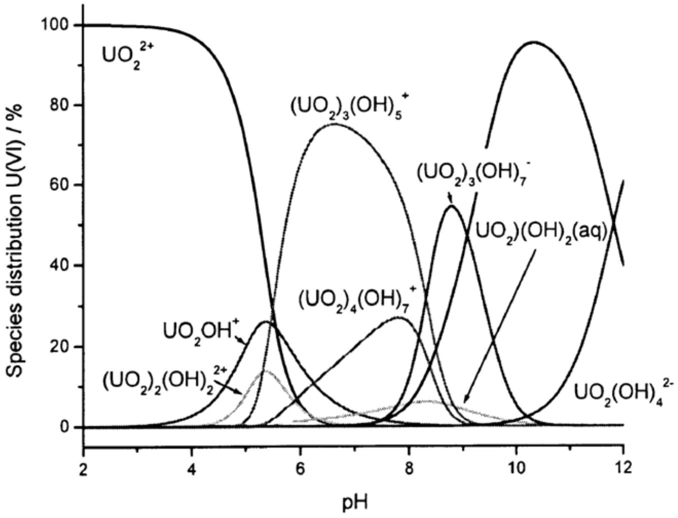

Figure 2.1 Different species of uranyl in pure water related to pH (Giammar, 2001)

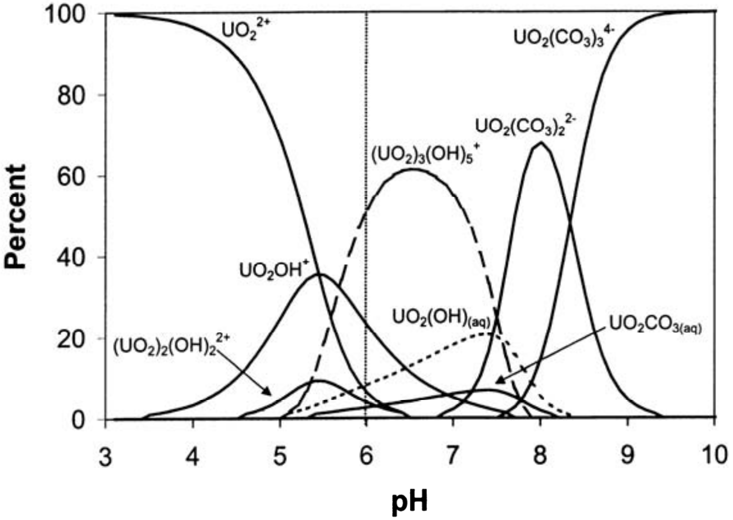

Figure 2.2 Speciation of dissolved uranium as a function of pH for $[U]_{total}$=5µM, I= 0.1 M, and $P_{CO_2}=10^{-3.5}$ atm. Calculations were made without considering the precipitation of any solid phases (Giammar, 2001)

Figure 2.3 shows the speciation of uranium in an aqueous solution in the presence of phosphate. Uranyl complexation with phosphate can be formed in a different range of pH, but abundance and kind of ions vary with pH. At pH of less than 3.5, predominant species are uranyl and uranyl dihydrogen phosphate ions, and in pH about 4.5, predominant species is uranyl monohydrogen phosphate; in pH more than 6, predominant species is uranyl phosphate ion. Above pH 7.5, uranyl phosphate concentration starts decreasing.

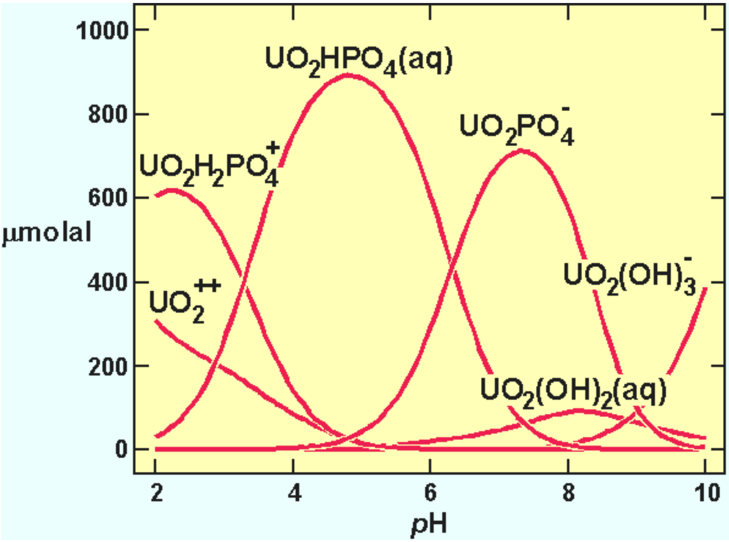

Figure 2.3 Uranyl speciation in presence of phosphate at different pHs (Rockware.com)

-Speciation of uranium in soil solution: Uranium is present in the soil solution primarily (80- 90%) in the +VI oxidation state as uranyl cation (Sheppard and Evenden, 1988; Mortvedt, 1994; Ebbs *et al.*, 1998; Laroche *et al.*, 2005). Most forms of soluble uranium in soil solution, over a broad range of soil pH, are hydrated uranyl (UO_2^{2+}) cations, and uranyl complexes. Also, several organic acids may increase the solubility of uranium in soils (Kabata-Pendias and Pendias, 1984). The highest concentration of free uranyl in solution is found at a pH of less than 5, that is the first abundant of soluble uranium (+VI) (Ebbs *et al.*, 1998; EPA, 1999; Langmuir, 1997; Giammar, 2001). With increasing pH, complexes of uranyl hydroxide and carbonate increase in soil solution or nutrient solution in equilibrium with air CO_2. Uranyl hydroxide complexes are found in positive or negative charges depending on the pH of soil solution. Because of the low concentration of uranium in soil solution, uranium mobility and transferring to sub layers and groundwater is low, and it is accumulated in topsoil in the A horizon. The K_ds (distribution coefficients) of uranium in the range of pH between 5 and 8 are greater than at other pHs; the highest values for K_d are reported at pH about 6 in different soils (EPA, 1999). This implies that uranium concentration, in the pH range of 5 to 8 in soil solution is low and uranium would be precipitated or adsorbed onto solid phase.

The dominant species of uranium in soil solution are different ions and complexes of uranyl. Chemical speciation of uranium (+VI) in soil is highly dependent on soil composition and on the pH of soil solution. Complexation reactions rule the fate of uranyl in soil systems. Uranyl can combine with chloride, sulphate, phosphate, hydroxyl, some organic acids, and carbonates in soil solution related to composition of soil solution and soil pH. In addition to pH, speciation of soluble uranium can be affected by redox potential and ionic strength (EPA, 1999; Ervanne, 2004; Hossner *et al.,* 1998; Giammar, 2001).

The negative charge of soil colloids can hold uranyl and other cationic forms of uranium as exchangeable cations. In this way, uranyl cation and its complexes can adsorb onto clays, insoluble organic matter, Fe and Al oxides and hydroxides, and some of other solid components (EPA, 1999; Giammar, 2001; Szecsody *et al.,* 1998). This decreases uranium concentration in soil solution and decreases uranium leaching to the groundwater. The cation exchangeable capacity (CEC) has direct relationship with soil pH. Uranium (+VI) sorption onto soil surfaces tends to increase with increasing pH (up to pH 7), and it is readily reversible by decreasing the pH (Hossner *et al.,* 1998). In low ionic strength solution with low concentration of uranium (+VI), uranyl concentration will be controlled by cation exchange and adsorption processes (Hossner *et al.,* 1998; EPA, 1999). Generally, the least amount of uranium is found in the exchangeable and soluble forms, thereby the available amount of uranium is limited for plant uptake (Hossner *et al.,* 1998).

Some components of organic matter in the soil can form insoluble complexes with uranium (+VI), thus uranium concentration in soil solution decreases (Kabata-Pendias Pendias, 1984; Hossner *et al.,* 1998; Lamas, 2005).

Dissolution and precipitation have greater effect on the uranium (+IV) concentration than on the uranium (+VI) concentration. Under reducing conditions, these processes tend to become increasingly important and several precipitates may form depending on the environmental conditions (EPA, 1999). Dissolution and precipitation of uranium (+VI) components are affected by pH, ion composition, and ion strength of soil solution. Precipitation-sorption reactions of uranium increase with increased soil pH (Mortvedt, 1994).

Microorganisms have a great capability to accumulate uranium in their tissues. The bioconcentration factor for uranium has been reported to be up to 300 times compared with its content in soils (Kabata-Pendias and Pendias, 1984).

2.3 Uranium transfer to plants

Some factors affect the uranium uptake by plants. These parameters may be the uranium concentration in soil solution, species of uranium in soil solution, ability of plant for adsorbing, uranium transferring to surface of roots, and so on. In the following, some parameters that can affect the transferring of uranium to plants are discussed.

2.3.1 The effect of uranium concentration in root environment

It has been repeatedly reported that uranium uptake by plants is increased with increasing of uranium concentration (Lamas, 2005; Rivas, 2005; Vandenhove, 2002; Environment Canada, 2002; Meyer *et al.*, 2004; Gulati *et al.*, 1980; Eapen *et al.*, 2003; Laroche *et al., 2005*). Laroche *et al.* (2005) reported a linear relationship between total uranium concentration in the range of 0 to 5 µmol l^{-1}, in the hydroponic solution and total uranium content in roots of *Phaseolus vulgaris* at three different pHs (*Figure 2.4*). Also, Pettersson *et al.* (1993) reported a linear relationship between uranium concentration in roots, rhizomes, and foliages of water lily and uranium concentration of water media and uranium concentration of sediments, in the range of 20 to 400 Bq kg^{-1} wet weight of sediments. Like other elements and heavy metals, uranium uptake by plants increases with increasing uranium availability in substrates, but a linear relationship can not be found over all concentration levels. With increasing uranium concentration in contaminated soils, the transfer factor (TF) will decrease (critical values vary dependin on the soil properties and the type of plants). Lakshmanan *et al.* (1988) reported that transfer factor of uranium in some vegetables like brinjal, bottle gourd, lady's finger, radish, and potato decreased with increase of uranium level in soil as well as in irrigation water. Also, they reported only an increase of less than 2 times in the uranium level in different parts of rice plant though uranium level in soil increased 10 times.

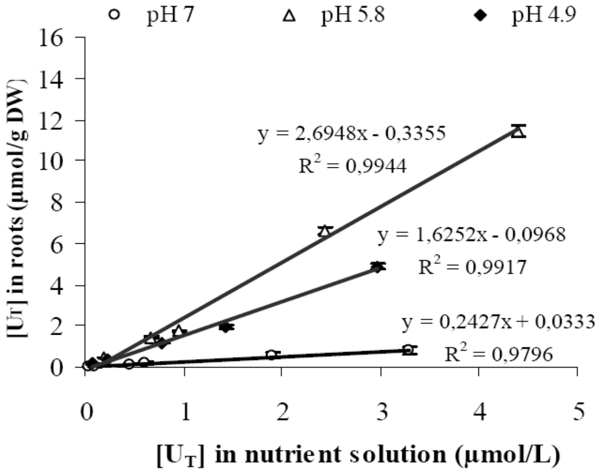

Figure 2.4 Relationship between total uranium content in roots of bean (*Phaseolus vulgaris)* and total uranium concentration in nutrient solution at three pHs (Laroche *et al.*, 2005)

2.3.2 The effect of uranium speciation

As stated in *Section 2.3*, there are different uranium speciations in soil solution. These speciation are free uranyl (UO_2^{2+}), hydroxyl uranyls [$(UO_2)_2(OH)_2^{2+}$, UO_2OH^+, $(UO_2)_3(OH)_5^+$, $UO_2(OH)_{(aq)}$, $UO_2(OH)_3^-$], uranyl carbonates [$UO_2CO_{3(aq)}$, $UO_2(CO_3)_2^{2-}$, $UO_2(CO_3)_3^{4-}$], uranyl phosphates, uranyl sulphate, uranyl nitrate, and uranyl-organo compounds. Generally it is reported that the uranyl cation is readily taken up by plants. Though uranyl concentration decreases with an increase of pH, uranium uptake does not decrease until pH 9 because of increasing the concentration of other soluble uranyl complexes in soil solution. Laroche *et al*. (2005) reported that free uranyl ion content in medium was less considerable at pH 5.8 than that of pH 4.9.

Nevertheless, other species of uranium, e.g., hydroxide complexes, were taken up by beans, and uranium root uptake by bean was not only linked with free ion uranyl but also with other species of uranium in solution, and so with total uranium in solution.

Results of Vandenhove *et al.* (2007b) confirmed that the uranyl cation, uranyl carbonate complexes, and $UO_2PO_4^-$ complex are most of readily uranium forms taken up by ryegrass and may for other high plants too. Also, the plant availability of uranyl carbonate and phosphate complexes, and organic ligand complexes were reported by others (Laroche *et al.*, 2005; Sheppard *et al.*, 1988; Sheppard *et al.*, 2005).

Ebbs *et al.* (1998) reported that due to complexation of the uranium with phosphate, uranium content in roots and shoots of pea in hydroponic solution decreased. This negative effect of phosphate on uranium uptake can be related to the kind of uranyl phosphate complexes. The pH of hydroponic solution, in the experiment of Ebbs *et al.*, was 5 and in this pH major amount of uranyl phosphate complexes are in neutral or positive forms. These results show different availability of various types of uranyl phosphate complexes. According to these reports, it seems only $UO_2PO_4^-$ species of uranyl phosphate complexes can be easily taken up by plants. Of course, differences between various types of plants must be considered and investigated.

2.3.3 The effect of pH

The pH affects uranium bioavailability in the soil and nutrient solutions in different ways. Plant uptake of uranium is affected by soil pH. The most important parameters affected by pH are uranium speciation, soluble uranium concentration, and concentration of competing cations with uranium. Moreover, uranium taken up by plants varies with plant type and species.

Concentrations of Ca^{2+} and Mg^{2+} increase with soil pH and uranium uptake by plants decreases by competition with these cations as well (Mortvedt, 1994). Increasing pH also increases precipitation-sorption reactions that can decrease uranium uptake by plants.

The pH is the most effective parameter on kind of uranium speciation and uranium complexes in solution (Giammar, 2001; EPA, 1999; Ervanne, 2004; Vandenhove, 2002; Ebbs, 1998; Laroche *et al.,* 2005; Hossner *et al.,* 1998). Any change in concentration of uranium in soil or nutrient solution, as well as the kind of uranium complexes and uranium speciation (see previous chapter of this study for more details) can affect uranium uptake by plants.

The effect of pH on uranium content in roots of bean (*Phaseolus vulgaris)* was clearly distinguished in result of Laroche *et al*. (2005). The maximum and the minimum concentration of uranium in roots of bean were found at pH 5.8 and pH 7, respectively. According to that report, the root Transfer Factor (TF) of bean increased with increasing of pH (*Figure 2.5*).

The pH can reverse the effect of phosphate ions on plant uranium uptake. This effect of pH is discussed in more details in the next section (*Section 2.3.4*).

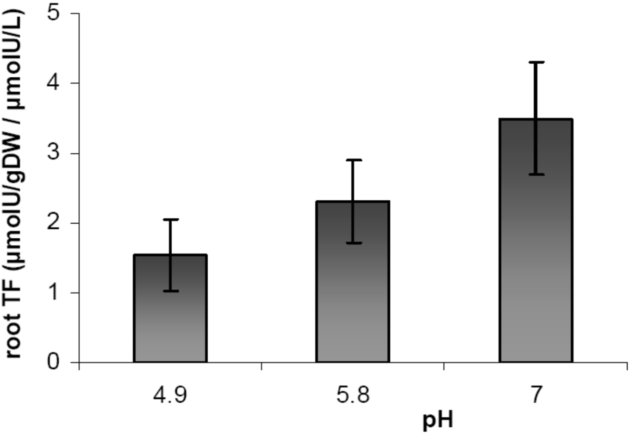

Figure 2.5 Relationships between the mean root Transfer Factor (TF) of uranium in range of uranium concentration in hydroponics solution and pH of solution (in *Phaseolus vulgaris*) (Laroche *et al.*, 2005)

2.3.4 The effect of phosphate

There are contrasting reports on the effect of phosphate in the soil solution on uranium plant uptake. Laroche *et al.* (2005) reported that increasing phosphate concentration, from 0 to 15 µM in nutrient solution in a hydroponics culture, decreased free uranyl concentration about 43%, 68%, and 12% at pH 4.9, 5.8, and 7, respectively. This report showed that despite reduction of free uranyl cation concentration, uranium uptake by bean (*Phaseolus vulgaris*) was not affected by phosphate treatments in any pHs. In addition, Transfer Factor (TF), the ratio between the concentration in plant and the concentration in solution, did not change considerably (*Figure 2.6*). Also, Rivas (2005) reported no significant effect of phosphate on the uranium concentration and uranium uptake of faba bean (*Vicia faba*), and uranium concentration of maize (*Zea mays*) on a contaminated soil.

On the other hand, Ebbs *et al.* (1998) reported that addition of phosphorus had largely overcome the toxic effects of uranium in a hydroponic solution at pH 5.0, most likely due to complexation of the uranium with phosphate. Uranium-phosphate complexation may have reduced the bioavailability of uranium to peas, as uranium concentration of roots and shoots was decreased more than 50% with phosphate treatment in nutrient solution at pH 5. Eapen *et al.* (2003) also reported negative effect of phosphate on uranium concentration and uranium uptake by the *Brassica juncea* hairy root tissues. These hairy root tissues were grown *in vitro* (Murashige and Skoog’s basal medium with 10 µM uranium nitrate and pH of 5.6). Uranium concentration decreased from 291.5 to 181.0 $\mu g\ g^{-1}$ dry weight when the phosphate had been added to the medium in comparison to the medium with no phosphate. They mentioned that total uranium uptake by hairy root tissue of *Brassica juncea* decreased from 97% to 40% of total present uranium in the medium.

Negative effect of phosphate in soil culture on uranium concentration in plant tissues or total plant uranium uptake has been reported by some authors. Adding phosphate fertiliser to soil extremely decreased concentration and total uptake of uranium in ryegrass (*Lolium perenne*), and sunflower (*Helianthus annuus*) in contaminated soils (Lamas, 2005; Rivas, 2005). Also, total uranium uptake by maize was decreased by phosphate fertiliser applying in a contaminated soil (Rivas, 2005). However, increasing effects of phosphate (by applying P-fertiliser) on uranium concentration and uptake by hay on a weakly acidic soil, with a pH of 5.3-6.7, was reported by Ananyan (1991).

Contrasting effects of phosphate on uranium plant uptake can be explained by different types of uranium phosphate complexes that may form depending on soil or solution pH. As mentioned above, phosphate can decrease concentration of free uranyl cation in solution and the type of complexes varies according to kind and amount of electrical charge (see *Figure 2.3*). Then in a pH of higher than 6, the major uranium phosphate complex is $UO_2PO_4^-$ and it can be readily taken up by plants. In the pH range of 4 to 6, and less than 4, the most abundant uranium phosphate complexes are $UO_2HPO_{4(aq)}$ and $UO_2H_2PO_4^+$, respectively, and it seems that they are not available to plants.

About the positive effect of phosphate on uranium uptake by hay, reported by Ananyan (1991), it may be related to micro amounts of soluble uranium complexes which were introduced to the soil as impurities with superphosphate.

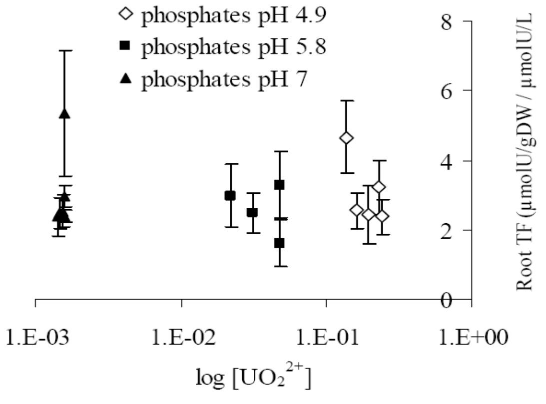

Figure 2.6 Relationships between root Transfer Factor of uranium and UO_2^{2+} content in nutrient solution in presence of phosphate at three pHs (Laroche *et al.*, 2005)

2.3.5 The effect of nutrients

In addition to phosphate, other nutrients were also observed to influence uranium plant uptake. Applying nitrogen fertilisers in soil treated with uranium increased uranium concentration and uranium uptake by sunflowers. It also increased the uranium concentration of maize (Rivas, 2005). Also, a weak positive effect of nitrate on uranium uptake by ryegrass was reported by Vandenhove *et al*. (2006). Positive effects of nitrogen fertilisers on uranium concentration and total uranium uptake by plants are probably related to increasing concentration of mobile uranium in soil solution as soluble uranium nitrate complexes. Ananyan (1991) reported nitrogen treatment decreased uranium concentration of hay in one part of experiments while in the same time, the yield and the total uranium uptake increased about 30% and 27%, respectively; the NP and NPK treatments increased uranium concentration of hay about 34% and 68%, also the yield was increased 232% and 268% (total uranium uptake about 4.5 and 6.3 times more than that of the Control) in the same time.

About the effect of lime on uranium uptake, Ananyan (1991) reported that NPK+$CaCO_3$ treatment, despite increasing yield by 2.7 times, decreased uranium concentration in the hay; in the treatment with a single application of $CaCO_3$ the uranium content of the plants decreased by 37% compared with the Control, and with two applications of $CaCO_3$ the uranium content was within the sensitivity limits of the detection method. The results of Lamas (2005) also showed, with application of calcite, the uranium content of ryegrass (*Lolium perenne*) decreased by 32% to 37% in different levels of uranium application in the soil. The negative effect of calcite and lime on uranium uptake is related to pH increase and its effect on uranium speciation, and competition effect with higher level of available Ca.

The effect of sulphur application has been pointed out in several reports, but K_2SO_4 was used as source of sulphur. Thus, it is not possible to decide whether the observed result is related to sulphur or potassium. Whicker *et al.* (1999) reported that application of K_2SO_4 decreased the content of the radionuclides americium (Am) and curium (Cm) in the bush bean, corn husk, and corn kernel, but uranium, plutonium(Pu), and thorium(Th) content were not affected significantly. Rivas (2005) reported that uranium content and uranium uptake by faba bean (*Vicia faba.*) were decreased by potassium sulphate application, significantly. Also, uranium concentration of maize decreased while a positive effect on the yield was observed. In the case of sunflowers, potassium sulphate application also decreased uranium concentration, but uranium uptake was increased. It seems decrease of uranium concentration was mainly the result of an increase in dry matter.

2.4 Assessing solubility and plant availability of phosphorus in fertilisers

2.4.1 Chemical extractants for assessment of phosphorus availability

One important point about phosphorus sources in agriculture is the plant availability of their phosphorus. The solubility and plant availability of phosphorus in phosphate fertilisers is different and varies considerably from one type of phosphorus-containing fertiliser to another. The recommendation of phosphate fertilisers is strongly dependent on solubility and availability of their phosphorus.

There are different chemical methods to assess and describe solubility and availability of phosphorus, according to the different types of phosphate fertilisers, in the German and European Fertiliser Ordinances (*Table 2.4*). Phosphorus of fertilisers can be found in three portions: soluble in water, soluble in diluted acids or bases, and insoluble/residual phosphorus. Aside from the chemical interactions between phosphate and soil colloids/solution, the water soluble portion and some of soluble phosphate in other chemical extractants can be taken up by plants. The bioavailability of soluble phosphorus portion of various fertilisers in chemical extractants, is strongly dependent on the type of fertilisers, soil chemical properties, and the type of plants.

Until now, several different chemical extractants have been applied for evaluation of the phosphorus solubility in fertilisers. There are many chemical solutions used by various countries for assessing the plant availability of phosphorus in fertilisers. Several countries used water and a single citrate extraction as a measure of phosphorus solubility in all types of phosphate fertilisers (Deeley *et al*., 1987). The European Union Fertiliser Ordinance 2003 listed eight chemical measures of the solubility for various phosphate fertiliser types. In the official handbook of the VDLUFA, a German handbook for the chemical analysis of fertilisers, several chemical solutions are listed, including concentrated sulphuric acid, mixed concentrated sulphuric acid and nitric acid with copper sulphate, mixed concentrated sulphuric acid with copper sulphate, *aqua regia*, 2% formic acid, 2% citric acid, neutral ammonium citrate, alkaline ammonium citrate, warm water, and water in the room temperature (Ostmann, 1995).

Table 2.4 Chemical extraction methods for assessing phosphorus solubility in the various types of phosphorus-fertilisers (Kratz and Schnug, 2009)

Method	**The types of fertilisers (according to the VDLUFA or EU fertiliser ordinance)**
Water	All types of fertilisers
Alkaline ammonium citrate at 65 °C (Petermann)	Dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$)
Alkaline ammonium citrate at 20 °C (Petermann)	Mixtures of $CaNaPO_4$ and Ca_2SiO_4 (VDLUFA: also mineral fertilizers, Al-Ca-phosphate, and special fertilisers suspensions
Alkaline ammonium citrate at 20 °C (Joulie)	Al-Ca-phosphate
Neutral ammonium citrate	Superphosphates (SSP), Triple superphosphates (TSP)
Water and neutral ammonium citrate (Fresenius-Neubauer)	Superphosphate, mineral fertilisers
2% citric acid	Thomas phosphate or other fertilisers manufactured from it
2% citric acid and alkaline ammonium citrate (Petermann)	Mixtures of phosphatic basic slag and DCP and/or MCP
2% formic acid	Partially acidulated and soft ground phosphate rock (PR) and their mixtures
Mineral acids:	
H_2SO_4	Fertilisers with no or only very small amounts of organic material
A mixture of HNO_3 and H_2SO_4 (VDLUFA: with copper sulphate)	Unity method according to EU-VO, VDLUFA: for fertilisers with larger proportions of organic material
H_2SO_4 and copper sulphate	Fertilisers with larger proportions of organic material, but without nitrates
Dry ashing with calcium carbonate, dissolving with HNO_3	Fertilisers with a higher content of organic material

The most important factor for selecting an extractant is the type of phosphorus fertiliser. The mineral acids are usually used for assessing the total amounts of phosphorus contents. On the other hand, water, organic acids, and other organic compound solutions are used to evaluate plant available phosphorus of fertilisers. There was a significant relationship between solubility of phosphorus in water and neutral ammonium citrate, with plant availability of phosphorus in mono ammonium phosphate and triple superphosphate fertilisers when the principle constituents of the citrate insoluble fraction were calcium phosphates. The results of Sikora and Mullins (1995) showed that the neutral ammonium citrate method (The Association of Official Analytical Chemists, AOAC, method) also gave correct predictions of phosphorus availability when the insoluble fraction of phosphorus is Fe, Al, or Mg phosphate compounds. This method is also used in New Zealand to estimate the proportion of phosphate rock present.

Based on a world wide literature review, Kratz and Schnug (2009) classified the plant availability of different phosphate compounds as follows:

-Monocalcium phosphate (MCP), complete and immediate plant availability;

-Dicalcium phosphate (DCP), good plant availability but slow acting;

-Ammonium phosphates (MAP, DAP), complete and immediate plant availability;

-Siliceous phosphates (also Ca-Na-silico-carnotite), good plant availabability;

-Fe/Al-phosphates (i.e. $AlNH_4PO_4F_2$, $FeNH_4(HPO_4)_2$, and $MgAl(NH_4)_2H(PO_4)_2F_2$ in MAP and $Fe_3(K,Na,H)H_8(PO_4)_6.6H_2O$ and CaAlH $(HPO_4)_2F_2.2H_2O$ in SSP), variable plant availability depending on the type of their chemical bands as well as their degrees of crystallization (amorphous compound of Fe and Al phosphates are more soluble than their crystalline forms) and the pH of soil; impurities of MAPs are evidently less available than those of MCPs, but more readily available and easily soluble than those of DCP, variscite ($AlPO_4.2H_2O$) and strengite ($FePO_4.2H_2O$);

-Apatites, low plant availability; phosphorus plant availability of apatites depends on the free carbonate in them, degree of crystallinity, and grain size in the ground powder (see the review paper of Kratz and Schnug, 2009, for more details).

Each chemical extractant can dissolve different form of phosphorus from phosphorus-containing fertilisers; according to a worldwide literature survey, the solubility of various phosphorus forms in different extractants has been reported by Kratz and Schnug (2009) (*Table 2.5).*

Table 2.5 Soluble phosphorus form in different chemical extractants according to literature (from Kratz and Schnug, 2009)

Extractant	**Extracted kind/form of phosphorus**	**Reference**
Water (W)	Monocalcium phosphate (MCP) $Ca(H_2PO_4)_2.H_2O$, ammonium phosphate	Scheel (1968), Braithwaite (1987), Hignett and Brabson (1961)
Alkaline ammonium citrate (AAC)	Dicalcium phosphate (DCP) $CaHPO_4$ or "not soluble in water, but plant-available P fraction ", i.e. bio-available Fe-Al-phosphates	Hignett and Brabson (1961), Werner (1967), Junge and Werner (1989)
Neutral ammonium citrate (NAC) or sequential extraction by water and Neutral ammonium citrate (W+NAC)	MCP, DCP, about 80% of Al-/Fe-phosphate, basic calcium phosphate (hydroxyapatite / tricalcium phosphate), unresolved PR residues or PR reversed by ammonization;	Braithwaite (1987), Hammond *et al*. (1989), Hignett and Brabson (1961), Schmitt (1969)
	Apatites depending on carbonate substitution in their crystals or the proportion of free carbonates in PR	Chien and Hammond (1978), Leon *et al.* (1986)
Citric acid	MCP, DCP, about 20% of Al-/Fe-phosphates, about 20% of unresolved residues of PR in partially acidulated PRs;	Braithwaite (1987)
	P-siliceous compounds, e.g. lime-silica phosphate (silicocarnotite, $5CaO.P_2O_5.SiO_2$)	Maercker (1895), Martens (1943), Gericke (1952, 1968)
Formic acid	Apatites depending on carbonate substitution in their crystals or the proportion of free carbonates in PR	Chien and Hammond (1978), Leon *et al.* (1986)
Mineral acids	Total phosphate	Finck (1992)

Extraction time has a positive effect on the dissolution amounts of phosphorus in all fertiliser types. Dissolution of ground phosphate rock is reduced in citrate extractants as the pH of solutions increases in citric acid, neutral ammonium citric, and ammonia solution ammonium citrate (Deeley *et al.*, 1987).

The results from the literature reveal that none of chemical extraction methods can assess the agronomic effectiveness of phosphorus-containing fertilisers in a wide range of growth environments. Also, physical characteristics of fertilisers, e.g., granule size, placement of the fertiliser, and physical conditions during fertiliser manufacturing, e.g., time and degree of dryness and cooling, may greatly affect crop response (Terman *et al.*, 1964; Archer and Thomas, 1956). In addition, duration of the growing season and residual effects of phosphorus-fertilisers in next growth seasons can affect real bioavailability of phosphorus. On the other hand, most of the chemical extraction methods for assessing phosphorus bioavailability are generally developed on empirical bases, and some factors, such as sample weight, time of digestion, fineness of sample, and agitation, can affect the relative phosphorus solubility of fertilisers. Only some of these factors can be standardised (Braithwaite, 1987; Mackay *et al.*, 1990; Deeley *et al.*, 1987; Kratz and Schnug, 2009). The origin of phosphate rocks used as a direct phosphorus source application or as original source of phosphorus for manufacturing fertilisers may affect the relations between the results of one extraction method and plant response because of some differences in type and amounts of impurities. There are contradictory reports on the suitability of chemical extractants for assessment of phosphorus bioavailability that confirm undeniable effects of mentioned factors on the crop response to phosphorus supplied by fertilisers. Thus, none of laboratory methods based on chemical extractants may give results on bioavailability of phosphorus in agreement with the crop response in a wide range of plant, soil, and environment conditions.

The sequential extraction method with water and neutral ammonium citrate is an evident example in case of variability of a correlation between a soluble proportion of phosphorus in an extractant and the agronomical effectiveness of fertilisers. There are a large number of reports that revealed that the sum of water soluble and ammonium citrate soluble phosphorus is suitable for an assessment of phosphorus availability in various superphosphates and ammonium phosphate fertilisers, while there are other reports that disclosed contradictory results. For instance, Brabson and Burch (1964) reported that neutral ammonium citrate is such a powerful extractant that it dissolves most calcium phosphates that occur in fertilisers, including precipitated apatites, regardless of their agronomic effectiveness. But they found a good correlation between agronomic response and phosphorus solubility in water plus in alkaline ammonium citrate in ammoniated super phosphate fertilisers. The results of Mullins *et al*. (1990) revealed also an overestimation of available phosphorus using the neutral ammonium citrate assessment method, in five triple superphosphate fertilisers' residues (*water-insoluble* fractions). The same drawbacks are found for other chemical extractants and phosphorus-containing fertilisers as well.

2.4.2 Assessment of heavy metal bioavailability

Solubility of heavy metals in soil is one of the main parameters affecting their transferability to plants. The bioavailable fraction of heavy metals in soil mainly consists of their soluble, exchangeable and complex forms. Thus, a chemical extracting solution should be sufficiently acidic to solubilise a solid phase fraction which contributed to plant uptake, contain a displacing ion in order to exchange a fraction of adsorbed ions, and have the property to extract soluble organo-mineral complexes (Kiekens and Cottenie, 1985). Evaluation of heavy metals in one step extraction is named single extraction, and it reflects the elements' situation at the given moment and does not assess their effects over a longer lapse of time.

Until now, numerous chemical extractants have been employed to assess bioavailability of a number of heavy metals or one single heavy metal. According the world wide literature some of these extractants are as follows:

-Water
-0.1 M and 0.01 M $CaCl_2$
-0.1 M $MgCl_2$
-2 M KCl
-1 M NH_4Cl
-1 M NH_4NO_3
-1 M KNO_3
-0.1 M $NaNO_3$
-0.1 M $Ca(NO_3)_2$
-0.03-1 M $NaHCO_3$
-0.1 M HCl
-2.5% AAc
-1 M NH_4OAc, pH = 7 and 4.8
-$(NH_4)_2C_2O_4$
-0.05 M EDTA
-0.05 M NH_4-EDTA, pH = 7
-0.05 M Na_2-EDTA, pH = 7

- 1 M NH_4NO_3+ 0.025 M Na_2-EDTA
- 1 M $CaCl_2$ + 0.025 M Na_2-EDTA
- 0.005 M DTPA
- 0.005 M DTPA + 0.1 M TEA, pH = 5.3
- 0.5 M NH_4OAc + 0.02 M EDTA, pH = 4.65
- NaOH

Beside the single extractions, sequential extraction schemes represent different fractions of heavy metals in soil for better understanding their distribution over different forms including soluble, exchangeable, organic, adsorbed, and precipitated (may in different subdivisions) forms. Filgueiras *et al.* (2002) classified the heavy metal proportions in soil into 6 main fractions as: water-soluble fraction, exchangeable fraction, acid soluble fraction, reducible fraction, oxidisable fraction, and residuals. Many types of chemical solutions have been applied in various sequential extraction procedures by researchers around the world during the last four decades. Some of these sequential extraction schemes are presented in *Table 2.6*.

As was shown for heavy metals, various single and sequential extraction methods have been used for the description of uranium status and uranium bioavailability in soils, sediments, or other environmental samples, too. Anyhow, the decision to select one or some of these chemical solutions for single extracting or sequential extraction methods is arbitrary, and the suitability of these extraction procedures for assessment of uranium or other heavy metals bioavailability relies on empirically found correlations only.

Table 2.6 Some representative sequential extraction schemes used between 1973 and 2000 (Filgueiras *et al*., 2002)

Scheme[b]	**Stage**[a]						
	A	**B**	**C**	**D**	**E**	**F**	**G**
MacLaren and Crawford (1973)	$CaCl_2$	HOAc	—	$K_4P_2O_7$	NH_4Ox/HOx	—	DCB
Gibbs (1977)	$MgCl_2$	—	—	NaOCl/DCB^C	—	—	DCB^B
Engler *et al*. (1977)	NH_4OAc	—	$NH_2OH.HCl$	—	—	H_2O_2/NH_4OAc	DCB
Tessier et al. (1979)	$MgCl_2$	NaOAc	—	—	$NH_2OH.HCl/$ HOAc	H_2O_2/NH_4OAc	—
Meguellati *et al*. (1983)	$BaCl_2$	NaOAc^C	—	—	$NH_2OH.HCl/$ HOAc^D	H_2O_2/NH_4OAc^B	—
Shuman (1983)	$Mg(NO_3)_2$	—	$NH_2OH.HCl^C$	NaOCl^B	NH_4Ox/HOx	—	—
Salomons and Förtsner (1984)	NH_4OAc	NaOAc	$NH_2OH.HCl$	—	NH_4Ox/HOx	H_2O_2/NH_4OAc	—
Miller *et al*. (1986)	$Ca(NO_3)_2/$ $Pb(NO_3)_2$	HOAc/ $Ca(NO_3)_2$	$NH_2OH.HCl$	$K_4P_2O_7$	NH4Ox/HOx	—	NH4Ox/ HOx
Elliot *et al*. (1990)	$MgCl_2$	NaOAc	—	$Na_4P_2O_7^D$	NH_4Ox/HOx^C	—	—
Ure *et al*. (BCR) (1993)	—	HOAc	$NH_2OH.HCl$	—	—	H_2O_2/NH_4OAc	—
Krishnamurti *et al*. (1995)	$Mg(NO_3)_2$	NaOAc	$NH_2OH.HCl^D$	$Na_4P_2O_7^C$	NH_4Ox^F	$H_2O_2/Mg(NO_3)_2^E$	$NH_4Ox/$ AA
Campanella *et al*. (1995)	—	NH_4OAc	$NH_2OH.HCl/$ HOAc	—	—	HCl^C/NaOH^D/ HNO_3^E	—
Sahuquillo *et al*. (1999) (Modified BCR)[c]	—	HOAc	$NH_2OH.HCl^c$	—	—	H_2O_2/NH_4OAc	—

[a] When the order of attack differs from that shown in the table, this is indicated by the superscript. Phases: A, exchangeable; B, acid soluble; C, easily reducible (i.e. Mn oxides); D, easily oxidisable (i.e. humic and fulvic acids); E, moderately reducible (i.e. amorphous Fe oxides); F, oxidisable oxides anf sulfides; G, poorly-reducible (i.e. crystalline Fe-oxides). The residual fraction is not included in the table. [b] See the list of abbreviation. [c] This scheme differs mainly from that of Ure *et al*. in the hydroxylamine hydrochloride concentration (0.5 instead of 0.1 mol l^{-1} and the pH of this extractant (1.5 instead of 2).

The comparison between extraction methods for assessing phosphorus availability of phosphorus-containing fertilisers and bioavailability of heavy metals in soil or other components shows that both assessing method groups are based on empirical results and an arbitrary selection of solutions. Therefore in this research we tested some of the chemical extractants used for phosphorus solubility in various types of phosphorus-containing fertilisers to investigate their capability for assessing uranium transferability to plants. With regard to complexity of extraction methods, finding a common chemical extractant for both elements is very helpful to decrease the necessary time for analyses and the costs.

2.5 *The aims of this study*

As it was reviewed, according to the literature, uranium concentration in contaminated soil or other media in a considerable concentration level higher than natural soil affects plant uranium concentration and uptake, and also it has been confirmed that uranium can be loaded to the soil with phosphorus-fertilisers applied in different amounts, but it is not clear how much of uranium loaded onto soil can transfer to plant tissues. It has repeatedly been reported that uranium concentration in the roots of plants is considerably higher than in their shoots, and it seems that an investigation of root vegetables is necessary if fertiliser-derived uranium could influence uranium taken up by crops.

Besides the total amount of uranium in phosphorus-fertilisers, solubility of uranium present is an important factor affecting the uranium transferability to plants. There are different chemical extractants for assessing phosphorus solubility of various fertiliser types and heavy metal bioavailability. Finding a common extractant for assessing phosphorus solubility and uranium bioavailability is very important to decrease laboratory analyses and costs. With regard to all these, this research focused on the following objectives:

- Investigating the uranium solubility of different phosphorus sources and comparing it with solubility of phosphorus in conventional and standard extractants which are used to assess phosphorus solubility of phosphorus-containing fertilisers,
- Investigating the effect of different U-containing P-fertilisers on uranium concentration in shoots and roots of different types of plants (monocotyledon: maize, dicotyledon: sunflower, root crop: carrot) and uranium transferability to them,
- Investigating relations between chemical extractability and plant uptake of uranium in different U-containing P-fertilisers, and finally, finding a common extractant for assessing them in different P-fertilisers.

3 Material and Methods

3.1 Solubility of P and U in P sources

This experiment was conducted to study solubility of phosphorus and uranium of various phosphorus sources in different chemical extractants. The results of this experiment lead us to an opinion on uranium solubility of various phosphorus sources and their relations to phosphorus solubility with regard to the kind of extractants. The first step for studying uranium transferability to plants was to investigate uranium solubility in fertilisers that was followed by pot experiments as the second step.

3.1.1 Characterization and type of phosphorus-containing fertilisers

Different types of phosphorus-containing fertilisers including straight phosphorus-fertilisers (SPF), compound fertilisers (CF), organo-mineral fertilisers (OMF) and phosphate rocks (PR) were selected from the fertiliser sample collection of Julius Kühn-Institut (JKI) (*Table 3.1*).

Table 3.1 General characteristics of fertilisers and phosphate rocks used in the present study

No.	Name of sample (in collection samples of JKI)	Type of nutrient	Type of samples	Description/Origin
1	MD1	P	SPF	TSP
2	MD2	P	SPF	TSP
3	MD3	P	SPF	DCP
4	SP18	P	SPF	SP
5	MD4	P	PR	USA, Florida
6	MD15	NPK(Mg)	CF	12-12-17(2)
7	MD16	NPK(Mg)	OMF	5-4-5(3)
8	MD17	NPK(Mg)	OMF	9-5-13(4)
9	MD18	NPK(Mg)	OMF	8-7-12(2)
10	MD19	NPK(Mg)	OMF	8-8-10(2)
11	MD27	NPK	CF	18-6-12
12	MD28	NP	CF	

Table 3.1 continued

No.	Name of sample (in collection samples of JKI)	Type of nutrient	Type of samples	Description/Origin
13	MD32	NPK	CF	
14	MD33	NPK	CF	
15	MD35	P	PAPR	Partially acidulated PR
16	MD36	NPK(S)	CF	
17	MD38	PK	CF	
18	MD37	P	PAPR	Partially acidulated PR
19	MD39	NPK	CF	
20	MD40	PK	CF	
21	MD42	NPK	CF	13-9-16
22	MD43	PK	CF	12-24
23	MD67	NPK(Mg)	CF	12-12-17(2) with B and Zn
24	MD75	NPK(Mg+S)	OMF	10-5-7(2-5)
25	PR5	P	PR	Algeria
26	PR11	P	PR	Israel
27	PR12	P	PR	Morocco, Khoribga
28	PR14	P	PR	Morocco, Khoribga
29	PR17	P	PR	Senegal, Taiba
30	PR19	P	PR	Israel
31	PR52	P	PR	Morocco, Khoribga
32	PR54	P	PR	USA
33	PR56	P	PR	Israel
34	PR62	P	PR	Russia, Kola
35	PR68	P	PR	Morocco
36	PR84	P	PR	Togo
37	PR93	P	PR	Morocco, Khoribga

3.1.2 Preparation of fertiliser and phosphate rock samples

All fertiliser samples were oven-dried at 45ºC for 24 h, and then ground with a vibrating disc mill (Retsch RS100) with a zirconia grinding set. The ground samples were kept in polyethylene bottles for chemical analyses. All ground samples were oven-dried before weighing for extraction via different methods.

3.1.3 Extraction methods for assessing phosphorus and uranium solubility

So-called total phosphorus and uranium contents in all samples were determined in *aqua regia* digests. In addition to *aqua regia*, several commonly used extractants to assess phosphorus solubility of fertilisers according to the German and European Fertiliser Ordinances, including 2% formic acid (FA), 2% citric acid (CA), water and neutral ammonium citrate (NAC), and alkaline ammonium citrate (AAC), were selected for assessing the solubility of uranium and phosphorus in samples (*Table 3.2*). In all extraction and dilution steps, twofold deionized water was used.

Table 3.2 Comparing digestion and extraction methods for assessing phosphorus and uranium solubility

Extractant	pH	Temperature (*°C*)	Sample/solution ratio (*g ml*$^{-1}$)	Reference
Aqua regia	Strongly acidic	103	5/100	AbfKlaerV (1992)
Water	5.5-6.5	20	1/250	Ostmann (1995)
Neutral ammonium citrate	7	40	1/250	Ostmann (1995)
Alkaline ammonium citrate	9.5	20	2.5/250	Ostmann (1995)
2% citric acid	2.1	20	2.5/250	Ostmann (1995)
2% formic acid	1.9	20	2.5/250	Ostmann (1995)

3.1.4 Extraction of available amounts of macronutrients in fertiliser samples

Total amount of nitrogen (N) was measured by Elementar vario MAX CNS (Manual method), and was considered as available amount of nitrogen in different fertilisers. Plant available amounts of potassium (K) were extracted according to the German fertiliser analysing methods (Moosmüller, 1995). Available amounts of calcium (Ca), magnesium (Mg), and sulphur (S) in fertilisers and phosphate rocks were extracted using hot water extraction method (Schwarzer and Runge, 1995). These elements were analysed according to conventional methods.

3.1.5 Analytical methods

Phosphorus in extractants was measured by colorimetric determination using a Perkin-Elmer 550SE UV/VIS spectrophotometer at 882 nm (John, 1970). In this study uranium was analyzed by means of Inductively Coupled Plasma-Quadrupole Mass Spectroscopy (ICP-QMS), employing a VG Elemental Plasma Quad 3 (Thermo Elemental, United Kingdom) (Sparovek *et al.*, 2001; Lamas *et al.*, 2002).

All organic compounds in extractions and samples must be removed before every ICP-QMS analysing. For removing organic matter at first 40±0.05 *ml* of each extraction sample was transferred to a clean crucible and dried in a sand-bath at 170 *°C*. After drying extractions, the crucibles moved to an electrical furnace and were heated for 4 hours at 490 *°C* to ash sample residuals. The crucibles were cooled and the ashes were dissolved in 10 *ml* nitric acid 10% during 2 hours, 30 *ml* deionised water was added, and the digested solutions were filtrated to polyethylene bottles with folded filter paper (S&S 593 1/2).

The standard solutions of uranium for ICP-MS measuring were prepared in concentrations of 0.010, 0.025, 0.050, 0.100, 0.150, 0.200, 0.250 $\mu g\ l^{-1}$ using the *ICP multi-element standard solution VI for ICP-MS* (a product of MERCK Company, containing 10 $mg\ l^{-1}$ U) and 2% nitric acid as dilutor in the all dilution steps. The limitation detect of apparatus was 0.01 $\mu g\ l^{-1}$ for uranium. The standardising of the apparatus was done two times per day, i.e. at the start and end of analysing. Also the qualification test was done according to the quarterly report of ISE (International Soil-Analytical Exchange) three times per day regularly. Ashed and solubilised extraction samples were diluted using deionised water from 50 to 200 times depending on the type of fertilisers and extraction methods before analysing by ICP-QMS.

3.2 Pot experiments

Pot experiments were conducted to study phosphorus and uranium transferability from the different phosphorus sources to plants and to assess the suitability of extractants for predicting the plant available portion of these elements. The present study was conducted as a “case study” with a limited number of samples to identify some trends and patterns; however, it was not within the scope of this work to include a sufficient number of samples for a thorough statistical evaluation. To this end, a two-step experimental design was planned.

The pot experiments were done following two different methods including the Neubauer method and an experiment in large pots (Kick-Brauckmann) in both steps. Every pot experiment was conducted in a factorial experiment design with two or three factors including phosphorus-containing fertilisers, kind/variety of plants, and substrates (*Table 3.3*). Four replicates were considered for each phosphorus treatment. The total phosphorus amount application were equal in all pots in each experiment and quantity of applied phosphorus was not the factor rather, the total amount of uranium loaded to substrates and its solubility with regard to the types of fertilisers was real independent factor.

Table 3.3 Experimental design of the pot experiments

Experimental design	Substrate	Test plant	Fertiliser treatment	Replication	Duration (days)	Time
Step 1: Pilot Neubauer experiment	Sand	Maize (*Zea mays L.*) & Sunflower (*Helianthus annuus L.*)	Blank	4	22	Jun. & Jul., 2007
			KH_2PO_4	4	22	
			MD37 (PAPR)	4	22	
			SP18 (SPF; SSP)	4	22	
Step 1: Pilot Kick-Brauckmann experiment	Sand & Soil	Carrot (*Daucus carota* cv. Napoli)	KH_2PO_4	4, 4	98, 99	May-Aug. 2007
			MD37 (PAPR)	4, 3	98, 99	
			SP18 (SPF; SSP)	4, 4	98, 99	
		Carrot (*Daucus carota* cv. Kazan)	KH_2PO_4	4, 3	114	
			MD37 (PAPR)	4, 4	114	
			SP18 (SPF; SSP)	4, 4	114	
Step 2: Main Neubauer experiment	Sand & Mixed soil/sand	Maize (*Zea mays L.*) & Sunflower (*Helianthus annuus L.*)	Blank	4	28	May 2008
			KH_2PO_4	4	28	
			SP18 (SPF; SSP)	4	28	
			MD2 (SPF; TSP)	4	28	
			MD19 (OMF; NPK+Mg)	4	28	
			MD28 (CF; NP)	4	28	
			MD37 (PAPR)	4	28	
			MD38 (CF; PK)	4	28	
Step 2: Main Kick-Brauckmann experiment	Sand	Carrot (*Daucus carota* cv. Napoli)	Blank	3	133	May- Sep. 2008
			KH_2PO_4	3	133	
			SP18 (SPF; SSP)	4	133	
			MD1 (SPF; TSP)	3	133	
			MD2 (SPF; TSP)	4	133	
			MD19 (OMF; NPK+Mg)	2	133	
			MD28 (CF; NP)	2	133	
			MD35 (PAPR)	3	133	
			MD37 (PAPR)	4	133	
	Mixed soil/sand	Carrot (*Daucus carota* cv. Napoli)	Blank	4	118	May-Aug. 2008
			KH_2PO_4	4	118	
			SP18 (SPF; SSP)	4	118	
			MD1 (SPF; TSP)	4	118	
			MD2 (SPF; TSP)	4	118	
			MD19 (OMF; NPK+Mg)	4	118	
			MD28 (CF; NP)	3	118	
			MD35 (PAPR)	4	118	
			MD37 (PAPR)	3	118	

Both pilot experiments were conducted using two phosphorus-containing fertilisers plus one Control using reagent grade KH_2PO_4. In the pilot Neubauer pot experiment a Blank treatment also was added to phosphorus source treatments. The scope of the present research work only allowed for a kind of case study, which could identify possible trends regarding the behaviour of different types of fertilisers. Based on these trends, future experiments can be planned. With regard to this statement, several phosphorus sources from various types of phosphorus-containing fertilisers were used in the main experiments (*Table 2.3*).

Test plants in Neubauer experiments were one monocotyle crop (maize) and one dicotyle crop (sunflower). These two plants were selected with regard to high demand phosphorus and it was expected that they will respond well to the supplied phosphorus fertilisers and potentially take up uranium loads with the fertilisers. The carrot as a root harvest crop was selected to be able to study uranium accumulation in an edible root crop.

In soil substrates chemical interactions between nutrients/heavy metals and soil mineral and organic colloids can affect their transferability to plants, and the behaviour of elements in soil is different from in sand substrate. With regard to these interactions, pure sand and mixed soil sand substrate were applied in both main experiments to compare the effect of substrate on uranium transferring to plants and also to have the possibility to study maximum transferability of uranium to plants in sand substrate. More details of these experiments are mentioned in the following sections.

3.2.1 Pilot pot experiments

3.2.1.1 Pilot Neubauer pot experiment

In the pilot Neubauer pot experiment, the suitability of this method for studying the availability and transferability of uranium and phosphorus was evaluated by applying only two phosphorus sources (a phosphate rock and a superphosphate fertiliser) with different solubility. Another aim of this pilot experiment was to evaluate maize and sunflower growth conditions and their suitability for this test.

- P treatments:

Two phosphorus-containing fertilisers (a phosphate rock and a straight phosphorus-fertiliser), and a Control using purified and soluble chemical compound (reagent grade KH_2PO_4) were selected as treatments. In every treatment 80 *mg* of phosphorus was added to each pot (*Table 3.4*). In addition, a Blank was prepared without any phosphorus addition.

Table 3.4 P treatments and applied quantities in the pilot Neubauer pot experiment

Treatment	**Total P content of fertiliser (*mg g^{-1}*)**	**Total U content of fertiliser (*mg kg^{-1}*)**	**U/P ratio in fertiliser (mg g^{-1})**	**Amount of fertiliser added per pot (*g*)**	**mg U per pot**
MD37	119.4	72.8	0.61	0.670	0.049
SP18	106.7	80.0	0.75	0.750	0.060
KH_2PO_4	227.9	0	0	0.352	0
Blank	0	0	0	0	0

- Plants:

Two kinds of plants, sunflower (*Helianthus annuus L.*) and maize (Z*ea mays L.*), were selected for Neubauer pot experiments. The number of seeds for sunflower pots and maize pots were 100 and 90, respectively.

- Substrates and preparation of pots:

The substrate used in the pilot Neubauer pot experiment was sand. Some characteristics of the sand used in this experiment are shown in *Table 3.5*.

The sand was washed with distilled water, dried at 150°C to sterilize, and sieved to 1 mm. A plastic tube, 12 mm in diameter and shorter than the internal height of pot, was settled vertically in the middle of the pot for aeration, watering, and nutrient solution supply (*Figure 3.1*). 400 *g* sand was homogeneously mixed with the phosphorus source and filled into the pot. The grains were picked up with tweezers and stuck into the sand with their pointed end (carrying the germ bud) downwards, their upper end was at level with the sand surface. Finally, the seeds were covered by 50 *g* washed and sieved (to 0.5 mm) sand.

Table 3.5 Some chemical and physical characteristics of the sand substrate used in the pilot Neubauer pot experiments

Parameter	Sand substrate	Method/Reference
pH	6.07	0.01 $CaCl_2$ (VDLUFA-Method, Hoffmann, 1991)
$\theta_{m, F.C}$ %	14	Stöven (1999)
N %	0.012	Manual method of Elementar, vario MAX CNS
O.M %	0.49	Manual method of Elementar, vario MAX CNS
K_{total} ($mg\ kg^{-1}$)	349	*Aqua regia* (AbfKlaerV, 1992)
$K_{ava.}$ ($mg\ kg^{-1}$)	0.0	Calcium-Acetat-Lactate, CAL (Schueller, 1969)
P_{total} ($mg\ kg^{-1}$)	102	*Aqua regia* (AbfKlaerV, 1992)
$P_{ava.}$ ($mg\ kg^{-1}$)	3.5	Calcium-Acetat-Lactate, CAL (Schueller, 1969)
U_{total} ($mg\ kg^{-1}$)	0.201	*Aqua regia* (AbfKlaerV, 1992)
$U_{ava.}$ ($mg\ kg^{-1}$)	0.030	AAAc-EDTA (Lakanen and Erviö, 1971/Sillanpää, 1990)

Figure 3.1 Pots of Neubauer experiment with distributed maize and sunflower seeds before covering by sands

- *Fertilisation:*

All the pots were fertilised with nitrogen (N), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), and iron (Fe) using nutrient solution three times per growth period (see *Table 3.6*). The concentrations of nutrient solutions were adjusted based on nutrient concentration of sunflower and maize seedlings reported by Reuter and Robinson (1997) and expected dry matter yield (*Table 3.6* and *Table 3.7*). Nutrient solutions were added to the pots using the settled glass tube in substrate (*Figure 3.2*).

- *Irrigation:*

The Neubauer pots were watered, using the plastic tube, up to 70% of field capacity by twofold deionized water after seeding. Water amount for first irrigation was 60 ml per pot. In the first week, the pots were irrigated 1 to 3 times per day, 10 to 15 ml each time, by twofold deionized water according to the lost water via evaporation, which was monitored by a scale. After growing seedlings during the second and third weeks, the amount of water was gradually increased per each irrigation turns. Also, in weight monitoring of water amount by scale, the basic weight of each pot was gradually increased from 600 *g* and 620 *g* for sunflower and maize pots to 690 *g* and 750 *g* during growing period, respectively.

Table 3.6 Composition, concentration, and timing of nutrient additions to the pilot Neubauer seedlings experiment (22 growing days)

Nutrient compound	**Concentration of compound** **($g\ l^{-1}$)**	**Volume supplied at each addition** **(*ml*)**	**Application time** **(*days after sowing*)**
NH_4NO_3	17.15	2.5	6, 13, 18
KNO_3	41.38	2.5	6, 13, 18
$Ca(NO_3)_2.4H_2O$	117.87	2.5	6, 13, 18
$Mg(NO_3)_2.6H_2O$	20.29	2.5	6, 13, 18
$MgSO_4.7H_2O$	18.45	2.5	6, 13, 18
Fe-EDTA	1.58	2.5	6, 13, 18

Table 3.7 Total amount of nutrients added to the pilot Neubauer seedlings experiment pots

Nutrient	**N**	**K**	**Ca**	**Mg**	**S**	**Fe**
Total amount applied (*mg* pot^{-1})	225	158	150	28.6	18.0	1.80

Figure 3.2 Adding nutrient solution and watering Neubauer pot experiments using a pipette (Eppendorf Multipipette Plus) via a settled glass tube in substrate

- Harvest and preparation of samples:

Plants were grown for 22 days. In that time, maize seedlings were at BBCH 12/13 (between 2 and 3 leaves), and sunflower plants were mostly at BBCH 12/14 (2 to 4 unfolded leaves) according to the BBCH code (Meier, 2001) (*Figure 3.3*).

Figure 3.3 Sunflower and maize seedlings at harvest time in Neubauer experiment 2007

At harvest, all seedlings were accurately cut about 3mm above the sand surface and the number of seedlings counted and fresh weight was determined. Shoots were oven-dried at 65 °C until constancy of weight and dry weights were determined. The substrates containing roots, husk of seeds, and non germinated seeds were transferred to clean plates. The roots were extracted from the semi-dried substrates by shaking them on a round plate, in that root particles were gathered on the top surface at the middle of plate. Some broken parts of roots were picked up with a pair of tweezers from the soil/sand substrates. Then, roots were washed with deionized water and dried at 65 °C until constancy of weight. The dry weight of roots was measured. The dry plant material was ground using a vibrating disc mill (Retsch RS100) with a zirconia grinding set and kept in sealed polyethylene containers until chemical analysis.

After separating the roots and remaining seeds/husks, the substrates were dried at room temperature and stored for chemical analysis.

3.2.1.2 Pilot carrot pot experiment

The pilot carrot pot experiment was done to evaluate the suitability of different substrates as well as two different varieties of carrot plants for the investigation. Again, only two phosphorus sources of varying solubility were used in the pilot carrot experiment. This test was conducted according to the following material and methods:

- *P treatments:*

Two phosphorus-containing fertilisers including a phosphate rock (MD37) and a straight phosphorus-fertiliser (SP18), and a Blank without phosphorus application were used as treatments. The fertiliser samples were ground and conditioned at 40°C for 24 hours and were kept in a desiccator before weighing. 1000 *mg* phosphorus was used in MD37 and SP18 treatments (see *Table 3.8*).

- *Test plants:*

Two varieties of carrot, *Daucus carota* L., “Napoli” and “Kazan”, were selected for the pilot carrot pot experiment. Growth period (complete growth duration from sowing till harvest) for Napoli and Kazan varieties is about 3-3.5 and 5 months, respectively. These two varieties were selected as test plants in the pilot experiment to study the probable effect of growth period on plant uranium concentration and uptake. 20 seeds were planted per pot. Thinning was done at BBCH 13/14 (3-4 leaves) according to the BBCH code (Meier, 2001), and only ten stronger seedlings were kept in each pot.

Table 3.8 P treatments in the pilot carrot pot experiment

Treatment	**Total P content of fertiliser** ($mg\ g^{-1}$)	**Total U content of fertiliser** ($mg\ kg^{-1}$)	**U/P ratio** ($mg\ g^{-1}$)	**Amount of fertiliser** ($g\ pot^{-1}$)	**P** ($mg\ pot^{-1}$)	**Fertiliser-derived U** ($mg\ pot^{-1}$)
MD37	119.4	72.8	0.61	8.375	1000	0.61
SP18	106.7	80.0	0.75	9.372	1000	0.75
Control (KH_2PO_4)*	227.9	0	0	4.388	1000	0

***** KH2PO4 was added to the pots of Blank treatment in solution form five weeks after sowing

- *Substrates and preparation of pots:*

For the first carrot pot experiment, soil and sand were used as substrates (some of their characteristics are shown in *Table 3.9*). 10 *kg* air dried soil, sieved to 2 mm, was mixed with the phosphorus-fertiliser and filled into the Kick-Brauckmann pot (*Figure 3.4*). For the sand variant, the sand was washed with deionized water and dried in oven at 105 °C. In order to allow for the same rooting depth in both substrates, 11.5 *kg* of sand were used in each pot (15% more than the soil substrate). 20 carrot seeds were seeded in a depth of about 0.5 cm.

Figure 3.4 Kick-Brauckmann pots applied for carrot pot experiment

Table 3.9 Some chemical and physical characteristics of soil and sand used in the pilot carrot pot experiment

Parameter	**Sand**	**Soil**	**Method/Reference**
pH	5.97	5.94	0.01 $CaCl_2$ (VDLUFA-Method, Hoffmann, 1991)
Clay %	-	9	
Silt %	-	49	
Sand %	-	42	
$\theta_{m, F.C}$ %	15.2	28.5	Stöven (1999)
$CaCO_3$%	n.d.	0.03	
N %	0.006	0.084	Manual method of Elementar, vario MAX CNS
O.M %	0.51	2.04	According to total C and $CaCO_3$%
K_{total} ($mg\ kg^{-1}$)	386	999	*Aqua regia* (AbfKlaerV, 1992)
$K_{ava.}$ ($mg\ kg^{-1}$)	n.d.	202.1	Calcium-Acetat-Lactate, CAL (Schueller, 1969)
P_{total} ($mg\ kg^{-1}$)	136	467	*Aqua regia* (AbfKlaerV, 1992)
$P_{ava.}$ ($mg\ kg^{-1}$)	4.4	50.0	Calcium-Acetat-Lactate, CAL (Schueller, 1969)
U_{total} ($mg\ kg^{-1}$)	0.252	0.659	*Aqua regia* (AbfKlaerV, 1992)
$U_{ava.}$ ($mg\ kg^{-1}$)	0.011	0.010	AAAc-EDTA (Lakanen and Erviö, 1971/Sillanpää, 1990)

-: not analyzed n.d.: not detectable

- Fertilisation:

Macronutrient fertilisation of carrot pots was done based on expected dry matter yield and nutrient concentration in carrot tissues using data of Wonneberg and Cellars (2004). All the pots were fertilised with nutrient solution three times per growth period (see *Table 3.10*). In Blank treatment pots of the sand substrate, phosphorus deficiency strongly affected seedlings growth as carrot seedlings in these pots were obviously smaller than those of Blank treatment pots in the soil substrate five weeks after sowing. Thus, for avoiding the dry matter effect on uranium uptake in the Blank treatment and having nearly the same dry matter for a better comparison of uranium transfer from phosphorus source to plants, 1000 *mg* phosphorus was added to Blank treatments using potassium hydrogen phosphate five weeks after seeding.

Micronutrient requirements were calculated based on the micronutrient concentration of carrots reported by Fleck *et al.* (2001) and expected dry matter yield. Micronutrient fertilisation was done once per growth season (see *Table 3.11*).

Table 3.10 Composition, quantities, and application time of macro-nutrient fertilisers in the pilot carrot pot experiment

Nutrient	**Applied nutrient form**	**Initial amount in MD37** (*g*)	**Initial amount in SP18** (*g*)	**Needed amount per pot**	**Applied in MD37 treatment** (*g pot^{-1}*)	**Applied in SP18 treatment** (*g pot^{-1}*)	**Applied in Blank treatment** (*g pot^{-1}*)	**Application time** (*days after sowing*)
N	KNO_3, $Ca(NO_3)_2.4H_2O$	-	-	1.65	1.65	1.65	1.65	32, 50, 70
P	KH_2PO_4	1.00	1.00	1.00	-	-	1.00	38
K	KNO_3, K_2SO_4, KH_2PO_4	0.21	-	4.42	4.21	4.42	4.42	32, 50, 70
Ca	$Ca(NO_3)_2.4H_2O$	2.91*	2.18	1.33	1.33	-	1.33	32, 50, 70
Mg	$MgSO_4.7H_2O$	0.07*	0.02	0.3	0.3	0.3	0.3	32, 50, 70
S	K_2SO_4, $MgSO_4.7H_2O$	0.06	1.05	1.45	1.4	0.4	1.45	32, 50, 70

* Mostly unavailable to plants

Table 3.11 Composition, amounts, and application time of micronutrients in the pilot carrot pot experiment

Nutrient	**Compound**	**Average concentration in carrot** (*µg g^{-1}*)	**Expected plant uptake** (*mg pot^{-1}*)*	**Applied nutrient amount** (*mg pot^{-1}*)	**Application time** (*days after sowing*)
Zn	$ZnSO_4.H_2O$	20	2.6	3	35
Fe	Fe-EDTA	120	15.6	18	35
Mn	$MnSO_4.H_2O$	20	2.6	3	35
Cu	$CuSO_4.7H_2O$	5	0.7	0.8	35
B	H_3BO_3	20	2.6	3	35
Mo	$(NH_4)_6Mo_7O_{24}.4H_2O$	3	0.4	0.5	35

* Expected plant uptake was calculated according to 130 g dry matter yield per pot plus 15% more.

- *Irrigation:*

After sowing, all pots were irrigated with 200 ml of deionized water per day. It was continued for 10 days. After that, irrigation was done according to daily evapotranspiration to keep soil and sand water content at about 50% to 70% of field capacity. Water content was controlled by weighing some of the pots every week and comparing with the initial weight after the first irrigation and a gradual increasing during the growth season with regard to fresh matter produced.

- *Harvest:*

The carrot cultivars were seeded on April 30, 2007. The Napoli and Kazan cultivars were grown for 14 and 16 weeks and were harvested on August 6 and 7, and August 22, respectively. The pots were watered to about field capacity the night before harvest, the carrots pulled out from the soil and sand substrates, washed with deionized water, and carrot shoots were separated from carrot roots by a pair of scissors. Fresh weight of shoots and roots were determined. The shoots were cut to less than 7 cm sticks, and the carrot roots also were cut to small pieces about 1cm. The shoots and roots were oven-dried at 65 °C until constancy of weight and dry weights were determined. The samples were ground (see *Section 3.2.1.1*) and kept in polyethylene containers for analyzing of elements.

After harvest, the substrates were dried at room temperature and a sample from mixed air dried substrate from each pot was sieved to <2 mm and stored for chemical analyses.

3.2.2 Main pot experiments

Main pot experiments were carried out in the both methods including Neubauer method and Kick-Brauckmann pot experiment method. Some changes in the number of phosphorus treatments, types and preparation of substrates, test plants, and finally pot fertilisation were made based on results of the preliminary pot experiments. The aim of these experiments was to study the effects of various phosphorus sources on uranium concentration and uptake in shoots and roots of carrot, maize, and sunflower plants. The effect of different substrates on uranium concentration and uptake in various types of plants and phosphorus-fertilisers was investigated using sand and mixed soil/sand substrates. Finally, finding a relationship between uranium content or soluble uranium portions of various phosphorus sources and plant uptake was done based on the results of these experiments. Some details on material and methods of these experiments are explained in the following sections, separately for each method.

3.2.2.1 P treatments

Seven and six phosphorus-containing fertilisers were used for carrot pot experiment and Neubauer pot experiment, respectively. These samples were selected from analyzed phosphorus sources for studying uranium and phosphorus solubility based on their types (i.e., completely water soluble phosphorus sources as well as poorly soluble phosphorus sources, pure mineral types as well as an organo-mineral one, pure phosphorus vs. compound (multi-nutrient) fertilisers), phosphorus and uranium contents, uranium-to-phosphorus ratios for considerable amounts of uranium adding onto substrates, and salt effects on seedlings. In both experiments, two treatments with no uranium application were considered, one without any phosphorus application (Blank treatment) and another one with application of a purified chemical phosphorus compound (KH_2PO_4 treatment). The amount of phosphorus in each experiment was the same as primary experiments (see *Section 3.2.1*). Total concentrations of uranium and phosphorus in fertilisers and applied amount of them as the treatments in the Neubauer and carrot pot experiments are presented in *Table 3.12*.

3.2.2.2 Test plants

In the main pot experiments, test plants for Neubauer experiment were the same as the pilot experiment. The number of seeds was reduced to 65 and 60 per pot for maize and sunflower, respectively. In the carrot experiment, only the “Napoli” cultivar was used. Seeding and thinning was done in the same way as in the pilot experiment.

Table 3.12 Type, total P and U content, the ratio of U to P, and available content of macronutrients for plants in P treatments of the main Neubauer and carrot pot experiments

Sample name	**Type of fertiliser** *	**Total P** ** (*g kg^{-1}*)	**Total U** ** (*mg kg^{-1}*)	**U/P ratio** (*mg g^{-1}*)	**Amount of fertiliser in Neubauer exp.** (*g pot^{-1}*)	**Amount of fertiliser in carrot pot exp.** (*g pot^{-1}*)
MD1	SPF (TSP)	185.3	52.3	0.282	ni	5.40
MD2	SPF (TSP)	185.6	160.3	0.864	0.431	5.39
MD19	OMF	30.1	27.6	0.900	2.611	32.64
MD28	CF (NP)	79.1	60.7	0.767	1.012	12.64
MD35	PR (PAPR)	80.4	33.6	0.418	ni	12.44
MD37	PR (PAPR)	119.4	72.8	0.610	0.670	8.37
MD38	CF (PK)	84.4	52.2	0.619	0.948	ni
SP18	SPF (SSP)	106.7	80.0	0.750	0.750	9.37
KH_2PO_4	-	227.6	0	0	0.351	4.39

* : SFP, OMF, CF, and PR are straight phosphorus-fertiliser, organo-mineral fertiliser, compound fertiliser, and phosphate rock, respectively.

** : U and P extracted by *aqua regia* (so-called total)

ni : not included

3.2.2.3 Substrates and preparation of pots

In the main Neubauer pot experiment, two substrates were used, including sand substrate and the standard substrate of Neubauer test (a mixed soil/sand). All sand used in the Neubauer pot experiment was sieved to 1 mm, washed with deionized water, and dried in an oven at 150 °C. Both substrates consisted of three layers as follows:

a) first layer: 250g mixed substrate with the phosphorus-fertiliser (standard substrate: 100g soil, 150g sand, and the phosphorus source; sand substrate: 250g sand mixed with the phosphorus source)

b) second layer: 100g sand added onto the first layer and flattened on the surface

c) third layer: 100g sand used for covering the seeds sown on the surface of second layer.

In the main carrot pot experiment, two substrates were also used, including sand and mixed soil/sand. As sand substrate, 10 *kg* sand washed, dried, and sieved to 2 mm was used in each pot. The mixed soil/sand substrate consisted of 5 *kg* soil (in 5.5% moisture and sieved to 2mm) and 5 *kg* sand (washed, dried, and sieved to 2 mm), which were mixed entirely and homogeneously for each pot. All substrates were mixed as well with phosphorus treatments and were filled into the pots.

The sand and soil used in the present study as substrate in the main pot experiments were analyzed for some chemical and physical parameters (*Table 3.13*). Data in *Table 3.13* show final characteristics of sand substrate after washing and drying in oven.

Table 3.13 Some chemical and physical characteristics of the sand and soil used in the main pot experiments

Parameter	**Neubauer pot experiment**		**Carrot pot experiment**		**Method/Reference**
	Sand	**Soil**	**Sand**	**Mixed soil/sand**	
pH	6.11	6.07	6.02	5.96	0.01 $CaCl_2$ (VDLUFA-Method, Hoffmann, 1991)
Clay %	-	8	-	6	
Silt %	-	52.5	-	24	
Sand %	-	39.5	-	70	
$CaCO_3$%	0	0.07	0	0.04	
$\theta_{m, F.C}$ %	15.5	28.7	14.3	23.9	Stöven (1999)
N %	0	0.066	0	0.040	Manual method of Elementar, vario MAX CNS
O.M %	0.05	1.45	0.08	0.81	According to total C and $CaCO_3$%
K_{total} ($mg\ kg^{-1}$)	390	1104	409	809	*Aqua regia* digestion
$K_{ava.}$ ($mg\ kg^{-1}$)	15.0	121.3	12.7	67.5	Calcium-Acetat-Lactate, CAL (Schueller, 1969)
P_{total} ($mg\ kg^{-1}$)	116	633	129	385	*Aqua regia* (AbfKlaerV, 1992)
$P_{ava.}$ ($mg\ kg^{-1}$)	5.9	54.4	4.8	32.1	Calcium-Acetat-Lactate, CAL (Schueller, 1969)
U_{total} ($mg\ kg^{-1}$)	0.283	0.767	0.288	0.524	*Aqua regia* digestion
$U_{ava.}$ ($mg\ kg^{-1}$)	0.009	0.008	0.010	0.008	AAAc-EDTA (Lakanen and Erviö, 1971/Sillanpää, 1990)

- not analyzed

Figure 3.5 Neubauer pot experiment pots in 2008

3.2.2.4 Fertilisation

- Fertilisation of the main Neubauer pot experiment:

With regard to preliminary Neubauer pot experiment, total amount of N, K, Ca, and Mg were decreased in fertilisation program of the main Neubauer pot experiment. The nutrient compounds, their concentrations in nutrient solutions, application times and total applied amount of nutrients in the main Neubauer pot experiments are shown in *Table 3.14* and *Table 3.15*.

Table 3.14 Composition and timing of nutrient additions to the main Neubauer maize (27 growing days) and sunflower (28 growing days) seedlings

Nutrient compounds	**Compound concentration ($g\ l^{-1}$)**	**Volume supplied at each addition (*ml*)**	**Application time (*days after seeding*)**
NH_4NO_3	17.15	2.5	7, 14, 21
KNO_3	35.18	2.5	7, 14, 21
$Ca(NO_3)_2.4H_2O$	47.15	2.5	7, 14, 21
$MgSO_4.7H_2O$	18.45	2.5	7, 14, 21
Fe-EDTA	1.58	2.5	7, 14, 21

Table 3.15 Total amount of nutrients added to the main Neubauer seedlings experiment pots

Nutrient	**N**	**K**	**Ca**	**Mg**	**S**	**Fe**
Total applied amount (*mg pot*$^{-1}$)	138	102	60.0	13.6	18.0	1.80

- *Fertilisation of the main carrot pot experiment*

In the main carrot pot experiment some changes were made in the micronutrients fertilisation plan based on results of the pilot carrot pot experiment. Total uptake of micronutrients by carrot plants (i.e., Napoli cultivar) in the pilot experiment on soil substrate was at least 2 times more than their total uptake on sand substrate, with regard to this in the main carrot pot experiment the amount of micronutrients was increased about 2-3 times comparing to the pilot carrot pot experiment (*Table 3.16*)

For macronutrients, the important parameters affecting the fertilisation were plant demand and levelling the concentration of available nutrients in all treatments and substrates. As the treatments, various phosphorus sources were applied in different quantities to add the same amounts of total phosphorus in all treatments and substrates. Also, a wide range of available macronutrients content was in phosphorus-containing fertilisers used (see *Table 3.17*). Therefore, macronutrient fertilisers should be added in different quantities to various treatments in order to equalize the macronutrient application rates for all treatments. In addition, plant available amount of these elements was different in sand and mixed soil/sand substrate.

Table 3.16 Composition, concentration, amounts, and application time of micronutrient solutions in the main carrot pot experiment

Nutrient	**Chemical compound**	**Concentration of compound (*g l^{-1}*)**	**Used solution (*ml pot^{-1}*)**	**Nutrient supply (*mg pot^{-1}*)**	**Appling time (*days after seeding*)**
Zn	$ZnSO_4.H_2O$	0.265	100	6	35
Fe	Fe-EDTA	2.887	100	40	35
Mn	$MnSO_4.H_2O$	0.184	100	6	35
Cu	$CuSO_4.7H_2O$	0.119	100	3	35
B	H_3BO_3	0.430	100	7.5	35
Mo	$(NH_4)_6Mo_7O_{24}.4H_2O$	0.028	100	1.5	35

Table 3.17 Available amount of some of macronutrients in the phosphorus-fertilisers

Sample name	**Type of fertiliser**	**Available nutrient** [*] **(*mg g*$^{-1}$ *P*)**				
		K	**S**	**N**	**Ca**	**Mg**
MD1	SPF (TSP)	10	75	2	620	38
MD2	SPF (TSP)	10	63	3	650	26
MD19	OMF	1620	1960	2180	360	74
MD28	CF (NP)	95	1230	1910	43	90
MD35	PR (PAPR)	33	183	23	50	137
MD37	PR (PAPR)	160	27	3	37	28
MD38	CF (PK)	1810	59	16	55	23
SP18	SPF (SSP)	30	420	16	1030	22
KH_2PO_4	-	1260	0	0	0	0

* : total N was considered as available N, for other nutrients warm water soluble fraction was considered as available amount

At first, according to expected dry matter yield and concentration of nutrients in carrot plant tissues, the available nutrients levels which were needed in sand and mixed soil/sand substrates were determined (*Table 3.18*). In the second step, the pure amounts of nitrogen (N), potassium (K), sulphur (S), calcium (Ca), and magnesium Mg) which must be supplied by fertilisation were calculated (*Table 3.19* and *Table 3.20*). Thus, the composition, type, and amounts of fertiliser compounds were determined according to initial available contents of nutrients in phosphorus-fertilisers (*Table 3.17*) and the type of substrates for each treatment. Anyhow, because of high initial available amounts of some nutrients in some of phosphorus sources, e.g., N and S in MD19 treatment or N in MD28 treatment, and limitation of compounds types as the source of nutrients, total amounts of some nutrients exceeded the defined level.

Macronutrient fertilisation application was split into three times per growth season and was done 48, 70, and 92 days after seeding in the mixed soil/sand substrate and 48, 77, and 110 days after seeding in the sand substrate. Dividing the amount of fertilisers for each application time was done according to initial amount of nutrients in phosphorus treatment, and the goal was to level the amounts of all macronutrients with the first or finally with second fertiliser application (e.g., total amount of K per pot on mixed soil/sand was 4.5 *g* and it was split to 1.5 *g* per each using time, in the Control, KH_2PO_4 treatment, 1.26 *g* coming from the treatment and 0.24 g, 1.5 g, and 1.5 *g* was supplied by the first, second, and third fertilisation, respectively; while about MD19 treatment 1.62 *g* K was initially added to pot by treatment and 0 g, 1.38 g, and 1.5g was supplied by the first, second, and third fertilisation, respectively).

Table 3.18 Total applied amount and compounds of macronutrients in mixed soil/sand and sand substrates in carrot pot experiment 2008

	N (*g pot*$^{-1}$)	**K** (*g pot*$^{-1}$)	**S** (*g pot*$^{-1}$)	**Ca** (*g pot*$^{-1}$)	**Mg** (*g pot*$^{-1}$)
Mixed soil/sand	1.65	4.50	1.50	1.35	0.35
Sand	1.80	5.00	1.70	1.50	0.40
Compound	NH_4NO_3, KNO_3, $Ca(NO_3)_2.4H_2O$	K_2SO_4, KNO_3, K_2CO_3*	K_2SO_4, $MgSO_4.7H_2O$	$Ca(NO_3)_2.4H_2O$, $CaCl_2$*	$MgSO_4.7H_2O$

* : was used when the other compounds were not possible to be applied

Table 3.19 Macronutrient fertilisation for the main carrot pot experiment in mixed soil/sand substrate

Treatment	**Nutrient portion**	**Nitrogen (N)**	**Potassium (K)**	**Sulphur (S)**	**Calcium (Ca)**	**Magnesium (Mg)**
Blank	Initial available amount per treatment *(g)*	-	-	-	-	-
	Supplied by fertiliser *(g)*	1.65	4.50	1.50	1.35	0.35
KH_2PO_4	Initial available amount per treatment *(g)*	-	1.26	-	-	-
	Supplied by fertiliser *(g)*	1.65	3.24	1.4	1.35	0.35
MD1	Initial available amount per treatment *(g)*	-	0.01	0.08	0.62	0.04
	Supplied by fertiliser *(g)*	1.65	4.49	1.32	0.73	0.31
MD2	Initial available amount per treatment *(g)*	-	0.01	0.06	0.65	0.03
	Supplied by fertiliser *(g)*	1.65	4.49	1.34	0.70	0.32
MD19	Initial available amount per treatment *(g)*	2.18	1.62	1.96	0.36	0.07
	Supplied by fertiliser *(g)*	-	2.88	0.36	0.99	0.28
MD28	Initial available amount per treatment *(g)*	1.91	0.10	1.23	0.04	0.09
	Supplied by fertiliser *(g)*	-	4.40	0.34	1.31	0.26
MD35	Initial available amount per treatment *(g)*	0.02	0.03	0.18	0.05	0.14
	Supplied by fertiliser *(g)*	1.69	4.47	1.22	1.30	0.21
MD37	Initial available amount per treatment *(g)*	-	0.16	0.03	0.04	0.03
	Supplied by fertiliser *(g)*	1.65	4.34	1.37	1.31	0.32
SP18	Initial available amount per treatment *(g)*	0.02	0.03	0.42	1.03	0.02
	Supplied by fertiliser *(g)*	1.63	4.47	0.98	0.31	0.33

Table 3.20 Macronutrient fertilisation for the main carrot pot experiment in sand substrate

Treatment	**Nutrient portion**	**Nitrogen (N)**	**Potassium (K)**	**Sulphur (S)**	**Calcium (Ca)**	**Magnesium (Mg)**
Blank	Initial available amount per treatment *(g)*	-	-	-	-	-
	Supplied by fertiliser *(g)*	1.80	5.00	1.72	1.50	0.40
KH_2PO_4	Initial available amount per treatment *(g)*	-	1.26	-	-	-
	Supplied by fertiliser *(g)*	1.80	3.74	1.70	1.50	0.40
MD1	Initial available amount per treatment *(g)*	-	0.01	0.08	0.62	0.04
	Supplied by fertiliser *(g)*	4.90	1.79	1.62	0.88	0.36
MD2	Initial available amount per treatment *(g)*	-	0.01	0.06	0.65	0.03
	Supplied by fertiliser *(g)*	1.80	4.99	1.64	0.85	0.37
MD19	Initial available amount per treatment *(g)*	2.18	1.62	1.96	0.36	0.07
	Supplied by fertiliser *(g)*	-	3.38	0.43	1.14	0.33
MD28	Initial available amount per treatment *(g)*	1.91	0.10	1.23	0.04	0.09
	Supplied by fertiliser *(g)*	-	4.90	0.47	1.46	0.31
MD35	Initial available amount per treatment *(g)*	0.02	0.03	0.18	0.05	0.14
	Supplied by fertiliser *(g)*	1.78	4.97	1.52	1.45	0.26
MD37	Initial available amount per treatment *(g)*	-	0.16	0.03	0.04	0.03
	Supplied by fertiliser *(g)*	1.80	4.84	1.67	1.46	0.37
SP18	Initial available amount per treatment *(g)*	0.02	0.03	0.42	1.03	0.02
	Supplied by fertiliser *(g)*	1.78	4.97	1.28	0.47	0.38

3.2.2.5 Irrigation

Irrigation was done in the same way as irrigation of preliminary pot experiment in both methods (see *Section 3.2.1*).

3.2.2.6 Harvest

- Neubauer test:

The maize and sunflower plants, in Neubauer pot experiment, were grown for 27 and 28 days, respectively. In that time, maize seedlings were at BBCH 13/14 (between 3 and 4 leaves), and sunflower plants were mostly at BBCH 13/15 (3 to 5 unfolded leaves), according to the BBCH code (Meier, 2001) (*Figure 3.7*). The harvest, preparation of samples, and recording initial data were done like the pilot Neubauer test except for the treatment of maize root samples. In the main Neubauer test (on 2008), all the roots, husk of seeds, and non germinated seeds were extracted accurately from the substrate after semi-drying substrate in clean aluminium plates at room temperature (*Figure 3.6*). After washing by deionized water and drying, the roots were separated from the husks and non germinated seeds and separately weighed, prepared, and kept for further analyses.

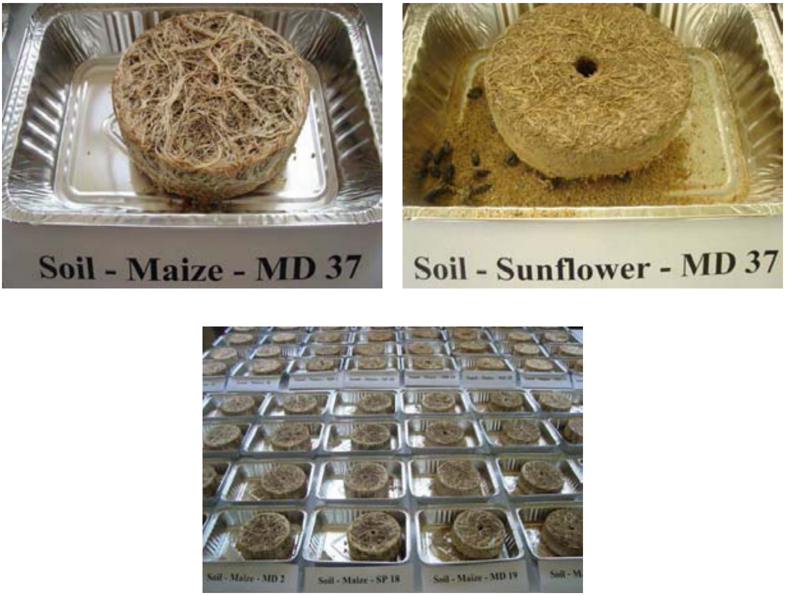

Figure 3.6 Drying Neubauer substrates at room temperature before extracting roots

Figure 3.7 Maize and sunflower seedling at harvest time in Neubauer experiment 2008 (Blank treatment in sand substrate)

- Carrot pot experiment

Carrot plants were grown for 17 and 19 weeks (120 and 135 days) in the mixed soil/sand and sand substrates, respectively. Harvest was done in the same way of the pilot experiment in 2007 (*Figure 3.8*).

Figure 3.8 Carrot yield in some treatments after harvest in 2008

3.3 Analysis of plant samples

3.3.1 Preparing plant samples

As mentioned before (see *3.2* and *3.3*), all plant samples including shoots, roots, and remained parts of seeds (from maize in the Neubauer test) were dried at 65°C to constant weight. The oven-dried samples were fine ground with a vibrating disk mill (Retsch RS100) with a zirconia grinding set and were kept in polyethylene container. The samples were put in oven at 65°C during the last night before weighing for digestion.

3.3.2 Digestion

The plant tissue samples were digested applying microwave digestion method (a microwave, CEM Mars Xpress, GmbH, Germany was employed). 0.5 *g* (±0.0005 *g*) ground and oven-dried plant material was weighed. 6.0 ml concentrated nitric acid (65%) and 1.5 ml hydrogen peroxide (30% concentration) was added to each sample in a special polyethylene tube. The set of tubes, including 15 samples and a Blank, were completely closed 10 minutes after adding hydrogen peroxide. The tubes were transferred to the microwave and digestion was done by adjusted program. The using program had been adjusted to five steps:

-raising temperature to 120°C, 5 minutes,
-staying at 120°C, 2 minutes,
-raising temperature to 200°C, 5 minutes,
-staying at 200°C, 15 minutes,
-cooling, 30 minutes.

The digested materials were entirely transferred to volumetric flasks (50 ml) and the tubes were rinsed using twofold deionized water and added to the flasks. The flasks were filled up to the volume with deionized water and mixed. The digested solutions were filtrated to polyethylene bottles with folded filter paper (Sartorius Stedim Biotech, 292). These digested solutions were kept and used for chemical analysis of elements.

3.3.3 Measuring

The concentration of uranium and other heavy metals were determined using Inductively Coupled Plasma-Quadrupole Mass Spectroscopy (VG Elemental PlasmaQuad 3, Thermo Elemental, United Kingdom) in all microwave digested solutions. The limitation detect, standardising the apparatus, qualifying test and other details was like measuring uranium in fertilisers extractions (see *Section 3.1.5* for more details). Digested plant tissues samples were diluted from 2 till 20 times depending on uranium concentration in the solutions that was less than that in fertilisers’ extraction samples. The concentrations of P, K, S, Ca, Mg, Fe, Zn, Mn, Al, Cu, and B in plant sample digestions were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (SpectroFlame M120 S, Germany) with a higher limitation detect comparing to measuring by ICP-QMS.

3.4 Substrates analysis

3.4.1 Preparation of substrate samples

After extracting the roots from the substrates in the Neubauer pot experiments, air dried and crushed samples passed through a 2mm sieve were used for chemical and physical analysis.

In the carrot pot experiments all substrate, sand and mixed sand/soil or soil, of each pot was air dried in a big plastic bag with mixing at least one time per day. Air dried substrate was mixed homogeneously. A sub-sample of about 400-500 *g* was taken from each substrate and was crushed by a plastic hammer and sieved to 2 mm for chemical and physical analysing.

3.4.2 Chemical digestion/extraction for substrate samples

For determination of available and total element contents in soil, sand, and mixed soil/sand substrates and the pH of substrates, standard and conventional extraction methods were used (see *Table 3.21*).

Table 3.21 Analytical methods for substrate analyses

Parameters	**Extraction/Digestion**	**Analysis**
pH	0.01M $CaCl_2$ (Hoffmann, 1991)	Potentiometry
$P_{ava.}$	Calcium-Acetate-Lactate (Schueller, 1969)	Colorimetry (John, 1970)
$K_{ava.}$	Calcium-Acetate-Lactate (Schueller, 1969)	Atomic emission photometry
P_{total}	*Aqua regia* (AbfKlaerV, 1992)	ICP-OES
K_{total}	*Aqua regia* (AbfKlaerV, 1992)	ICP-OES
$U_{ava.}$	AAAc-EDTA (Lakanen and Ervioe, 1971)	ICP-QMS

3.4.3 Measuring elements and pH

Uranium and other heavy metals in *aqua regia* and AAAc-EDTA extractions were measured by an ICP-QMS (VG Elemental PlasmaQuad 3, Thermo Elemental, United Kingdom) (see *Section 3.1.5* for more details). Phosphorus and potassium concentration in CAL extraction were determined colorimetrically using a Perkin-Elmer 550SE UV/VIS spectrophotometer and atomic emission photometry using a flame photometer Eppendorf-D, ELEX 6361, respectively.

P, K, S, Ca, Mg, Fe, Mn, and Al in *aqua regia* digestion of substrate samples were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (SpectroFlame M120 S, Germany).

The pH of soil and other substrates suspensions was determined potentiometrically using a Methrohm 605 pH meter.

Total C, N, and S were determined using an elemental analyzing (Elementar, vario MAX CNS, Germany).

3.5 Statistical analysis

Statistical analysis of data was performed using the Statistical Package for Social Sciences (SPSS) version 12.0 (SPSS, 2003). Correlations between uranium soluble in various extractants were calculated. Differences between means of uranium extractable and phosphorus in each type of phosphorus-containing fertilisers were tested in various extractants by Duncan’s test following one-factor univariate ANOVA.

The data of pot experiments were analyzed statistically by multi-factor univariate analysis of variance (GLM univariate ANOVA), and one-factor univariate ANOVA was applied to analyze data of each substrate and each kind of plant separately. All calculations of an element’s concentrations were made on a dry weight basis. Correlation and regression analysis were used to determine the relations between the factors.

4 Results

The main objectives of this research work were to study solubility of phosphorus and uranium in different phosphorus-containing fertilisers and to investigate availability of phosphorus and transferability of uranium to plants. To this aim, this study was done in three parts. The first part was studying the solubility of uranium and phosphorus in different phosphorus-containing fertilisers and various chemical extractants. So-called total uranium and phosphorus content digested by *aqua regia,* and uranium and phosphorus solubility in five chemical extractants including water, neutral ammonium citrate, alkaline ammonium citrate, 2% formic acid, and 2% citric acid were analysed and their results are presented in *Section 4.1*. In the second part, plant uptake of uranium and phosphorus by maize and sunflower seedlings, and carrot plants were studied in two different pot experiment methods. The results of these experiments are presented in *Section 4.2* and *4.3*, separately. Finally, correlations and regressions between the uranium solubilities in different extractants and uranium uptake by carrot plants, and maize and sunflower seedlings are introduced in *Section 4.4*.

4.1 Phosphorus and uranium solubility in P-fertilisers

The extractants and type of fertilisers significantly affected observed uranium solubility (in both expressions as concentration and percentage, i.e. referred to AR) in phosphorus-containing fertilisers, also interaction effect between extractant and type of fertiliser was significant (GLM univariate test, $P<0.001$). The same results were observed for the soluble phosphorus. The solubility of uranium and phosphorus differed strongly between extractants, with the solubility of uranium in water being significantly lower than that of phosphorus in water in all types of analyzed fertilisers. Averaged over all fertiliser types, the solubility of uranium and phosphorus in 2 % CA was quite similar (*Table 4.1*). Uranium showed somewhat lower relative solubility (i.e., referred to AR) than phosphorus in all extractants except in AAC (in that relative solubility of uranium was more than that of phosphorus) (*Table 4.1*).

Table 4.1 Differences between extractants with regard to mean U and P concentration and relative U and P solubility, calculated over all types of fertilisers by one-factorial ANOVA

Extractant	**n**	**Mean U concentration** *(mg kg^{-1})*	**Mean relative U solubility** *(% of U_{AR})*	**Mean P concentration** *(g kg^{-1})*	**Mean relative P solubility** *(% of P_{AR})*
Water	35	4.01 a	10.2 a	30.5 a	40.8 ab
Neutral ammonium citrate	35	20.4 ab	41.3 b	35.5 a	47.8 b
2% formic acid	35	20.8 ab	41.3 b	58.5 c	69.7 c
Alkaline ammonium citrate	35	21.1 ab	41.7 b	28.2 a	33.3 a
2% citric acid	35	29.2 b	60.6 c	50.1 b	62.1 c
Aqua regia	35	81.0 c	100 d	97.9 d	100 d

The different letters show significant differences by Dunkan’s test at the 0.05 level.

In both expressions as concentration and relative solubility (i.e. referred to AR), the highest mean solubility of uranium, after *aqua regia*, was found in 2 % citric acid (29.2 *mg kg^{-1}*, 60.6%) and the lowest in water (4.01 *mg kg^{-1}*, 10.2%). The relative solubility of uranium, calculated over all types of fertilisers analyzed here, in 2 % CA, AR, and water were significantly different from each other as well as from relative solubility in NAC, 2 % FA, and AAC. However, there were no significant differences between the relative solubility in NAC, 2 % FA, and AAC. Also, average percentages of soluble uranium in these extractants were approximately equal (ranging from 41.3% to 41.7%), while average soluble uranium fractions in water and 2 % citric acid were 10 % and 61 % of total uranium, respectively (*Table 4.1*).

Soluble uranium fractions extracted by different methods had significant correlation coefficients, ranging from 0.54 between water and 2% CA to 0.86 between 2 % FA and 2 % CA at the 0.01 level (*Table 4.2*). In the case of phosphorus solubility, there were significant correlation coefficients between all extractants at the 0.01 level. The correlation coefficients between all extractants, except between AAC and 2% FA, in the case of phosphorus solubility were stronger than in the case of uranium solubility, varying from 0.70 to 0.97 between AAC and 2% FA, and between NAC and 2% CA, respectively (*Table 4.3*).

Table 4.2 Correlation coefficients between soluble uranium (related to U in AR) in different extractants (Pearson correlation, n = 35)

Extractant	**2 % Formic acid**	**2 % Citric acid**	**Neutral ammonium citrate**	**Alkaline ammonium citrate**	**water**
2 % Formic acid	1				
2% Citric acid	0.86 **	1			
Neutral ammonium citrate	0.69 **	0.66 **	1		
Alkaline ammonium citrate	0.81 **	0.85 **	0.70 **	1	
Water	0.55 **	0.54 **	0.56 **	0.56 **	1

** Correlation is significant at the 0.01 level (2-tailed).

Table 4.3 Correlation coefficients between soluble phosphorus (related to P in AR) in different extractants (Pearson correlation, n = 35)

Extractant	**2 % Formic acid**	**2 % Citric acid**	**Neutral ammonium citrate**	**Alkaline ammonium citrate**	**water**
2 % Formic acid	1				
2% Citric acid	0.88 **	1			
Neutral ammonium citrate	0.79 **	0.97 **	1		
Alkaline ammonium citrate	0.70 **	0.87 **	0.88 **	1	
Water	0.71 **	0.85 **	0.91 **	0.82 **	1

** Correlation is significant at the 0.01 level (2-tailed)

Solubility of phosphorus in the tested extractants was different for various types of phosphorus-containing fertilisers (*Table 4.4*). Relative solubility of uranium varied significantly between different types of phosphorus-containing fertilisers. The lowest relative uranium solubility was in phosphate rocks, and the highest was found in compound fertilisers. In contrast, the highest mean relative solubility of phosphorus was found in straight phosphorus-fertilisers (*Table 4.4* and *Table 4.5*).

Table 4.4 Concentration and solubility of uranium and phosphorus in different extractants for various types of fertilisers

Extractant	**Type of fertiliser**	**n**	**Mean concentration**		**Mean relative solubility (% of *aqua regia* content)**	
			U *(mg kg^{-1})*	**P** *(g kg^{-1})*	**U** *%*	**P** *%*
Aqua regia	SPF	3	81.3	191.9	100	100
	CF	12	37.2	47.5	100	100
	OMF	5	18.7	26.2	100	100
	PR	15	136.8	43.5	100	100
2 % Formic acid	SPF	3	26.1	215.1	30	112
	CF	12	22.9	41.8	72	91
	OMF	5	8.6	21.3	50	83
	PR	15	22.2	53.0	17	39
2% Citric acid	SPF	3	58.6	215.8	62	112
	CF	12	31.8	42.1	107	93
	OMF	5	9.6	17.2	55	68
	PR	15	27.7	34.3	25	25
Neutral ammonium citrate	SPF	3	55.8	205.6	71	107
	CF	12	13.7	36.3	64	84
	OMF	5	5.0	11.7	34	49
	PR	15	23.7	8.7	20	7
Alkaline ammonium citrate	SPF	3	71.2	208.8	84	109
	CF	12	28.0	24.9	79	55
	OMF	5	3.8	8.4	22	32
	PR	15	11.3	1.4	10	1
Water	SPF	3	13.8	186.9	17	97
	CF	12	4.0	28.4	17	71
	OMF	5	2.2	9.6	14	40
	PR	15	2.7	7.8	3	5

Table 4.5 Differences between mean relative solubility of U and P for various types of P-containing fertilisers, tested over all extractants except *aqua regia*

Type of fertiliser	**n**	**Mean U solubility** *(% of U_{AR})*	**Mean P solubility** *(% of P_{AR})*
Phosphate rocks	75	14.7 a	15.5 a
Organo-mineral fertilisers	25	34.8 b	54.2 b
Straight P-fertilisers	15	52.5 c	107.4 d
Compound fertilisers	60	67.8 c	79.1 c

The different letters show significant differences by Duncan's test at the 0.05 level.

Table 4.6 Comparison of relative U solubility in different extractants, calculated separately for each group of P-containing fertilisers

Extractant	**Mean relative U solubility** *(in % of U_{AR})*			
	Straight P-fertilisers (n = 3)	**Compound fertilisers (n = 12)**	**Organo-mineral fertilisers (n = 5)**	**Phosphate rocks (n = 15)**
Water	16.8 a	16.6 a	13.8 a	2.6 a
2% Formic acid	29.5 a	71.5 b	49.6 ab	16.8 bc
Neutral ammonium citrate	71.1 b	64.1 b	33.8 ab	19.6 cd
Alkaline ammonium citrate	83.5 bc	79.4 bc	22.2 ab	9.5 ab
2% Citric acid	61.5 b	107 cd	54.9 b	24.9 d
Aqua regia	100 c	100 cd	100 c	100 e

Comparison columnar, i.e. different letters in one column denote significant differences by Duncan's test at the 0.05 level.

Mean relative uranium and phosphorus solubilities in each extractant were analyzed separately in different types of phosphorus-containing fertilisers. When looked at separately, all four types of phosphorus-containing fertilisers showed significant differences in their uranium and phosphorus solubility in various extractants (*Table 4.6* and *Table 4.7*). In compound fertilisers, organo-mineral fertilisers, and phosphate rocks, the highest uranium solubility was found in 2 % citric acid, while for straight phosphorus-fertilisers, the strongest extractant was AAC (without considering AR). The lowest uranium solubility was found in water varying from 2.6 % in phosphate rocks to 16.8 % in straight phosphorus-fertilisers (related to total uranium content measured in *aqua regia* digests; *Table 4.6*). In contrast to this, the lowest phosphorus extracting power in phosphate rocks, organo-mineral fertilisers, and compound fertilisers was for AAC, varying from 1.11% in phosphate rocks to 55.3% in compound fertilisers (*Table 4.7*).

Table 4.7 Comparison of relative P solubility in different extractants, calculated separately for each group of P-containing fertilisers

	Mean relative P solubility *(in % of P_{AR})*			
Extractant	**Straight P-fertilisers (n = 3)**	**Compound fertilisers (n = 12)**	**Organo-mineral fertilisers (n = 5)**	**Phosphate rocks (n = 15)**
Alkaline ammonium citrate	109 bc	55.3 a	32.1 a	1.1 a
Water	97 a	71.4 ab	39.6 a	5.4 a
Neutral ammonium citrate	107 bc	84.1 bc	48.8 ab	6.7 a
2% Citric acid	112 c	93.3 bc	67.9 abc	25.1 b
2% Formic acid	112 c	91.5 bc	82.6 bc	39.4 c
Aqua regia	100 ab	100 c	100 c	100 d

Comparison columnar, i.e. different letters in one column denote significant differences by Duncan's test at the 0.05 level.

The relative solubility of uranium in water extractant was not significantly different between four types of phosphorus-containing fertilisers (*Table 4.8*), but the relative phosphorus solubility in water varied significantly between different types of phosphorus sources (*Table 4.9*). In other extractants (2% FA, 2% CA, NAC, and AAC), significant differences were found between some type of phosphorus-containing fertilisers for both mean relative solubility of uranium and phosphorus, but not in the same way and trend (*Table 4.8* and *Table 4.9*).

Table 4.8 Comparisons of U solubility of different types of P-containing fertilisers, tested for 5 different extractants

Type of fertilisers	**U solubility** *(% of U_{AR})*				
	Water	**2% FA**	**NAC**	**AAC**	**2% CA**
Phosphate rocks (n = 15)	2.6 a	16.8 a	19.6 a	9.5 a	24.9 a
Organo-mineral fertilisers (n = 5)	13.8 a	49.6 bc	33.8 ab	22.2 a	54.9 a
Compound fertilisers (n = 12)	16.6 a	71.5 c	64.1 bc	79.4 b	107.3 b
Straight P-fertilisers (n = 3)	16.8 a	29.5 ab	71.1 c	83.5 b	61.5 a

Comparison columnar, i.e. different letters in one column denote significant differences by Duncan's test at the 0.05 level.

Table 4.9 Comparison of P solubility of different types of P-containing fertilisers, tested for 5 different extractants

Type of fertilisers	**P solubility** *(% of P_{AR})*				
	Water	**2% FA**	**NAC**	**AAC**	**2% CA**
Phosphate rocks (n = 15)	5.4 a	39.4 a	6.7 a	1.1 a	25.1 a
Organo-mineral fertilisers (n = 5)	39.6 ab	82.6 b	48.8 b	32.1 b	67.9 b
Compound fertilisers (n = 12)	71.4 bc	91.5 b	84.1 c	55.3 b	93.3 c
Straight P-fertilisers (n = 3)	97.4 c	112 c	107 c	109 c	112 d

Comparison columnar, i.e. different letters in one column denote significant differences by Duncan's test at the 0.05 level.

The strongest uranium extracting power of water, NAC, and AAC was observed for straight phosphorus-fertilisers, while the strongest uranium extracting power of 2% FA and 2% CA was found for compound fertilisers. The strongest phosphorus extracting power of all extractants (without considering *aqua regia*) was found for straight phosphorus-fertilisers. The lowest uranium and phosphorus extracting power of all five extractants was found in phosphate rocks (*Table 4.8* and *Table 4.9*).

- Summary of P and U solubility in P-fertilisers: It is evident that the uranium solubility in phosphorus-containing fertilisers is dependent both on the extractant and on the type of phosphorus-containing fertiliser. The solubility of uranium differed from that of phosphorus in the tested extractants. The differences between phosphorus solubility of various types of phosphorus-containing fertilisers, tested here, was more than that of uranium solubility. Relative uranium and phosphorus solubility were different in the same type of phosphorus-containing fertilisers and the same extractant.

4.2 Neubauer pot experiment

The aim of the Neubauer experiment was to investigate how much of the uranium added to substrate with phosphorus fertilisation would potentially be transferred to plant roots and shoots. This should be achieved by a high density of plant seedlings and roots in a small amount of substrate. A high seedling to substrate/soil ratio could rapidly exploit and uptake the available amounts of elements during a short growing period. By the results of this experiment, the available amounts of uranium and phosphorus in different phosphorus sources were evaluated and compared with each other. When selecting different substrate (mixed soil/sand and sand) and different kind of plant seedlings, the effect of substrate and kind of plant on uranium and phosphorus transferring were studied. The results of these experiments are presented in the following sections.

4.2.1 The pilot Neubauer experiment, 2007

The main aims of the pilot Neubauer pot experiment were to evaluate suitability of this method for studying the availability and transferability of uranium and phosphorus to plant tissues and studying the suitability of sunflower and maize as test plant seedlings in this method.

The results of GLM univariate analysis of variance of the Neubauer experiment data including two factors, the kind of plants and phosphorus treatments, are presented in *Table 4.10* and *Table 4.11*. The results showed a significant effect of phosphorus treatments on the uranium concentration of roots, uranium uptake by roots, and total uranium uptake at the 0.001 level. The kind of plant significantly influenced the uranium concentration of plant shoots and roots and also uranium uptake by plant shoots at the 0.05, 0.001, and 0.01 levels, respectively. With regard to phosphorus, all parameters except concentration of phosphorus in shoots were significantly affected by both, the kind of plant and phosphorus source, at the 0.001 level (*Table 4.11*).

Table 4.10 Results of univariate analysis of variance for the comparison of the effects of the type of plants and different P sources on plant uranium concentration and uptake in the pilot Neubauer experiment, 2007

Parameters	**Plant factor**	**P source factor**	**Plant × P source**
U concentration in shoots	*	ns	ns
U concentration in roots	***	***	***
U uptake by shoots	**	ns	ns
U uptake by roots	ns	***	ns
Total U uptake	ns	***	ns

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

Table 4.11 Results of univariate analysis of variance for the comparison of the effects of the type of plants and different P sources on plant phosphorus concentration and uptake in the pilot Neubauer experiment, 2007

Parameters	**Plant factor**	**P source factor**	**Plant × P source**
P concentration in shoots	ns	***	*
P concentration in roots	***	**	ns
P uptake by shoots	**	***	ns
P uptake by roots	***	***	*
Total P uptake	***	***	ns

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

4.2.1.1 Dry matter yield

The effects of phosphorus sources on dry matter of maize and sunflower were statistically analysed by one-factorial analysis of variance of the pilot Neubauer experiment data. These analyses showed no significant effect of phosphorus sources on dry matter of maize and sunflower separated as shoots, roots, and total dry matter yield (*Table 4.12*). Also, Duncan’s test for differences between mean of shoots, roots, and total dry matters of maize and sunflower in various phosphorus treatments showed no significant difference between treatments except for sunflower shoots’ dry matter in which was observed a significant difference between the two controls (no phosphorus and KH_2PO_4 treatments) at the 0.05 level (*Table 4.13*).

Table 4.12 Results of one-factorial analysis of variance for the comparison of the effects of P sources on dry matter yield in Neubauer test, 2007

Parameter	**Maize**	**Sunflower**
Dry matter of shoots	ns	ns
Dry matter of roots	ns	ns
Total dry matter	ns	ns

ns: not significant at the 0.05 level.

Table 4.13 Influence of different P sources on dry matter yield of maize and sunflower seedlings in the Neubauer test 2007, separated by shoots, roots, and total dry matter yield

P treatments	**Maize dry matter** *(g)*			**Sunflower dry matter** *(g)*		
	Shoots	**Roots**	**Total**	**Shoots**	**Roots**	**Total**
Blank	3.33 a	11.99 a	15.32 a	4.01 a	3.61 a	7.71 a
Control (KH_2PO_4)	3.77 a	12.16 a	16.02 a	4.67 b	3.17 a	7.84 a
Superphosphate (SP18)	3.79 a	11.88 a	15.67 a	4.27 ab	3.74 a	8.01 a
Phosphate rock (MD37)	3.86 a	12.20 a	15.97 a	4.41 ab	3.21 a	7.63 a

Values in the same column followed by the same letters are not significantly different by Duncan's test at the 0.05 level.

4.2.1.2 Plant uranium concentration and uptake

One-factorial analysis of variance of data from Neubauer experiment in 2007, showed significant effects of various phosphorus sources on uranium concentration of roots, uranium uptake by roots, and total uranium uptake by seedlings of maize and sunflower (P value less than 0.001), and no significant effects on uranium concentration of shoots (*Table 4.14*).

Table 4.14 Effects of P sources on U and P concentration and uptake in maize and sunflower seedlings in the pilot Neubauer test, 2007, tested by one-factorial ANOVA

Parameters	**Uranium**		**Phosphorus**	
	Maize	**Sunflower**	**Maize**	**Sunflower**
Concentration in shoots	ns	ns	**	***
Concentration in roots	***	***	**	ns
Uptake by shoots	ns	ns	ns	***
Uptake by roots	***	***	**	*
Total uptake	***	***	**	***

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

4.2.1.3 Plant phosphorus concentration and uptake

The result of one-factorial ANOVA for phosphorus parameters in maize and sunflower seedlings are presented in *Table 4.14*. These results can be summarized as follows: in maize seedlings, phosphorus concentration of shoots and roots, phosphorus uptake by roots, and total phosphorus uptake were significantly influenced by different phosphorus sources; in sunflower seedlings, phosphorus concentration of shoots, phosphorus uptake by shoots and roots, and total uptake of phosphorus were significantly affected by different phosphorus sources. Different phosphorus sources did not significantly affect phosphorus uptake by shoots of maize and concentration of phosphorus in roots of sunflower.

4.2.1.4 Summary of the pilot Neubauer experiment, 2007

The results of the pilot Neubauer experiment showed no significant effect of phosphorus source factor* on the dry matter yield of shoots, roots, and total dry matter yield in the both test plants. However, phosphorus source factor significantly influenced the root uranium concentration and uptake and total uranium uptake by plants seedlings, and also phosphorus concentration and uptake in plant seedlings.

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[*] The real factor is the uranium loads and uranium solubility that varied between different sources of phosphorus-fertilisers when the same amount of phosphorus was added to the pots.

Significant effects of phosphorus sources on uranium concentration and uptake without any significant effect on dry matter yield confirmed the suitability of this method for studying the transferability of uranium and phosphorus elements from the fertilisers/substrates to plants. In other words, the absence of any effect of phosphorus sources on dry matter yield proved that plant uranium uptake was not influenced by phosphorus deficiency, thus it is possible to interpret differences between uranium uptakes from various phosphorus sources as differences between fertilisers with regard to their uranium bioavailability and uranium transferability to plants.

4.2.2 The main Neubauer experiment, 2008

By the main Neubauer pot experiment, available amount of phosphorus and uranium in six different types of phosphorus sources was evaluated. Also, these phosphorus sources were compared with each other and with two controls with regard to concentration and uptake of phosphorus and uranium in shoots and roots, and total dry matter of maize and sunflower seedlings. The differences between maize and sunflower plants, and the effects of substrate on uranium and phosphorus uptake and transferability were studied. The results are presented in the next two sections for uranium and phosphorus, respectively.

4.2.2.1 Influence of P sources on dry matter yield and on plant uranium concentration and uptake

The GLM univariate analysis of variance was done for investigating the effects of three factors including plant type, substrate, and phosphorus source (as fertiliser) on dry matter yield (shoots, roots, and total), uranium concentration in shoots and roots, and uranium uptake (by shoots, roots, and total); the results are summed up in *Table 4.15*. Dry matter of shoots and roots and total dry matter yield were significantly influenced by all three factors at the 0.001 level (*Table 4.15*). With looking accurately in details it was clear that the significant effect of phosphorus sources on dry matter yield was a result of salt effect of the MD19 and MD28 fertilisers. The total amount of phosphorus in the MD19 and MD28 is less than other fertilisers (see *Table 3.12*), and finally high soluble amounts of them in substrate damaged the seedlings and decreased the dry matter yield. Thus, the significant effect of phosphorus source on dry matter yield is not related to phosphorus deficiency or low solubility of phosphorus in the applied fertilisers.

Uranium concentration in shoots and roots, uranium uptake by roots, and total uranium uptake were significantly affected by all three factors at the 0.001 or 0.01 level. However, uranium uptake by shoots was only influenced significantly by substrate factor at the 0.001 level. The first order interaction of phosphorus source factor with plant and substrate factors and between plant factor and substrate factor in uranium concentration of roots, uranium uptake by roots, and total uranium uptake were significant at the 0.001 level (*Table 4.15*).

Table 4.15 Significance of effects of P sources, type of plants, and substrates on dry matter yield and plant U concentration and uptake in the main Neubauer experiment (2008), tested by multi-factorial ANOVA

Parameters	**P source factor**	**Plant factor**	**Substrate factor**	**P source× Plant**	**P source× Substrate**	**Plant× Substrate**	**Plant× Substrate ×P source**
Shoots' dry matter	***	***	***	***	ns	*	ns
Roots dry matter	***	***	***	***	ns	ns	ns
Total dry	***	***	***	***	ns	*	ns
U concentration of shoots	**	**	***	**	*	ns	*
U concentration of roots	***	***	***	***	***	***	***
U uptake by shoots	ns	ns	***	ns	ns	ns	ns
U uptake by roots	***	***	***	***	***	***	ns
Total U uptake	***	***	***	***	***	***	**

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

By comparing the effect of different phosphorus sources on the produced dry matter of shoots, roots and total dry matter by the Duncan's test following the GLM univariate analysis of variance, it was found that the lowest significant difference was in the dry matter of shoots and the highest significant difference was between dry matter of roots. The mean dry matter of shoots in MD19 treatment (an organo-mineral fertiliser) was significantly less than other treatments, while the mean dry matter of shoots (counted over both substrates and both types of plants) in all other treatments, including no phosphorus treatment, were not significantly different from each other (*Table 4.16*).

Table 4.16 Influences of P source factor on dry matter yield in the Neubauer experiment 2008 (Duncan's test following multi-factorial ANOVA)

P treatment	Type of fertiliser/treatment [*]	n	Dry matter of shoots *(g)*	Dry matter of roots *(g)*	Total dry matter *(g)*
Blank	-	16	8.17 b	3.44 cd	11.61 c
Control	KH_2PO_4	16	8.32 b	3.54 d	11.86 c
MD19	OMF (NPK+Mg)	16	6.86 a	2.55 a	9.41 a
MD28	CF (NP)	16	7.89 b	2.64 a	10.53 b
MD38	CF (PK)	16	8.20 b	3.18 bc	11.38 bc
SP18	SPF (SSP)	16	8.50 b	3.09 b	11.59 c
MD2	SPF (TSP)	16	8.33 b	3.33 bcd	11.66 c
MD37	PAPR	16	8.61 b	3.34 bcd	11.95 c

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

Influences of different phosphorus sources on uranium concentration of shoots and roots, uranium uptake by shoots and roots, and total uranium uptake were statistically analyzed by Duncan's test following the GLM multi-factorial ANOVA (*Table 4.17*). About the effect of different phosphorus sources on uranium concentration of shoots, only one significant difference was observed in that the shoots' uranium concentration in the MD19 treatment was significantly higher than other treatments. When looking accurately at the initial data of the MD19 treatment on sand substrate, it was clearly different from all other pots in the case of seedlings number and subsequently in dry matter yield, and in three pots of four replications only several sunflower seedlings grew. This problem probably was caused by salinity effect of the fertiliser on sunflower germination (i.e., due to the low phosphorus content of MD19, a very high amount of fertiliser had to be added in order to supply 80 *mg* P per pot, leading to a very high chloride concentration as a consequence).

Influence of all other fertilisers and two controls (no phosphorus application and KH_2PO_4 application with no loaded uranium onto substrates) on uranium concentration of shoots were not significantly different from each other (*Table 4.17*).

In terms of uranium concentration of roots, the results were different. The lowest and highest uranium concentration of roots were found in the Blank and the Control group and superphosphates group including the SP18 and MD2 treatments, respectively. These two groups of phosphorus treatments were significantly different from each other and from other phosphorus-fertiliser treatments at the 0.05 level, while there was no significant difference between two treatments inside each group. Some significant differences were found between other fertiliser treatments too (*Table 4.17*).

In terms of the effect of phosphorus treatments on uranium uptake by shoots, there was no significant difference between most of them, and a significant difference was only found between the MD38 and MD28 treatments (compound fertilisers, PK and NP, respectively) at the 0.05 level (*Table 4.17*).

Table 4.17 Influences of P source factor on plant U uptake and concentration in the main Neubauer experiment 2008 (Duncan’s test following multi-factorial ANOVA)

P treatment	**Type of fertiliser/treatment** *	**n**	**Uranium uptake** *(µg pot^{-1})*			**Uranium concentration** *(µg g^{-1})*	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
Blank	-	16	0.924 a	0.828 a	0.096 ab	0.255 a	0.013 a
Control	KH_2PO_4	16	0.762 a	0.679 a	0.083 ab	0.206 a	0.011 a
MD19	OMF (NPK+Mg)	16	1.510 b	1.407 b	0.102 ab	0.571 b	0.035 b
MD38	CF (PK)	16	1.886 bc	1.809 bc	0.076 a	0.600 b	0.010 a
MD28	CF (NP)	16	2.085 c	1.979 c	0.106 b	0.782 c	0.016 a
MD37	PAPR	16	2.086 c	2.003 c	0.083 ab	0.662 bc	0.010 a
SP18	SPF (SSP)	16	2.864 d	2.770 d	0.094 ab	0.985 d	0.012 a
MD2	SPF (TSP)	16	3.508 e	3.412 e	0.097 ab	1.126 d	0.013 a

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

The influence of different phosphorus treatments on total uranium uptake was the same as their influence on uranium uptake by roots. The trend of increasing total uranium uptake and uranium uptake by roots in different phosphorus treatments was most similar to variance trend of uranium concentration of roots. Total uranium uptake and root uranium uptake in all phosphorus-fertilisers were significantly more than those of the Blank and Control. While, there was no significant difference between the Blank (no phosphorus application) and the Control (KH_2PO_4 treatment) with regard to root and total uranium uptake; it can imply that available phosphorus level in the substrates could not affect the uranium uptake by plant seedlings in this experiment thus, any difference between phosphorus treatments, with regard to plant uranium uptake, can be related to different levels of available uranium in phosphorus-fertilisers. The highest total uranium uptake and uranium uptake by roots was found in the MD2 treatment that was followed first by the SP18 and then by other fertiliser treatments (*Table 4.17*).

4.2.2.2 Influence of P sources on dry matter and on uranium concentration and uptake separated by test plants and substrates

According to multi-factorial ANOVA results (*Table 4.15*), the first order interactions between phosphorus source factor and plant factor were significant in the case of all dry matter and uranium parameters except for uranium uptake by shoots. In addition, in the case of uranium concentration of shoots and roots, uranium uptake by roots, and total uranium uptake, interactions between phosphorus source factor and substrate were significant. These significant interactions indicated the significant influences of plant factor and substrate factor on the effects of phosphorus source factor. In other words, the effects of phosphorus source factor varied significantly from maize to sunflower seedlings and from sand substrate to mixed soil/sand substrate. Thus, data of the main Neubauer experiment were split and statistically analyzed for maize and sunflower in each substrate separately by one-factorial ANOVA and were followed by Duncan’s test for significant differences between different phosphorus treatments. These results are presented in *Table 4.18* to *Table 4.22*.

- Dry matter yield:

The effects of different phosphorus sources on total dry matter and shoots dry matter of maize seedlings in both substrates were not significant. But, the effects of different phosphorus sources on dry matter of roots were significant for maize in both substrates at the 0.001 level (*Table 4.18*).

Table 4.18 The effect of P source factor on U uptake and concentration, and on dry matter yield of maize and sunflower seedlings in different substrates in the main Neubauer experiment (2008) by one-factorial ANOVA

Plant	**Substrate**	**Uranium uptake**			**Uranium concentration**		**Dry matter**		
		Total	**Roots**	**Shoots**	**Roots**	**Shoots**	**Total**	**Roots**	**Shoots**
Maize	Mixed soil/sand	***	***	ns	***	ns	ns	***	ns
	Sand	***	***	ns	***	ns	ns	***	ns
Sunflower	Mixed soil/sand	***	***	ns	***	*	*	**	*
	Sand	***	***	ns	***	*	***	ns	***

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

The effect of phosphorus treatments on total dry matter and shoots dry matter of sunflower seedlings was significant in the mixed soil/sand and sand substrates at the 0.05 and 0.001 levels, respectively. In the case of total dry matter and shoots dry matter of sunflower in mixed soil/sand substrate, significant differences were found only between the MD19 treatment and other treatments while in sand substrate differences between treatments were more significant (for more details see *Table 4.19*). The effect of phosphorus treatments on root dry matter of sunflowers in mixed soil/sand substrate only was significant.

Dry matter yield of maize and sunflower seedlings varied significantly between sand and mixed soil/sand substrates in the main Neubauer experiments (*Table 4.20*).

Table 4.19 Influences of P source factor on dry matter yield of maize and sunflower seedlings in different substrates in the main Neubauer experiment 2008 (by Duncan’s test following one-factorial ANOVA)

P treatment	**Type of fertiliser/ treatment** *	**n**	**Dry matter in mixed soil/sand substrate** *(g)*			**Dry matter in sand substrate** *(g)*		
			Total	**Roots**	**Shoots**	**Total**	**Roots**	**Shoots**
					Maize seedlings			
Blank	-	4	14.64	4.36 cd	10.28	13.13	4.15 e	8.98
Control	KH_2PO_4	4	16.19	4.59 d	11.61	12.31	4.03 e	8.28
SP18	SPF (SSP)	4	14.40	3.81 bc	10.59	12.98	3.23 bcd	9.75
MD19	OMF (NPK+Mg)	4	14.64	3.09 a	11.56	12.55	2.73 ab	9.83
MD28	CF (NP)	4	14.82	3.32 ab	11.50	11.25	2.44 a	8.81
MD2	SPF (TSP)	4	14.88	4.33 cd	10.55	12.91	3.69 cde	9.23
MD38	CF (PK)	4	15.29	4.03 cd	11.26	10.60	3.03 abc	7.57
MD37	PAPR	4	15.35	4.10 cd	11.26	13.20	3.77 de	9.43
					Sunflower seedlings			
Blank	-	4	9.90 b	2.81 ab	7.09 b	8.77 bc	2.45	6.32 bc
Control	KH_2PO_4	4	10.81 b	3.40 c	7.41 b	8.13 bc	2.13	6.00 bc
MD19	OMF (NPK+Mg)	4	6.87 a	2.36 a	4.51 a	3.56 a	2.03	1.53 a
MD28	CF (NP)	4	9.04 b	2.46 ab	6.58 b	7.00 b	2.36	4.65 b
MD37	PAPR	4	9.72 b	2.73 ab	7.00 b	9.52 c	2.75	6.77 c
MD2	SPF (TSP)	4	9.94 b	2.87 ab	7.07 b	8.89 bc	2.42	6.47 bc
SP18	SPF (SSP)	4	10.24 b	2.92 b	7.32 b	8.74 bc	2.40	6.34 bc
MD38	CF (PK)	4	10.28 b	2.84 ab	7.44 b	9.34 c	2.81	6.53 c

*: see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level. Duncan’s test was done only for those parameters for which the one-factorial ANOVA had shown a significant effect of phosphorus treatments

- Plant uranium concentration and uptake:

The effect of phosphorus source factor was significant on total uranium uptake, uranium uptake by roots, and roots’ uranium concentrations of maize and sunflower seedlings in mixed soil/sand and sand substrates by one-factorial ANOVA at the 0.001 level. There was no significant effect on uranium uptake by shoots of maize and sunflower seedlings in any substrate. And, shoots’ uranium concentration of sunflower seedlings was significantly affected by the phosphorus source factor in both substrates at the 0.05 level, but for maize seedlings, it was not significantly influenced by different phosphorus sources (*Table 4.18*).

Statistical analysis of data showed significant influence of substrate factor on uranium concentration and uranium uptake in maize and sunflower seedlings in the main Neubauer experiment (*Table 4.20*).

The mean uranium concentration in shoots and roots of sunflower on sand substrate was at least two times more than that of maize. Although the mean uranium uptake by shoots of maize was almost the same as that of sunflower, but the mean values of roots and total uranium uptake in sunflower seedlings were approximately 67% and 64% more than those of maize on the sand substrate. On the other hand, although in the mixed soil/sand substrate the mean concentration of uranium of shoots and roots in sunflower seedlings were more than maize seedlings (about 80% and 30%, respectively), but uranium uptake by roots and total uranium uptake in sunflower seedlings were about 5 percent less than those of maize seedlings (*Table 4.20*). These comparisons show that the effect of substrate on uranium uptake by sunflower seedlings is completely stronger than the effect of substrate on uranium uptake by maize seedlings.

Table 4.20 Dry matter yield, and U concentration and uptake in maize and sunflower seedlings in different substrates, and significance of effects of substrate factor on them in the main Neubauer experiment 2008, by one-factorial ANOVA

Parameter	**Maize**			**Sunflower**		
	Sand	**Mixed soil/sand**	**Sig.** [a]	**Sand**	**Mixed soil/sand**	**Sig.** [a]
Shoots dry matter *(g)*	8.98	11.08	***	5.58	6.80	**
Roots dry matter *(g)*	3.38	3.95	**	2.42	2.80	***
Total dry matter *(g)*	12.37	15.03	***	7.99	9.60	**
Shoots U concentration *(µg g^{-1})*	0.013	0.006	***	0.029	0.011	*
Roots U concentration *(µg g^{-1})*	0.567	0.349	**	1.212	0.465	***
Shoots U uptake *(µg pot^{-1})*	0.116	0.068	***	0.112	0.073	***
Roots U uptake *(µg pot^{-1})*	1.795	1.351	*	3.002	1.296	***
Total U uptake *(µg pot^{-1})*	1.910	1.419	*	3.114	1.369	***

a: significance of effects of substrate factor on the parameter

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

- Maize seedlings on different substrates: Significant differences were found between various phosphorus treatments in the case of the mean of uranium concentration and uptake in roots, and the mean of total uranium uptake in maize seedlings in both substrates. In all these comparisons with a significant difference between phosphorus sources, the lowest mean of uranium parameters was found in Control (KH_2PO_4 treatment) and the highest mean was found in the MD2 treatment (a triple superphosphate fertiliser), and most other phosphorus treatments were significantly different from them (*Table 4.21*).

Table 4.21 Influences of P source factor on U uptake and concentration of maize seedlings in different substrates in the main Neubauer experiment 2008 (by Duncan's test following one-factorial ANOVA)

P treatment	**Type of fertiliser/ treatment** *	**n**	**Uranium uptake** *($\mu g\ pot^{-1}$)*			**Uranium concentration** *($\mu g\ g^{-1}$)*	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
			In mixed soil/sand substrate				
Blank	-	4	1.13 b	1.06 b	0.063	0.246 b	0.006
Control	KH_2PO_4	4	0.74 a	0.68 a	0.062	0.148 a	0.005
MD37	PAPR	4	1.20 b	1.14 bc	0.068	0.277 bc	0.006
MD19	OMF (NPK+Mg)	4	1.25 bc	1.17 bc	0.079	0.381 d	0.007
MD38	CF (PK)	4	1.38 bcd	1.32 bcd	0.065	0.325 c	0.006
MD28	CF (NP)	4	1.62 cd	1.54 cd	0.082	0.464 e	0.007
SP18	SPF (SSP)	4	1.70 d	1.64 d	0.062	0.432 de	0.006
MD2	SPF (TSP)	4	2.33 e	2.27 e	0.065	0.519 f	0.006
			In sand substrate				
Blank	-	4	0.72 a	0.59 ab	0.126	0.142 a	0.014
Control	KH_2PO_4	4	0.63 a	0.52 a	0.106	0.130 a	0.013
MD38	CF (PK)	4	1.38 ab	1.28 bc	0.104	0.442 b	0.014
MD37	PAPR	4	1.80 bc	1.69 cd	0.117	0.451 b	0.013
MD19	OMF (NPK+Mg)	4	2.00 bc	1.87 cd	0.135	0.689 c	0.014
SP18	SPF (SSP)	4	2.20 c	2.08 d	0.123	0.638 bc	0.013
MD28	CF (NP)	4	2.42 c	2.31 d	0.110	0.960 d	0.013
MD2	SPF (TSP)	4	4.12 d	4.02 e	0.103	1.087 d	0.011

*: see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level. Duncan's test was done only for those parameters for which the one-factorial ANOVA had shown a significant effect of phosphorus treatments

More significant differences were found between phosphorus treatments with regard to their effect on uranium concentration in shoot and root tissues and uranium uptake by shoots in the case of sunflower seedlings in comparison to maize seedlings in both substrates (*Table 4.21* and *4.22*).

- *Sunflower seedlings on mixed soil/sand substrate:* Total uranium uptake by sunflower seedlings followed the same trend as their roots' uranium uptake in different phosphorus treatments on both substrates. In mixed soil/sand substrate, the lowest total uranium uptake and the lowest roots uranium uptake were found in the Blank, and the highest amounts were found in the SP18 (SSP) treatment. With regard to the total uranium uptake by sunflower seedlings, only the superphosphate and triple superphosphate fertilisers were significantly different (higher than) from other phosphorus treatments while these two treatments were not significantly different from each other (*Table 4.22*).

- *Sunflower seedlings on sand substrate:* The lowest and highest amount of total uranium uptake and root uranium uptake, in sunflower seedlings on the sand substrate, was found in the Control (KH_2PO_4) and MD2 (TSP) treatments, respectively. Total uranium uptake in all fertiliser treatments, except MD19, were significantly more than the Blank and Control (*Table 4.22*). The highest uranium uptake in sand substrate by sunflower seedlings (in the MD2 treatment, a TSP) was about 8 times more than the lowest one (in the Control) while the highest uranium uptake in mixed soil/sand was about 2 times more than the lowest uranium uptake. Also, differences between the highest and lowest uranium uptake of maize seedlings in sand substrate was considerably more than in mixed soil/sand.

The comparison of results in sand and mixed soil/sand confirmed that uranium transferability from fertilisers to plants in sand substrate (and probably in all soilless culture media) is much easier than soil substrate. With regard to chemical interactions like precipitation, adsorption, and competition, between uranium ions and complexes with soil colloids, and other free ions and complexes found in soil solution, the decrease of uranium transferability to plants was expected in mixed soil/sand substrate.

Table 4.22 Influences of P source factor on U uptake and concentration of sunflower seedlings in different substrates in the main Neubauer experiment 2008 (by Duncan's test following one-factorial ANOVA)

P treatment	**Type of fertiliser/ treatment** *	**n**	**Uranium uptake** *($\mu g\ pot^{-1}$)*			**Uranium concentration** *($\mu g\ g^{-1}$)*	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
			In mixed soil/sand substrate				
Blank	-	4	1.01 a	0.93 a	0.082	0.331 ab	0.012 abc
Control	KH_2PO_4	4	1.01 a	0.95 a	0.058	0.281 a	0.008 a
MD19	OMF (NPK+Mg)	4	1.19 a	1.12 a	0.071	0.471 c	0.017 c
MD37	PAPR	4	1.24 a	1.17 a	0.064	0.433 bc	0.009 a
MD38	CF (PK)	4	1.24 a	1.17 a	0.075	0.408 bc	0.010 ab
MD28	CF (NP)	4	1.30 a	1.19 a	0.104	0.486 c	0.016 bc
MD2	SPF (TSP)	4	1.93 b	1.87 b	0.063	0.646 d	0.009 a
SP18	SPF (SSP)	4	2.04 b	1.97 b	0.068	0.663 d	0.010 ab
			In sand substrate				
Blank	-	4	0.84 a	0.73 a	0.113	0.303 a	0.018 a
Control	KH_2PO_4	4	0.67 a	0.56 a	0.107	0.264 a	0.018 a
MD19	OMF (NPK+Mg)	4	1.59 ab	1.47 ab	0.125	0.744 ab	0.101 b
MD28	CF (NP)	4	3.00 bc	2.87 bc	0.126	1.217 b	0.028 a
MD38	CF (PK)	4	3.54 c	3.48 c	0.062	1.223 bc	0.010 a
MD37	PAPR	4	4.10 cd	4.02 cd	0.084	1.488 c	0.012 a
SP18	SPF (SSP)	4	5.52 d	5.40 d	0.124	2.206 d	0.020 a
MD2	SPF (TSP)	4	5.64 d	5.49 d	0.155	2.252 d	0.024 a

Values in the same column followed by the same letters are not significantly different at the 0.05 level. Duncan's test was done only for those parameters for which the one-factorial ANOVA had shown a significant effect of phosphorus treatments

- Summary of plant U concentration and uptake in the main Neubauer test, 2008:

The main Neubauer pot experiment results showed significant effects of phosphorus sources on uranium uptake by maize and sunflower seedlings, in most treatments more than 95% accumulated in roots. Uranium concentration and uptake were more strongly affected by phosphorus sources in roots than in shoots in both plant seedlings tested. In comparing the substrates effect on uranium uptake by seedlings, it was found that total uranium uptake in sand substrate was higher than in mixed soil/sand substrate in the fertiliser and phosphate rock treatments, but in the Blank and Control treatments it changed inversely. Total uranium uptake by sunflower seedlings in all phosphorus treatments except the MD19 treatment (OMF, NPK+Mg) was higher than that by maize seedlings in sand substrate. The highest uranium taken up by both plant seedlings was observed in completely water soluble, straight phosphorus-fertilisers, i.e., simple and triple superphosphates.

4.2.2.3 Influence of P sources on plant phosphorus concentration and uptake

GLM univariate analysis of variance showed a significant effect of phosphorus cources on plant phosphorus concentration and uptake in the main Neubauer experiment at the 0.001 level (*Table 4.23*). Also, substrate factor and plant factor had significant effect on all plant phosphorus concentration and uptake variables except in the case of plant factor effect on root phosphorus uptake. The interaction effects between type of plant and substrate in all phosphorus parameters were not significant. While interaction effects of plant factor and phosphorus source factor were significant plant phosphorus concentrations and uptake in roots and shoots at the 0.001 level.

Table 4.23 Significance of effects of P source factor, type of plants, and substrate on plant P concentration and uptake in the main Neubauer experiment (2008) by multi-factorial ANOVA

Parameters	**P source factor**	**Plant factor**	**Substrate factor**	**P source × Plant**	**P source × Substrate**	**Plant × Substrate**	**Plant × Substrate × P source**
P concentration of shoots	***	***	***	***	***	ns	ns
P concentration of roots	***	***	*	***	ns	ns	*
P uptake by shoots	***	***	***	***	***	ns	ns
P uptake by roots	***	ns	***	***	ns	ns	**
Total P uptake	***	***	***	***	***	ns	ns

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

In a total view, with regard to analyzing all data of both substrates and plants together, the means of phosphorus parameters for all phosphorus treatments and the Control were absolutely higher than those of the Blank in the main Neubauer pot experiment. The most of these differences also were significant by Duncan’s test at the 0.05 level (*Table 4.24*). The highest mean total phosphorus uptake by plant seedlings was in the MD2 treatment, a triple superphosphate fertiliser, which had a little difference (not significant) with the Control (reagent grade KH_2PO_4 treatment). The mean of total phosphorus uptake of these two treatments (MD2 and KH_2PO_4) were significantly different from (higher than) those of all other treatments (based on both plants and substrates data). There were observed some other significant differences between the rest of treatments in the case of the mean total phosphorus uptake by plant seedlings as well (*Table 4.24*). On the other hand, the lowest mean total phosphorus uptake was found in the Blank treatment that was significantly different from the mean phosphorus uptake of all other treatments except that of the MD38 (compound fertiliser, PK) treatment.

Table 4.24 Influences of P source factor on plant P uptake and concentration in the main Neubauer experiment 2008 (Duncan's test following multi-factorial ANOVA)

P treatment	**Type of fertiliser/ treatment** *	**n**	**Phosphorus uptake** *(mg pot^{-1})*			**Phosphorus concentration** *(mg g^{-1})*	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
Blank	-	16	33.7 a	6.4 a	27.3 ab	1.90 a	3.29 a
Control	KH_2PO_4	16	68.1 e	12.6 e	55.4 ef	3.62 c	6.89 d
MD38	CF (PK)	16	35.0 a	8.3 b	26.7 a	2.70 b	3.33 a
MD37	PAPR	16	38.9 b	8.0 b	30.9 b	2.51 b	3.72 b
MD19	OMF (NPK+Mg)	16	49.2 c	9.6 c	39.6 c	3.98 e	5.99 c
MD28	CF (NP)	16	59.4 d	9.9 c	49.5 d	3.84 cd	6.26 c
SP18	SPF (SSP)	16	63.0 d	11.0 d	52.0 de	3.66 cd	6.27 c
MD2	SPF (TSP)	16	68.5 e	11.9 de	56.7 f	3.71 cd	6.91 d

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

Table 4.25 Significance of the effects of substrate factor on plant P concentration and uptake in different substrates and the mean of them in the Neubauer experiment in 2008 by one-factorial ANOVA

Parameter	**n**	**Maize**			**Sunflower**		
		Sand	**Soil/sand**	**Sig. [a]**	**Sand**	**Soil/sand**	**Sig. [a]**
Shoots P concentration *(mg g^{-1})*	32	5.3	4.8	ns	6.0	5.3	ns
Roots P concentration *(mg g^{-1})*	32	2.7	2.6	ns	3.9	3.7	ns
Shoots P uptake *(mg pot^{-1})*	32	48.2	53.2	ns	32.4	35.3	ns
Roots P uptake *(mg pot^{-1})*	32	9.1	10.1	ns	9.3	10.3	ns
Total P uptake *(mg pot^{-1})*	32	57.2	63.3	ns	41.7	45.6	ns

a: significance of the effect of substrate factor on the parameter
ns: not significant

The effect of substrate factor on plant phosphorus concentration and uptake was not significant for maize and sunflower seedlings in the main Neubauer experiment (*Table 4.25*). However, the mean phosphorus concentration in shoots and roots of both plant seedlings in sand substrate was more than that in mixed soil/sand substrate. In contrast, the phosphorus uptake by shoots, roots, and total in sand substrate were less than those in mixed soil/sand substrate. This contrast can be explained by the effect of substrate factor on dry matter yield in maize and sunflower seedlings (see *Table 4.20* and previous section).

The highest mean of total phosphorus uptake by maize seedlings was found in the MD2 (TSP) and MD28 (CF, NP) treatments in sand and mixed soil/sand substrates, respectively (*Table 4.26*). The lowest mean of phosphorus uptake, by maize, was observed in the MD38 (CF, PK) treatment on both substrates, and it was significantly lower than that of the Blank on sand substrate. The low phosphorus uptake by maize from the compound PK-fertiliser (MD38) is due to low phosphorus concentration in shoots and roots of maize seedlings in both substrates and lower dry matter yield in sand substrate (*Table 4.19* and *4.26*). Phosphorus solubility of the MD38 fertiliser in all extractant was lower than other P-fertiliser except the PAPR fertiliser (MD37) and it could be the reason for lower phosphorus concentration in maize seedlings.

Anyhow lower phosphorus uptake in the MD38 treatment in comparison to the Blank is strange and may be related to other factors except its negligible phosphorus solubility.

Table 4.26 Influences of P source factor on plant P uptake and concentration in maize seedlings in mixed soil/sand and sand substrate in the main Neubauer experiment 2008, by Duncan's test following one-factorial ANOVA

P treatment	**Type of fertiliser/ treatment ***	**n**	**Phosphorus uptake** *(mg pot^{-1})*			**Phosphorus concentration** *(mg g^{-1})*	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
			In mixed soil/sand substrate				
Blank	-	4	42.0 a	8.4 ab	33.5 a	1.94 a	3.25 a
Control	KH_2PO_4	4	79.7 c	14.6 d	65.1 cd	3.19 c	5.61 bc
MD38	CF (PK)	4	41.6 a	7.7 a	33.9 a	1.92 a	3.01 a
MD37	PAPR	4	44.0 a	8.0 ab	36.0 a	1.97 a	3.22 a
SP18	SPF (SSP)	4	68.0 b	11.7 c	56.2 b	3.09 bc	5.36 b
MD2	SPF (TSP)	4	75.5 c	12.6 c	62.8 c	2.97 bc	5.97 cd
MD19	OMF (NPK+Mg)	4	75.8 c	8.5 ab	67.3 cd	2.76 b	5.85 bcd
MD28	CF (NP)	4	80.2 c	9.4 b	70.7 d	2.85 bc	6.15 d
			In sand substrate				
Blank	-	4	41.0 b	6.9 ab	34.3 b	1.61 a	3.82 c
Control	KH_2PO_4	4	76.0 e	12.7 d	61.1 cde	3.72 f	7.40 f
MD38	CF (PK)	4	24.8 a	5.9 a	18.9 a	1.96 ab	2.38 a
MD37	PAPR	4	36.3 b	6.7 ab	28.6 b	2.05 b	3.03 b
MD19	OMF (NPK+Mg)	4	61.4 c	7.7 b	54.5 c	2.55 c	5.58 d
MD28	CF (NP)	4	66.7 cd	8.1 b	58.6 cd	3.29 de	6.65 e
SP18	SPF (SSP)	4	72.1 de	9.7 c	62.4 de	3.01 d	6.43 e
MD2	SPF (TSP)	4	79.5 e	14.8 e	66.8 e	3.45 ef	7.24 f

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

Table 4.27 Influences of P source factor on P uptake and concentration in sunflower seedlings on different substrates in the main Neubauer experiment 2008, by Duncan's test following one-factorial ANOVA

P treatment	Type of fertiliser/ treatment*	n	Phosphorus uptake (mg pot^{-1})			Phosphorus concentration (mg g^{-1})	
			Total	Roots	Shoots	Roots	Shoots
			In mixed soil/sand substrate				
Blank	-	4	29.5 a	6.0 a	23.5 a	2.14 a	3.40 a
Control	KH_2PO_4	4	58.8 c	13.2 d	45.6 b	3.88 cd	6.19 b
MD37	PAPR	4	37.3 b	8.1 ab	29.2 a	2.97 b	4.23 a
MD38	CF (PK)	4	37.6 b	9.4 bc	28.2 a	3.31 bc	3.82 a
MD19	OMF (NPK+Mg)	4	38.2 b	11.2 cd	27.1 a	4.78 e	5.89 b
MD28	CF (NP)	4	50.5 c	11.6 cd	38.9 b	4.70 e	5.92 b
SP18	SPF (SSP)	4	54.5 c	11.3 cd	43.2 b	3.87 cd	5.98 b
MD2	SPF (TSP)	4	58.6 c	11.8 cd	46.8 b	4.13 de	6.61 b
			In sand substrate				
Blank	-	4	22.4 a	4.7 a	17.8 ab	1.92 a	2.84 a
Control	KH_2PO_4	4	57.8 c	7.8 b	50.0 d	3.67 bcd	8.34 f
MD19	OMF (NPK+Mg)	4	21.3 a	11.7 d	9.6 a	5.80 e	6.66 cd
MD38	CF (PK)	4	35.9 b	10.0 bcd	25.8 bc	3.60 bc	3.96 b
MD37	PAPR	4	38.0 b	8.3 bc	29.7 c	3.04 b	4.40 b
MD28	CF (NP)	4	40.3 b	10.7 bcd	29.6 c	4.52 cd	6.34 c
SP18	SPF (SSP)	4	57.4 c	11.2 cd	46.3 d	4.67 d	7.30 de
MD2	SPF (TSP)	4	60.6 c	10.2 bcd	50.4 d	4.29 cd	7.81 ef

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

The lowest phosphorus concentration and phosphorus uptake in shoots and roots and total phosphorus uptake in sunflower seedlings on mixed soil/sand substrate were found in Blank treatment. The highest total phosphorus uptake by sunflower seedlings on mixed soil/sand and sand substrate were found in the KH_2PO_4 and MD2 treatments, respectively (*Table 4.27*). Anyhow, there was no significant difference between the SP18 treatment, the MD2 treatment, and the Control in the case of total phosphorus uptake by sunflower seedlings on both substrates (*Table 4.27*). Comparing the phosphorus parameters in sunflower seedlings on different substrates showed more significant differences between the various phosphorus sources in sand substrate than in mixed soil/sand substrate by Duncan’s test at the 0.05 level.

- Summary of P source effects on the P concentration and uptake of maize and sunflower seedlings, 2008:

As it was expected, the effect of P source factor on P concentration and uptake in shoots and roots of both maize and sunflower seedlings was significant, and the total phosphorus uptake by maize and sunflower seedlings in all P-fertiliser treatments was higher in comparison to the Blank except in the compound PK-fertiliser (MD38) in maize. In all cases (crops and substrates) the highest plant P uptake in Neubauer experiment was found in completely water soluble fertiliser, TSP, except in case of maize on mixed soil/sand that it was found in the compound NP-fertiliser. The effect of the kind of substrate on phosphorus uptake and concentration in none of maize and sunflower seedlings was significant.

4.3 Carrot pot experiments

According to the literature, most uranium taken up by plants is accumulated in plant roots tissues. In the cereal plants, leaf vegetables, and fruits this could prevent uranium from entering to the human food chain. But in the case of root crops and root vegetables, uranium accumulation in roots can intensify uranium entering the food chain. The effects of different phosphorus sources on uranium and phosphorus concentration and uptake and their transferability to carrot plants as a root vegetable was studied here by carrot pot experiments. The effects of substrate on uranium and phosphorus transferability from different phosphorus sources to carrot plants were studied, too.

4.3.1 The pilot carrot pot experiment, 2007

Carrot pot experiment was started by a pilot pot experiment using two various phosphorus sources plus control, two varieties, and two substrates including soil and sand. This experiment was done to identify probable effects of three mentioned factors on phosphorus and uranium uptake and transferability to carrot plants, and managing the main carrot pot experiment with more samples of phosphorus sources.

The GLM univariate analyses of variance showed that the phosphorus sources, the carrot varieties, and the substrates significantly affected the produced dry matter of shoots, roots, and total dry matter of carrot plants with exception of the roots dry matter that was not significantly affected by the variety factor (*Table 4.28*).

About the uranium parameters, the effects of the phosphorus source factor were significant on the uranium uptake by roots and the total uranium uptake at the 0.01 level. The total uranium uptake, and the uranium uptake by roots and shoots were significantly different in the soil and sand substrates (P<0.001). The effect of the variety factor was only significant on the uranium concentration and uranium uptake in the carrot roots at the 0.01 level (*Table 4.29*).

Table 4.28 Significance of effects of variety, substrate, and P source factors on dry matter yield in the carrot pot experiment 2007, analyzed by multi-factorial ANOVA

Parameters	**Variety factor**	**Substrate factor**	**P source factor**	**Variety × Substrate**	**Variety × P source**	**Substrate × P source**	**Variety × Substrate × P source**
Dry matter of shoots	***	***	***	***	ns	***	**
Dry matter of roots	ns	***	***	ns	ns	***	ns
Total dry matter	***	***	***	ns	ns	***	ns

, *, and ns: significant effect at the 0.01, 0.001 levels and not significant, respectively.

Table 4.29 Significance of effects of variety, substrate, and P source factors on plant U concentration and uptake in the carrot pot experiment 2007, tested by multi-factorial ANOVA

Parameters	**Variety factor**	**Substrate factor**	**P source factor**	**Variety × Substrate**	**Variety × P source**	**Substrate × P source**	**Variety × Substrate × P source**
U concentration of shoots	ns	ns	ns	**	ns	ns	ns
U concentration of roots	**	ns	ns	ns	ns	ns	*
U uptake by shoots	ns	***	ns	ns	*	***	ns
U uptake by roots	**	***	***	*	ns	**	ns
Total U uptake	ns	***	**	**	ns	***	ns

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

Table 4.30 Significance of effects of variety, substrate, and P source factors on plant P concentration and uptake in the carrot pot experiment 2007, tested by multi-factorial ANOVA

Parameters	**Variety factor**	**Substrate factor**	**P source factor**	**Variety × Substrate**	**Variety × P source**	**Substrate × P source**	**Variety × Substrate × P source**
P concentration of shoots	ns	*	***	ns	ns	***	ns
P concentration of roots	***	***	***	ns	*	***	ns
P uptake by shoots	***	***	***	***	***	***	ns
P uptake by roots	ns	***	***	ns	ns	**	ns
Total P uptake	***	***	***	**	ns	***	ns

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

All phosphorus parameters including uptake and concentration of phosphorus in carrot shoots and roots, and also total phosphorus uptake were affected significantly by the phosphorus source factor and the substrate factor, but the variety effect was not significant on the phosphorus concentration of shoots and phosphorus uptake in the carrot roots at the 0.05 level (*Table 4.30*).

Because of significant effect of the substrate factor and also significant interaction effects with variety and fertiliser factors on most studied parameters, carrot experiment data were statistically analysed in the sand and soil substrates separately. The GLM univariate analysis of variance of data, in each substrate, confirmed significant differences between effects of the phosphorus source factor on most of the parameters investigated in the present study. Shoots dry matter, roots dry matter, total dry matter, uranium concentration in the shoots and roots, uranium uptake by carrot shoots and roots, total uranium uptake, and phosphorus uptake by roots were the parameters which were not affected by the various phosphorus sources in the soil substrate while, in sand substrate, significant effects of the phosphorus source factor were observed on all of them except on uranium concentration of roots (in uranium concentration of shoots at the 0.05 level and for other parameters at the 0.001 level). More details of these results are presented in the next sections.

4.3.1.1 Influence of P sources on dry matter yield

In sand substrate, the effect of phosphate rock (the MD37 treatment) on shoots and roots dry matter, and total dry matter of carrot plants was significantly different from the effects of the Control and single superphosphate (the SP18 treatment) at the 0.05 level, and there was no significant difference between these last two treatments (*Table 4.31*). In soil substrate, no significant difference was observed between phosphorus treatments on total dry matter and shoots dry matter of carrot plants, but a significant difference between the effect of phosphate rock and the Control on roots dry matter was observed at the 0.05 level (*Table 4.31*).

Table 4.31 Influences of different P source applications on dry matter yield in the pilot carrot pot experiment, by Duncan's test following one-factorial ANOVA

Treatments	**Sand substrate (n=8)**			**Soil substrate (n=8)**		
	Shoots dry matter *(g)*	**Roots dry matter** *(g)*	**Total dry matter** *(g)*	**Shoots dry matter** *(g)*	**Roots dry matter** *(g)*	**Total dry matter** *(g)*
Control (KH_2PO_4)	26.78 b	43.83 b	70.61 b	37.48 a	89.46 b	126.93 a
Phosphate rock (MD37)	3.49 a	6.03 a	9.52 a	44.00 a	80.78 a	124.78 a
Superphosphate (SP18)	27.40 b	50.64 b	78.04 b	37.99 a	84.09 ab	122.08 a

Values in the same column followed by the same letters are not significantly different by Duncan's test at the 0.05 level.

4.3.1.2 Influence of P sources on uranium concentration and uptake

One-factorial statistical analyses of data in soil substrate not only showed no significant differences between uranium parameters in phosphate rock and superphosphate treatments, but also confirmed no significant difference between effects of these fertilisers and the Control (KH_2PO_4) on uranium concentration and uptake of shoots and roots of carrot plants (*Table 4.32*).

In contrast to soil substrate, in sand substrate significant differences between superphosphate and phosphate rock treatments in shoots and roots uranium uptake and total uranium uptake were observed, while there was no significant difference between them in the case of uranium concentration in shoots and roots of carrot plants (*Table 4.33*). On the other hand, there was no significant difference between the superphosphate and KH_2PO_4 treatments with regard to uranium uptake parameters and also uranium concentration of roots at the 0.05 level. It shows those differences between shoots' and roots' uranium uptake and total uranium uptake in phosphate rock and superphosphate treatments may have been caused by differences of dry matter yield of shoots and roots in those treatments.

In sand substrate, there was no significant difference between various phosphorus sources for uranium concentration of carrot roots, but a significant difference was observed between the superphosphate treatment and the Control in the case of uranium concentration of shoots (*Table 4.33*).

Table 4.32 Influences of P source factor on plant U concentration and uptake in soil substrate in the pilot carrot pot experiment, by Duncan's test following one-factorial ANOVA

Treatments	**Shoots U con.** *($\mu g\ g^{-1}$)*	**Roots U con.** *($\mu g\ g^{-1}$)*	**Shoots U uptake** *(μg)*	**Roots U uptake** *(μg)*	**Total U uptake** *(μg)*
Control (KH_2PO_4), n=7	0.024 a	0.020 a	0.856 a	1.808 a	2.664 a
Superphosphate (SP18) , n=8	0.023 a	0.027 a	0.849 a	2.231 a	3.080 a
Phosphate rock (MD37), n=7	0.038 a	0.024 a	1.338 a	1.934 a	3.273 a

Values in the same column followed by the same letters are not significantly different by Duncan's test at the 0.05 level.

Table 4.33 Influences of P source factor on plant P concentration and uptake in sand substrate in the pilot carrot pot experiment, by Duncan's test following one-factorial ANOVA

Treatments	**Shoots U con.** *($\mu g\ g^{-1}$)*	**Roots U con.** *($\mu g\ g^{-1}$)*	**Shoots U uptake** *(μg)*	**Roots U uptake** *(μg)*	**Total U uptake** *(μg)*
Control (KH_2PO_4), n=8	0.021 a	0.027 a	0.550 b	1.206 b	1.757 b
Phosphate rock (MD37), n=8	0.026 ab	0.027 a	0.092 a	0.149 a	0.241 a
Superphosphate (SP18), n=8	0.028 b	0.030 a	0.783 b	1.517 b	2.300 b

Values in the same column followed by the same letters are not significantly different by Duncan's test at the 0.05 level.

4.3.1.3 Influence of P sources on phosphorus concentration and uptake

Plant phosphorus concentration and uptake were investigated in carrot pot experiments. The lowest and highest amounts of all phosphorus parameters in soil substrate were observed in the phosphate rock (MD37) and Control (KH_2PO_4) treatments, respectively. There was significant difference between every two phosphorus treatments in the case of shoots' phosphorus concentration, roots' phosphorus uptake, and total phosphorus uptake by carrot plants, while no significant effect of various phosphorus sources was observed on shoots' phosphorus uptake. The phosphate rock (MD37) treatment was significantly different from other two treatments in terms of roots' phosphorus concentration (*Table 4.34*).

In sand substrate, the lowest amount of the all phosphorus parameters was found in the phosphate rock treatment, that was significantly different from both other treatments at the 0.05 level (*Table 4.35*); and the highest amounts of the all phosphorus parameters were observed in the Control (KH_2PO_4). In the case of sand substrate, the effect of superphosphate treatment on phosphorus concentration of shoots and roots and phosphorus uptake by shoots of carrot plants was significantly different from the Control (*Table 4.35*).

Table 4.34 Influences of P source applications on plant P concentration and uptake in soil substrate in the pilot carrot pot experiment, by Duncan's test following one-factorial ANOVA

Treatments	**Shoots P con.** *($mg\ g^{-1}$)*	**Roots P con.** *($mg\ g^{-1}$)*	**Shoots P uptake** *(mg)*	**Roots P uptake** *(mg)*	**Total P uptake** *(mg)*
Control (KH_2PO_4), n=7	2.78 c	3.72 b	105.8 a	331 c	437 c
Phosphate rock (MD37), n=7	1.54 a	2.59 a	69.3 a	209 a	278 a
Superphosphate (SP18), n=8	2.21 b	3.46 b	85.8 a	289 b	375 b

Values in the same column followed by the same letters are not significantly different by Duncan's test at the 0.05 level.

Table 4.35 Influences of P source applications on plant P concentration and uptake in sand substrate in the pilot carrot pot experiment, by Duncan's test following one-factorial ANOVA

Treatments	**Shoots P con.** *(mg g^{-1})*	**Roots P con.** *(mg g^{-1})*	**Shoots P uptake** *(mg)*	**Roots P uptake** *(mg)*	**Total P uptake** *(mg)*
Control (KH_2PO_4), n=8	4.06 c	3.71 c	107.6 c	158 b	266 b
Phosphate rock (MD37), n=8	0.91 a	0.84 a	3.4 a	5.2 a	8.6 a
Superphosphate (SP18), n=8	2.44 b	3.09 b	66.5 b	157 b	224 b

Values in the same column followed by the same letters are not significantly different by Duncan's test at the 0.05 level.

- Summary of the pilot carrot pot experiment, 2007:

The effects of P source, plant variety, and substrate factors on dry matter yield of carrot plants were significant. Because of very low initial available P in sand substrate, the effect of P sources on dry matter yield in sand substrate was more strongly significant than in soil substrate. The roots and total uranium uptake of carrot plants were significantly influenced by fertiliser-derived uranium, while it seemed that the role of dry matter yield variation on that was very important, and in contrast to sand substrate in the case of soil substrate were not found any significant differences between P sources regarding to plant uranium concentration and uptake. Total uranium uptake was not significantly affected by variety factor while in the case of roots' uranium uptake the effect of variety was significant. Anyhow with regard to some problems like powdery mildew disease that affected the Kazan variety, only the Napoli variety was selected for the main carrot experiment in 2008.

4.3.2 The main carrot pot experiment, 2008

By the main carrot pot experiment, uranium and phosphorus transfer to carrot plants from seven different phosphorus sources, also their effects on dry matter, uranium concentration, and phosphorus concentration of shoots and roots of carrot plants were studied in sand and mixed soil/sand substrates. The results are presented in the following sections.

4.3.2.1 Influence of P sources on dry matter yield

P source factor and substrate factor significantly affected plant dry matter yield of carrot plants; the first order interaction of phosphorus source and substrate factors also was significant at the 0.001 level (*Table 4.36*). Significant effect of phosphorus source factor on dry matter yield was found in sand and mixed soil/sand substrates separately at the 0.001 level, too (*Table 4.39*). The highest means of dry matter yield were found in the KH_2PO_4 treatment, and the lowest means were in the MD28 treatment. Also, this trend was found on mixed soil/sand substrate. When looking separately in substrates; however, the highest and lowest mean of dry matter yield in sand substrate were found in the MD19 treatment and Blank, respectively (*Table 4.40*). Some significant differences were found between total dry matters in different phosphorus treatments. These differences were not in the same trend when looking in each substrate separately (*Table 4.37* and *Table 4.40*).

Table 4.36 Significance of effects of P source and substrate factors on dry matter yield and plant U concentration and uptake in the main carrot pot experiment 2008, tested by multi-factorial ANOVA

Parameters	P source factor	Substrate factor	P source× Substrate
Dry matter of shoots	***	***	***
Dry matter of roots	***	***	***
Total dry matter	***	***	***
Uranium concentration of shoots	***	*	***
Uranium concentration of roots	*	ns	ns
Uranium uptake by shoots	***	*	***
Uranium uptake by roots	***	***	***
Total uranium uptake	***	*	***

*, **, ***, and ns: significant effect at the 0.05, 0.01, 0.001 levels and not significant, respectively.

Table 4.37 Influences of P source factor on dry matter yield in the main carrot pot experiment 2008, tested by Duncan's test following multi-factorial ANOVA

P treatment	**Type of fertiliser/ treatment** *	**n**	**Dry matter of shoots** *(g)*	**Dry matter of roots** *(g)*	**Total dry matter** *(g)*
Blank	-	7	11.13 ab	35.18 b	46.31 b
Control	KH_2PO_4	7	19.85 d	60.86 e	80.71 e
MD28	CF (NP)	5	8.33 a	19.48 a	27.81 a
MD37	PAPR	7	8.51 a	30.84 b	39.35 ab
MD35	PAPR	7	11.66 b	40.18 bc	51.84 bc
MD19	OMF (NPK+Mg)	6	15.90 c	47.84 cd	63.74 cd
SP18	SPF (SSP)	8	17.22 cd	51.30 cde	68.52 de
MD1	SPF (TSP)	7	18.92 d	56.20 de	75.11 de
MD2	SPF (TSP)	8	19.53 d	56.47 de	76.00 de

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

4.3.2.2 Influence of P sources on uranium concentration and uptake

The influence of phosphorus source factor on uranium concentration in shoots and roots of carrot was significant at the 0.001 and 0.05 level, respectively. Various phosphorus sources significantly affected the uranium uptake by shoots and roots, and total uranium uptake in carrot plants at the 0.001 level (*Table 4.36*).

In the case of uranium concentration in shoots and roots of carrot, a few significant differences were found between 9 various phosphorus treatments, applied in the present study. Uranium concentration of shoots in the MD19 (the highest one) and MD28 were significantly different from each other, and significantly more than shoots uranium concentration in all other phosphorus treatments, while there was no significant difference between other treatments in the case of uranium concentration in carrot shoots.

For uranium concentration in roots of carrot, only the MD19 was significantly higher than that of other treatments, and there was no significant difference between other phosphorus treatments. The lowest uranium concentration of shoots and roots of carrot plants were found in the Control (KH_2PO_4 treatment), but they were not significantly different from that of all other phosphorus treatments except from that of the MD19 and MD28 treatments in the case of uranium concentration in shoots, and from that of the MD19 treatment in the case of roots uranium concentration. Anyhow looking at replications of the MD19 and MD28 showed much more variation comparing to the Control (*Figure 4.1*), the most reason for the variations is the salt effect of these two fertilisers with regard to the amounts applied in this experiment (see *Table 4.48*) and sensitivity of carrot plant to the salinity (Blaylock, 1994; Grattan, 2002). It seems that another study should be done with a lower amount of these fertilisers for a better decision on the effect of these fertilisers on uranium concentration in root vegetables like carrot, and the uranium transferring to their edible parts.

Carrot-Soil-MD19 Carrot-Sand-MD19

Carrot-Soil-MD28 Carrot-Sand-MD28

Carrot-Soil-Control (KH_2PO_4) Carrot-Sand- Control (KH_2PO_4)

Figure 4.1 Comparing carrot plants harvested of the MD19 and MD28 fertilisers with the Control in the main carrot pot experiments

Table 4.38 Significance of differences between carrot U uptake and concentration in various P treatments in the main carrot pot experiment 2008, by Duncan's test following multi-factorial ANOVA

P treatment	Type of fertiliser/ treatment *	n	Uranium uptake (*µg pot^{-1}*)			Uranium concentration (*µg g^{-1}*)	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
Blank	-	7	0.61 ab	0.44 ab	0.17 a	0.017 a	0.017 a
Control	KH_2PO_4	7	0.92 bcd	0.66 bc	0.26 bc	0.011 a	0.013 a
MD37	PAPR	7	0.51 a	0.37 a	0.14 a	0.015 a	0.018 a
MD28	CF (NP)	5	0.63 abc	0.35 a	0.29 bc	0.019 a	0.049 b
MD35	PAPR	7	0.73 abcd	0.53 abc	0.20 ab	0.021 a	0.026 a
MD1	SPF (TSP)	7	0.95 cd	0.66 bc	0.29 bc	0.012 a	0.015 a
SP18	SPF (SSP)	8	1.03 d	0.74 c	0.29 bc	0.016 a	0.017 a
MD2	SPF (TSP)	8	1.05 d	0.75 c	0.30 c	0.014 a	0.016 a
MD19	OMF (NPK+Mg)	6	2.46 e	1.29 d	1.17 d	0.035 b	0.072 c

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

Table 4.39 The effect of P source factor on plant U concentration and uptake, and dry matter yield in different substrates in the main carrot pot experiment 2008, tested by one-factorial ANOVA

Substrate	n	U concentration		U uptake			Dry matter		
		Roots	**Shoots**	**Total**	**Roots**	**Shoots**	**Total**	**Roots**	**Shoots**
Mixed soil/sand	34	ns	***	***	***	***	***	***	***
Sand	28	***	***	***	***	***	***	***	***

*** and ns: significant effect at the 0.001 level and not significant, respectively.

In comparison to uranium concentrations in carrot plants, more significant differences were found between the mean of uranium uptake in various phosphorus treatments caused by high differences between dry matter yield in various fertiliser treatments, especially between completely water soluble fertilisers and poorly water soluble fertilisers, i.e., between straight fertilisers and partially acidulated phosphate rocks, respectively (*Table 4.38*).

Table 4.40 Influences of P source factor on dry matter yield in different substrates in the main carrot pot experiment 2008, by Duncan's test following one-factorial ANOVA

P treatment	**Type of fertiliser/ treatment** *	**Mixed soil/sand substrate**				**Sand substrate**			
		n	**Total DM**** *(g)*	**Roots DM** *(g)*	**Shoots DM** *(g)*	**n**	**Total DM***(g)*	**Roots DM** *(g)*	**Shoots DM** *(g)*
Blank	-	4	79.12 c	60.30 c	18.82 cd	3	2.55 a	1.68 a	0.87 a
Control	KH_2PO_4	4	99.21 d	75.57 d	23.64 e	3	56.04 cde	41.25 cd	14.79 bc
MD28	CF (NP)	3	24.57 a	18.82 a	5.75 a	2	32.69 bc	20.48 ab	12.21 b
MD19	OMF (NPK+Mg)	4	56.15 b	41.56 b	14.59 b	2	78.91 e	60.40 d	18.51 c
MD35	PAPR	4	84.03 cd	65.48 cd	18.55 cd	3	8.92 ab	6.45 a	2.47 a
MD37	PAPR	3	85.39 cd	67.16 cd	18.23 c	4	4.82 a	3.60 a	1.22 a
MD1	SPF (TSP)	4	87.21 cd	66.12 cd	21.09 cde	3	58.98 de	42.97 cd	16.01 bc
SP18	SPF (SSP)	4	90.61 cd	68.72 cd	21.89 de	4	46.43 cd	33.88 bc	12.55 b
MD2	SPF (TSP)	4	92.30 cd	69.22 cd	23.08 e	4	59.70 de	43.72 cd	15.98 bc

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

* see the list of abbreviations

** dry matter yield

For studying the effect of fertiliser-derived uranium on uranium concentration and uptake in carrot plants in mixed soil/sand and sand substrates the data of carrot experiment were analysed for each substrate separately. The results were as follows:

- *Mixed soil/sand substrate:* In mixed soil/sand substrate the influence of phosphorus source factor on carrot uranium concentration and uptake was significant at the 0.001 level (*Table 4.39*). For more details of differences between various phosphorus treatments, the results of Duncan's test are presented in *Table 4.41*. In mixed soil/sand substrate no significant difference was observed between the Blank, Control (KH_2PO_4), MD1, MD2, SP18, MD35, and MD37 treatments for uranium concentration and uptake in carrot plants. There were a few significant differences between various phosphorus treatments in the case of carrot uranium uptake and concentration in mixed soil/sand substrate, as follows:

- the mean uranium uptake by shoots of carrot plant in the MD19 treatment was significantly more than all other treatments;

- the lowest and highest mean of uranium uptake by roots and total uranium uptake, which were respectively found in the MD28 and MD19 fertilisers, were significantly different from each other and other treatments;
- the mean uranium concentration of shoots in the MD28 and MD19 were significantly higher than that of other phosphorus treatments; nevertheless, there was no significant difference between these two treatments or between all other treatments in the case of uranium concentration of carrot shoots (*Table 4.41*).

Table 4.41 Influences of P source factor on plant U uptake and concentration in mixed soil/sand substrate in the main carrot pot experiment 2008, by Duncan's test following one-factorial ANOVA

P treatment	**Type of fertiliser/ treatment***	**n**	**U uptake** (*µg pot^{-1}*)			**U concentration** (*µg g^{-1}*)	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
Blank	-	4	1.027 b	0.750 b	0.278 a	0.012	0.015 a
Control	KH_2PO_4	4	1.091 b	0.789 b	0.301 a	0.010	0.013 a
MD28	CF (NP)	3	0.593 a	0.300 a	0.293 a	0.018	0.067 b
MD1	SPF (TSP)	4	1.013 b	0.720 b	0.293 a	0.011	0.014 a
MD35	PAPR	4	1.052 b	0.778 b	0.274 a	0.012	0.015 a
MD2	SPF (TSP)	4	1.055 b	0.740 b	0.315 a	0.011	0.014 a
MD37	PAPR	3	1.077 b	0.774 b	0.303 a	0.012	0.017 a
SP18	SPF (SSP)	4	1.159 b	0.813 b	0.347 a	0.012	0.016 a
MD19	OMF (NPK+Mg)	4	1.832 c	1.127 c	0.705 b	0.039	0.049 b

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

- Sand substrate: In sand substrate, phosphorus source factor significantly influenced all uranium parameters of carrot plants, including uranium concentration of shoots and roots, uranium uptake by shoots and roots, and total uranium uptake at the 0.001 level (*Table 4.39*). Differences between phosphorus treatments, which were statistically analyzed by Duncan's test at the 0.05 level, are presented in *Table 4.42*. The mean of uranium concentration in carrot shoots in the MD19 treatment was the highest, and significantly different from that of all other treatments (in agreement with results of mixed soil/sand substrate), and that of the MD35 (PAPR) treatment also was significantly different from the others; while, no significant difference was found between the rest of phosphorus treatments in the case of shoots' uranium concentration (*Table 4.41*).

Table 4.42 Influences of P source factor on plant U uptake and concentration in sand substrate in the main carrot pot experiment 2008, by Duncan's test following one-factorial ANOVA

P treatment	**Type of fertiliser/ treatment**[*]	**n**	**U uptake** *($\mu g\ pot^{-1}$)*			**U concentration** *($\mu g\ g^{-1}$)*	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
Blank	-	3	0.055 a	0.036 a	0.018 a	0.022 cd	0.021 a
Control	KH_2PO_4	3	0.686 abc	0.478 abc	0.208 bc	0.012 a	0.014 a
MD37	PAPR	4	0.084 a	0.062 a	0.023 a	0.018 abc	0.019 a
MD35	PAPR	3	0.304 ab	0.208 ab	0.095 ab	0.032 e	0.041 b
MD28	CF (NP)	2	0.693 abc	0.420 abc	0.273 c	0.021 cd	0.022 a
MD1	SPF (TSP)	3	0.875 bc	0.590 bc	0.286 c	0.014 ab	0.018 a
SP18	SPF (SSP)	4	0.904 bc	0.677 bc	0.227 bc	0.020 bcd	0.018 a
MD2	SPF (TSP)	4	1.042 c	0.750 c	0.291 c	0.017 abc	0.018 a
MD19	OMF (NPK+Mg)	2	3.712 d	1.608 d	2.104 d	0.026 de	0.116 c

[*] see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

In sand substrate in comparison to mixed soil/sand substrate, many more differences were found between phosphorus treatments with regard to uranium concentration in roots, uranium uptake by shoots and roots, and total uranium uptake in carrot plants. The highest mean of uranium concentration in carrot roots on sand substrate was found in the MD35 treatments, and was significantly different from others. The highest mean of uranium uptake by shoots, roots, and total were found in the MD19 treatment; these data were 2.1, 1.6, and 3.7 micrograms per pot, respectively (*Table 4.42*). Although the lowest mean uranium concentration in shoots and roots of carrot plants were found in the Control, the lowest mean uranium uptake by shoots and roots, and total uranium uptake were found in the Blank (this was caused by low dry matter yield of carrot plants on sand substrate under extreme phosphorus deficiency, see *Table 4.40* for more information).

Although the effect of various phosphorus-fertilisers (fertiliser-derived uranium) on uranium concentration in roots of carrot in sand substrate was significant but the variation of roots' uranium concentration values in carrot plants on sand substrate (from 0.012 $\mu g\ kg^{-1}$ in the Control to 0.032 $\mu g\ kg^{-1}$ in the MD35 fertiliser) was absolutely less than those of maize and sunflower on sand substrate (*Table 4.21*, *4.22*, and *4.42*).

4.3.2.3 Influence of P sources on phosphorus concentration and uptake

Plant phosphorus concentration and uptake by carrot plants were significantly affected by phosphorus source factor and substrate factor in the main carrot pot experiment at the 0.001 level (*Table 4.43*). With regard to significant effect of substrate factor and significant interaction between substrate factor and phosphorus source factor, data of the main carrot pot experiment were statistically analyzed separately. The results of these analyzing are presented in *Table 4.44, Table 4.46,* and *Table 4.47*. The effect of various phosphorus sources on phosphorus concentration and uptake of carrot plants were significant in mixed soil/sand and sand substrate at the 0.001 level as well (*Table 4.44*).

Table 4.43 Significance of effects of P source and substrate factors on plant P concentration and uptake in the main carrot pot experiment 2008, analyzed by multi-factorial ANOVA

Parameters	**P source factor**	**Substrate factor**	**P source × Substrate**
P concentration of shoots	***	***	***
P concentration of roots	***	***	***
P uptake by shoots	***	***	***
P uptake by roots	***	***	***
Total P uptake	***	***	***

***: significant effect at the 0.001 level

Table 4.44 The effect of P source factor on plant P uptake and concentration in different substrates, analysed by one-factorial ANOVA

Substrate	**n**	**P uptake**			**P concentration**	
		Total	**Roots**	**Shoots**	**Roots**	**Shoots**
Mixed soil/sand	34	***	***	***	***	***
Sand	28	***	***	***	***	***

***: significant effect at the 0.001 level by one-factorial ANOVA

No significant differences were found between the Blank, and the two partially acidulated phosphate rocks (PAPR) (MD35 and MD37 fertilisers) in the case of plant phosphorus concentration and uptake (in/by shoots and roots, and total). In the case of roots' and total phosphorus uptake by carrots, the compound fertiliser (MD28, NP) also had no significant difference with the Blank, and two PAPR treatments while phosphorus concentration in this treatment was significantly higher than those of the Blank, and two PARPs (*Table 4.45*). Low root and total phosphorus uptake in the MD28 fertiliser was resulted by low dry matter yield due to salinity effect of this fertiliser treatment (for more details see *Table 3.12* and *Table 4.40*).

The highest mean of total phosphorus uptake and shoots phosphorus uptake by carrot plants were found in a triple superphosphate treatment (the MD1 fertiliser) with amount of 291 and 77 $mg\ pot^{-1}$, respectively, and the highest mean of phosphorus uptake by roots of carrot plant was 214 $mg\ pot^{-1}$ found in the KH_2PO_4 (Control). Anyhow, the differences between these 3 treatments (KH_2PO_4, MD1, and MD2) were not significant in any case (neither root nor shoot P uptake, nor total P uptake) (*Table 4.45*).

Table 4.45 Influences of P source factor on plant P uptake and concentration in the main carrot pot experiment 2008, by Duncan's test following the multi-factorial ANOVA

P treatment	**Type of fertiliser/ treatment** *	**n**	**P uptake** *(mg pot^{-1})*			**P concentration** *(mg g^{-1})*	
			Total	**Roots**	**Shoots**	**Roots**	**Shoots**
Blank	-	7	86.2 a	71.6 a	14.6 a	1.51 a	1.14 a
Control	KH_2PO_4	7	281.6 cd	214.2 c	67.4 cd	3.45 c	3.30 c
MD37	PAPR	7	71.9 a	60.5 a	11.4 a	1.32 a	1.08 a
MD35	PAPR	7	88.6 a	74.1 a	14.5 a	1.40 a	1.08 a
MD28	CF (NP)	5	102.1 a	68.5 a	33.6 b	3.53 cd	3.90 d
MD19	OMF (NPK+Mg)	6	189.7 b	146.0 b	43.7 b	3.13 b	2.73 b
SP18	SPF (SSP)	8	241.3 c	181.7 c	59.5 c	3.58 cd	3.42 c
MD2	SPF (TSP)	8	275.0 cd	200.8 c	74.2 d	3.57 cd	3.76 d
MD1	SPF (TSP)	7	290.5 d	213.8 c	76.7 d	3.77 d	4.03 d

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

- *Mixed soil/sand:* Comparison of various phosphorus effects on phosphorus concentration and phosphorus uptake by carrot plants separately in each substrate was better clarified by comparing them without differentiating substrates. In mixed soil/sand substrate, the lowest phosphorus uptakes (by shoots, roots, and total) were found in the MD28 treatment, and significantly different from all other treatments in roots and total phosphorus uptake. While phosphorus concentration of shoots in the MD28 treatment was significantly more than that of the Blank, MD35, MD37, and MD19 treatments, and also phosphorus concentration of carrot roots in the MD28 treatment was significantly higher than roots' phosphorus concentration in the Blank, MD35, MD37 treatments (*Table 4.46*). With regard to the type and composition of the MD28 fertiliser (*Table 3.12*) and produced dry matter (*Table 4.40*), it can be concluded that low total phosphorus uptake was related to negative salt effects of the fertiliser on carrot seedlings and on the carrot growth resulting in low dry matter yield.

Table 4.46 Influences of P source factor on plant P uptake and concentration on mixed soil/sand substrate in the main carrot pot experiment 2008, by Duncan's test following one-factorial ANOVA

P treatment	**Type of fertiliser/ treatment** *	**n**	**P uptake** *(mg pot^{-1})* Total	Roots	Shoots	**P concentration** *(mg g^{-1})* Roots	Shoots
Blank	-	4	149.3 b	124.3 b	25.0 a	2.06 a	1.34 a
Control	KH_2PO_4	4	365.0 c	277.8 c	87.2 c	3.68 cd	3.68 c
MD28	CF (NP)	3	83.5 a	63.7 a	19.7 a	3.41 bc	3.53 c
MD35	PAPR	4	150.2 b	126.3 b	23.9 a	1.93 a	1.29 a
MD37	PAPR	3	162.5 b	137.4 b	25.1 a	2.05 a	1.37 a
MD19	OMF (NPK+Mg)	4	171.8 b	131.9 b	39.9 b	3.26 b	2.71 b
SP18	SPF (SSP)	4	323.3 c	244.8 c	78.5 c	3.57 bc	3.58 c
MD2	SPF (TSP)	4	333.0 c	244.6 c	88.4 c	3.53 bc	3.83 c
MD1	SPF (TSP)	4	350.9 c	262.0 c	88.9 c	4.00 d	4.21 d

* see the list of abbreviations

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

In the case of total phosphorus uptake, all various phosphorus treatments, except compound fertiliser NP (the MD28 fertiliser), were separated in two groups statistically different from each other. The first group included the Blank (no phosphorus), the PAPRs (the MD35 and MD37), and the organo-mineral fertiliser (the MD19) treatments; and the second group included the completely water-soluble pure phosphorus-compounds including the Control (KH_2PO_4), the superphosphate (the SP18), and the triple superphosphate (the MD1 and MD2) treatments (*Table 4.46*), i.e., there were no significant differences between phosphorus uptake from the compound fertilisers, partially acidulated phosphate rocks and the Blank. Also, there were no significant differences between superphosphate fertilisers and the Control, as a completely soluble phosphorus source, in the case of total phosphorus uptake by carrot plants on mixed soil/sand substrate. Total phosphorus uptake in second group of treatments varied from 323 to 365 *mg pot^{-1}* and was about two times more than first group. In the phosphorus concentration in shoots and roots of carrot plants, more significant differences were found between the phosphorus treatments, although the Blank and the PAPRs (the MD35 and MD37) treatments were not significantly different from each other.

Table 4.47 Influences of P source factor on P uptake and concentration in sand substrate in the main carrot pot experiment 2008, by Duncan's test following one-factorial ANOVA

P treatment	Type of fertiliser/ treatment *	n	P uptake (*mg pot*$^{-1}$) Total	Roots	Shoots	P concentration (*mg g*$^{-1}$) Roots	Shoots
Blank	-	3	2.0 a	1.3 a	0.7 a	0.76 a	0.87 a
Control	KH_2PO_4	3	170.3 bc	129.3 bc	41.0 b	3.15 bc	2.78 b
MD37	PAPR	4	3.9 a	2.9 a	1.1 a	0.78 a	0.86 a
MD35	PAPR	3	6.5 a	4.5 a	2.0 a	0.70 a	0.81 a
MD28	CF (NP)	2	129.9 b	75.6 b	54.3 b	3.72 d	4.44 e
SP18	SPF (SSP)	4	159.2 bc	118.6 bc	40.6 b	3.58 d	3.26 bc
MD1	SPF (TSP)	3	210.0 c	149.5 c	60.4 b	3.46 cd	3.79 d
MD2	SPF (TSP)	4	217.0 c	157.0 c	60.0 b	3.60 d	3.70 cd
MD19	OMF (NPK+Mg)	2	225.6 c	174.3 c	51.4 b	2.87 b	2.76 b

Values in the same column followed by the same letters are not significantly different at the 0.05 level.

- Sand substrate: the lowest and highest mean total phosphorus uptake by carrot plants were 2.0 and 225.6 *mg pot*$^{-1}$ which were found in the Blank and MD19 treatments, respectively. Duncan's test over total phosphorus uptake by carrot plants, in sand substrate, showed no significant differences between the Blank, MD37, and MD35 treatments with a total phosphorus uptake less than 6.5 *mg pot*$^{-1}$. Also, there was no significant difference between total phosphorus uptake in the Control, SP18, MD1, MD2, and MD19 treatments varying from 159 to 226 *mg pot*$^{-1}$ (*Table 4.47*).

Phosphorus concentration and uptake in shoots and roots of carrot plants and total phosphorus uptake on sand substrate did not show any significant differences between the Blank, MD37, and MD35 treatments. Anyhow, significant differences between the rest of phosphorus treatments in the case of phosphorus concentration of shoots and roots were somehow more than in the case of phosphorus uptake by shoots and roots (*Table 4.47*).

- Summary of the main carrot pot experiment, 2008:

Dry matter yield of carrot plants was affected significantly by P-fertiliser treatments because of considerable differences in the case of phosphorus solubility. P source treatments significantly affected U and P concentration and uptake by carrot plants too. Significant differences between various P-fertilisers in the case of carrot U and P concentration and uptake in sand substrate were found for a larger numbers of P source treatments in comparison to mixed soil/sand substrate. The highest carrot U uptake was found in the organo-mineral fertiliser, NPK+Mg (MD19), in both substrates that was significantly higher than other treatments. This can represent that some organic complexes of uranium in this fertiliser are more available for carrot plants. The highest carrot P uptake in mixed soil/sand and sand substrates were found in the triple superphosphate (MD1) and the organo-mineral fertiliser (MD19), respectively.

Low transferability of uranium derived from straight and mineral P-fertilisers to carrot roots showed low risk of uranium entrance from these fertilisers to food chain by this crop.

4.4 Relationship between solubility of U and P and their transferability to plants in various P sources

One aim of this study was to find a probable common extraction method for assessing the solubility and bioavailability of phosphorus and uranium in phosphorus-containing fertilisers. To this aim relations between uranium extractable and phosphorus by different extractants and their uptake by test plants in the pot experiments are presented in this section. For correlations and regression equations, the extractable portion of fertilisers' uranium and phosphorus added to the pots and the mean uranium and phosphorus uptake by plants were used. Because of significant differences between uranium and phosphorus uptake in different substrates as well as in different plants (see *Section 4.2* and *4.3*), all correlations and regressions were studied separately for each substrate and plant.

Carrot:

The various soluble portions (extractable by 5 different chemical extractants) and so-called total amount (digested by *aqua regia*) of uranium and phosphorus in fertilisers used in the carrot pot experiment are shown in *Table 4.48*.

Table 4.48 Total and soluble amounts of U and P of different P sources per pots in the main carrot pot experiment

Fertiliser	**Type of fertiliser/ treatment** *	**Total content (AR)**	**Soluble amount in different extractants**				
			Water	**NAC**	**AAC**	**2% FA**	**2%CA**
			U (*µg pot*$^{-1}$)				
SP18	SPF (SSP)	750	139	711	617	338	403
MD1	SPF (TSP)	282	50	264	253	74	196
MD2	SPF (TSP)	863	147	543	779	302	692
MD19	OMF (NPK+Mg)	900	235	406	394	625	779
MD28	CF (NP)	767	2	178	280	279	512
MD35	PAPR	418	n.d.	45	50	69	294
MD37	PAPR	610	0.3	130	60	64	131
			P (*mg pot*$^{-1}$)				
SP18	SPF (SSP)	1000	861	1003	921	925	917
MD1	SPF (TSP)	1000	969	1030	974	1126	1107
MD2	SPF (TSP)	1000	989	1054	1129	1095	1085
MD19	OMF (NPK+Mg)	1000	601	690	710	822	810
MD28	CF (NP)	1000	366	740	529	899	903
MD35	PAPR	1000	31	184	54	766	405
MD37	PAPR	1000	0.2	140	11	511	381

n.d.: not detectable

In mixed soil/sand substrate, the highest correlation coefficient was found between the water extractable form of uranium and phosphorus and their uptake by carrot plants; also, only the relationship between uranium uptake and water uranium extractable was significant (*Table 4.49*). In sand substrate, only correlation coefficients of water and 2% formic acid uranium extractable forms with uranium uptake were significant at the 0.05 and 0.01 levels, respectively; and in the case of phosphorus, the relationship between water, alkaline ammonium citrate, neutral ammonium citrate, and 2% citric acid extractable portions and total phosphorus uptake were significant at the 0.01 level, and the relationship between 2% formic acid phosphorus extractable form and total phosphorus uptake was significant at the 0.05 level. However, water extractant can be considered as a common extractant for assessing bioavailability of both elements for carrot plants in both substrates. The relationships between water extractable forms of uranium and phosphorus are shown in *Figure 4.2* and *Figure 4.3*.

Table 4.49 Correlation coefficients between different extractable amounts of U and P of various P-fertilisers (n=7) and their uptake by carrot plants in different substrates

Extractable form	**U uptake**		**P uptake**	
	Mixed soil/sand	**Sand**	**Mixed soil/sand**	**Sand**
Water	0.79 *	0.85 *	0.83 *	0.90 **
Alkaline ammonium citrate	0.16 ns	0.31 ns	0.76 *	0.93 **
Neutral ammonium citrate	0.32 ns	0.36 ns	0.71 ns	0.90 **
2% Formic acid	0.65 ns	0.89 **	0.67 ns	0.79 *
2% Citric acid	0.43 ns	0.75 ns	0.63 ns	0.91 **
Aqua regia	0.32 ns	0.53 ns	-	-

*, **, and ns: significant correlation at the 0.05, 0.01 levels and not significant, respectively.

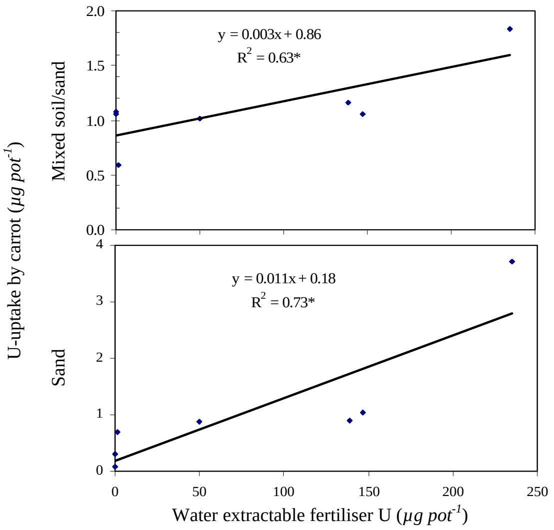

Figure 4.2 Relationships between water extractable fertiliser U and U-uptake by carrot plants in different substrates

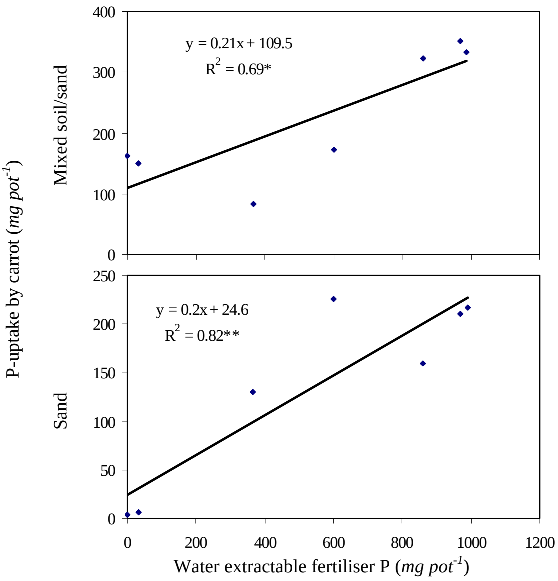

Figure 4.3 Relationships between water extractable fertiliser P and P-uptake by carrot plants in different substrates

Maize and sunflower:

The various soluble portions (extractable by 5 different chemical extractants) and so-called total amount (digested by *aqua regia*) of uranium and phosphorus in fertilisers used in the main Neubauer experiment are shown in *Table 4.50*. Correlation coefficients between uranium and phosphorus uptake by maize and sunflower seedlings and various extractable forms of them in different phosphorus-fertilisers are presented in *Table 4.51*. These correlations have been statistically analyzed for each substrate separately. For most extractants, the relationship between uranium solubility and uptake was out of range in the case of MD19, the organo-mineral fertiliser with magnesium. With ignoring the MD19’s data from the data set, the correlation coefficients between extractable forms and total uptake of uranium by maize and sunflower seedlings strongly increased. Thus, assessing suitability of different extraction methods for predicting uranium and phosphorus was done after excluding the MD19.

Table 4.50 Total and soluble amounts of U and P of different P sources per pots in the main Neubauer pot experiment

Fertiliser	Type of fertiliser/ treatment *	Total content (AR)	Soluble amount in different extractants				
			Water	**NAC**	**AAC**	**2% FA**	**2%CA**
		U (*µg pot^{-1}*)					
SP18	SPF (SSP)	60	11	57	49	27	32
MD2	SPF (TSP)	69	12	43	62	24	55
MD19	OMF (NPK+Mg)	72	19	32	32	50	62
MD28	CF (NP)	61	0.1	14	22	22	41
MD37	PAPR	49	n.d.	10	5	5	10
MD38	CF (PK)	50	n.d.	17	5	9	24
		P (*mg pot^{-1}*)					
SP18	SPF (SSP)	80	69	80	74	74	73
MD2	SPF (TSP)	80	79	84	90	88	87
MD19	OMF (NPK+Mg)	80	48	55	57	66	65
MD28	CF (NP)	80	29	59	42	72	72
MD37	PAPR	80	n.d.	11	1	41	30
MD38	CF (PK)	80	3	13	2	64	38

n.d.: not detectable

In agreement with the result of carrot plants, for sunflower seedlings the strongest relationship was found between water extractable form and uranium uptake in mixed soil/sand and sand substrate with significant correlation coefficients at 0.01 and 0.05 levels, respectively (*Table 4.51* and *Figure 4.4*). Concerning the relationship between phosphorus uptake by sunflower and various extractable forms, water extractable form had the strongest relationship with phosphorus uptake in sand substrate and it was the single significant relationship at the 0.01 level (r=0.98**). In the mixed soil/sand substrate, the correlation coefficient between water extractable phosphorus and phosphorus uptake (r= 0.96) was a little lower than those of phosphorus extractable by alkaline ammonium citrate, neutral ammonium citrate, and 2% citric acid, but significant at the 0.01 level. Thus, water extractant can be considered as a suitable extractant for assessing transferability of phosphorus and uranium from the studied phosphorus-fertiliser to sunflower seedlings too (*Table 4.51*).

In contrast to carrot plants and sunflower seedlings, the maize seedlings in both substrates showed no significant relationship between uranium uptake and uranium extractable by water. In both substrates the strongest relationship was found between uranium uptake and uranium extractable by *aqua regia* (so-called total uranium content) significantly at the 0.05 level (*Figure 4.6*). Also, the relationships of uranium uptake with uranium extractable by alkaline ammonium citrate and 2% citric acid were stronger than that of uranium extractable by water (*Table 4.51*).

Table 4.51 Correlation coefficients between different extractable amounts of U and P in various P-fertilisers and their uptake by maize and sunflower seedlings in mixed soil/sand and sand substrates

Substrate	Extractable form	U uptake		P uptake	
		Maize	**Sunflower**	**Maize**	**Sunflower**
Mixed soil/sand (n=6)	Water	0.24 ns	0.36 ns	0.75 ns	0.81 *
	Alkaline ammonium citrate	0.83 *	0.85 *	0.82 *	0.82 *
	Neutral ammonium citrate	0.57 ns	0.87 *	0.86 *	0.88 *
	2% Formic acid	0.06 ns	0.09 ns	0.70 ns	0.82 *
	2% Citric acid	0.44 ns	0.19 ns	0.91 *	0.86 *
	Aqua regia	0.46 ns	0.30 ns	-	-
Sand (n=6)	Water	0.36 ns	-0.08 ns	0.92 **	0.58 ns
	Alkaline ammonium citrate	0.82 *	0.53 ns	0.95 **	0.55 ns
	Neutral ammonium citrate	0.46 ns	0.53 ns	0.97 **	0.58 ns
	2% Formic acid	0.21 ns	-0.42 ns	0.76 ns	0.56 ns
	2% Citric acid	0.56 ns	-0.26 ns	0.96 **	0.52 ns
	Aqua regia	0.62 ns	-0.16 ns	-	-
		Without the MD19 fertiliser			
Mixed soil/sand (n=5)	Water	0.80 ns	0.99 **	0.75 ns	0.96 **
	Alkaline ammonium citrate	0.92 *	0.94 *	0.82 ns	0.99 **
	Neutral ammonium citrate	0.65 ns	0.98 **	0.89 *	0.99 **
	2% Formic acid	0.75 ns	0.80 ns	0.77 ns	0.88 ns
	2% Citric acid	0.94 *	0.59 ns	0.93 *	0.99 **
	Aqua regia	0.94 *	0.72 ns	-	-
Sand (n=5)	Water	0.70 ns	0.94 *	0.92 *	0.98 **
	Alkaline ammonium citrate	0.84 ns	0.82 ns	0.95 *	0.95 *
	Neutral ammonium citrate	0.49 ns	0.88 *	0.97 **	0.89 *
	2% Formic acid	0.63 ns	0.50 ns	0.77 ns	0.74 ns
	2% Citric acid	0.86 ns	0.38 ns	0.96 *	0.82 ns
	Aqua regia	0.91 *	0.52 ns	-	-

*, **, and ns: significant correlation at the 0.05, 0.01 levels and not significant, respectively.

2% citric acid and neutral ammonium citrate extractants had the best prediction of phosphorus uptake by maize seedlings in mixed soil/sand and sand substrate, respectively (*Figure 4.7*). Correlation coefficients of phosphorus extracted by these two extractants and phosphorus uptake were 0.93 and 0.97 and significant at the 0.05 and 0.01 levels, respectively (*Table 4.51*). Thus, for maize seedlings a common extractant was not found for assessing uranium and phosphorus transferability.

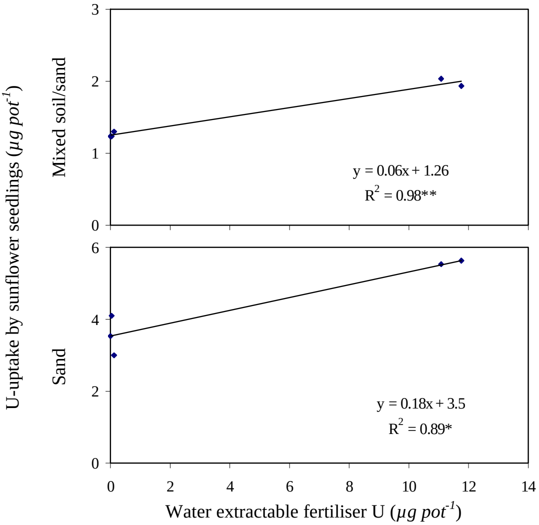

Figure 4.4 Relationships between water extractable fertiliser U and U-uptake by sunflower seedlings in different substrates

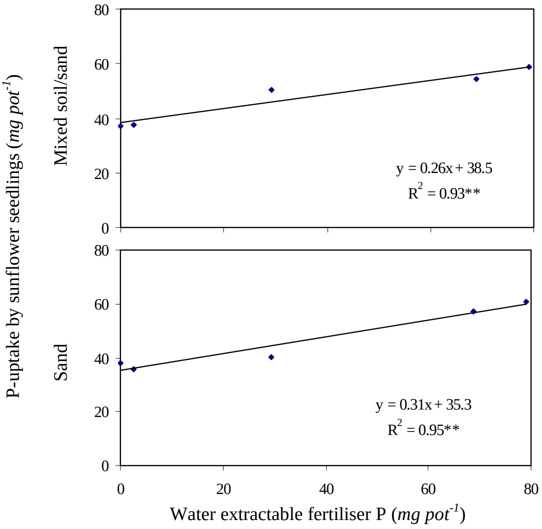

Figure 4.5 Relationships between water extractable fertiliser P and P-uptake by sunflower seedlings in different substrates

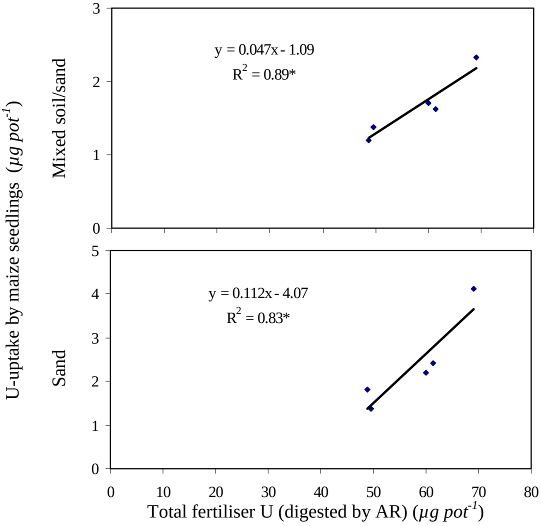

Figure 4.6 Relationships between total fertiliser U (i.e., digested by *aqua regia*) and U-uptake by maize seedlings in different substrates

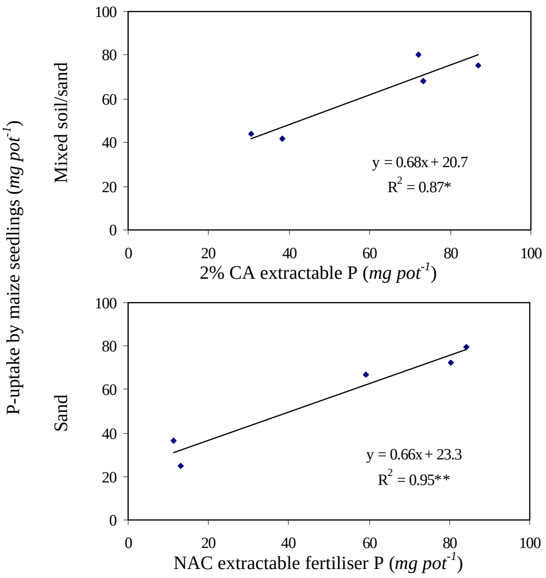

Figure 4.7 Relationships between NAC and 2% CA extractable fertiliser P and P-uptake by maize seedlings in sand and mixed soil/sand substrates, respectively

In the case of MD19 fertiliser, an organo-mineral fertiliser, total uranium and uranium soluble in 2% formic acid, 2% citric acid, and water was the highest amount per pot comparing to other phosphorus sources while uranium uptake, by maize and sunflower seedlings from that, was near to the lowest uranium uptake amount in the main Neubauer experiment. Although there was no other organo-mineral fertiliser in the experiment to make a statistical decision about them, it seems that no one of mentioned extractant (2% FA, 2% CA, water, and *aqua regia*) is suitable for assessing bioavailability of uranium in organo-mineral fertilisers. Anyhow, when MD19's data was included in the data set, the relationship between uranium uptake by maize or sunflower seedlings and uranium extractable by alkaline ammonium citrate or neutral ammonium citrate was the strongest in comparison to its relationship with other extractable forms of uranium. Also, significant relations were found in the maize uranium uptake in mixed soil/sand and sand substrate, and sunflower uranium uptake in mixed soil/sand at the 0.05 level with uranium extractable by alkaline ammonium citrate (correlation coefficient, r,: 0.83*, 0.82*, and 0.85*, respectively). It seems that among extractants used in the present study, ammonium citrate solutions, especially alkaline ammonium citrate, may be suitable for assessing the uranium and phosphorus transferability from organo-mineral fertilisers to maize and sunflower seedlings. However, in order to draw further conclusion about the suitability of these extractants for estimating the amount of potentially plant available uranium, further studies should be done on several organo-mineral fertilisers in a bio response experiment.

- Summary of relationship between U and P solubility and their transferability to plants:

Relations between U and P extractable portions by various chemical extractants and their uptake by plants varied between different plants (i.e., carrot plant, and sunflower and maize seedlings) as well as in different substrates. In sunflower and maize seedlings for most extractants, the relationship between U solubility and uptake was out of range in the case of organo-mineral fertiliser (MD19, NPK+Mg), and it was excluded from the data set. For carrot and sunflower seedlings the relations between water soluble form and plant uptake of U and P were significant in both substrates, and in several cases in sand or mixed soil/sand the relation between water soluble form and plant uptake had the strongest significant level and the highest correlation coefficient. Thus, water extractant can be considered as a common extractant for assessing P and U bioavailability in various P-fertilisers in carrot and sunflower plants except for organo-mineral fertilisers in sunflower plants.

In the case of maize seedlings, a common extractant was not found for assessing U and P bioavailability in various P-fertilisers. Moreover, for assessing P availability the best extractant was different in various substrates. So-called total U content, digested by *aqua regia* had the highest significant correlation coefficient with maize U uptake in both substrates. For maize P uptake the best extractants were 2% CA and NAC in mixed soil/sand and sand substrates, respectively.

5 Discussion

The main objective of the present research work was to investigate solubility of uranium in different phosphorus-containing fertilisers to predict its transferability to plants. To achieve this aim, solubility of uranium in various types of phosphorus-containing fertilisers was first investigated in different chemical extractants which are used for assessing phosphorus solubility according to the type in standard and conventional methods. In addition, a comparison of uranium and phosphorus solubility was performed to find a common extraction method for both elements. Therefore, the discussion of the results of this thesis starts with the result of uranium and phosphorus solubility in different chemical extractants.

Uranium solubility in chemical extractants alone is not enough to assess transferability of uranium to plants. Therefore, several phosphorus-containing fertilisers were tested for their effect on uranium uptake and uranium concentration in carrot plant tissues in a green house experiment. A second experiment was done with maize and sunflower in special small pots containing 450 *g* substrate and with high root density to find the potential available amount of phosphorus and uranium in each fertiliser (Neubauer bioassay method). In this experiment, differences between two types of plant seedling (monocotyle and dicotyle) in the case of uranium uptake and the effect of substrate on uranium transferability was investigated. Discussion on these results is given in *Chapter 5.2*.

Chapter 5.3 comprises some discussion about relationship between uranium and phosphorus solubility in chemical extractants and their uptake by carrot plants and maize and sunflower seedlings.

5.1 P and U solubility of P-fertiliser in chemical extractants

-Phosphorus: solubility of phosphorus in a chemical extractant varies among different types of phosphorus-fertilisers. Also, phosphorus solubility of one type of phosphorus-fertiliser in different extractants is not equal. Thus, different extraction methods are proposed for assessing solubility and bioavailability of phosphorus in different phosphate fertilisers (Deeley *et al*., 1987; Sikora and Mullins, 1995; Ostmann, 1995, Kratz and Schnug, 2009). As expected, significant differences between the various types of phosphorus-containing fertilisers tested here with regard to their relative phosphorus solubility, i.e. referred to *aqua regia*, in water, neutral ammonium citrate, alkaline ammonium citrate, 2% citric acid and 2% formic acid were confirmed by results of present research too. On the other hand, relative phosphorus solubility of each type of phosphorus-containing fertiliser in 5 extractants tested in the present study, were also significantly different.

Phosphorus solubility of straight phosphorus-fertilisers and compound fertilisers in 2% formic acid and 2% citric acid were approximately the same, but in organo-mineral fertilisers and phosphate rocks, phosphorus dissolution in 2% formic acid was considerably more than in 2% citric acid. In addition, in the case of phosphate rocks, phosphorus solubility in 2% formic acid and 2% citric acid were significantly different from each other (*Table 4.7*). Braithwaite *et al.* (1987) reported equal solubility of residual phosphorus (water insoluble) in 2% citric acid and 2% formic acid extractants in partially acidulated phosphate rock when the level of acidulation was 35% (related to stoichiometeric acidulation). Thus, with supposing the residues as phosphate rocks, these results are in contrast to that report. While, they are in accordance with the results of Braithwaite *et al.* (1993) saying that 2% formic acid was stronger than 2% citric acid to dissolve residual phosphorus of some partially acidulated phosphate rocks (i.e., phosphorus portion insoluble in water, neutral ammonium acetate, cold and hot neutral ammonium citrate, and alkaline ammonium citrate extractants).

-Uranium: 2% citric acid was the strongest extractant for uranium in compound fertilisers, organo-mineral fertilisers, and phosphate rocks. But in straight phosphorus-fertilisers uranium dissolution in neutral ammonium citrate and alkaline ammonium citrate was more than in 2% citric acid (*Table 4.4*). Uranium solubility of all phosphorus-fertiliser types in 2% citric acid was more than in 2% formic acid (*Table 4.6*). In the present study uranium had a mean solubility of about 25 % in 2% citric acid (about 0.1 M) among all 15 phosphate rock samples, while according to the report of Bashir *et al.* (2000) uranium solubility of phosphate rocks can be increased to more than 90% of total uranium content with increasing the citric acid concentration to 1M.

The results showed that uranium does not behave in the same way as phosphorus does in 5 tested chemical extractants. Also, it was concluded that uranium solubility of various P-containing fertilisers, like phosphorus, changes according to the kind of extractants and the type of fertilisers. Different solubility of uranium and phosphorus in various chemical extractants showed that it is not possible to draw conclusions about uranium solubility according to phosphorus solubility of P-containing fertilisers. Differences between uranium and phosphorus solubility can be related to their differences in chemical properties, behaviour, and natural characteristics. For instance, phosphorus in fertilisers is commonly in the anionic form of phosphate bound mainly to Ca and ammonium, or to Al, Fe, etc. (as impurities) in straight P-fertilisers, while uranium in fertilisers mostly can be found in the cationic form of uranyl bound to oxides, hydroxides, carbonates, silicates, and phosphates (see *Section 2.1*).

There are some reports in the literature on comparable tests between chemical extractants to assess their suitability for evaluation of phosphorus bioavailability. In most of these researches comparison between extractants has been done for only one type of phosphorus-fertilisers (e.g. Braithwaite, 1986; Mullins, 1988; Braithwaite *et al*., 1993). Anyhow, up to now there is no literature known by the author in the case of comparing extractants for assessment of phosphorus in various types of P-containing fertilisers or for assessing the solubility of phosphorus and a heavy metal of fertilisers in a common chemical extractant, and it seems that different chemical behaviours of various types of phosphorus-fertiliser’s compounds make it difficult to assess their phosphorus bioavailability using a common extractant. Also impurities in phosphorus-fertilisers have a wide range of properties depend on the origin and source of phosphate rocks and manufacturing methods that can affect solubility and bioavailability of heavy metals.

5.2 Influence of different P sources on plant uranium concentration and uptake

Increasing uranium concentration in the roots' environment causes more uranium uptake by plants. A lot of reports confirm increasing uranium uptake by different plants and crops with increasing uranium concentration in soil solution or hydroponic solution (Eapen *et al.*, 2003; Gulati *et al.*, 1980; Lakshmanan *et al.*, 1988; Lamas, 2005; Laroche *et al.*, 2005; Meyer *et al.*, 2004; Pettersson *et al.*, 1993; Rivas, 2005; Vandenhove, 2002), of course in some levels of uranium concentration which are much higher than natural levels of uranium concentration in comparison to soil solution. However, only a few reports address the effects of fertiliser-derived uranium on plant uranium concentration and uptake, and mostly concentrated on uranium balance in long fertilised soils by phosphorus-fertilisers (Jones, 1992; Mortvedt, 1994; Rogasik *et al*., 2007; Takeda *et al*., 2006; Makweba and Holm, 1993; Rothbaum *et al*., 1979). The direct effects of phosphorus-containing fertilisers, i.e., fertiliser-derived uranium, on plant uranium concentration and uptake in maize and sunflower seedlings and carrot plants were investigated in the present study. The results of this investigation are discussed in the next sections.

5.2.1 Effect of P sources on plant uranium concentration and uptake in maize and sunflower seedlings

- Uranium concentration: The effect of phosphorus sources (different in total uranium content and uranium solubility) on uranium concentration in roots of maize and sunflower seedlings on sand and mixed soil/sand substrate (in Neubauer test) was strongly significant (P value less than 0.001) (*Table 4.18*). This significant effect of phosphorus sources is due to various amounts of uranium introduced to substrates as impurities in different solubility, as can be seen by the fact that roots uranium concentration in the Control treatment (reagent grade KH_2PO_4) in both plants and both substrates was the lowest amount, i.e., roots' uranium concentrations in fertiliser treatments were significantly higher than not only that of the Blank but also that of the Control. However this result is in agreement with the result of Laroche *et al.* (2005) and Pettersson *et al.* (1993) about positive effect of uranium concentration in hydroponic solution (0-5 $\mu mol\ l^{-1} \approx$ 0-1.19 $mg\ l^{-1}$) and sediments (20-400 $Bq\ kg^{-1} \approx$ 1.6-33 $mg\ kg^{-1}$) on roots uranium concentration in *Phaseolus vulgaris* and water lily, respectively. Of course, the positive effect of higher concentrations of uranium in root environments on uranium concentration in roots has been reported repeatedly by authors. The significant influence of different phosphorus sources on uranium concentration of roots found in the present study means that fertiliser-derived uranium can affect the uranium concentration of maize and sunflower roots in a short growing period.

In contrast to uranium concentration in roots, uranium concentration in shoots of maize seedlings was not affected significantly by fertiliser-derived uranium coming from different phosphorus sources. In the case of sunflower, only the organo-mineral fertiliser (the MD19) treatment showed a significant difference in uranium concentration with other fertiliser treatments on both substrates (*Table 4.22*). However, this significant difference was presumably not the effect of phosphorus or uranium concentration or solubility of the fertiliser, but could have been a consequence of the reduced dry matter yield caused by the salinity effects of the MD19 treatment described earlier (*Figure 5.1*) (for more details see *Section 4.2.2.1*).

Figure 5.1 Sunflower seedlings on sand substrate of the MD19 treatment in the main Neubauer pot experiment

Mortvedt (1994) reported no significant difference between uranium concentration in maize (*Zea mays* L.) leaves or grains, wheat straws or grains, soybean [*Glycine max* (L.) Merr.] leaves or grains, and timothy (*Phleum pratense* L.) forage grown on non-fertilised and TSP-fertilised (long-term, >50 yr, with a phosphorus rate of about 30 $kg\ ha^{-1}$) soil. The results of the present study confirmed no significant effect of fertiliser-derived uranium on uranium concentration in shoots of maize, and are in agreement with results of Mortvedt (1994). Contrasting effects of fertiliser-derived uranium on uranium concentration of shoots versus roots of maize and sunflower can be explained by very low transferability of uranium from roots to shoots of plants, and it has been repeatedly reported by researchers (Morishima *et al.*, 1977; Netten and Morley, 1983; Lamas, 2005; Laroche *et al.*, 2005; Chen *et al.*, 2005; Ribera *et al.*, 1996). In agreement with other reports, in the present study also the roots/shoots uranium concentration ratios varied from 10 to 124 for maize and sunflower seedling, and the lowest value for each plant in different substrates was found in the Blank treatment. Increasing roots/shoots uranium concentration ratio with increasing the fertiliser-derived uranium or its solubility explains why phosphorus sources (fertiliser-derived uranium) could not affect significantly shoots' uranium concentration of maize and sunflower seedlings.

- Uranium uptake: Influence of different phosphorus-fertilisers on uranium uptake by maize and sunflower was significant in various substrates at the 0.001 level (*Table 4.18*). This significant effect could possibly be interpreted a result of different phosphorus concentration in roots' environment because of various solubility of phosphorus-fertilisers, but comparing different phosphorus treatments with the Blank and the Control rejects this assumption. Total uranium uptake by maize and sunflower seedlings on sand and mixed soil/sand substrate in all treatment was higher than that in the Blank and the Control (completely soluble and purified KH_2PO_4) (*Table 4.21* and *Table 4.22)*. On the other hand, uranium uptake by maize and sunflower seedlings in the Control (with the highest available amount of phosphorus) was lower than or equal to uranium uptake in the Blank (with the lowest available amount of phosphorus). Thus it can be observed that significant differences between uranium uptakes from various phosphorus-fertilisers have been caused by different levels of fertiliser-derived uranium. These results showed that the fertiliser-derived uranium can affect significantly uranium uptake by maize and sunflower seedlings and can transfer to the plant tissues. These results are in agreement with report of Ananyan (1991) in that uranium concentration and uptake by hay, on a weakly acidic soil, in the treatment with applying superphosphate fertiliser was significantly higher than that of the treatments with no phosphorus application. Uranium uptake by roots of maize and sunflower seedling varied from 82% to 94% of total uranium uptake in the Blank and the Control while in phosphorus-fertiliser treatments with increasing uranium uptake it varied from 92% to 98% of total uranium uptake, this implies that most of fertiliser-derived uranium transferred to maize and sunflower seedlings have been accumulated in their roots, and uranium uptake by shoots could not be affected significantly by fertiliser-derived uranium factor (phosphorus source factor).

The highest amount of uranium transferred to maize seedlings in both substrates was observed in a triple superphosphate fertiliser (MD2) and it was significantly higher than that of all other fertilisers (*Table 4.21*). For sunflower seedlings, the highest amount of uranium uptake was observed from the triple superphosphate fertiliser (MD2) and the single superphosphate fertiliser (SP18) in sand and mixed soil/sand substrates, respectively (*Table 4.22*). Moreover, total uranium uptake by sunflower seedlings from TSP and SSP fertilisers had no significant differences, while in maize seedlings uranium uptake from TSP fertiliser was significantly higher than SSP fertiliser in both substrates. These differences between maize and sunflower plants and between substrates in the case of sunflower demonstrated that transferability of fertiliser-derived uranium to plants is dependent on the type of plant and substrate as well as uranium solubility and uranium content of fertilisers.

In all phosphorus-fertilisers used in the present study as treatments, the total uranium uptake by maize and sunflower seedlings on sand substrate was more than on mixed soil/sand substrate. The decreasing effect of mixed soil/sand substrate on uranium uptake by maize and sunflower may be related to chemical interactions between uranyl cations/complexes and soil colloids like remaining in exchangeable phase (Hossner *et al*., 1998), combining with soil organic colloids, or adsorption onto solid phase (EPA, 1999; Szecsody *et al*., 1998; Giammar, 2001). The other parameter that can affect the uranium bioavailability in soil solution is ionic strength of soil solution that might affect the uranium speciation in soil solution (Ervanne, 2004; Hossner *et al.*, 1998; Giammar, 2001) and it also can affect roots uranium uptake by competition between uranyl and other cations like Ca^{2+}, Mg^{2+}, K^{+}, and other heavy metals in a higher concentration of them in soil solution comparing to sand substrate (Mortvedt, 1994).

5.2.2 Effect of P sources on plant uranium concentration and uptake in carrot plants

-Uranium concentration: Comparing the uranium concentration in shoots and roots of carrot, maize, and sunflower distinguished a big difference between the carrot and the other two plants. While the uranium concentration in roots of maize and sunflower seedlings was from 10 times (in the Blank and Control) to about 100 times (in some of fertiliser treatments) more than uranium concentration in their shoots, in carrot plants these concentrations were much closer to each other and in some treatments it even varied inversely. A higher uranium concentration in leaves of carrot in comparison to uranium concentration of roots was reported by Tracy *et al.* (1983) on contaminated soils. In the report of Tracy *et al.* (1983) there is some data showing contrast result in the case of comparison between uranium concentration in shoots and roots of carrot in some contaminated soils as uranium concentration in roots of carrot on a contaminated soil (27 $\mu g\ kg^{-1}$ in the depth of 0-30 cm) was about threefold higher than that of shoots, while in another contaminated soil (590 $\mu g\ kg^{-1}$ in the depth of 0-30 cm) this ratio was inversely less than 1 (0.85). Of course, because of detection limit of 0.2 $\mu g\ kg^{-1}$ (it was 0.01 $\mu g\ kg^{-1}$ in the present study), there was no data for comparing uranium concentration in roots and shoots of carrot plants in the Control soil in that report. There are similar results for radish, another root crops; Lakshmanan and Venkateswarlu (1988) reported a uranium concentration factor (defined as the ratio of uranium concentration in the fresh weight to that in soil/irrigation water) of leaves higher than that of roots of radish plants in contaminated and natural soils as well as in contaminated and natural irrigation water treatments.

The mobility of uranium and other heavy metals is largely restricted in plants because of adsorption on cell wall materials, and as a result, their concentration in aboveground tissues is commonly lower than in roots. There is a barrier to translocation of uranium towards aerial parts that may be attributed to multiple causes such as uranium binding to mucilage, its retention in cellular walls, and its accumulation in the root's apoplast and simplast. Uranium as a heavy metal could be bound to the root surface and/or adsorbed in the apoplast. However, closed or elevated levels of uranium of shoots in comparison to roots of carrot plants indicated that the excess of uranium is expected to be passed through the root endodermis to enter the transpiration streams and then to transfer to shoots. The concentration of heavy metals varies in different types of vegetables, and also it depends on the heavy metal element. For example, the concentration of lithium (Li) in carrot roots is less than most vegetables except from onion bulbs, the concentration of cesium (Cs) in carrot roots is less than potato tubers and cucumber fruits while it is higher than its concentration in tomato fruits, and the concentration of chromium (Cr) in carrot root and onion bulbs was higher than leafy vegetables (Kabata-Pendias and Mukherjee, 2007). Based on the available data, uranium contents in various vegetables vary from 5 to 20 $\mu g\ kg^{-1}$ (Kabata-Pendias and Pendias, 2001). Based on data of *Agency for Toxic Substances and Disease Registry* of USA, Kabata-Pendias and Mukherjee (2007) reported a uranium content of 7.7 $\mu g\ kg^{-1}$ in fresh weight of carrot roots which was considerably lower than that in parsley with a U concentration of 60 $\mu g\ kg^{-1}$. Sarkar (2002) reported that the concentration of ^{238}U in fresh vegetables equals about three times of its value in root vegetables.

Root’s type of carrot is taproot that elongates mainly downward into the soil and is expanded and formed a storage organ during storing of photosynthetic organic substances transferred from the leaves. Like typical taproot plants, carrot also develops some fibrous roots to uptake oxygen, water, and nutrients mostly in the area below the modified storage taproot (Streich, 2007). Most of these fibrous roots and hairy roots are destroyed when the carrot roots are dug from the soil. With regard to storage characteristic of carrot roots, most parts of fleshes in them are similar to the tissues of plants’ fruit. Anyhow, as it is expected, uranium and heavy metals concentration in carrot roots, despite fruits and grains, is not the lowest concentration among different plant tissues because of direct contact to the soil solution and particles, and also consistence of some tissues including the xylem and phloem vessels that may have a higher heavy metal concentration than storage tissues.

Sheppard *et al.* (1989) reported a higher concentration ratio (CR) of uranium and two other heavy metals (Th and Pb) in potato peels versus potato fleshes. The higher concentration of these heavy metals in peels of potato could be occurred via passive adsorbing (in the apoplast) and/or soil particle pollutants in the peel samples. The same result can be expected in carrot roots as well; anyhow in the present study whole edible roots of carrot were analysed and the mean uranium concentration of them was approximately equal to its concentration in the shoots (presumably a lower uranium concentration in the fleshes and a higher uranium concentration in the peels comparing to the shoots' uranium concentration). If the carrot roots be considered as normal roots, the lower uranium concentration of them can be explained by the lower ratio of peel/flesh in edible part of carrot roots (carrot roots without hairy and fibrous roots developed) comparing to normal taproots or fibrous roots. As a result, the apoplast/simplast ratio in carrot roots also is strongly lower than that of the other types of plant roots, and it can be a strong reason for nearly the same uranium concentration values in shoots and roots of carrot and probably other root crops.

Although low uranium concentration in carrot roots shows a lower risk of fertiliser-derived uranium to enter the food chain by carrot roots, it does not imply that real uranium uptake by carrot plants is less than other plants because in the present study, the hair roots of carrot plants were cut when the roots were pulled out from the substrate or they were eliminated by washing and cleaning the roots. The hairy roots are the first organelle of higher plants responsible for uptake of elements, and due to their highly branched nature, have a large surface area which can remove heavy metals and uranium (Eapen *et al*., 2003), thus in root crops like carrot hair roots can perform the role of roots in other plants to accumulate uranium and prevent its transfer to edible roots and above ground tissues.

In shoots of carrot plants uranium concentration in the organo-mineral fertiliser (the MD19) was significantly higher than other treatments in both substrates except that of a compound fertiliser (NP, MD28) in mixed soil sand substrate. Bioavailability of uranyl complexes with some of organic compounds has been reported in the literature (Kabata-Pendias and Pendias, 1984; Sheppard *et al.*, 1988; Sheppard *et al.*, 2005), thus the higher uranium concentration in shoots of carrot may be related to organic complexes of uranium that could be easily taken up by carrot roots and be transferred to above ground tissues. In the case of the compound fertiliser MD28 (NP), the higher uranium concentration in shoots of carrot than that of the other P-fertiliser treatments was a surprising result with regard to its uranium soluble portion that was much less than those of other treatments in most extractants. With regard to the low total uranium uptake as a result of the low dry matter yield in the MD28 treatment which has been caused by salt effect (see *Section 4.3.2.2*), the higher uranium concentration of shoots could be a result of low dry matter yield not a higher bioavailability of uranium derived from the fertiliser.

- Uranium uptake: Total uranium uptake and roots uranium uptake by carrot plants on mixed soil/sand substrate was not significantly different in the Control, Blank, straight phosphorus-fertilisers (the MD1, MD2, and SP18), and partially acidulated phosphate rocks (the MD35 and MD37) treatments. Only uranium uptakes in the MD28 (CF, NP) and MD19 (OMF, NPK+Mg) treatments were significantly different from each other as well as from other treatments (the lowest and highest uranium uptake by roots and total uptake were observed in these two treatments, respectively). As it was discussed in the last section, in both of these treatments dry matter yield of carrot plants was significantly affected by the negative salinity effect of the phosphorus-fertiliser source, and due to this negative influence, dry matter yield of carrot plant on mixed soil/sand substrate in them was significantly lower than all other treatments including the Blank too (of course, dry matter yield in the MD28 treatment was considerably less than that of the MD19 treatment) (*Table 4.40*). Despite of lower carrot dry matter yield in the organo-mineral fertiliser (the MD19) compared to other treatments (except the compound NP-fertiliser, MD28), its uranium uptake by shoots and roots were significantly higher than those of all other treatments. Higher carrot uranium uptake from the MD19 treatment can be related to the highest amounts of total uranium content and the highest amounts of water soluble uranium (60 % more than the TSP fertiliser, the MD2, see *Table 4.48*) supplied to the pots from different phosphorus sources applied in the present study. As it is known from the literature, some organic complexes are soluble and can be taken up by plants (Kabata-Pendias and Pendias, 1984; Sheppard *et al.*, 1988; Sheppard *et al.*, 2005). The organic component in the MD19 could be a reason for higher carrot uranium uptake in the present study as well. However, in maize and sunflower seedlings despite of higher total and water-soluble amount of uranium in the organo-mineral fertiliser (MD19), the highest uranium plant uptake was found in superphosphates. This difference between test plants confirms a very important the role of plant type in uranium uptake.

5.2.3 The effect of phosphate ions/complexes on uranium uptake by test plants

As was mentioned in the *Section 5.2.1*, in both substrates uranium uptake by maize seedlings in the KH_2PO_4-Control was significantly less than that in the Blank. This confirms the attenuating effect of applied phosphate on uranium uptake by maize seedlings in a natural soil. The decreasing effect of phosphorus application on uranium plant uptake has been reported by Ebbs *et al*. (1998) for peas in hydroponic solution, Eapan *et al*. (2003) for uranium uptake by hairy roots of *Brasica juncea* on *in vitro*, Lamas (2005) for ryegrass in contaminated soil, and Rivas (2005) for total uranium uptake by sunflower and maize on a contaminated soil. Of course, it should be considered that initial uranium concentration in mixed soil/sand substrate used here was considerably lower than that of all substrates mentioned above. On the other hand, this effect was not found for sunflower in mixed soil/sand; in that case, uranium uptake from the Blank and Control were approximately equal.

The effect of phosphate availability in media on plant uranium uptake by carrot in mixed soil/sand was similar to sunflower and there was no significant difference between the Control (KH_2PO_4 application) and the Blank. Anyhow, for carrot in sand substrate the root uranium concentration in the KH_2PO_4-Control was significantly less than that in the Blank; but the total, roots, and shoots uranium uptake in the Control were higher than that in the Blank because of considerable differences between their dry matter yield caused by severe phosphorus deficiency in the Blank.

In contrast to the results of maize in mixed soil/sand, no significant effects of pure phosphorus application on uranium uptake by sunflower seedlings were observed in the same substrate. This result is in agreement with the report of Laroche *et al*. (2005) expressing no significant effect of phosphate ions on uranium uptake by bean plants on hydroponic culture, and the results of Rivas (2005) in the case of faba bean plants in contaminated soils.

However, despite the attenuating effect of phosphate ions/complexes on uranium plant uptake in some cases, the increasing effect of phosphorus-fertilisers on uranium uptake because of impurities and loading fertiliser-derived uranium should be considered.

5.3 Relationships between U and P solubilities and their transferability to plants

According to results of the present study, water was the best extractant for assessing the uranium solubility of various types of phosphorus-fertilisers (except the organo-mineral fertiliser for sunflower) and their transferability to sunflower and carrot plants growing on a mixed soil/sand or sand substrate. For assessing the phosphorus solubility and transferability, water extractant was also the best in the case of carrot plants on mixed soil/sand substrate and sunflower on sand substrate. Water extractant was also one of the more suitable extractants for assessing phosphorus transferability in the case of carrot plants on sand substrate and sunflower plants on mixed soil/sand substrate with high significant correlation coefficients (*Table 4.49* and *4.49*). A good relationship between water soluble phosphorus and plant phosphorus uptake from superphosphates, ammonium phosphates and partially acidulated phosphate rocks (acidulated at levels higher than 50% of stoichiometric) has been reported in the literature (Braithwaite, 1987; Mullins, 1988). Anyhow it has been also reported that with a proportion of soluble phosphorus in water higher than 80% of total phosphorus, in fully acidulated phosphorus-fertilisers like triple superphosphate and mono ammonium phosphates, variable amounts of water soluble phosphate has not affected yields, and fertiliser performance has been poorly affected (Mullins and Evans, 1990; Mullins and Sikora, 1990; Prochnow *et al*., 2001).

When looking in each plant and substrate separately, the best chemical extractant varied for assessing uranium and/or phosphorus transferability. However, water extractant was better than other extractants that could be selected as a common chemical extractant to assess bioavailability of U and P elements in various types of phosphorus-containing fertilisers for only carrot and sunflower plants in the present study. In contrast to sunflower and carrot, uranium uptake by maize seedlings neither in mixed soil/sand substrate nor in sand substrate showed any significant relation with water soluble uranium portion of fertilisers. Also, in the present study was not found any significant relation between water soluble phosphorus and maize seedlings' phosphorus uptake in mixed soil/sand substrate. These results confirmed that none of these chemical extraction procedures can be used satisfactorily to assess bioavailability of phosphorus and uranium of all phosphorus containing fertilisers for all crops. In addition to contrary results between maize and the other two plants in their agronomic response to water soluble portion of phosphorus in the present work, there are some other contrary reports found in the literature for agronomic response to water soluble phosphorus portion of phosphorus-fertilisers. While a large number of reports reveal a good relationship between agronomic response and water soluble phosphorus in superphosphates and ammoniated superphosphates (Terman *et al.*, 1964; Webb and Pesek, 1959) and partially acidulated phosphate rocks (Hammond *et al.*, 1980; Braithwaite, 1987), there are some other reports showing the bioavailability of some water insoluble phosphorus components in superphosphates or ammoniated superphosphates (Mullins *et al.*, 1990; Bartos *et al.*, 1991; Prochnow *et al.*, 2001). Anyhow water extraction method was selected as a common chemical procedure for assessment bioavailability of U and P in various types of fertilisers with regard to strong relations with sunflower and carrot response in most cases. More accurate decision for introducing a common extractants to assess bioavailability of U and P together or at least only uranium of various types of P-containing fertilisers need more investigations. Up to now there is no literature known by the author in the case of assessment heavy metal solubility and bioavailability only by water extraction, and water soluble amount of heavy metals in fertiliser and sewage sludge and soil solution is commonly negligible. Water soluble fraction assesses only easily available fraction of heavy metals (Filgueiras *et al.*, 2002).

In maize seedlings the strongest relation was found between uranium uptake and so-called total uranium content (digested by *aqua regia*) of fertilisers in sand substrate. Anyhow for phosphorus in maize, the strongest relation between total uptake and soluble forms in mixed soil/sand and sand substrate was not the same: on mixed soil/sand the strongest relation was found between phosphorus extractable by 2% citric acid and plant uptake, while on sand substrate it was between neutral ammonium citrate soluble form and total uptake. This is in agreement with the capability of 2% CA and NAC extractants for extracting the available amounts of phosphorus compounds of some types of P-fertiliser like superphosphates and partially acidulated phosphate rocks. Based on a world wide literature review by Kratz and Schnug (2009), these two extractants (2% CA and NAC) can dissolve monocalcium and dicalcium phosphate components and about 80% of Al-/Fe-phosphate, also a portion of ammoniated RP residues and about 20% of PARP residues can be dissolved in NAC and 2% CA extractants, respectively (for more details see *Table 2.5*).

Although the results of the present study showed strongly significant differences between various types of P-fertilisers on uranium uptake by maize and sunflower and also some differences between them in the case of uranium uptake by carrot plants, but in all cases only a small portion of fertiliser-derived uranium could to be taken up by plants. The total uranium uptake by tested plants was also considerably less than water soluble portion of uranium in the straight P-fertilisers and the organo-mineral fertiliser (the MD19). It can be inferred that water soluble amount of uranium loaded onto soil by straight P-fertilisers and organo-mineral fertilisers is enter to soil solution and might be transferred to the ground water. Though uranium can be adsorbed by clay minerals and soil organic matter, there is a risk of its transfer to the ground water resources (Sparovek *et al*., 2001). In addition to clay minerals, there are some other parameters affecting the adsorption of uranium soils including: pH, the types of soil minerals, dissolved carbonate concentration, and CEC (EPA, 1999). However, the risk of uranium transfer to the ground water in light soils is higher than heavy and/or organic soils.

As it was mentioned before, the present study was conducted as a case study to identify trends and patterns of uranium and phosphorus solubility in extractants and their transferability to plants. The results showed some differences between the various types of phosphorus-containing fertilisers, e.g. organo-mineral fertilisers with other fertiliser types, and further investigations can be done using more phosphorus-fertiliser samples from each type in a simple or factorial experimental design to find a probable common extractant for assessing phosphorus and uranium transferability from various phosphorus-fertilisers to plants. Also time effects on transferability of fertiliser-derived uranium can be investigated using a plant with long growth period and preferentially with several harvests.

6 Summary/Zusammenfassung

Phosphate fertilisers are commonly used in agricultural lands to supply phosphorus nutrients for enhancing quantity and quality of crop products around the world. According to the literature, phosphorus-fertilisers may contain considerable amounts of uranium varying from less than 10 to more than 360 $mg\ kg^{-1}$, and uranium can be loaded onto soils from 7 to 23 $g\ ha^{-1}\ yr^{-1}$ with applying of 22 kg P $ha^{-1}\ yr^{-1}$ (50 kg P_2O_5 ha^{-1}) from various phosphorus-containing fertilisers. With increasing uranium content and concentration in soil, uranium concentration may increase in plant tissues, animal bodies, and the human body in the long run; it can cause damage to health of human and animals mainly based on its chemical toxicities as a heavy element. Total amount of uranium, uranium-to-phosphorus ratio, and uranium solubility and transferability vary in different P-containing fertilisers.

The objectives of the present study were three points: comparison of uranium and phosphorus solubility in chemical extractants commonly used for P-fertilisers, investigation of the effect of U-containing P-fertilisers on the uranium concentration and uptake of different crops (a monocotyledon: maize, a dicotyledon: sunflower, and a root crop: carrot), and investigation of relationships between chemical extractability and plant uptake of uranium coming from different P-containing fertilisers. To this end, the following experiments were carried out:

a) Determination of U and P solubility of 37 P-containing fertilisers in 5 standard chemical extractants for P solubility defined in the EU Fertiliser Ordinances (W, NAC, AAC, 2% FA, and 2% CA) and in *aqua regia* for so-called total amounts.
b) Neubauer pot experiments with maize and sunflower.
c) Kick-Brauckmann pot experiments with carrot.

The most important findings of the research work presented here on chemical extractions were:

- Relative uranium solubility (i.e. referred to AR) significantly varied in the different extractants as well as in the various types of P-fertilisers, the same results were found for phosphorus. The highest and lowest mean relative

uranium solubility were equal to 60.6 % and 10.2 % of so-called total uranium content (digested by *aqua regia*) and were found in 2 % citric acid and water, respectively.

- The lowest and highest mean relative uranium solubility over all extractants were found in phosphate rocks and compound fertilisers which were equal to 14.7 % and 67.8 %, respectively. In contrast, the highest mean relative solubility of phosphorus was found in straight phosphorus-fertilisers with a value of about 100 %.
- The results of the present research work showed significant differences between uranium and phosphorus solubility in various extractants when looking separately at all four types of phosphorus-containing fertilisers. Uranium showed somewhat lower relative solubility than phosphorus in all extractants except in AAC.

In the first year, two pilot experiments were carried out to test the suitability of the Neubauer method for the research objective, suitability of maize and sunflower crops as test plants in Neubauer method, suitability of different carrot varieties, and behaviour of different substrates. The pilot experiments revealed that:

- Maize and sunflower seedlings could grow under special condition of the method in Neubauer pots in a high plant density for about 3 weeks. The results of the pilot Neubauer pot experiments also showed significant effects of fertiliser-derived uranium on uranium concentration and uptake while the dry matter yield was independent from phosphorus availability in substrate in a short term growing period of 22 days.
- The results of pilot Kick-Braukmann pots with carrot showed significant effects of P-fertilisers and substrate on dry matter yield, and U uptake by carrot plants, while the effect of variety on roots’ dry matter yield and total U uptake was not significant. The effect of fertiliser-derived U on carrot uranium concentration and uptake in soil substrate was not significant.

In the second year, a number of phosphorus sources varying in composition (RP, straight P-fertiliser, mineral compound P, organo-mineral compound P), P-solubility, uranium content, U/P-ratio, and uranium solubility were tested in a Neubauer and Kick-Braukmann pot experiments, yielding the following results:

- The effect of different phosphorus sources (fertiliser-derived uranium) on uranium concentration in roots of maize and sunflower seedlings on sand and mixed soil/sand substrate was strongly significant ($P<0.001$). In contrast to uranium concentration in roots, uranium concentration in shoots of maize seedlings was not affected significantly by different phosphorus sources. In sunflower seedlings on sand substrate, only shoots uranium concentration in the organo-mineral fertiliser, NPK+Mg (MD19), was significantly higher than that of other treatments.
- The effects of different phosphorus sources on uranium concentration of carrot shoots were significant in both substrates, i.e U concentration of carrot shoots in the organo-mineral fertiliser, NPK+Mg (the MD19) and the NP-compound fertiliser (the MD28) were significantly higher than in other treatments. The effects of different phosphorus sources on uranium concentration in roots of carrot were only significant in sand substrate varying from 12 to 32 ng g^{-1} in the Control and the MD35 treatment (a PARP fertiliser), respectively.
- Uranium concentration in roots of maize and sunflower seedlings was about 10-100 times (varying in different treatments) higher than uranium concentration in their shoots, but in carrot plants these concentrations were completely similar and even on the contrary, uranium concentration in roots of carrot plants was lower than that in their shoots in the most of treatments. These results can show lower risk of fertiliser-derived uranium for entering to the food chain via carrot roots.
- Uranium uptake by roots and total uranium uptake were significantly influenced by fertilizer-derived uranium in maize seedlings at the 0.05 level and in sunflower seedlings at the 0.001 level.
- In maize and sunflower plants total uranium uptake from all P-fertilisers (except PK-fertiliser, MD38, in that uranium uptake on both substrates was equal) on sand substrate was considerably higher than on mixed soil/sand. With regard to decreasing the concentration of uranyl ions and complexes in soil solution due

to precipitation, adsorption, and cation exchange reactions in presence of soil colloids, the attenuating effect of soil substrate on uranium transferring to plants was expected. In contrast to P-fertiliser treatments, in the KH_2PO_4-Control and Blank total and root uranium uptake in mixed soil/sand was higher than in sand substrate, caused by a low initial amount of uranium in the sand substrate.

- The highest total uranium uptake by maize and sunflower seedlings (2.33 and 4.12 $\mu g\ pot^{-1}$ for maize and 2.04 and 5.64 $\mu g\ pot^{-1}$ for sunflower in mixed soil/sand and sand substrate, respectively) were observed in straight phosphorus-fertilizers (superphosphates).
- Uranium uptake by carrot roots and shoots was affected significantly by phosphorus sources. The highest mean uptake by carrot plants (shoots, roots, and total amount) was found in the OMF, NPK+Mg (MD19), and significantly different from those of other treatments, this could imply that some organic component in the MD19 fertilisers can be easily taken up by hair roots and transferred to carrot roots.
- Total uranium uptake by carrot plants on sand substrate was lower than that on mixed soil/sand substrate in all phosphorus-fertilisers except the organo-mineral fertiliser which contained highest total and water soluble amounts of uranium.

The results of investigation of relationships between chemical extractability and plant uptake of uranium coming from different P-containing fertilisers were:

- The best prediction of uranium and phosphorus bioavailability for carrot plants from various P-fertilisers tested in this research was found by water extractant. The relations between water extractable form of uranium and phosphorus and their uptake by carrot plants were significant on both substrates with correlation coefficient values of 0.79 and 0.85 for U, and 0.83 and 0.90 for P in mixed soil/sand and sand substrate, respectively.
- As for carrot plants, for sunflower seedlings also the best extraction for assessing bioavailability of uranium and phosphorus was water extraction. Correlation coefficients between sunflower uranium uptake and water soluble proportion of uranium in both substrates and between sunflower phosphorus

uptake and its water extractable form in sand substrate were the highest among all extractants, and statistically significant. The relationship between phosphorus uptake by sunflower and its water solubility proportion on mixed soil/sand was strongly significant ($P<0.01$, $r=0.96$).

- The results revealed that a common extractant can not be introduced for assessing phosphorus and uranium bioavailability in various type of P-Fertiliser for maize seedlings. Anyhow, the best assessing extractants for uranium and phosphorus bioavailability for maize were *aqua regia* and neutral ammonium citrate (NAC), respectively.

Based on the results of this study it was concluded that uranium and phosphorus behave differently in various chemical extractants, and solubility of uranium and phosphorus of P-containing fertilisers depend on both the extractant and the type of P-containing fertilisers. Also, it was concluded that fertiliser-derived uranium can significantly affect plant uranium uptake in maize and sunflower seedlings with regard to total uranium concentration and its solubility. On the other hand, total uranium uptake by all tested plants was considerably less than water soluble portion of uranium in straight P-fertilisers and the organo-mineral fertiliser (the MD19) implying a potential risk of fertiliser-derived uranium transferring to the ground water. Transferability of fertiliser-derived uranium to carrot roots and shoots was not significant except in the case of an organo-mineral fertiliser (NPK+Mg, the MD19). These results suggest that the risk of uranium transferring from mineral phosphorus-fertiliser to the food chain via root crops is rather low; however, further studies should be carried out to confirm this.

Vergleichende Untersuchungen zur Löslichkeit von Uran und Phosphor in Phosphatdüngern und zum Urantransfer in Pflanzen

Zusammenfassung

Phosphordünger werden weltweit in der Landwirtschaft eingesetzt, um Menge und Qualität der angebauten Feldfrüchte zu erhöhen. Nach Literaturangaben können die eingesetzten Phosphordünger mit Gehalten zwischen ≤ 10 und ≥ 360 mg kg^{-1} jedoch beträchtliche Mengen an Uran (U) aufweisen. Pro Jahr können somit bei einer jährlichen Applikationsrate von 22 kg P ha^{-1} (50 kg P_2O_5 ha^{-1}) je nach Dünger zwischen 7 und 23 g U ha^{-1} ausgebracht werden. Mit steigendem U-Gehalt im Boden kann auch die U-Konzentration in Pflanzenteilen, Tieren und letztendlich im menschlichen Körper ansteigen, wo es aufgrund seiner toxischen Wirkung als Schwermetall Schäden der menschlichen und tierischen Gesundheit hervorruft. Die Gesamtgehalte von U, U:P- Verhältnis, U-Löslichkeit und Verfügbarkeit schwanken dabei in Abhängigkeit von der Herkunft der P-haltigen Dünger.

Die hier vorgestellte Arbeit hatte folgende Ziele: Vergleich der Löslichkeit von Uran und Phosphor in verschiedenen üblicherweise für P-Dünger eingesetzten chemischen Extraktionsmitteln, Untersuchung der Auswirkung U-haltiger P-Dünger auf Urankonzentration und –aufnahme durch unterschiedliche Kulturpflanzen (Monokotyle: Mais, Dikotyle: Sonnenblume, Wurzelfrucht: Karotte) sowie der Beziehungen zwischen chemischer Extrahierbarkeit und Pflanzenaufnahme von Uran aus verschiedenen P-haltigen Düngemitteln. Zu diesem Zweck wurden folgende Experimente durchgeführt:

d) Bestimmung der Löslichkeit von U und P von 37 P-haltigen Düngemitteln in 5 Standardextraktionsmitteln für P-Dünger gemäß EU-Düngemittelverordnung (Wasser, neutrales Ammoniumcitrat, alkalisches Ammoniumcitrat, 2%ige Ameisensäure, 2%ige Zitronensäure) und in Königswasser (“scheinbare Gesamtgehalte”).

e) Neubauer-Gefäßversuche mit Mais- und Sonnenblumenkeimlingen.

f) Kick-Brauckmann-Gefäßversuche mit Karotte.

Hinsichtlich der Untersuchungen zur chemischen Extraktion wurden folgende Ergebnisse erzielt:

a. Die relative Uranlöslichkeit (d.h. bezogen auf den Königswassergehalt) variierte sowohl zwischen den verschiedenen Extrakten als auch zwischen den verschiedenen Düngemitteln signifikant. Gleiches galt für die Löslichkeit von Phosphor. Die höchste bzw. niedrigste relative Uranlöslichkeit wurde mit 60,6% bzw. 10,2% des Königswassergehaltes in 2%iger Zitronensäure bzw. in Wasser gefunden.

b. Im Mittel über alle Extraktionsmittel wurde die niedrigste relative Uranlöslichkeit mit 14,7% für Rohphosphate, die höchste mit 67,8% für Mehrnährstoffdünger ermittelt. Im Gegensatz dazu wurde die höchste relative P-Löslichkeit mit einem Wert von rund 100% für reine P-Dünger gefunden.

c. Bei separater Betrachtung der vier verschiedenen Düngemittelgruppen zeigten die Ergebnisse dieser Arbeit signifikante Unterschiede zwischen der Uran- und der Phosphorlöslichkeit in verschiedenen Extrakten. Mit Ausnahme von alkalischem Ammoniumcitrat (AAC) zeigte Uran in allen Extrakten eine etwas geringere Löslichkeit als Phosphor.

Im ersten Jahr wurden zwei Vorversuche durchgeführt, um die Brauchbarkeit der Neubauermethode für die vorliegende Fragestellung, die Verwendbarkeit von Mais und Sonnenblume im Neubauerversuch, die Eignung verschiedener Karottensorten sowie das Verhalten verschiedener Substrate zu testen. Die Vorversuche erbrachten folgende Ergebnisse:

- Mais- und Sonnenblumenkeimlinge sind in der Lage, unter den speziellen Bedingungen der Neubauermethode mit hoher Pflanzendichte und einer Versuchsdauer von nur 3 Wochen zu wachsen.

- Die Neubauer-Vorversuche zeigten signifikante Wirkungen des aus Düngemitteln stammenden Urans auf U-Konzentration und U-Pflanzenaufnahme, während der Trockenmasseertrag von der P-Verfügbarkeit im Substrat innerhalb der kurzen Versuchsperiode von 22 Tagen unbeeinflusst blieb.

- Der Kick-Brauckmann-Vorversuch mit Karotte zeigte signifikante Wirkungen der P-Dünger und des Substrates auf Trockenmasseertrag sowie U-Aufnahme durch die Karotten, während der Effekt der Pflanzensorte auf Wurzel-Trockenmasseeertrag und Gesamt-Uranaufnahme nicht signifikant war. Auch der Effekt von aus Düngern stammendem U auf die U-Konzentration in Karotten sowie auf die U-Aufnahme aus dem Boden war nicht signifikant.

Im zweiten Jahr wurden eine Reihe von P-Quellen, die sich hinsichtlich ihrer Zusammensetzung (Rohphosphate, reine P-Dünger, mineralische und organo-mineralische Mehrnährstoffdünger), P-Löslichkeiten, U-Gehalte, U/P-Verhältnis und U-Löslichkeiten unterschieden, in einem Neubauer- und einem Kick-Brauckmann-Gefäßversuch getestet. Folgende Ergebnisse wurden erzielt:

- Die Wirkung verschiedener P-Quellen (bzw. des aus dem Dünger stammenden Urans) auf die Urankonzentration in den Wurzeln von Mais- und Sonnenblumenkeimlingen war sowohl auf Sand als auch auf dem gemischten Boden/Sand-Substrat stark signifikant ($p<0.001$). Im Gegensatz zur Urankonzentration der Wurzeln wurde die Urankonzentration in den Sprossen von Mais nicht von den unterschiedlichen P-Quellen beeinflusst. Bei den auf Sand gezogenen Sonnenblumenkeimlingen war nur die Urankonzentration in den Sprossen der Variante mit organisch-mineralischem NPK+Mg-Dünger (MD 19) signifikant höher als die der anderen Varianten.

- Die Wirkungen verschiedener P-Quellen auf die Urankonzentration des Karottenkrautes waren auf beiden Substraten signifikant, namentlich war die U-Konzentration im Kraut der Varianten mit organisch-mineralischem NPK+Mg (MD 19) und mit mineralischem NP (MD 28) signifikant höher als in den anderen Varianten. Die Urankonzentration in den Wurzeln (Karotten) zeigten nur auf Sand signifikante Unterschiede, mit einer Spanne von 12 bis 32 ng g^{-1} (Kontrolle bzw. teilaufgeschlossenes Rohphosphat MD 35).

- Die Urankonzentration in den Wurzeln von Mais- und Sonnenblumenkeimlingen war zwischen 10-100fach (je nach Düngevariante) höher als jene in den Sprossen. Dagegen zeigten die Karotten in Wurzel und Kraut Urankonzentrationen in vergleichbarer Größenordnung bzw. in den meisten Düngevarianten sogar niedrigere Konzentrationen in den Wurzeln.

Diese Ergebnisse deuten auf ein niedriges Risiko des Transfers von düngebürtigem Uran in die Nahrungskette durch Karotten hin.

- Die Uranaufnahme durch die Wurzeln bzw. durch die gesamte Pflanze wurde vom Urangehalt der Dünger signifikant beeinflusst (bei Maiskeimlingen $p<0{,}05$, bei Sonnenblumenkeimlingen $p<0{,}001$).
- Sowohl bei Mais als auch bei Sonnenblume war die Gesamt-Uranaufnahme für alle P-haltigen Dünger (mit Ausnahme des PK-Düngers MD38, bei dem keine substratbedingten Unterschiede zu sehen waren) auf Sand deutlich höher als auf dem gemischten Boden/Sand-Substrat. Vor dem Hintergrund sinkender Konzentrationen von Uranylionen und -komplexen aufgrund von Fällung, Adsorption und Kationenaustauschreaktionen in Gegenwart von Bodenkolloiden war dieser reduzierende Effekt des Bodenmischsubstrates auf den Pflanzentransfer von Uran erwartet worden. Anders als bei den P-Düngervarianten wurde für die KH_2PO_4-Kontrolle sowie für die Nullvariante eine höhere Wurzel- bzw. Gesamtaufnahme von Uran auf dem Mischsubstrat als auf Sand gefunden, was auf den niedrigeren Ausgangsgehalt von Uran in Sand (gegenüber dem Bodenmischsubstrat) zurück geführt wurde.
- Die höchste Urangesamtaufnahme von Mais- und Sonnenblumenkeimlingen (2,33 und 4,12 µg Topf^{-1} für Mais und 2,04 und 5,64 µg Topf^{-1} für Sonnenblume im Mischsubstrat bzw. in Sand) wurden bei reinen P-Düngern (Superphosphat) beobachtet.
- Die Uranaufnahme von Karottenwurzeln und –kraut wurde von den P-Quellen signifikant beeinflusst. Die höchste mittlere Aufnahme durch Karotten (Kraut, Wurzeln und Gesamtpflanze) wurde für die Variante mit dem organisch-mineralischen NPK+Mg (MD19) gefunden, sie unterschied sich signifikant von den anderen Varianten. Das legt nahe, dass dieser Dünger eine gut lösliche bzw. pflanzenaufnehmbare organische Komponente enthielt.
- Die Gesamturanaufnahme der Karotten war mit der Ausnahme des organo-mineralischen Düngers, der den höchsten Urangesamtgehalt und den höchsten wasserlöslichen Urangehalt aufwies, für alle Dünger auf Sand geringer als auf dem gemischten Boden/Sand-Substrat.

Hinsichtlich der Beziehungen zwischen chemischer Extrahierbarkeit und Pflanzenaufnahme von Uran aus unterschiedlichen P-haltigen Düngern wurden folgende Ergebnisse erzielt:

- Als bester Indikator der Uran- und P-Verfügbarkeit für Karotten wurde für die hier untersuchten Dünger das Wasserextrakt identifiziert. Die Zusammenhänge zwischen wasserlöslichem U und P und ihrer Aufnahme durch Karotten waren auf beiden Substraten signifikant, mit Korrelationskoeffizienten von 0,79 und 0,85 für U, und 0,83 und 0,90 für P im Mischsubstrat bzw. in Sand.

- Wie für Karotten war auch für die Sonnenblumenkeimlinge die Wasserextraktion am besten zur Einschätzung der Bioverfügbarkeit von U und P geeignet. Die Korrelationskoeffizienten zwischen den entsprechenden Parametern waren auf Sand für das Wasserextrakt am höchsten und in allen Fällen statistisch signifikant. Für das Mischsubstrat wurde eine stark signifikante Korrelation zwischen P-Aufnahme und P-Löslichkeit in Wasser ermittelt ($p<0{,}01$, $r=0{,}96$).

- Aus den Ergebnissen für Mais ist ersichtlich, dass die Einführung eines gemeinsamen Extraktionsmittels für die Abschätzung der Verfügbarkeit von P und U aus diversen P-haltigen Düngemitteln in diesem Fall nicht möglich ist. Für Uran war hier Königswasser das am besten geeignete Extrakt, für Phosphor neutrales Ammoniumcitrat.

Basierend auf den Ergebnissen der vorliegenden Untersuchung lässt sich schließen, dass sich Uran und Phosphor in den getesteten chemischen Extrakten unterschiedlich verhalten. Die Löslichkeit von U und P aus Düngemitteln hängt sowohl vom gewählten Extraktionsmittel, als auch vom Typ des Düngemittels ab. Weiterhin wurde fest gestellt, dass der Gehalt an düngerbürtigem Uran seine Löslichkeit und Pflanzenaufnahme sowie die Urankonzentration in der Pflanze im Fall von Mais- und Sonnenblumenkeimlingen signifikant beeinflussen kann. Allerdings war die Gesamtpflanzenaufnahme von Uran im Fall der reinen P-Dünger und des organo-mineralischen Düngers (MD 19) deutlich geringer als die Menge des zugeführten wasserlöslichen Urans, was auf ein potentielles Risiko des Transfers von düngebürtigem Uran in das Grundwasser hindeutet. Ein signifikanter Transfer von düngebürtigem Uran in Karotten fand nicht statt, mit Ausnahme der Variante mit dem organo-mineralischen NPK+Mg (MD19). Dies legt nahe, dass das Risiko eines Urantransfers aus mineralischen P-Düngern in die Nahrungskette über Wurzelfrüchte eher gering ist, was allerdings durch weitere Untersuchungen bestätigt werden sollte.

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