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Interactions between soil uranium contamination and fertilization with N, P and S on the uranium content and uptake of corn, sunflower and beans, and soil microbiological parameters

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LIST OF ABBREVIATIONS

BBCH-Code:	**B**iologische **B**undesanstalt, Bundessortenamt and **CH**emical industry
ACGIH:	American Conference of Governmental and Industrial Hygienists
ANOVA:	Analysis of Variance
CHPPM.	Center for Health promotion and Preventive Medicine
DHA:	Dehydrogenase Activity
DU:	Depleted Uranium
FAO:	Food Agricultural Organization of the United Nations
GLM:	General Linear Model
ICP-QMS:	Inductively Coupled Plasma-Quadropole Mass Spectrometry
LAI:	Leaf Area Index
LLD:	Lower Detection Limit
LSD:	Least Significant Difference
LW:	Leaf Weight
MTs:	Metallothioneins
NCRP:	National Council on Radiation Protection
NIOSH:	National Institute of Occupational Safety and Health
OSHA:	Occupational Safety and Health Administration
PAHs:	Polycylic Aromatic Hydrocarbons
PCBs:	Byphenyls
PCs:	Phytochelatins
RfD:	Reference Dose
Sig.:	Significance
TCE:	Trichloroethylene
TNT:	Trinitrotoluene
TSCF	Transpiration Stream Concentration Factor
TPF:	Triphenyl Formazan
TTC:	2, 3, 5-triphenyltetrazolium
UNEP:	United Nations Environment Programme
USEC:	United States Enrichment Corporation
US-EPA:	United States Environmental Protection Agency
WHO:	World Health Organization

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1 Introduction

Uranium is the heaviest chemical element to be found in the nature. It is a radioactive alpha emitter and a toxic heavy metal, which endangers environment and human health. This hazard potential is still misjudged frequently. As natural element, uranium is present in all spheres of life in varying concentrations. In the past 50 years the quantities of uranium, which have been set free into the environment by human activities increased, and the danger of importing uranium into the food chain increased simultaneously.

Natural uranium is a mixture of three isotopes (^{234}U, ^{235}U, and ^{238}U) which are in stable equilibrium. The big input of uranium into the environment comes from mining operations, nuclear industry, industrial and medical wastes, the use of phosphate fertilizers in agriculture (Kratz, 2004), and last but not least important depleted uranium (DU) ammunition used during the wars (Azuoazi et al., 2001; Barisic el al., 1992; Kobal et al., 1990; Schnug et al., 1996). The latter uranium source contains predominantly ^{238}U with the longest half live (4.5×10^9 years) of the three isotopes of the group, owing the same chemical proprieties and chemical toxicity (Burkart, 1988; 1991). The Figure 1.1 shows natural uranium sources and anthropogenic input of uranium into the environment; at which the great contribution to soil contamination is due to the wars.

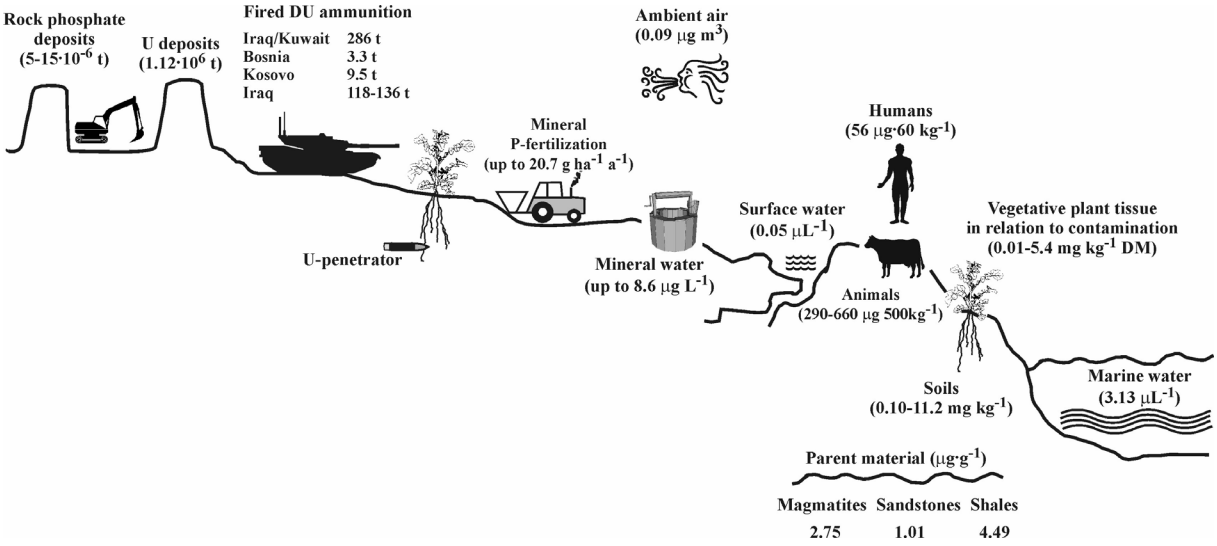


Figure 1.1: Natural uranium sources and anthropogenic input of uranium into the environment.

The potential risk of uranium soil contaminations is a global problem since about every country is affected by one or more activities mentioned before. The main geographical locations of minerals containing uranium are: Australia, Canada (Ontario), Czech Republic, France, Great Britain (Cornwall), Russia, South Africa, US (Colorado, Utah, New Mexico), and Zaire (HSDB, 1994) (Figure 1.2)

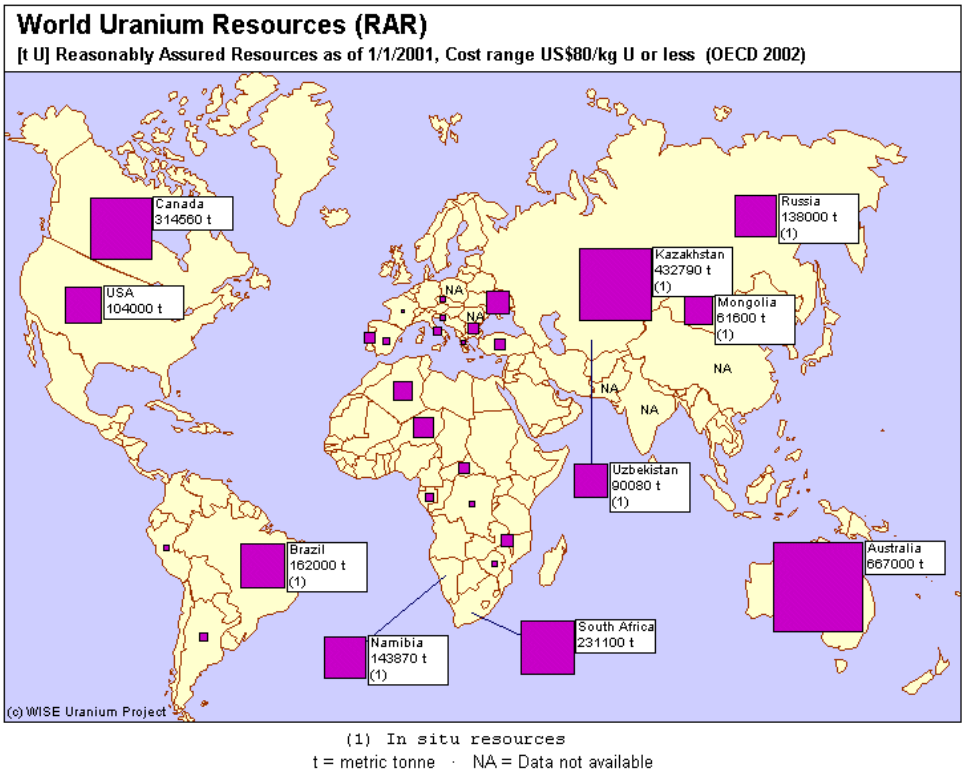


Figure 1.2: World uranium resources map (Source: http://www.antenna.nl/wise/uranium/).

Ten years ago, the military started the increasing employment of depleted uranium (DU) ammunition (Anderson, 2003), which has material properties to destroy armored vehicles; especially the last two Iraq wars (1991, 2003) lead to an increase of uranium in the environment.

DU is described by Moret (2004) as the "Trojan Horse of Nuclear War" because DU ammunitions does not only kill instantly, but also afterwards by its radiological and chemical toxicity. An estimated 286 t U was used in the Iraq/Kuwait (1991) wars, 3.3 t U in Bosnia (1994-1995), 9.5 t in Kosovo (1999), and between 118 and 136 t U in Iraq (2003) (Brand, 2004). All regions are characterized by a low supply of fertilizers and low soil quality (UNEP, 2003) (Figure 1.3).

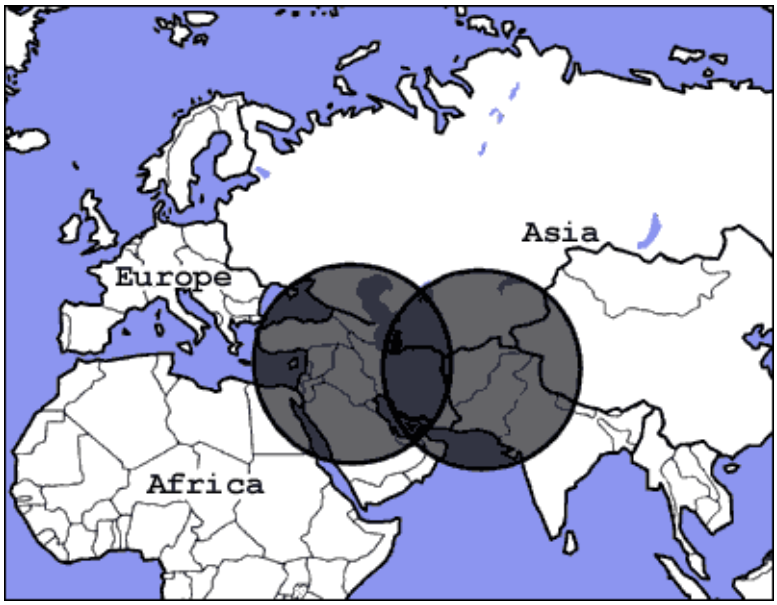


Figure 1.3: Map of regions within a 1,000 miles radius of Baghdad and Afghanistan that have been contaminated with depleted uranium since 1991 (Moret, 2004).

Uranium and its decomposition products are getting in direct contact with mineral and organic soil components, groundwater, microorganisms and plant roots.

The solubility of uranium in soils depends on many factors like pH, redox potential, temperature, texture, organic, and inorganic compounds, moisture and microbial activity. Microorganisms appear to be excellent indicators of soil health because they respond to changes in the soil ecosystem quickly. Close relationships with their surrounding environment are based on their high surface to volume ratio. Changes in microbial populations or activity can precede detectable changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil degradation.

Since microorganisms are involved in many soil processes, they may also give an integrated measure of soil health, an aspect that cannot be obtained with physical or chemical measures alone.

Soil enzyme activities are very sensitive to both natural and anthropogenic disturbances and show a quick response to the induced changes. Therefore, enzyme activities can be considered as effective indicators of soil quality changes resulting from environmental stress or management practices. Changes in enzyme activity have been found to be very responsive to measures such as non-tillage, organic fertilization, crop rotation and practices of organic farming. Likewise, restoration of degraded arid soils in marginal areas strongly influenced

soil enzyme activities (Quilchano and Marañón, 2002). However, so far no information exists about enzyme activity in DU contaminated soil.

Heavy metals are often accumulated in the top layer of the soil and therefore accessible for the roots of crops. In plants occur two stages in ion uptake and it is generally agreed that these also operate for metal ions, passive uptake via apoplast and active uptake via the symplast. Since primary cell walls consist of a network of cellulose, hemicellulose (including pectins), and glucoprotein, negative charges act as cation exchangers and anion repellers, for example generated on carboxylic groups (-R-COO$^-$) (Ross, 1994).
Uranium can adopt different valences (U^{3+} (III), U^{4+} (IV), UO_2^+ (V), and UO_2^{2+}(VI)), but the predominant status type in the environment is U (IV) and U (VI). These different valences are also an explanation for the toxicity potential of uranium compared to other heavy metals. Plants can only absorb organic nutrients in form of ions. The plant available UO_2^{2+} ion will be extracted from soil colloids by ion exchange procedures (Figure 1.4), as well as by the release of organic compounds of microorganisms or of the roots themselves (e.g. phenol acids, amino acids).

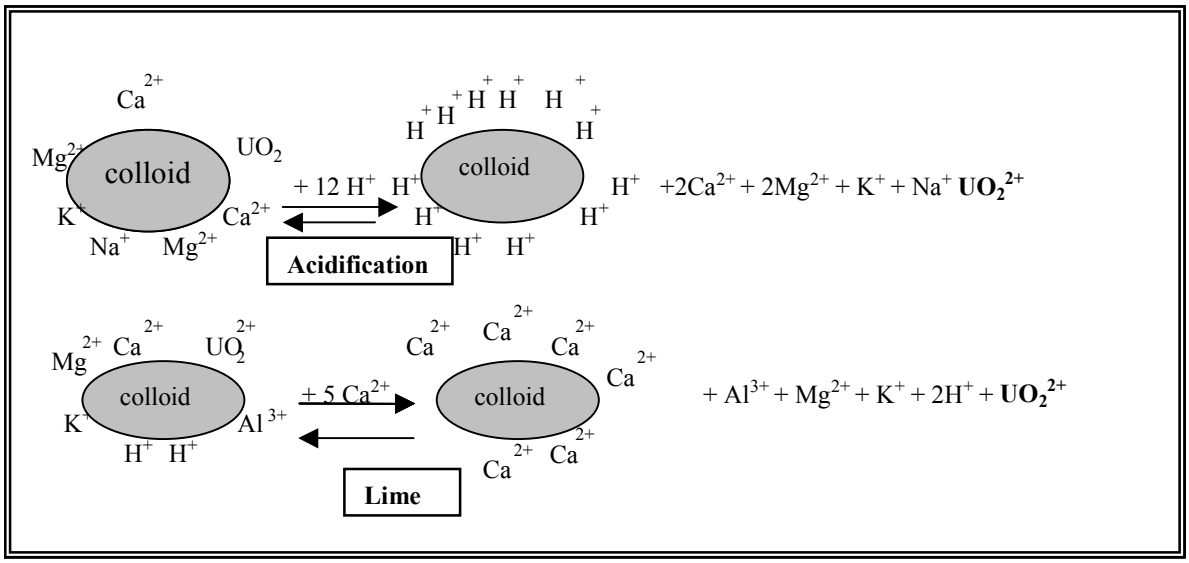


Figure 1.4: Ion exchange procedures in soil (Schroetter, 2004; adopted from Schröder, 1969).

Heavy metals are retained by soil in three ways (Baird et al., 2005):

1. By adsorption onto the surface of mineral particles
2. By complexation by humic substances in organic particles
3. By precipitation reactions.

Heavy metals belong to the trace elements group. For plants, they can be classified as non-essential or essential. Non-essential metals disturb the normal operational sequence of metabolic processes in the plant, even if present in smallest quantities. They can act toxically, depending on the dose (Figure 1.5). Soil organisms can also accumulate metals in their tissues with concentrations up to 50 times higher than in the surrounding soil. Ross et al. (1994) and Luckey (1980, 1982, 1991) found that biological functions are stimulated by low dose radiation, which is called "hormesis".

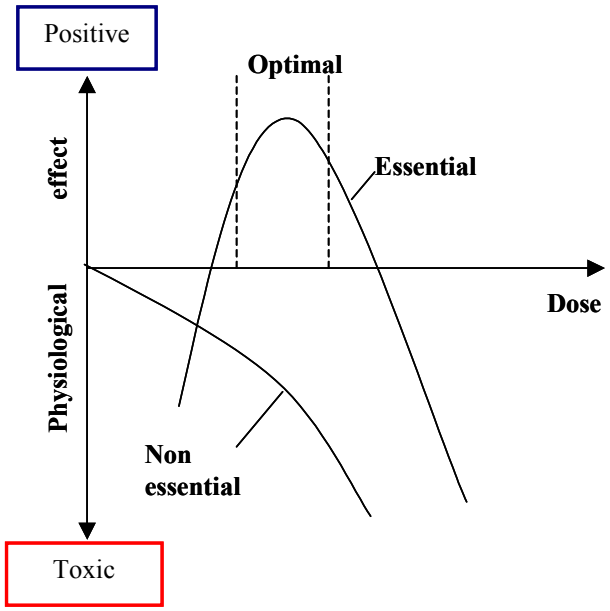


Figure 1.5: Dose – effect relationship of essential and non–essential metals for plant growth (Bliefert, 1994)

Uranium is a non-essential element. Toxic levels of metal in soil can detrimentally affect the number, diversity, and activity of soil organisms with side effects on soil organic matter decomposition and nitrogen liberalization processes. For soils in Germany the German Federal Soil Protection Ordinance (BBodSchV) defines precautionary threshold values for the most environmentally relevant heavy metals (Table 1.1), but so far, no threshold value has been established for uranium.

Table 1.1: Soil guide respectively threshold values for soils.

Soil guide value	Cd	Cr	Cu	Hg	Ni	Pb	Zn
	[mg g^{-1} DM]						
Sand	0.4	30	20	0.1	15	40	60
Loam/Silt	1.0	60	40	0.5	50	70	150
Clay	1.5	100	60	1.0	70	100	200

Source: German Federal Soil Protection Ordinance, BBodSchV, 1999

As it was mentioned before one of the main entry paths of DU in the environment is its military use. Although the agriculture sector relatively few contributed to the economy of Iraq before the Gulf War, recent investigations by United Nations Environment Programme (UNEP) showed that it plays an increasingly important role in the last years. Given serious import supply constraints, the government has implemented a number of measures aimed at achieving greater self-sufficiency in food. However, economic sanctions have limited access to foreign investment and imported supplies, including spare parts for farm machinery as well as fertilizers, pesticides and herbicides. In addition, the region suffered from a major drought at the end of 2000 (UNEP, 2003). Unsustainable water management practices, including construction of large dams and irrigation schemes, have resulted in deterioration of the soil quality and land productivity.

Phosphorus fertilization can reduce the uranium soil-plant transfer (Lamas, 2005), however there are no studies on the influence of nitrogen and sulfur fertilization on uranium soil plant transfer.

The larger part of nitrogen and sulfate taken up by plants is used for protein synthesis. Over a wide range of plant species and in different tissues the N/S ratio is between 20 and 40 on a molar basis. This means, that the efficient use of nitrogen in plant growth strongly depends on the absorption of appropriate amounts of sulfur.

The current database of the U.S. Environmental Protection Agency contains nearly 25,000 records on 21 metals in plants, related to the uptake, accumulation, and translocation by vascular plants. The largest numbers of records (> 1000) are for *Zea*, *Phaseolus,* and *Triticum* families (Nellesser, 1993). A relative high proportion of all metal records are for Cu (18.6%), Zn (17%), Cd (14.4%), and Pb (9%). Below 1% are records for V, Cs, Th, Sb, Pt, Be, Sn and U (Kabata-Pendias, 2000).

In this context the objectives and key questions of this work were developed:

1. Quantification of the influence of nitrogen, sulfur, and phosphorus fertilization on uranium content in plant material.

2. Characterization of differences in plant growth and uranium uptake between dicotyledonous and monocotyledonous crop species in dependence on the uranium contamination levels of the soil substrate.
3. Does uranium soil contamination have effects on the soil microorganism population?
4. Is the microbial activity affected by uranium soil contamination?

2 Review

2.1 Properties of uranium and its natural occurrence

Uranium is a heavy, ductile and slightly paramagnetic metal, silvery-white in color and pyrophoric when finely divided. It is slightly softer than steel and reacts with cold water when present in a finely divided state. It easily oxidizes in air and becomes coated with a layer of oxide (Bleise et al., 2002).

Natural uranium is present in earth crust material in concentrations between 2-3 mg kg^{-1}, (Table 2.1). Uranium is a mixture of three isotopes ^{234}U, ^{235}U, and ^{238}U, in a proportion of 0.01 %, 0.72 %, and 99.27 % respectively. The isotopes distribute different proportions of radioactivity. About 48.9 % of the radioactivity is associated with ^{234}U, 2.2 % is associated with ^{235}U and the remaining 48.9 % with ^{238}U. Each isotope has a different physical half-life, the time that it takes for half of that uranium isotope to release its radiation and change into a different element (US Environmental Agency Protection, 1999).

Table 2.1: Abundance of heavy elements in rocks (modified after Yaron, 1984).

Element	**Magmatites**	**Sandstones**	**Shales** (hydrolysates)	**Precipitates** (carbonate and sulfate rocks)	**Evaporites** (salt deposits)	**Seawater**
	[mg kg^{-1}]					
Cd	0.19	0.02	0.05	-	-	1.1×10^{-4}
Co	23.00	0.33	8.06	0.12	1.60	3.9×10^{-4}
Cr	198.00	120.00	423.00	7.08	10.60	2.0×10^{-4}
Cu	97.40	15.40	44.70	4.44	2.00	9.1×10^{-7}
Fe	42.20	18.60	38.80	8.19	265.00	3.4×10^{-3}
Hg	0.33	0.06	0.27	0.04	-	1.5×10^{-4}
Mn	93.00	392.00	573.00	842.00	4.40	4.0×10^{-4}
Ni	93.80	2.57	29.40	12.80	1.40	6.6×10^{-3}
Pb	15.60	13.50	80.00	16.50	0.90	3.0×10^{-5}
Tl	1.10	1.50	1.60	0.06	-	-
U	**2.75**	**1.01**	**4.49**	**2.20**	**0.20**	**3.3×10^{-3}**
Zn	80.00	16.30	130.00	15.60	0.60	5.0×10^{-3}

In the Table 2.2 properties of the natural uranium isotopes and the natural abundance are summarized.

Table 2.2: Properties of the isotopes of natural uranium.

Nuclide	Atomic mass	Natural abundance	Half-life
^{234}**U**	234.04 g	0.72 %	2.47 x 10^5 yrs*
^{235}**U**	235.04 g	0.005 %	7.00 x 10^8 yrs
^{238}**U**	238.05 g	99.28 %	4.51 x 10^9 yrs
Source: http://www.epa.gov/radiation/radionuclides/uranium.htm * yrs: years			

Uranium can be found within almost all natural materials in traces (Table 2.3, Table 2.4).

Table 2.3: Concentrations of natural uranium in the environment.

Total content in:		Value
Air		0.09 [µg m^{-3}]
Soil	Minimum	0.10 [mg kg^{-1}]
	Maximum	11.20 [mg kg^{-1}]
Fresh water		0.05 [µg L^{-1}]
See water		3.13 [µg L^{-1}]
Source: The Handbook of Trace Elements, 1997		

The daily intake of uranium is estimated to be 1 – 2 µg by food and 1.5 µg by water consume (Agency for Toxic Substances and Disease Registry, 1999).

Table 2.4: Concentrations of natural uranium in foods.

Concentration of natural uranium in food		
Type of food	**Fresh weight [ng g^{-1}]**	**Reference**
Whole grain products	1.45	NCRP 1984a
Potatoes	2.66-2.92; 15-18	NCRP 1984a; EPA1985j
Carrots	7.7	EPA 1985j
Root vegetables	0.94-1.20	NCRP 1984a
Cabbage	4.7	EPA 1985j
Meat	0.58-1.32; 20	NCRP 1984a; EPA 1985j
Poultry	0.14-0.42	NCRP 1984a
Beef	14	EPA 1985j
Beef liver	26	EPA 1985j
Beef kidney	70	EPA 1985j
Eggs	0.23; 9.6	NCRP 1984a; EPA 1985j
Cow milk	4	EPA 1985j
Fresh fish	0.43-0.85; 11	NCRP 1984a; EPA 1985j
Welsh onion	69	EPA 1985j
Wheat bread	19	EPA 1985j
Baked products	1.32-1.5; 12	NCRP 1984a; EPA 1985j
Polished rice	1.43-6.0; 15	NCRP 1984a; EPA 1985j
Macaroni	0.4-0.63	NCRP 1984a
Tea	5	EPA 1985j
Coffee	6	EPA 1985j
Red pepper	5	EPA 1985j
Mustard	0.2	EPA 1985j
Table salt	40	EPA 1985j
Canned vegetables	0.09-0.18	NCRP 1984a
Fruit juices	0.04-0.12	NCRP 1984a
Fresh fruits	0.71-1.29	NCRP 1984a
Dried beans	1.5-3.67	NCRP 1984a
Fresh vegetables	0.52-0.92	NCRP 1984a
Source: "Toxicological profile for uranium". US Department of Health and Human Services, 1999.		

The uranium in the human body is mostly derived from uranium in food, especially from vegetables, cereals, and table salt (Priest, 2001; Fisenne et al., 1987).

Table 2.5: Concentrations of natural uranium in human tissues.

Human body[1)]	**Value [µg]**
Total	56
Skeleton	32
Muscle tissue	11
Fat	9
Blood	2
Lung, liver and kidneys	< 1

[1]Human body = relating to a weight of 70 kg
Source: Fisenne et al., 1987

Natural uranium is considered as a weak radioactive element. In addition, uranium is categorized as a heavy metal with a chemo toxic potential (Burkart, 1988; 1991).

All natural uranium isotopes emit alpha particles, namely positively charged ions composed of two protons and two neutrons. Due to their relative large size and charge, alpha particles lose their kinetic energy rapidly and have little penetrating power. They are unable to penetrate even the superficial keratin layer of human skin. Uranium principally represents an internal radiation hazard. Uranium isotopes decay to other radioactive elements that eventually decay into stable lead isotopes. In the decay process, beta and gamma radiation will be emitted. Beta particles have greater ability to penetrate the skin than alpha particles. Gamma rays are extremely penetrating and can make up both, an internal and external hazard. In nature, uranium is in general equilibrium with the daughter of the decay chain. The decay products of ^{238}U (^{234}Th and ^{234}Pa) and ^{234}U (^{231}Th) are responsible for the presence of beta and gamma radiation in purified natural uranium (Bleise et al., 2002).

2.2 Use of uranium

In the 1940s, virtually all the uranium mined was used in the production of nuclear weapons, this production ceased in the 1970s. Today the substantial use of uranium is as fuel in nuclear reactors, mostly for electricity generation. Only the uranium isotope ^{235}U (app. 0.72 % of mass) is fissile. Consequently, during the production of nuclear fuel for most types of reactors, the relative concentration of ^{235}U needs to be increased. A by-product of this enrichment process is depleted uranium (DU). Metallic uranium (including DU) is 65 % (about twice times) more dense than lead (11 g cm^{-3} compared to 19 g dm^{-3}), has a high melting point (1,132 °C), is highly pyrophoric, and has a tensile strength comparable to most steels. These properties, as well as the relative high availability and low costs led to various civilian and military applications of DU.

One intended use of DU for example, has been as a cladding material in fast-breeder reactors, where its interactions with neutrons should produce additional reactor fuel as ^{239}Pu. DU has also been used as a fluorescent additive in dental porcelain crowns (now discontinued), as X-ray radiation shielding in hospitals, as containers for the transport of radioactive material, as chemical catalysts, as counterweights for rudders and flaps in commercial aircraft and fork lift and in the keels of sailing yachts.

In the early 1970s the US Army started to test the use of depleted uranium metal in kinetic energy penetrators and tank armors. High-density materials such as tungsten and DU were considered. DU has been finally selected due to its availability, price, and pyrophoricity (Bleise, 2002). Tungsten has a much higher melting point (3,410°C) compared to uranium (1,132°C) and has no pyrophoric properties (Figure 2.1). The surface of a DU penetrator ignites on impact (especially with steel) due to the high temperature generated by the impact and the relatively low melting point of uranium. In addition, the projectile sharpens; it melts and pierces heavy armors. DU impacts are often characterized by a small, round entry hole (US ACS, 1995).

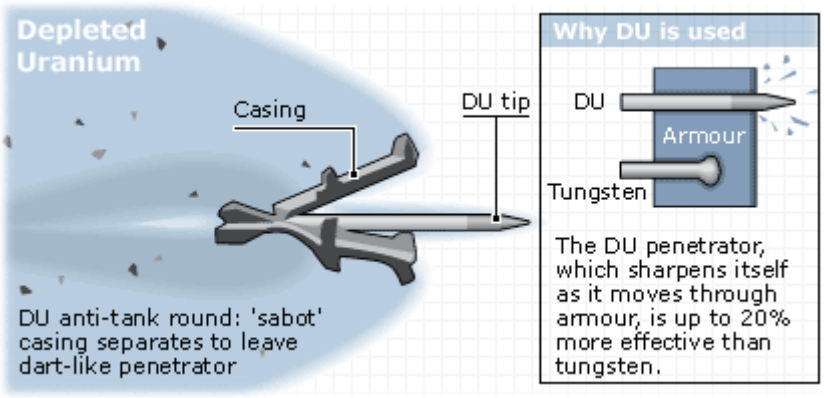


Figure 2.1: DU military advantage versus tungsten (BBC News, 2003).

2.3 Toxicology of uranium

Only the beta and gamma components of DU contribute to the external radioactivity dose. DU can be harmful to the health dependent on external or internal exposure to radioactivity. The main affected organ is the skin.

Internal exposure to DU can occur through three pathways:

- Ingestion (food and water)
- Inhalation (aerosol) and embedded fragments
- Contaminated wounds (soldiers).

Uranium and its oxides can be mobilized in the soil solution and contaminate drinking water or enter plants by their roots and finally go into the food chain (Birchall and Clark, 2001). When it enters the food chain, particularly the internal organs are affected, due to its pronounced toxicity. Gastrointestinal absorption of uranium can vary from < 0.1 to 6 %, depending on the solubility of the uranium compound (Leggett and Harrison, 1995; Wrenn et al., 1985) and the concomitant administration of oxidizing agents, such as the iron (III) ion and quinhydrone (Sullivan et al., 1986). The mayor uranium compounds produced are U_3O_8, UO_2, and UO_3 (Harley et al., 1999). These three uranium oxides are relatively insoluble and only dissolve slowly in body fluids (weeks for UO_3, years U_3O_8 and UO_2). Uranium compounds are classified according to their solubility as type fast (F) [UF_6], medium (M) [UO_3] and slow (S) [U_3O_8 and UO_2]. The solubility of U_3O_8 lies between type M and S. In the body fluids, uranium is dissolved as uranyl-ion (UO_2^{2+}), an ionic form that may react with biological molecules (Lin et al., 1993).

Following ingestion, uranium rapidly appears in the bloodstream (La Touge et al., 1987), where it is associated primarily with red cells (Fisenne and Perry, 1985). A non-diffusible uranyl-albumin complex also forms in equilibrium with a diffusible ionic uranyl-hydrogen carbonate complex (UO_2HCO^{3+}) in the plasma (Moss, 1985). Because of their high affinity for phosphate, carboxyl, and hydroxyl groups, uranyl compounds readily combine with proteins and nucleotides to form stable complexes (Moss, 1985). Clearance from the bloodstream is also rapid and the uranium subsequently accumulates in the kidneys and the skeleton, whereas only little is found in the liver (La Touche et al., 1987). The skeleton is the major location of uranium accumulation (Wrenn et al., 1985). The uranyl-ion replaces calcium in the hydroxyapatite complex of bone crystals (Moss, 1985). Once equilibrium is attained in the skeleton, uranium is excreted in the urine and faeces. Urinary excretion of humans has been found to account for approximately 1 % of total excretion, averaging 4.4 $\mu g\ day^{-1}$ (Singh et al., 1990). The rate is partly depended on the pH of tubular urine (Berlin and Rudell, 1986). Under alkaline conditions most of the uranyl-hydrogen carbonate complex is excreted in the urine. If the pH is low, the complex dissociates to a variable degree and the uranyl-ion may then bind to cellular proteins in the tubular wall, which may then impair tubular function (Royal Society, 2001).

The toxic hazard risk of uranium is not in its radiation effects but in its chemical effects on the renal tubules (Hamilton and Hardy, 1974). Uranyl-nitrate hexahydrate ($UO_2\ (NO_3)_2 \cdot 6H_2O$) inhibited the sodium transport and the independent adenosine triphosphate (ATP) utilization

as well as the mitochondrial oxidative phosphorylation in the renal proximal tubule. The chemical toxicity of uranium is as high as for example arsenic (Kirk - Othmer, 1984).

2.4 Regulations and proposed rules

The following values are suggested by different international scientific organizations, and some of them are used directly in European and French regulations.

The proposed values and limits are guides to determine an appropriate protection under exposure conditions at work and in the environment. They are not danger limits; caution is necessary when using them to estimate the risk of occurrence of a biological effect or a disease; real conditions of exposure must be taken into account

2.4.1 Inhalation

French regulations state that, considering the chemical toxicity of soluble uranium compounds, quantities inhaled in a single day must not exceed 2.5 mg of uranium regardless of the isotopic composition of the uranium.

Data in the legislation or recommendations for working environments in the United States are given in Table 2.6. These values given by the NIOSH (National Institute of Occupational Safety and Health), the OSHA (Occupational Safety and Health Administration), and the ACGIH (American Conference of Governmental and Industrial Hygienists) are determined for an exposure of 8 hours per day.

Table 2.6: Reference values for uranium, suggested by different international scientific organizations

Organization	**ACGIH**	**NIOSH**	**OSHA**
Soluble U compounds		0.05 mg m^{-3}	0.05 mg m^{-3}
Insoluble U compounds	0.2 mg m^{-3}	0.25 mg m^{-3}	0.2 mg m^{-3}

Given that the inhalation rate for a standard man at his workstation is 1.2 m^3 per hour corresponding to approximately 10 m^3 for an 8-hour work shift, it can be seen that the values in the Table 2.6 give limiting values for inhaled quantities equal to 2 to 2.5 mg per day for insoluble compounds of uranium and 0.5 mg per day for soluble compounds.

2.4.2 Oral ingestion

French regulations state that quantities ingested in a single day must not exceed 150 mg of uranium, regardless of its isotopic composition, due to the chemical toxicity of soluble compounds of uranium.

The EPA (Environmental Protection Agency) suggests a value of 3 μg $kg^{-1}d^{-1}$ as a reference dose (RfD). It is the quantity of soluble uranium salts that can be ingested without an appreciable risk of an effect harmful to health.

The World Health Organization (WHO) fixes guide values for chemical elements to guarantee lack of toxicity in drinking water consumed daily (based on 2 liters per day for an adult). The WHO has suggested a provisional guide value equal to 2 μg uranium per liter of water.

2.5 The problem of depleted uranium

The 30 mm DU rounds, which were used by the US air force in the Gulf and Kosovo wars, can pierce steel armor up to a thickness of 9 cm. The A-10 aircraft is equipped with a gun firing 3,900 rounds per minute. A typical burst of fired of 2-3 seconds involves 120-195 rounds. Normally, the DU ammunition is present in about 75 % of the rounds, the rest consist of non-DU ammunition. The shots hit the ground in a straight line. Depending on the angle of approach, they hit the ground 1-3 m apart and cover an area of about 500 m^2. The number of penetrators hitting a target depends upon the type of target. In most cases, not more than 10 % of the penetrators hit the target (CHPPM, 2000).

The DU dusts that may be formed during impact can dispersed and contaminate the environment. It is estimated that normally 10-35 % (maximum of 70 %) of the DU penetrator becomes an aerosol on impact or when the DU catches fired (Harley et al., 1999). It has been reported that most of the dust particles are smaller than 5 μm in size that keeps them in the air for an extended time and spread them over larger areas according to wind direction (US AEPI, 1995). DU dust can travel up to 40 km and remain airborne for a considerable time. The armor-piercing ammunition was claimed to contribute to health problems, known as the Gulf War Syndrome, cancer deaths, and birth defects and recently as the Balkan Syndrome (Durakovic, 2001). It has been estimated that **320 tons** of DU were used in weapons during the 1991 Gulf War and about **12 tons** were used in the Balkan in the late 1990s (Royal Society, 2002). During the activities of the US army in Afghanistan weapons systems that usually work with DU containing ammunition were used. Therefore, it can be assumed that this region is also contaminated (Fahey, 2003). Much of the DU ammunition

was likely fired near urban areas that would create a higher risk of exposure for the civilian population (Fahey, 2003).
The United States Enrichment Corporation (USEC) estimates a production of **85,000 tons** of DU through 2005, their disposal has not yet been determined (Byrd, 2000; Durakovic, 2001).
The United Nations Environment Programme (UNEP) accomplished environmental measurements on targeted DU sites in Kosovo in 2000, Serbia and Montenegro in 2001, and Bosnia and Herzegovina in 2002. In addition, UNEP was involved in the IAEA DU assessment in Kuwait in the spring of 2002. All these studies confirm that DU has environmental impacts.

2.6 Possible solutions to match DU contamination

Contaminated soil can be remediated by chemical, physical, or biological techniques. The available techniques may be grouped into two categories:

- *Ex situ* techniques which require removal of the contaminated soil for treatment on- or off-site
- *In situ* methods, which remediate without the excavation of contaminated soil. *In situ* techniques are favored over the *ex situ* techniques due to their lower costs and reduced impact at the concerned ecosystem (Khan, 2000).

On-site management of heavy metal contaminated soil can be achieved by diluting the contaminant to levels below given threshold values by using clean soil. Immobilization of inorganic contaminants is also a possible strategy (Mench et al., 1994). Immobilization can be achieved by complexing the contaminant (Wills, 1998) or increasing the soil pH by liming.
Soil washing or extraction for removing inorganic compounds from contaminated soil is the only alternative to the off-site burial method (Tuin and Tels, 1991). As with organic compounds, this technique produces a residue with high heavy metal contents that require further treatment (Dennis et al., 1994). This method though effective, is costly.

Furthermore, the physical-chemical technologies used for soil remediation decrease the usability of soil for plant growth as they also remove all biological activities, including useful microbes, such as nitrogen fixing bacteria and mycorrhizal fungi as well as fauna (Khan et al., 2000).

The majorities of plant species are sensitive to metal toxicity and thus are restricted to soils with low metal concentrations. They are strict or obligate non-metallophytes. Strict metallophytes (also called eumetallophytes, obligate metallophytes or absolute metallophytes) are taxa growing exclusively on soils with high metal concentrations. Between these two extremes are many intermediate species. These are the facultative metallophytes (sometimes called pseudometallophytes) (Pollard et al., 2002). Plants have a constitutive (present in most phenotypes) and adaptative (present only in tolerant phenotypes) mechanism for the accumulation or toleration of a high contaminant concentration in their rhizospheres. The physiological mechanisms of tolerance have been categorized as either exclusion, blocking the movement of metals at the soil/root or root/shoot interface or accumulation, allowing uptake of metals into aerial parts and rendering them non-toxic through chemical binding or intracellular sequestration (Baker, 1981; Baker and Walker, 1990; Ernst et al., 1992). The use of such plants to cleanup soils and water contaminated with organic and inorganic pollutants is a technique termed as phytoremediation. It is emerging as a new tool for *in situ* remediation.

There are two different types of soil pollutants. One is of mineral nature and the other one is of organic nature. Elemental pollutants include toxic heavy metals and nuclides, such as arsenic, cadmium, cesium, chromium, lead, mercury, strontium, technetium, tritium, and uranium (Dushenkov et al., 1997; Salt et al., 1998; Salt and Kramer, 1999).
Organic pollutants that are important targets for phytoremediation include polychlorinated biphenyls (PCBs) such as dioxin, polycylic aromatic hydrocarbons (PAHs) such as benzopyrene, nitro aromatics like trinitrotoluene (TNT) and linear halogenated hydrocarbons such as trichloroethylene (TCE). Many of these compounds are not only toxic and teratogenic, but are also carcinogenic (Cunnigham et al., 1996; Dushenkov et al., 1995).

The ideal phytoremediation crop should combine rapid growth and high biomass with high metal accumulation in the shoot tissues (Chaney et al., 2000; Lasat, 2002). Many of the known hyper accumulators are both small and slow growing and often species of limited population size and very restricted distributions (Pollard et al., 2002). Most heavy metal accumulating plants have roots penetrating only shallow depths. To allow remediation within a reasonable period (e.g. < 5 years) the plant yield and metal uptake have to be enhanced dramatically.

Another limitation of the phytoremediation is the potential contamination of the food chain, if animals graze on the heavy metal contaminated vegetation (Khan et al., 2000). Also, the disposal of the harvested biomass still needs to be resolved. Various techniques including air-drying, ashing or incineration, composting, pressing and compacting for landfill and leaching are some of the options (Salt et al., 1999). The primary factor limiting the potential success of U phytoextraction is the availability of U to plants. The free uranyl-cation (UO_2^{2+}), which predominates at a pH of 5.0 – 5.5 is the form of uranium that will most readily accumulated by plants. This means that it is present in solution and potentially mobile, so it is posing a threat to groundwater. Therefore, a long time is needed before phytoremediation is achieved at an acceptable level.

The pollution of contaminated land is generally heterogeneous. There are highly polluted areas and also less polluted areas that can be contaminated by the former. By chemotherapy, it is possible to reduce the transfer of pollutants among the several parts of the environment (soil, air, water, plants, etc.) and to increase their transformation. The parameters involved are those related to the pollutant: Physicochemical state (speciation), location, mobility, bioavailability, and degradation. Moreover, there are parameters related to the soil: pH, Eh, and agronomic conditions for microorganisms, plants, etc. to grow. The two sets parameters interact (Yaron et al., 1996).

The fixation of heavy metals by microorganisms, plants, or soil organic matter occurs on a short time scale. On the one hand, after the death of the microorganisms or the transformation of organic matter, toxic elements will again be mobile and bioavailable. In addition, if heavy metals are incorporated in mineral structures, a long-term fixation may be expected.

Several soil conditioners or fertilizers may be used to fix toxic material or to change the physicochemical properties of the soil (pH, Eh, organic matter, clay, etc.) in order to improve the fixation of pollutants by the solid phase of the soil or by chemicals (Yaron et al., 1996).

3 Material and methods

3.1 Interaction between uranium contamination and fertilization on uranium content and uranium plant uptake

3.1.1 Characterization of the soil substrate

The uranium-contaminated soil used for this experiment has been derived from a previous pot experiment (Lamas et al., 2005). It consisted of two kinds of soil, a silty-loamy sand soil extracted from a grassland site and a sandy soil from a forest site. Samples from different soil depths (0-25 cm and 25-50 cm) have been taken (Table 3.1). The original soil substrates, used in the experiment before, had the following treatments:

3 contamination levels of U (170, 360 and 650 mg kg^{-1} U) and one non contaminated initial soil substrate as control (U_t content 0.34 mg kg^{-1})
2 P fertilization rates given as $CaHPO_4$ (0 and 1,200 mg kg^{-1} P)
2 liming doses given as $CaCO_3$ (1,177 and 3,097 mg kg^{-1} Ca).

The existent root balls were cut, separated, and sieved in order to prepare the soil substrate for the microbial investigations. For the following analytical and microbiological analyses approximately 100 ml of the soil substrate were separated and stored in small plastic bags closed with cotton plugs to guarantee aerobic conditions until the experimental test.
For the greenhouse studies both kinds of soil (grassland and forest soil), both layers and lime doses were mixed in a way to preserve the U and P treatment (Figure 3.1).

Table 3.1: Grassland and forest soil properties.

Sample site	FAO classification	Soil depth	Carbon content [% C_{org}]
Grassland	Dystric Cambisol/ Orthic Luvisol	Top soil (0-25 cm)	1.9
		Sub soil (25-50 cm)	0.3
Forest	Leptic Podzol	Top soil (0-25 cm)	4.4
		Sub soil (25-50 cm)	3.2

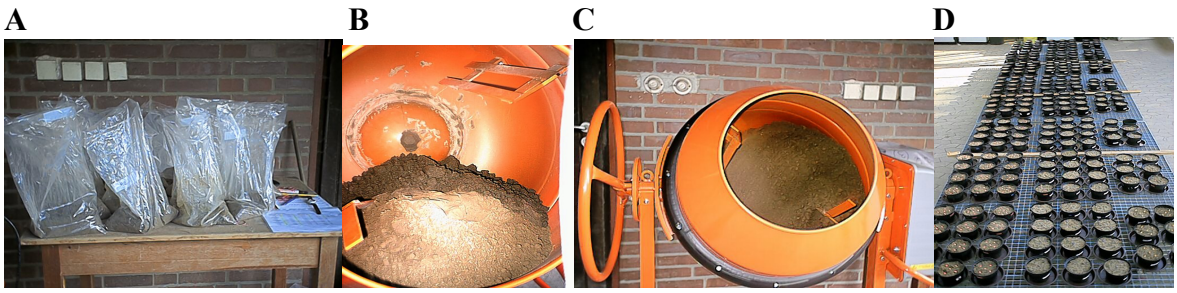


Figure 3.1: Sequence of the soil substrate preparation. A: Bags with grassland and forest soil, B: mixer machine with both soils inside, C: mixer machine working for a homogeneous mixture and D: filled pots (photos: D. Gardiman).

The uranium, phosphorus, sulfur and nitrogen contents and pH values used in the experiment are summarized in Table 3.2.

Table 3.2: Uranium, phosphorus, sulfur, nitrogen contents, and pH values of the initial soil substrate.

Treatments		**U_{total} [mg kg^{-1}]**	**$U_{available}$ [mg kg^{-1}]**	**P_{total} [mg kg^{-1}]**	**$P_{available}$ [mg kg^{-1}]**	**N_{total} [%]**	**SO_4-S [mg kg^{-1}]**	**pH**
U_1	Without P	Control	Control	334	0.06	70	9.35	6.5
U_2		166	64	334	0.04	71	9.95	6.8
U_3		329	126	334	0.06	75	10.90	6.6
U_4		660	251	334	0.07	77	10.40	6.5
U_1	With P	Control	Control	1,558	0.06	406	14.85	6.5
U_2		173	16	1,558	0.06	416	14.30	6.6
U_3		385	27	1,558	0.07	430	14.00	6.6
U_4		644	63	1,558	0.06	422	13.35	6.7

3.1.2 Experimental design

Three agricultural crops with different growth properties were tested:

- Corn, in the following will be referred as maize, (*Zea mays L.*)
- Sunflower (*Helianthus annuus L.*)
- Faba bean (*Vicia faba L.*).

Pot experiments had been conducted in the greenhouse under controlled water conditions. The pots (capacity 1 liter) contained 750 g of soil substrate and were seeded with 5 seeds per pot on June 25th and harvested on August 4th 2003 before the generative stage began.

Several treatments have been performed using three different uranium contaminations (plus control) combined with two different fertilizer levels and three different fertilizers (Table 3.3).

Table 3.3: Characterization of the U, P levels and N, S treatments.

U level in soil[1)]		**N rate[2)]**	**P level in soil[3)]**	**S rate[4)]**
Without $CaHPO_4$ supply	**With $CaHPO_4$ supply**			
[mg kg^{-1}]				
U_1: 0.34	U_1: $0.2 \cdot 10^{-4}$	N_1: 250	P_1: 334	S_1: 0
U_2: 166	U_2: 173	N_2: 500	P_2: 1,558	S_2:50
U_3: 329	U_3: 385	-	-	-
U_4: 660	U_4: 644	-	-	-

[1] U level in soil: added as U_3O_8
[2] N rate: added as NH_4NO_3
[3] P level in soil: added as $CaHPO_4$
[4] S rate: added as K_2SO_4

In the case of faba bean, no nitrogen fertilization had been applied. Nitrogen fertilizer was applied two times; the second portions 2 weeks after the first (June 30th and July 14th, respectively). In addition, 10 ml of Mg and micronutrients were supplied at each pot according to Table 3.4.

Table 3.4: Composition of the Mg and microelements solution.

Concentration of the parent solution	**Nutrient (chemical formula)**	**Initial weight for 1 L parent solution**	**Quantity required for 1 L nutrient solution**
5000 mg kg^{-1}	Fe-EDTA	3,286 mg	
0.5	$MgCl_2 \cdot 6H_2O$	102 g	40ml
100	$MnCl_2 \cdot 4H_2O$	295 mg	100ml
100	$ZnCl_2$	208 mg	100ml
100	$CuCl_2 \cdot 2H_2O$	268 mg	20ml
100	H_3BO_3	572 mg	50ml
		Total volume: (replenish to 1 L)	310ml

Each treatment combination was carried out with 3 replications, resulting in a total of 96 pots of maize, 96 pots of sunflowers and 48 pots of faba bean which sums up to a total of 240 pots in the experiment (Figure 3.2).

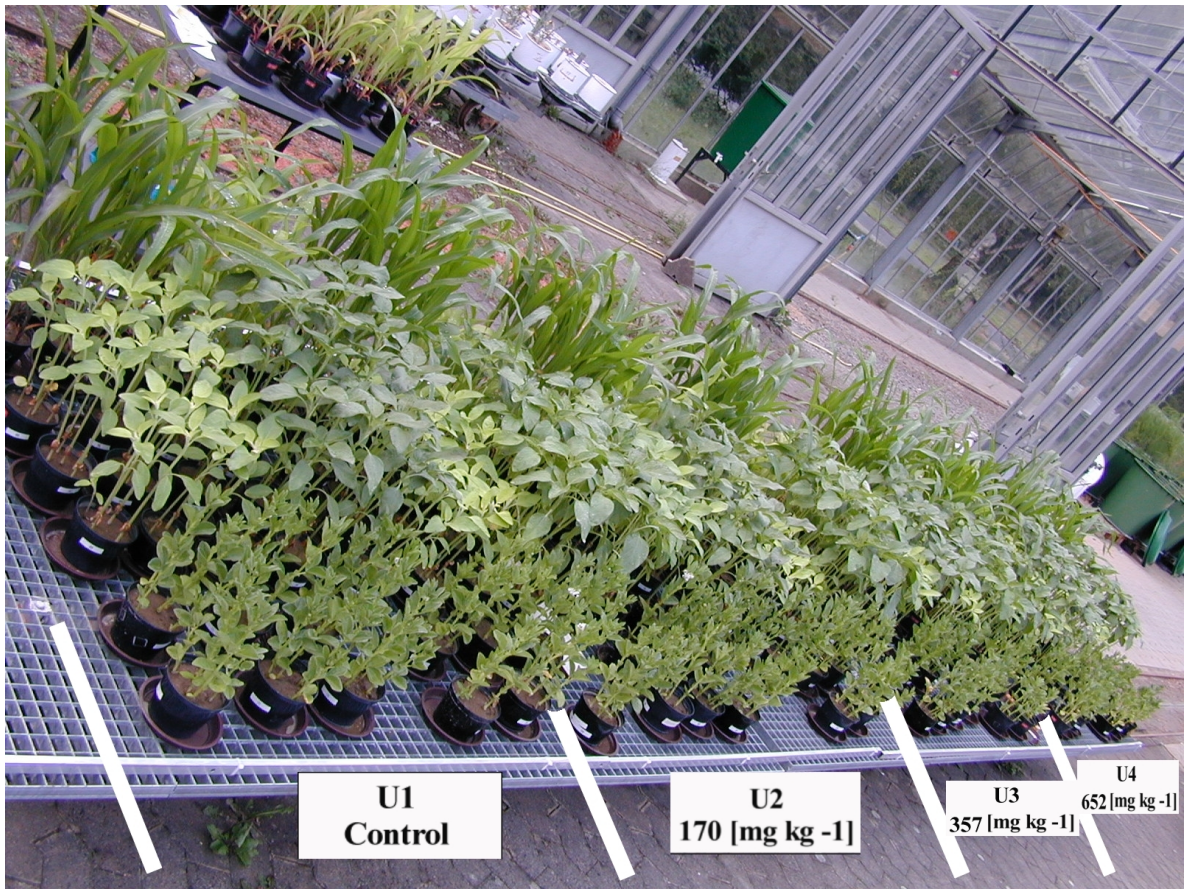


Figure 3.2: Experimental design: The influence of the N, P and S application rates in relation to the uranium contamination on biomass production and uranium uptake of maize (*Zea mays L.*), sunflower (*Helianthus annuus L.*) and faba bean (*Vicia faba L.*) (photo: D. Gardiman).

The experiment lasted 40 days. During this time, the pots were watered daily with deionized water two times a day to guaranty an optimal water supply for the growing plants.

At harvest shoots were cut from the roots, leaf area index (sunflower), height, and fresh weight were determined. Roots were extracted and stored according to safety procedures for future experiments. Shoots were oven-dried at 65°C for a minimum of 48 hours and the dry weight was measured. The dry plant material was ground to pass at 0.5 mm screen and kept in sealed plastic boxes until chemical analysis.

3.1.3 Analytical methods

Plant analysis

The development stages of the plants as well as the state of plant health were continuously controlled and assessed. Differences in germination time between crops, the number of plants per pot, the plant height, fresh and dry weight, and the numbers of leaves per plant were determined. Additionally the leaf area indexes of the youngest leaves of the sunflower plants were measured by the software package *image* (H & K Meßsysteme, Berlin). Nutrient concentrations of plant tissues were analyzed for calculating the total nutrient uptake.

Growth stages were determined according to the BBCH code (Weber and Bleiholder, 1990; Lancashire et al., 1991). At harvesting, the maize plants were at BBCH 15 (five leaves). The sunflowers were at BBCH 18/32 (between 8 and 10 leaves) and the faba bean were at BBCH 6 (main branch), 61 (beginning of the flowering), respectively.

All plant analytical data referred to the dry weight expressed as dry matter (DM).

In order to determine the total mineral ion concentration 50 mg of each plant sample were weight into a several Teflon container. 4 ml of HNO_3 and 1 ml of H_2O_2 were added and a microwave digestion procedure had been performed. Digested solutions were brought to a final volume of 50 ml with deionized water, filtered, and stored in plastic tubes. By the Inductively Coupled Plasma-Quadropole Mass Spectrometry (ICP-QMS) the content of elements, shown in Table 3.5 were determined. The total nitrogen content (%) in the shoots was determined by the *Kjeldahl* method (Table 3.5).

Table 3.5: Chemical analysis of plant tissues.

Parameter	Method
U, P, S, Ca, Mg, Fe, Mn, Zn, Cu, B, Mo	ICP-QMS. Lower limit of detection (LLD) was 15 ng L^{-1} (Sparovek et al., 2001)
N_t	*Kjeldahl* extraction (Hoffman, 1991)

Soil analysis

The soil analyses were carried out according to Lamas (2005). Soil samples from the pot experiment were air dried and sieved (mesh size 2 mm). All chemicals used were of "pro analysis" grade.

Solution extraction and procedures

The used solution was AAAcEDTA-extraction solution according to Sillanpää (1982)

The pH was adjusted to 4.65 with CH_3COOH or CH_3COONH_4.

For soils, a solution ratio 1:10 (5 g of dry soil and 50 ml of the extracting solution) had been shaken in polyethylene bottles for 1 hour at 27 rpm. The suspensions had been filtered through Schleicher & Schuell N 593 ½ filter paper, afterwards.

To avoid the high concentration of organic compounds in the extracts, which resulted in instabilities of the calibration of the ICP-QMS system; the following treatment was applied to the filtrated solutions: 10 ml of the AAAcEDTA filtrate were transferred to a ceramic crucible, evaporated on a sand bed at 200 °C to dryness, and then ashed in a muffle furnace at 550 °C over night. After cooling 0.2 ml of concentrated HNO_3 were added to each crucible and evaporated. Ashes were then dissolved with 10 ml of 2.5 % HNO_3 for ICP-QMS determination.

The P determination in soil was carried out in two steps:
Calcium-Acetat- Lactac (CAL) extraction according to Schüller (1969); followed by the colorimetrical analysis using a Perkin-Elmer 550SE UV/VIS spectrophotometer.

The SO_4-S determination was conducted according to Bloem (2002). The method consists in a modification of BLAIR methods, where 10 g air dry and sieved (<2 mm) soil was shaken with 50mL 0.0025 *M* KCl for 3 hr on a horizontal shaker. The samples were filtered (Schleicher and Schuell, N° 593) and the extracts ready for measurement with ICP-AES. Table 3.6 summarize the methods for soil analytical analyses.

Table 3.6: Analytical methods for soil analyses.

Parameter	Method
pH	Potentiometricaly in 0.01 M $CaCl_2$ suspension (Hoffmann, 1991)
N_t	*Kjeldahl* (Hoffman, 1991)
P_t, U_t	Digestion with aqua regia and ICP-QMS
P_{CAL}, $U_{available}$	P: extraction by Calcium-Acetate-Lactate (CAL) and final determination photometricaly U: extraction by AAAcEDTA (Sillanpää, 1982) and final determination by ICP-QMS
SO_4-S	According to Bloem et al. (2002)

3.2 Soil microbiological parameters

The soil substrate used in this experiment has the characteristics reported in Table 3.1.

3.2.1 Dehydrogenase activity

The soil microbial activity was estimated by the measurement of dehydrogenase activity (DHA). DHA reflects a broad range of oxidative activities. Free dehydrogenases in soil systems are not expected since they are intracellular enzymes (Rossel and Tarradelas, 1991).
Soil samples were suspended in a triphenyltetrazolium chloride solution and incubated for 24 hours at 30°C. The triphenyl formazan (TPF) produced was extracted with acetone and measured photometrically at 546 nm (modified from Thalman, 1968).

Solution extractions and procedures:

The solution utilized were the following:
Tris buffer (0.1M)
Substrate solution: 0.5 TTC (w/v), 2, 3, 5-triphenyltetrazolium chloride (TTC) in Tris buffer was dissolved and stored in the dark at 4°C
Acetone p.a
Standard solution (0.1 mg TPF ml^{-1}).

TTC (2, 3, 5-triphenylterazolium chloride) and TPF are sensitive to light, to avoid bright light during the entire reaction the Erlenmeyer flakes were covered with aluminium paper.
Procedure: In darkness conditions, 2 g of fresh soil were placed into four tubes. 5 ml of substrate solution to three tubes (samples) and 5 ml of Tris buffer to the fourth tube (control) were added; mixed and closed with rubber stoppers, afterwards incubated for 24 hours at 30°C in the dark.

To extract the produced triphenyl formazan, 10 ml of acetone (to both samples and control) were added, then mixed and shaken every 30 minutes, during 2 hours in the dark.
Subsequently, the solutions have been filtrated in a semi dark room. The extinction of the filtrates and calibration standards has been measured photometrically at 546 nm within 1 hour.

Calculation of the results

Determination of the µg TPF in the filtrates from the calibration curve:

$$TPF = \frac{(S - C) \cdot 100}{2\ \%\ DM}$$

Where:

TPF: [µg g^{-1} DM 24 h]

S: extinction value (average of the replications) estimated on the base of the calibration curve (photometrically at 546 nm, 1 hr) [µg TPF]

C: control, it was also calculated on the base of calibration curve mentioned before [µg TPF]

2: initial soil weight [g];

100· $\%^{-1}$dm: factor for soil dry matter

3.2.2 Microorganism count

In the present study the population of fungi, aerobic heterotrophic bacteria and actinomycetes were measured using the agar spread techniques, which rely on the growth of a microbial population to levels that are visible. This is achieved under specific conditions, e.g. time, temperature, oxygen content and pressure in liquid or on solid media containing specified nutrients. This technique assumes that each colony derived from an individual cell and that the incubation conditions allowed the recovery of all cells present.

Solutions and procedures:

Agar spread technique: A soil sample was suspended and a series of decimal dilutions were prepared. Aliquots of appropriate dilutions were spread to Petri dishes with a glass spatula Drigalski, on solidified, sterilized and at room temperature nutrient agar. During incubation at 20°C and controlled humidity the colonies of the three kinds of microorganisms were grown. The colonies were counted and the number of viable fungi, aerobic heterotrophic bacteria, and actinomycetes per gram of soil were estimated by considering the soil dilution.

Fungi

Nutrient solution (Table 3.7): 16.5 g of Worth Broth (Merck) were dissolved in a liter of bidest water and mixed. The pH was fit at **4.5,** afterwards 20 g of agar-agar (Roth) was added.

Table 3.7: Nutrient solution: Worth broth (Merck).

Ingredients	Amounts
Malt extract	7.500 [g]
Universal peptone	0.375 [g]
Maltase	6.375 [g]
Dextran	1.375 [g]
KH_2PO_4	0.375 [g]
NH_4Cl	0.500 [g]
Rose Bengal	1 [ml]
Agar-agar	20 [g]
Bidest water	1,000 [ml]
pH 4.5 (fit with 2N NaOH or 2N HCl)	

The nutrient solution was sterilized in an autoclave for 15 min at 121°C and 100 kPa. Afterwards, the solution was cooled down to about 50°C and placed in Petri dishes.

Aerobic heterotrophic bacteria

Nutrient solution: 2.5 g of Standard I Broth (Merck) nutritive (1/10) (Table 3.8) has been dissolved in a liter of bidest water and mixed.

Table 3.8: Nutrient solution: Standard I nutrient Broth (Merck).

Ingredients	Amounts [g]
Peptones	15
Yeast extract	3
Sodium chloride	6
D (+) glucose	1
Agar-agar (not present in the broth)	12
pH 7.5 (fit with 2N NaOH or 2N HCl)	

The pH was set to 7.5, afterwards 20 g of agar-agar (Roth) were added. The nutrient solution was sterilized in an autoclave for 15 min at 121°C and 100 kPa. After cooling to about 50°C the solution was placed in Petri dishes.

Actinomycetes

In order to prevent infection with fungi and specially yeast, Nystatin and Actidion were added to the agar nutrient solution. To avoid vegetative bacteria growth dilutions series were prepared with phenol. In this condition only is expected to found arthrospore, which are the spore of actinomycetes.

In Table 3.9 the ingredient of the nutrient solution for the growth of actinomycetes are summarized.

Table 3.9: Nutrient solution for actinomycetes.

Ingredients	Amount
Glucose	0.2 [g]
Casein	0.2 [g]
KH_2PO_4	0.5 [g]
$MgSO_4 \cdot 7\ H_2O$	0.2 [g]
Trace elements solution (Drew, 1983)	5 [ml]
Agar-agar	15 [g]
Bidest water	1,000 [ml]
pH 6.7 (fit with 2N NaOH or 2N HCl)	

Extraction: 10 g of soil (natural moisture content) and 90 ml of $Na_4P_2O_7$ solution were placed in a 200 ml bottle, 5 glass beads (Ø 3 mm, sterilized) was added. The suspension were mixed for 20 min. After 10 min, the supernatant was decanted and diluted with physiological NaCl but only for bacteria and fungi. Special solution with phenol was prepared for actinomycetes.

Dilution: A serial dilution was carried out, 1 ml of supernatant was added at 9 ml of physiological NaCl and became range of dilution 10^{-2}, 1 ml of dilution 10^{-2} was added at 9 ml of physiological NaCl and became range of dilution 10^{-3} etc. The following dilutions were employed:

- For fungi: 10^{-1}, 10^{-2}, and 10^{-3}
- Aerobic heterotrophic bacteria: 10^{-3}, 10^{-4}, and 10^{-5}
- Actinomycetes: 10^{-2} and 10^{-3}.

Incubation:

Fungi and aerobic heterotrophic bacteria were incubated for 7 days at 20°C ± 2°C, whereas the incubation for actinomycetes was 14 days at the same temperature.

Calculation of the results

Determination of the colony forming units per grams of dry soil:

$$CFU = \frac{N° \cdot C \cdot 10 \cdot 100}{100 - \%\ M}$$

Where:

CFU: colony forming units [CFU g^{-1} dry soil]

N°: count of colonies per gram of fresh soil

C: concentration of dilution used

$\frac{100}{100-\%M}$:Conversion factor to express in dry soil

10: concentration of soil extraction dilution

% M: percentage moisture

3.3 Statistical methods

The results were analyzed statistically by a General Linear Model procedure, 3 and 4 way ANOVA using the statistical software-package SPSS (1998). The mean standard, the analysis of variance, and the linear regression coefficients were determined. Least significant difference value (LSD) between mean values were significant at $p < 0.05$ level.

3.4 Safety measures

Uranium and its compounds are hazardous materials, for both the radioactivity and chemical point of view. Despite the total amount of uranium employed in the experiments was below the threshold values for which the German law request permission by government authorities; good practices of work were adopted. For instance, work with dry soil was performed in aired place and protection clothes, respiratory protection and gloves were used.

Good practices of safety work employed:

Staff always wore disposable dust protection masks, overalls and latex gloves when attending the experiment or during sampling and sample preparation.

All plant material harvested was used in analyses.

The soil surfaces of the vegetation pots were covered with a layer of quartz sand to avoid dust development from dry surfaces.

Filtration residues from soil extractions were collected and disposed according to the regulations for low radioactive wastes.

After the experimentation the soil of each individual pot was transferred to a polyethylene bag, sealed after air-drying and stored in a refrigerator for further experiments.

4 Results

4.1 Influence of U contamination and N, P and S rates on biomass production and uranium uptake of crop plants

Growth and development of plants are characterized by carbon assimilation and morphological changes expressed as differentiation processes during the plant cycle. Crop productivity covers the total production of plant material by a crop, above and below ground. In total six macronutrient and seven micronutrients are essential for plant growth according to the rules of Arnon (1954), these are: Nitrogen (N), phosphorus (P), sulfur (S), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), molybdenum (Mo) and chlorine (Cl). Nevertheless, some heavy metals are also required micronutrients. Toxic effects of these elements are, thus, largely a function of concentration. These elements are beneficial and have nutritional values lower than some critical dosages but become inhibitory to toxic with an increase in concentration, as shown in Figure 1.5. The threshold toxic concentrations differ for each heavy metal and are governed primarily by the chemistry of each heavy metal in question and associated physiologic effects. On the contrary, nonessential heavy metals are inhibitory at all concentrations.

In the presented study the influence of the N-, P- and S rates in relation to the uranium contamination rates on biomass production and uranium uptake of maize (*Zea mays L.*), sunflower (*Helianthus annuus L.*), and faba bean (*Vicia faba L.*) was determined and results are presented individually for each crop.

4.1.1 Biomass production and root morphology of maize (*Zea mays L.*)

Two rates of N, P and S were chosen for experimentation; with lower rates of 250 mg kg^{-1} N, 334 mg kg^{-1} P and 0 mg kg^{-1} S ($N_1P_1S_1$) being insufficient for optimum plant growth and higher application rates of 500 mg kg^{-1} N, 1,558 mg kg^{-1} P and 50 mg kg^{-1} S ($N_2P_2S_2$) fully marginally satisfying the nutritional demand.

Uranium contaminations rates were at the following doses: 170, 357, 652 mg of U kg^{-1} soil, plus control: 0.34 mg kg^{-1}. All four factors were factorials combined. Reference values for sufficient ranges for N, P and S of maize are summarized in Table 4.1.

Table 4.1: Sufficiency ranges for the N, P and S supply of maize (whole shoot plant material, BBCH-code 15).

Nutrient	Sufficiency range			Deficiency range
	[%]			
N	3.50	-	4.50	< 3.50
P	0.30	-	0.50	< 0.30
S	0.21	-	0.75	< 0.08

Source: Reuter et al. (1997)

The mean N, P and S concentrations are shown for the low and high nutritional levels in Table 4.2.

Table 4.2: Influence of P, N and S rates on the N, P and S concentrations of maize grown on a soil without U contamination (control).

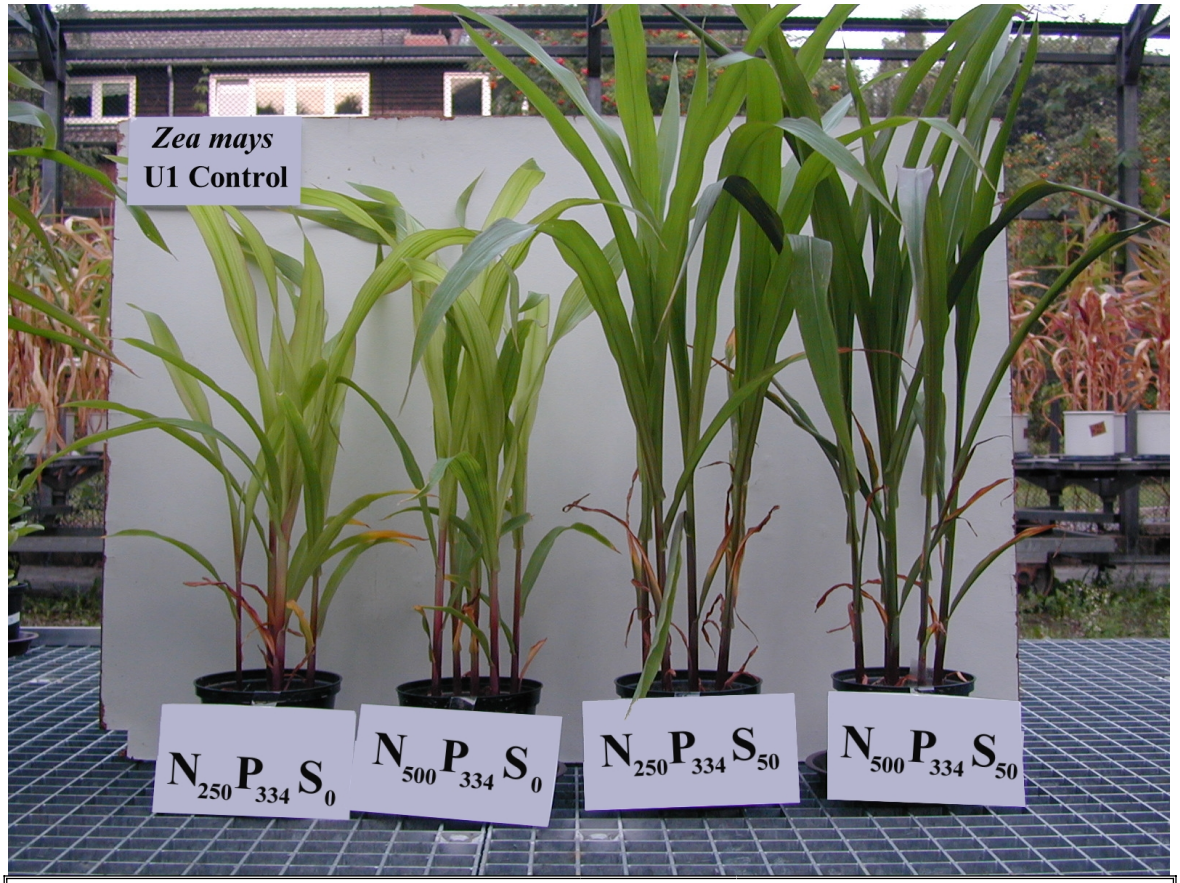
Nutrient	Treatments							
	$N_1P_1S_1$	$N_1P_1S_2$	$N_1P_2S_1$	$N_1P_2S_2$	$N_2P_1S_1$	$N_2P_1S_2$	$N_2P_2S_1$	$N_2P_2S_2$
	[%]							
N	2.34*	1.57*	2.75*	1.53*	4.19	2.77*	5.13	2.32*
P	0.18*	0.13*	0.39	0.28*	0.19*	0.13*	4.51	0.36
S	0.05*	0.12	0.05*	0.10	0.05*	0.16	0.05*	0.15

* Deficient according to data in Table 4.1
N rate [mg kg^{-1}] 1 = 250, 2 = 500
P rate 1 = 334, 2 = 1,558,
S rate 1 = 0, 2 = 50

The results presented in Table 4.2 reveal that no combination of treatments yielded a sufficient N, P and S nutrition, whereby the lower N concentration in the $N_2P_2S_2$ and $N_2P_1S_2$ treatment can be explained by a dilution effect caused by the enhanced biomass production in these pots. These deficient and/or marginal levels of the N, P and S nutrition were chosen in order to provide an estimate about the U uptake by plants of different crops in extensive farming systems.

From the three nutrients tested, the S rate had the distinctly strongest influence on the biomass production with a mean increase by 333% (Figure 4.1 and 4.3). The higher N and P rates led accordingly to a relatively higher crop productivity of 115 % and 131 %, respectively. Sulfur is related to plant growth and the nutritive value in crops such as forages (Wang et al., 2002), wheat (Haneklaus and Schnug, 1992; Zhao et al., 1996, 1997), pea (Zhao et al., 1999) and rape-seed (Helal and Schnug, 1995; Zhao et al., 1997) by increasing grain yield, promoting general vegetative growth and enhancing protein and chlorophyll content, dry matter digestibility and intake of animal feeds and baking quality of cereals. The distribution of S in

sulfur deficient plants is also closely related to the N supply, S deficiency symptoms are most severe under high N-input (Schnug, 1990).



Biomass [g pot^{-1}]	6.8	6.2	10.6	11.8

N rate [mg kg^{-1}] 1 = 250, 2 = 500
P rate 1 = 334
S rate 1 = 0, 2 = 50

Figure 4.1: Influence of the N and S rate at low P rate on growth of maize (photo: D. Gardiman)

Biomass [g pot^{-1}]	4.9	4.6	13.2	15.3

N rate [mg kg^{-1}] 1 = 250, 2 = 500
P rate 2 = 1,558,
S rate 1 = 0, 2 = 50

Figure 4.2: Influence of the N, P and S rates at high P rate on growth of maize (photo: D. Gardiman)

Root morphology

Mineral nutrients supply can strongly affect root growth, morphology and distribution of root systems in the substrate. This effect is particularly marked for nitrogen, but less distinct for phosphorus (Marschner, 2002). Helal and Schnug (1995) reported that an increasing S supply not only prolonged root length and retarded root mortality of *Brassica napus*, but also enhanced S and N uptake by plant and utilization. A qualitative assessment of the influence of P, N and S rates on root development of maize grown on a soil without U contamination was carried out.

Many plants produce finer roots when grown at low nutrient supply rate (Fitter, 1987) that corresponds with the results found in the presented experiments (Figure 4.3).

In oilseed rape for example, with decreasing nitrate concentration root hairs become much more frequent all along the root axis. When N is limited, increasing root hair length can also

be found for grasses (Robinson and Rorison, 1987) and was determined for maize (Figure 4.3).

The effect of phosphorus on root hair formation is similar to that of nitrate. The plants shown in Figure 4.4 were grown at the higher P rate than those presented in Figure 4.3. The images reveal that a lower P rate had no effect on the root hair length, but it distinctly increased the density of root hairs per unit root length.

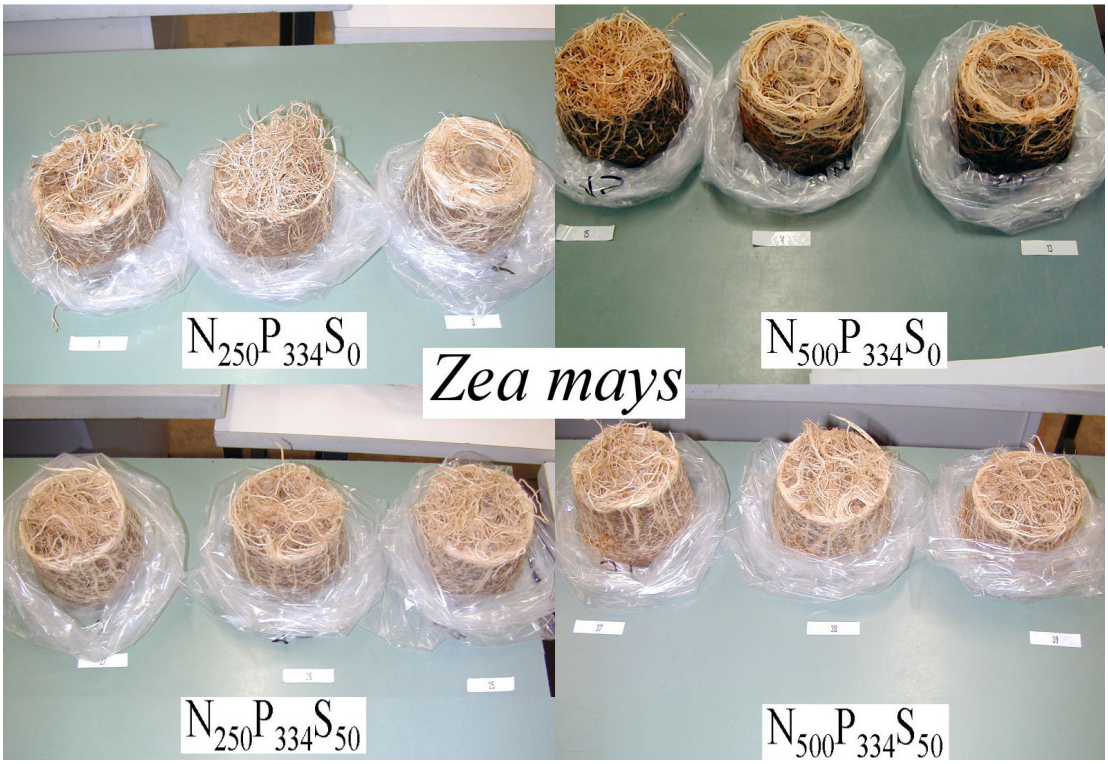


Figure 4.3: Influence of increasing N and S rates at a low P rate in soil on root density of maize grown on a soil without U contamination (control) (photos: D. Gardiman).

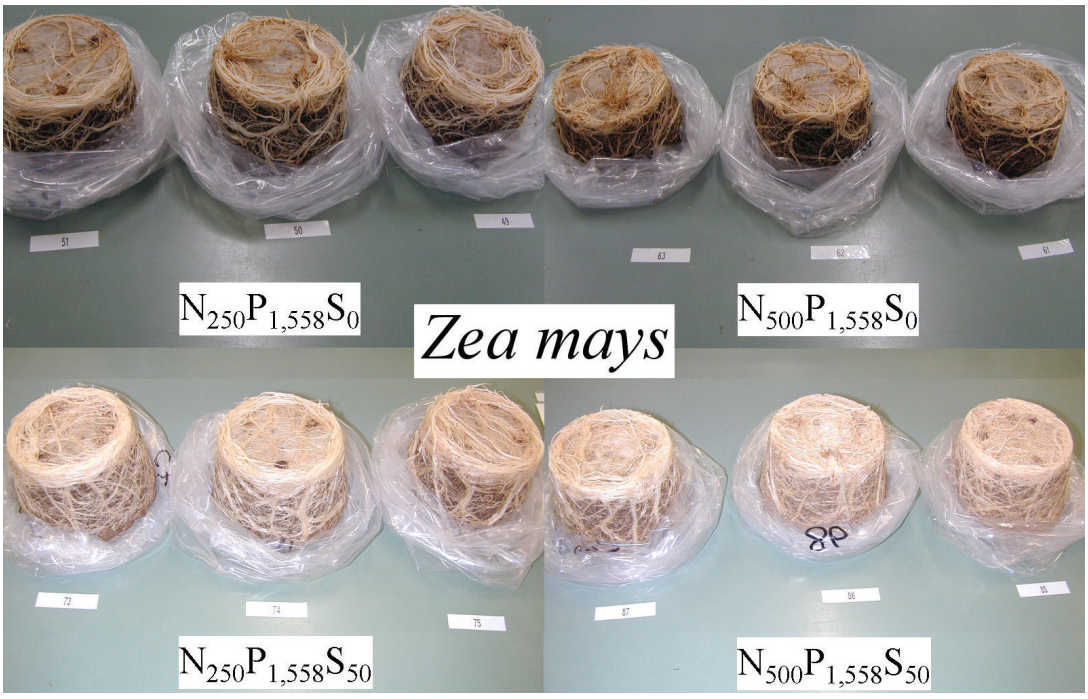


Figure 4.4: Influence of increasing N and S rates at a high P rate in soil on root density of maize grown on a soil without U contamination (control) (photos D.Gardiman).

4.1.2 Influence of U contamination levels and P, N and S rates on biomass, U concentration, U uptake, and the concentration of macro and micronutrients of maize

U contamination levels significantly decreased the biomass production in the treatments U_2 (170 mg kg^{-1}) and U_4 (652 mg kg^{-1}) compared to control (Table 4.3). The P concentration was significantly lower in the U_2 (170 mg kg^{-1}) treatment then at the higher U rates (U_4). Additionally with increasing U rate the U concentration (Figure 4.5) and the concentrations of Fe, Mn, Cu and B increased, while that of Zn decreased (Table 4.3).

Table 4.3: Influence of the U-contamination levels in soil on biomass production, U concentration, U uptake and the concentration of macro and micronutrients in maize (4 way ANOVA).

Variable / Factor	Biomass	U concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
U rate[2)]	[g pot^{-1}]	[mg kg^{-1}]	[µg pot^{-1}]	[%]					[mg kg^{-1}]					
1	9.17	0.01	0.10	2.83	0.26	0.090	0.49	0.12	123.2	42.1	21.6	5.3	6.7	1.6
2	7.53	1.21	7.39	2.65	0.25	0.100	0.47	0.12	149.2	43.4	20.7	4.3	6.7	1.5
3	8.33	1.66	10.88	2.83	0.28	0.090	0.50	0.13	91.0	47.8	23.9	6.7	7.7	1.8
4	7.76	3.98	23.24	2.90	0.28	0.090	0.58	0.14	99.6	51.6	19.1	6.8	9.1	1.6
LSD $_{5\%}$	0.90	0.87	4.50	0.14	0.02	0.005	0.11	0.02	22.5	8.7	2.9	1.2	2.4	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652

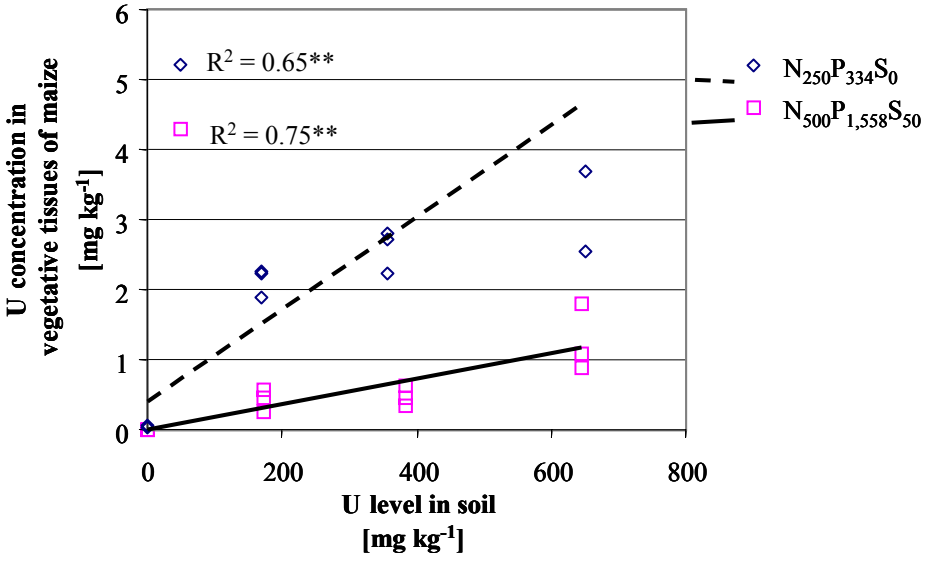


Figure 4.5: Influence of the U rate on the U concentration in vegetative tissue of maize in relation to the P, N and S rates.

The higher N rate significantly increased ($p < 0.05$) the concentrations of U, P, S, Ca, Fe, Zn and the U uptake (Table 4.4). Miller (1974) found in this context that N fertilization yielded a higher translocation rate of P to the shoot, while the uptake itself was not influenced.

Table 4.4: Influence of the N rate on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients in maize (4 way ANOVA).

Variable / Factor	Biomass	U-concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
N rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	8.27	1.29	8.88	2.07	0.25	0.080	0.44	0.13	101.3	46.8	19.5	5.6	7.2	1.7
2	8.13	2.14	11.92	3.53	0.28	0.100	0.58	0.13	130.3	45.6	23.1	5.9	7.9	1.5
LSD $_{5\%}$	0.64	0.62	3.18	0.10	0.01	0.003	0.08	0.01	15.9	6.2	2.0	0.8	1.7	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]N rate [mg kg^{-1}]: 1 = 250, 2 = 500

The rate of P significantly increased ($p < 0.05$) the concentrations of N, Ca, Fe and Mo, while it led to a significant decrease of the S, Mn, Zn, B-concentrations and the U uptake (Table 4.5).

Table 4.5. Influence of P rate on biomass production, U concentration, U uptake and the concentration of macro and micronutrients in maize (4 way ANOVA).

Variable / Factor	Biomass	U concentration	U Uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
P rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	8.37	1.93	13.97	2.68	0.16	0.090	0.44	0.13	108.6	55.9	23.0	5.9	8.4	1.5
2	8.02	1.50	6.84	2.93	0.38	0.090	0.57	0.13	123.0	36.5	19.6	5.7	6.7	1.8
LSD $_{5\%}$	0.64	0.62	3.18	0.10	0.01	0.003	0.08	0.01	15.9	6.2	2.0	0.8	1.7	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

The rates of S significantly increased ($p < 0.05$) the biomass production (Figure 4.7) and the concentration of Mo, whereas the concentrations of U, N, P, Fe, Zn and B significantly decreased. Decreasing values can be explained by a dilution effect caused by the growth promoting influence of N, P and S (Table 4.6).

Table 4.6. Influence of S rate on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients in maize (4 way ANOVA).

Variable / Factor	Biomass	U-concentrations	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
S rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	4.62	2.50	10.16	3.59	0.31	0.050	0.53	0.13	130.8	47.4	22.7	5.9	8.4	1.5
2	11.70	0.94	10.65	2.02	0.23	0.130	0.49	0.13	100.7	45.1	20.0	5.7	6.7	1.8
LSD $_{5\%}$	0.64	0.62	3.18	0.10	0.01	0.003	0.08	0.01	15.9	6.2	2.0	0.8	1.7	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]S rate [mg kg^{-1}]: 1 = 0, 2 = 50

Regarding to the main parameters measured (biomass production; U concentration in plant tissues, and U-plant uptake), in relationship with the four main effect of: U, N, P, and S rates; the result from General Linear Model (GLM) procedure revealed that:
Biomass production significantly decreased by the U rate, whereas by S rate was markedly higher than the control. N and P rates did not influenced on the biomass production. This implies that other factors were yielded limiting; for instance, sulfur (S) deficiency (Table 4.2).

U concentration in plant tissues was affected, obviously, by the U rate but also N rate increased significantly this parameter. Moreover, P rate did not influence on U concentration in plant tissues. Nevertheless, the increments of biomass, due to S fertilization, decrease the U concentration in plant in more than 62 % compared to the control.

U plant uptake has been increased by the U and N rates. As expected, the application of P significantly decreased the U plant uptake; whereas S rate has not influenced on this parameter.

Besides the main effect produced by U contamination, interactions between N, P, and S rates have been also noted, which are shown in Table 4.7.
It can be seen that biomass production was also affected by S rate*P rate interaction, U concentration in plant tissues by U rate*N rate, U rate*S rate, and N rate*S rate*P rate interactions as well as U plant uptake by U rate*P rate interaction (Table 4.7).

Table 4.7: Statistical significance (F test) for the comparison of the influence of U, P, N and S rates on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients in maize.

	Biomass	**U concentration**	**U uptake**	**N**	**P**	**S**	**Ca**	**Mg**	**Fe**	**Mn**	**Zn**	**Cu**	**B**	**Mo**
				Concentrations										
U rate	**	***	***	**	***	ns	ns	ns	***	ns	*	***	ns	ns
N rate	ns	**	ns	***	***	***	**	ns	***	ns	***	ns	ns	ns
P rate	ns	ns	***	***	***	*	**	ns	ns	***	**	ns	*	***
S rate	***	***	ns	***	***	***	ns	ns	***	ns	*	ns	ns	***
U rate*N rate	ns	*	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
U rate*P rate	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
U rate*S rate	ns	**	ns	ns	ns	ns	ns	ns	*	*	ns	ns	ns	ns
N rate*P rate	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	*	ns	ns	ns
N rate*S rate	ns	ns	ns	***	**	***	ns	ns	ns	ns	*	ns	ns	**
S rate*P rate	***	ns	ns	***	**	**	ns	ns	ns	ns	*	ns	ns	***
U rate*N rate*P rate	ns	ns	ns	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns
U rate*N rate*S rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
U rate*S rate*P rate	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
N rate*S rate*P rate	ns	*	ns	*	ns	ns	ns	ns	**	ns	ns	ns	ns	ns
U rate*N rate*S rate*P rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively

Therefore, in the results from Table 4.4, Table 4.5, and Table 4.6 (4-ways ANOVA) were also considered the effects of the interactions on the parameters measured, which needs to be taken into account with view to the interpretation of the results obtained. For better understanding of the results from the main effects of increasing U rate, low ($N_1P_1S_1$) and high ($N_2P_2S_2$) nutrition levels were separated for regression analysis (Table 4.8). The same procedure was applied for sunflower and faba bean.

Table 4.8: Regression coefficients for the relationships between U rate and biomass, U concentration, U uptake, and nutrient content of maize in relation to the N, P, and S nutritional levels.

X = U rate Y = Parameter	**Treatment**	**Regression equation**	**Coefficient of determination (R^2)**	**Significance**
Biomass	$N_1P_1S_1$	Y = -0.0021 X + 6.66	0.26	ns
	$N_2P_2S_2$	Y = -0.0026 X + 14.67	0.15	ns
U concentration	$N_1P_1S_1$	Y = 0.0065 X + 0.46	0.65	**
	$N_2P_2S_2$	Y = 0.0018 X - 0.0023	0.75	**
U uptake	$N_1P_1S_1$	Y = 0.0325 X + 3.75	0.65	**
	$N_2P_2S_2$	Y = 0.0259 X - 0.97	0.72	**
N concentration	$N_1P_1S_1$	Y = 0.0002 X + 2.34	0.17	ns
	$N_2P_2S_2$	Y = 0.0003 X + 2.30	0.07	ns
P concentration	$N_1P_1S_1$	Y = $5 \cdot 10^{-05}$ X + 0.18	0.48	*
	$N_2P_2S_2$	Y = $3 \cdot 10^{-05}$ X + 0.35	0.03	ns
S concentration	$N_1P_1S_1$	Y = $-1 \cdot 10^{-05}$ X + 0.05	0.73	**
	$N_2P_2S_2$	Y = $-3 \cdot 10^{-06}$ X + 0.14	0.00	ns
Fe-concentration	$N_1P_1S_1$	Y = -0.0270 X + 123.6	0.02	ns
	$N_2P_2S_2$	Y = -0.0835 X + 129.91	0.60	*

$N_1P_1S_1$-treatment [mg kg^{-1}]: N_1 = 250, P_1 = 334, S_1 = 0
$N_2P_2S_2$-treatment [mg kg^{-1}]: N_2 = 500, P_2 = 1,558, S_2 = 50
*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively

The regression coefficients for the relationships between U rate and the concentrations of Ca, Mg, Mn, Zn, Cu, B, and Mo are presented in the appendix (Table A.6).

The results in Table 4.8 reveal that about 70% of the variation of U concentration and U uptake by maize could be explained by the U rate. Striking is furthermore that the U rate accounted for 60% of the variation of the Fe in the plant tissue at the high nutritional level.
In case of the relationship between U rate and biomass, one of the replicates had a distinctly lower biomass and supposedly some other factors than those that were tested yielded this effect. Therefore, this sample was excluded from regression analysis.

Despite the result from the Table 4.3, which shows that biomass decrease significantly by the U rate (comparison of the mean values), the regression coefficients show (Table 4.8) that the percentage of variance of biomass could not be explained by the U rate for the extreme situation of nutritional level. The Figure 4.6 shows that not visible relationship exists among the U rate and the biomass.

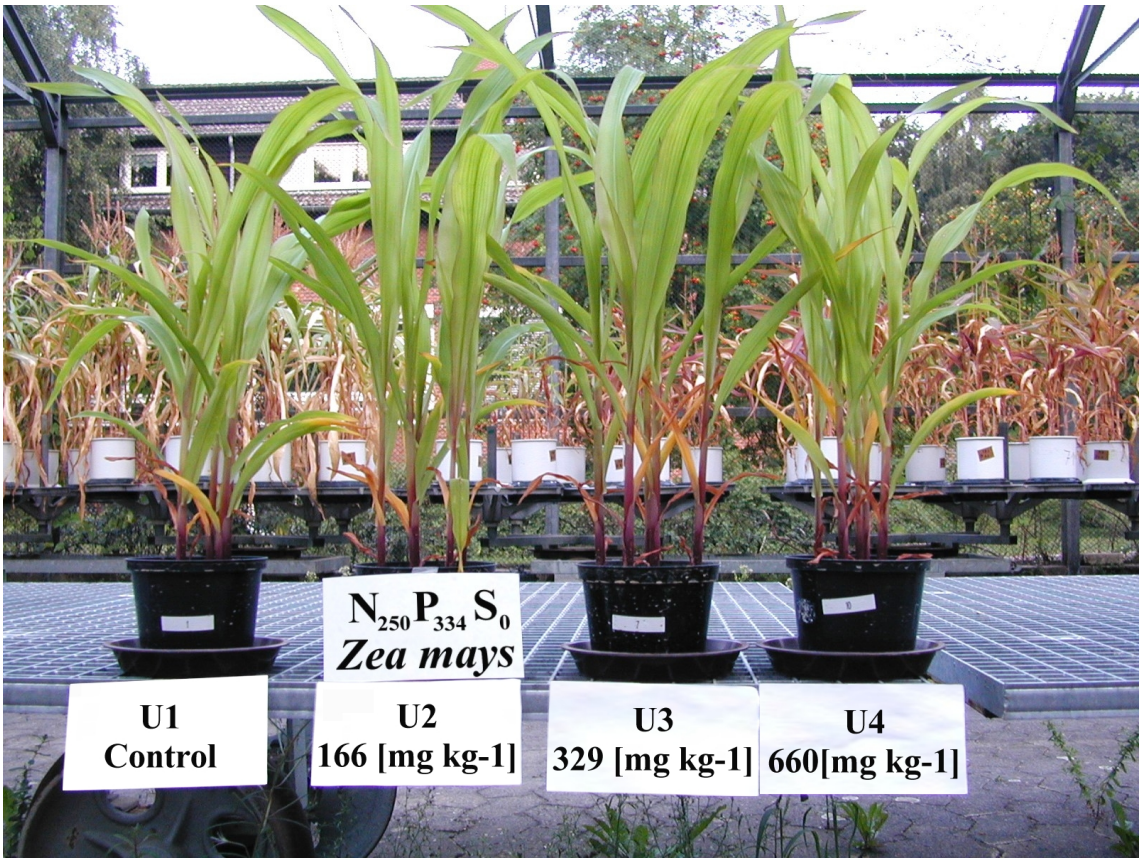


Figure 4.6: Influence of the U rate on biomass of maize in relation to low P, N and S rates (photos D. Gardiman).

4.1.3 Biomass production and root morphology of sunflower (*Helianthus annuus L.*)

The same factors (three U rates, two rates of N, P and S) and parameters like maize have been applied to the sunflower experiment. The lower rate of 250 mg kg^{-1} N was between critical and adequate for plant growth, 334 mg kg^{-1} P and 0 mg kg^{-1} S was insufficient optimum for plant growth. Moreover, the higher application rates of 500 mg kg^{-1} N, 1,558 mg kg^{-1} P and 50 mg kg^{-1} S fully marginally satisfying the nutritional demand. All four factors were factorials combined. The sufficiency ranges for N, P and S of sunflower in the whole shoot plant samples 40 days after sowing are summarized in Table 4.9.

Table 4.9: Sufficiency ranges for the N, P, and S supply of sunflower (BBCH code 18/32, between 8 and 10 leaves)

Nutrient	Sufficiency range	Deficiency range
	[%]	
N	2.02	1.63
P	-	0.39
S	0.43	< 0.29

Source: Reuter et al., (1997)

In Table 4.10 the mean N, P and S concentrations are shown for the low and high nutritional levels.

Table 4.10: Influence of N, P and S rates on the N, P and S concentration of sunflower grown on a soil without U contamination (control).

Nutrient	Treatment							
	$N_1P_1S_1$	$N_1P_1S_2$	$N_1P_2S_1$	$N_1P_2S_2$	$N_2P_1S_1$	$N_2P_1S_2$	$N_2P_2S_1$	$N_2P_2S_2$
	[%]							
N	2.59	1.87	3.29	1.87	3.66	3.43	4.60	3.71
P	0.17*	0.12*	0.43	0.30*	0.15*	0.11*	0.40	0.39
S	0.07*	0.23*	0.07*	0.24*	0.07*	0.25*	0.07*	0.31

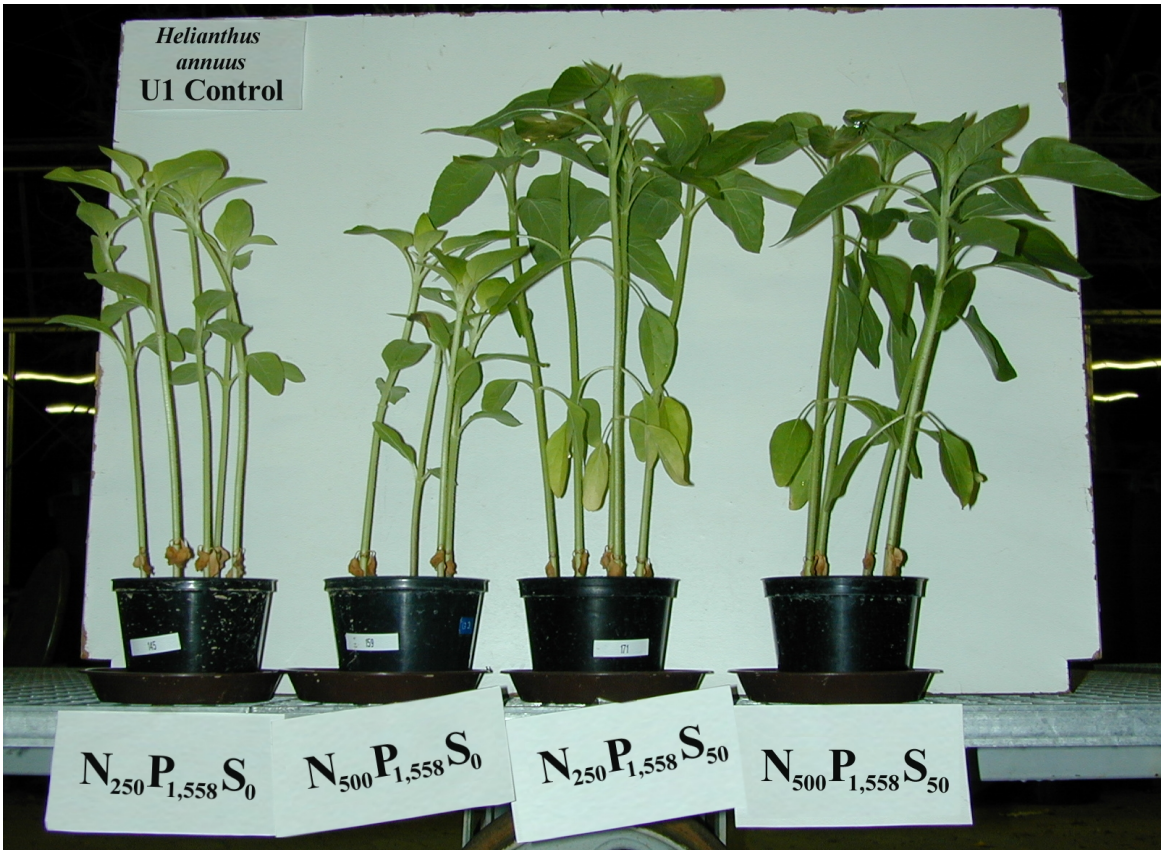
*deficient according to data in Table 4.8
N rate [mg kg^{-1}] 1 = 250, 2 = 500
P rate 1 = 334, 2 = 1,558,
S rate 1 = 0, 2 = 50

The results presented in Table 4.10 reveal that only the combination of $N_2P_2S_2$ treatment yielded a sufficient N, P and S supply. From the three nutrients tested, the S rate had the distinctly strongest influence on the biomass production with a mean increase by 222% (Figure 4.7 – 4.8). The higher N rate led accordingly to a relatively lower crop yield of 12%, however, the higher P rate led a crop productivity of and 107 %.

Biomass [g pot^{-1}]	4.2	4.0	7.6	7.0

N rate [mg kg^{-1}] 1 = 250, 2 = 500
P rate 1 = 334, 2 = 1.558.

Figure 4.7: Influence of the P, N and S rate at low P rate on growth of sunflower (photo: D. Gardiman)



Biomass [g pot^{-1}]	3.3	3.3	8.5	7.5

N rate [mg kg^{-1}] 1 = 250, 2 = 500
P rate 2 = 1,558,
S rate 1 = 0, 2 = 50

Figure 4.8: Influence of the P, N and S rates at high P rate on growth of sunflower (photo: D. Gardiman).

Leaf weight and leaf area index

Environmental factors, which limit crop growth may act through a reduction in the leaf interception of the incoming photosynthetically active radiation or the ability of a plant to transform the intercepted radiation into biomass production or even through a combination of both (Plenet et al., 2000).

The fraction of the incoming photosynthetically active radiation, which is absorbed by the canopy mainly depends on the leaf area index (LAI) and crop geometry (Wang et al., 2003). However, so far, no research on the effect of U contamination on LAI and on the leaf weight (LW) of sunflower has been conducted.

The Table 4.11 shows that U rate significantly decrease the leaf weight in the 170 mg kg^{-1} U rate compared to the control, whereas that S rate has the distinctly strongest influence on the LW with a mean increase by 264%.

Table 4.11: Influence of U, P, N and S rate on leaf weight of sunflower (4-way-ANOVA).

LEAF WEIGHT								
	U rate[1)]	**LW[2)]**	**N rate[3)]**	**LW**	**P rate[4)]**	**LW**	**S rate[5)]**	**LW**
	1	0.64 a	1	0.61 a	1	0.62 a	1	0.34 a
	2	0.58 b	2	0.63 a	2	0.62 a	2	0.90 b
	3	0.62 a						
	4	0.64 a						
$LSD^{6)}_{5\%}$		0.04		0.03		0.03		0.03

[1]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[2]LW [g pot^{-1}]: leaf weight
[3]N rate: 1 = 250, 2 = 500
[4]P rate : 1 = 334, 2 = 1,558
[5]S rate: 1 = 0, 2 = 50
[6]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different mean at p<0.005

The leaf area index was significantly higher in the 652 mg kg^{-1} U rate compared that of control and the lower U rates. Besides S rate, which had also the strongest influence of 240% on LAI; the P rate had an important significantly increment of 113% on the mentioned parameter (Table 4.12).

Table 4.12: Influence of U, P, N and S rate on leaf area index (LAI) of sunflower (4-way-ANOVA).

LEAF AREA INDEX								
	U rate[1)]	**LAI[2)]**	**N rate[3)]**	**LAI**	**P rate[4)]**	**LAI**	**S rate[5)]**	**LAI**
	1	202.80 a	1	201.86 a	1	193.18 a	1	120.92 a
	2	199.56 a	2	210.12 a	2	218.80 b	2	291.06 b
	3	203.62 a						
	4	217.99 b						
$LSD^{6)}_{5\%}$		11.89		8.41		8.41		8.41

[1]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[2]LAI [cm^2 pot^{-1}]: leaf area index
[3]N rate: 1 = 250, 2 = 500
[4]P rate : 1 = 334, 2 = 1,558
[5]S rate: 1 = 0, 2 = 50
[6]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different mean at p<0.005

The Table 4.13 shows that despite P-and N rate, individually, had not influenced on LW, interactions in a 2 ways levels have been observed. It can be seen that LAI parameter was affected by several interactions as well (Table 4.12).

Table 4.13: Statistical significance (F test) for the comparison of the influence of U, P, N and S rates on leaf weight and leaf area index of sunflower.

	Leaf weight	**Leaf area index**
U rate	**	**
P rate	ns	***
S rate	***	***
N rate	ns	ns
U rate * N rate	ns	ns
U rate * P rate	*	ns
U rate * S rate	ns	ns
P rate * S rate	***	***
P rate * N rate	*	***
S rate * N rate	***	***
U rate * P rate * N rate	ns	ns
U rate * P rate * S rate	**	*
U rate * S rate * N rate	ns	ns
P rate * S rate * N rate	ns	*
U rate * P rate * S rate * N rate	ns	ns

*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively

However, at the extremes of deficient ($N_1P_1S_1$) and sufficient ($N_2P_2S_2$) nutritional level no relationships between U rate and LW, and U rate and LAI were found (Table 4.14)

Table 4.14: Regression coefficients for the relationships between U rate and leaf weight and leaf area index in relation to the nutrient content of sunflower.

X = U- rate Y = Parameter	**Treatment**	**Regression equation**	**Coefficient of determination (R^2)**	**Significance**
Leaf weight	$N_1P_1S_1$ [1)]	Y = 0.0001 X + 0.38	0.30	ns [3)]
	$N_2P_2S_2$ [2)]	Y = 0.0002 X + 1.07	0.13	ns
Leaf area index	$N_1P_1S_1$	Y = 0.0271 X + 134.07	0.15	ns
	$N_2P_2S_2$	Y = 0.0158 X + 353.52	0.01	ns

[1] $N_1P_1S_1$-treatment [mg kg^{-1}]: N_1 = 250, P_1 = 334, S_1 = 0

[2] $N_2P_2S_2$-treatment [mg kg^{-1}]: N_2 = 500, P_2 = 1,558, S_2 = 50

[3] ns: not significant difference

Root morphology

Under the condition of nutrient deprivation, significant changes in the dimension of roots and the extent of lateral root development exist. A qualitative assessment of the influence of N, P and S rates on root development of sunflower cultivated on soil without U contamination was carried out.

The Figure 4.9 shows that N-deficiency had little effect on the formation of adventitious roots or first orders laterals, but reduced the second order laterals. Starvation in N increases the density of root hairs (Robinson and Rorinson, 1987). Under natural conditions, such developmental changes serve to increase the likelihood that roots may reach a source of the limiting nutrient.

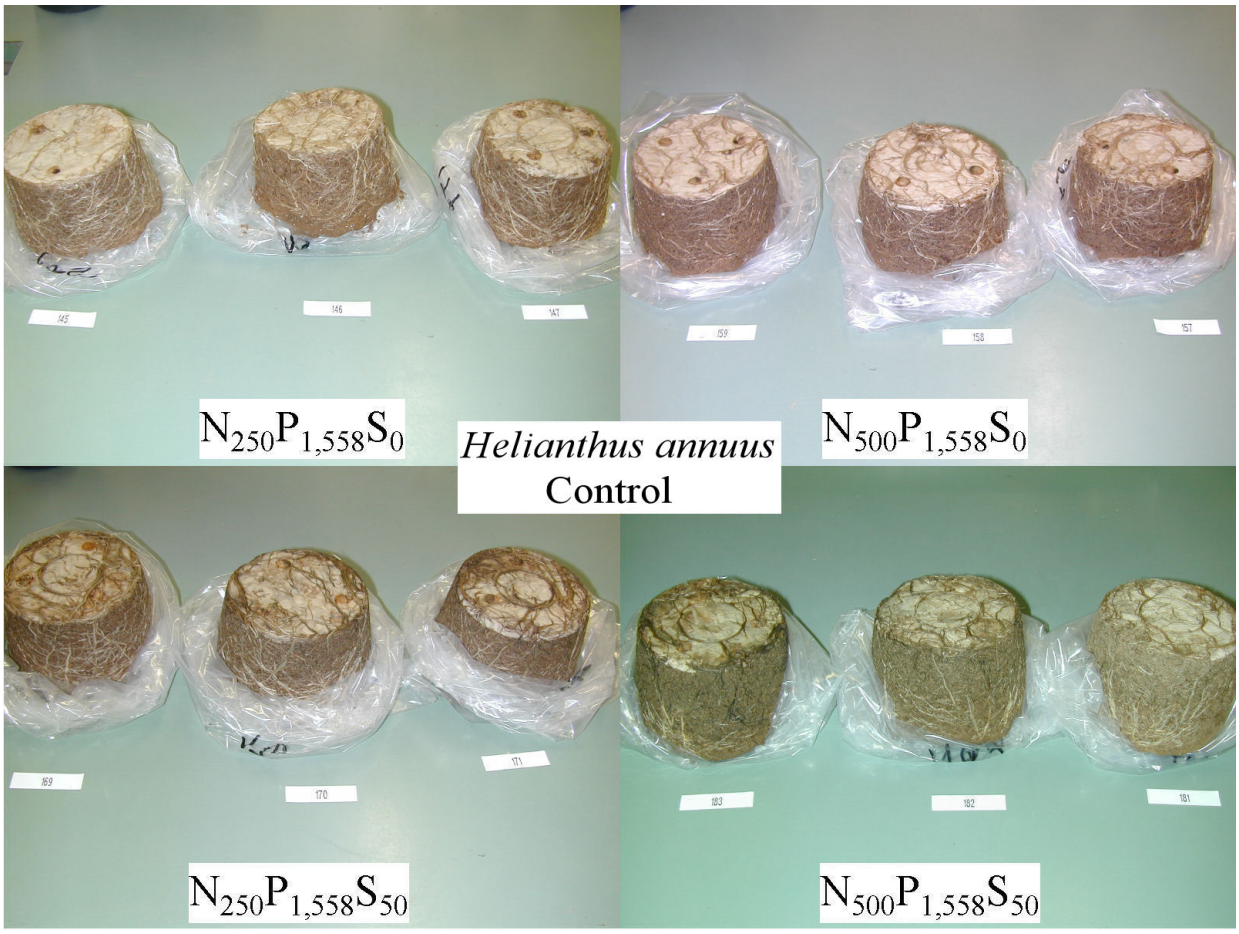


Figure 4.9: Influence of increasing N and S rates on root density of sunflower cultivated on soil without U contamination (control) at P_2 rate (photos: D. Gardiman).

4.1.4 Influence of U contamination levels and P, N and S rates on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients of sunflower

The Table 4.15 shows the U contamination levels significantly decreased the biomass production in U_2 (170 mg kg^{-1}), U_3 (357 mg kg^{-1}), and U_4 (652 mg kg^{-1}) treatments compared to the control. The P concentration was significantly higher in the U_3 (357 mg kg^{-1}) and U_4 (652 mg kg^{-1}) treatments compared to the control.

The N concentration was significantly higher in the U_4 (652 mg kg^{-1}) treatment compared with the rest of U rates. Additionally at U_3 (357 mg kg^{-1}) rate the Cu and Mo concentrations increased, while that of Fe decreased in the U_2 (170 mg kg^{-1}) rate (Table 4.15).

Table 4.15: Influence of the U rate on biomass production, U concentration, U uptake and the concentration of macro and micronutrients in sunflower (4 way ANOVA).

Variable / Factor	Biomass	U concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
U rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	5.7	<LLD[3)]	<LLD	3.1	0.26	0.16	1.77	0.19	96.9	123.5	32.1	7.5	24.4	0.7
2	4.5	0.9	3.6	3.3	0.27	0.16	1.81	0.20	62.5	109.9	31.6	6.6	26.5	0.8
3	4.7	2.3	9.8	3.2	0.29	0.16	1.79	0.20	84.8	116.2	34.7	13.9	25.1	1.2
4	4.5	4.3	17.3	3.5	0.31	0.17	1.87	0.22	89.4	108.5	28.2	8.8	26.4	0.9
LSD[4)] 5%	0.4	0.8	3.9	0.2	0.02	0.02	0.15	0.02	12.4	21.9	4.6	1.5	2.8	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[3]<LLD: lower limit of detection (15 ng L^{-1})
[4]LSD: least significant difference

The higher N rate significantly increased the concentrations of U, P, S and U uptake ($p<0.05$), while the biomass production significantly decreased, due to the very strong effect of S-deficiency (Table 4.16).

Table 4.16: Influence of the N rate on biomass production, U concentration, U uptake and the concentration of macro and micronutrients in sunflower (4 way ANOVA).

Variable / Factor	Biomass	U concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
N rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**						**[mg kg^{-1}]**				
1	5.02	1.50	6.01	2.55	0.27	0.15	1.75	0.21	79.5	120.8	31.7	9.5	25.8	0.9
2	4.63	2.29	9.32	4.01	0.29	0.18	1.86	0.20	87.4	108.3	31.7	8.9	25.4	1.0
LSD[3)] 5%	0.30	0.56	2.76	0.11	0.01	0.01	0.11	0.01	8.7	15.5	3.3	1.0	2.0	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]N rate [mg kg^{-1}]: 1 = 250, 2 = 500
[3]LSD: least significant difference

The P rate significantly increased the concentrations of N, S and Ca ($p<0.05$), while it led to a significant decrease of the biomass production, U, Mg, Mn and ZN concentrations and U uptake (Table 4.17).

In both maize and sunflower crops, it was observed that the P rate significantly decrease the biomass, the U concentration, and the U uptake by the treatment. P fertilization have been well demonstrated to be effective reducing heavy metals availability in soils, which is shown in the Figure 4.10. It is important to recognize that depending on the nature of P compounds and the heavy metal species some of these materials contain high levels of metals and can act as an agent of metal introduction to soils. Accordingly these materials should be scrutinized before their large scale use as immobilizing agent in contaminated sites.

During the dissolution of P compounds like for instance, $CaHPO_4$ the soil pH around the fertilizer grain is lowered down to 2, the acidification causes the dissolution of metal compounds resulting in its increase in the soil solution which are then precipitated by P as $((UO_2)_3(PO_4)_2)$ complex (Lamas, 2005).

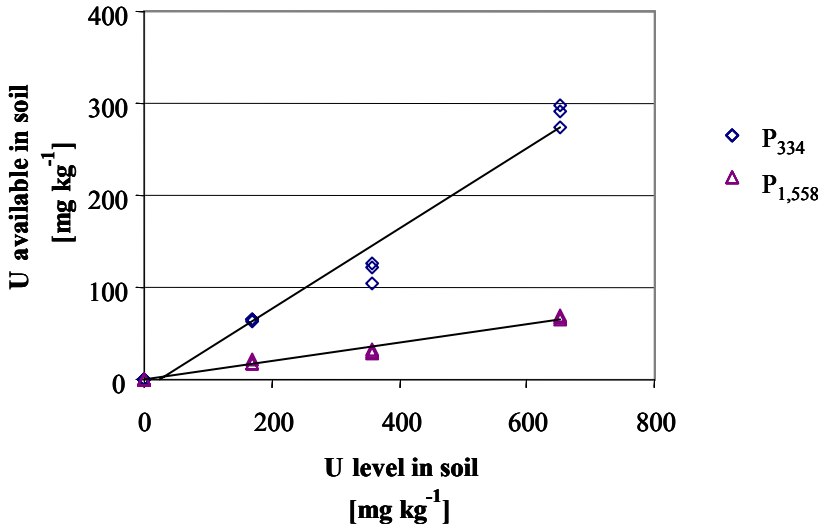


Figure 4.10: Relationship between U available in soil and U rate in relationship with two different phosphorus rates (P_1; P_2).

Table 4.17: Influence of the P rate on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients in sunflower (4 way ANOVA).

Variable / Factor	Biomass	U concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
P rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	5.04	2.26	10.13	3.15	0.15	0.16	1.60	0.21	81.2	136.7	34.1	9.0	26.1	0.8
2	4.61	1.53	5.20	3.41	0.42	0.17	2.02	0.20	85.6	92.4	29.2	9.3	25.1	1.0
LSD[3)] 5%	0.30	0.56	2.76	0.11	0.01	0.01	0.11	0.01	8.7	15.5	3.3	1.0	2.0	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]P rate: [mg kg^{-1}]: 1 = 334, 2 = 1,558
[3]LSD: least significant difference

The S rate significantly ($p<0.05$) increased the biomass production (Figure 4.10 and Figure 4.11) and U uptake whereas the concentrations of N, P, Ca, Mg, Zn and B significantly decreased. Decreasing values can be explained by a dilution effect caused by the growth promoting influence of N, P and S (Table 4.18).

Table 4.18: Influence of the S rate on biomass production, U concentration, U uptake and the concentration of macro and micronutrients in sunflower (4 way ANOVA).

Variable / Factore	Biomass	U concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
S rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	2.93	2.26	5.88	3.86	0.31	0.07	1.95	0.22	81.2	78.4	33.6	9.5	32.1	1.0
2	6.72	1.52	9.45	2.70	0.25	0.25	1.66	0.18	85.7	150.7	29.7	8.9	19.1	0.8
LSD[3)] 5%	0.30	0.56	2.76	0.11	0.01	0.01	0.11	0.01	8.7	15.5	3.3	1.0	2.0	0.2

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]S rate [mg kg^{-1}]: 1 = 0, 2 = 50
[3]LSD: least significant difference

In the case of sunflower, no so many interactions like in maize were found. For instance, no interactions on U concentrations in plant tissues were observed. Nevertheless, N rate*P rate and S rate*P rate interactions affected the biomass production. In addition, the U plant uptake was influenced by U rate *P rate and U rate *S rate interactions as well (Table 4.19).

Table 4.19: Statistical significance (F test) for the comparison of the influence of U, P, N and S rates on biomass production, U concentration, U uptake and the concentration of macro and micronutrients in sunflower.

	Biomass	U concentration	U uptake	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
U rate	***	***	***	***	***	ns	ns	*	***	ns	ns	***	ns	***
N rate	**	*	*	***	*	***	*	ns	ns	ns	ns	ns	ns	ns
P rate	**	*	***	***	***	***	***	ns	ns	***	**	ns	ns	*
S rate	***	*	*	***	***	***	***	***	ns	***	*	ns	***	*
U rate *N rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
U rate *P rate	ns	ns	*	ns	*	ns	ns	ns	**	ns	ns	ns	ns	ns
U rate *S rate	ns	ns	*	**	ns	ns	ns	ns	**	*	ns	ns	ns	ns
N rate*P rate	*	ns	ns	ns	***	*	ns	ns	**	***	ns	ns	*	ns
N rate*S rate	ns	ns	ns	ns	***	***	***	***	**	ns	***	ns	**	*
S rate*P rate	***	ns	ns	***	ns	***	*	***	ns	**	ns	ns	**	ns
U rate *N rate*P rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
U rate *N rate*S rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
U rate *S rate*P rate	ns	ns	ns	*	ns	ns	ns	ns	**	ns	ns	ns	ns	ns
N rate*S rate*P rate	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
U rate *N rate*S rate*P rate	ns	ns	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively

As it was mentioned before, low ($N_1P_1S_1$) and high ($N_2P_2S_2$) nutritional levels were separated for regression analysis (Table 4.20).

Table 4.20: Regression significance for the relationships between U rate and biomass, U concentration, U uptake, and nutrient content of sunflower in relation to the N, P and S nutritional level.

X= U rate Y= Parameter	**Treatment**	**Regression equation**	**Coefficient of determination (R^2)**	**Significance**
Biomass [g pot^{-1}]	$N_1P_1S_1$	Y= -0.0016 X + 4.26	0.39	ns
	$N_2P_2S_2$	Y= -0.002 X + 7.49	0.27	ns
U concentration [mg kg^{-1}]	$N_1P_1S_1$	Y= 0.0062 X + 0.21	0.91	***
	$N_2P_2S_2$	Y= 0.0062 X - 0.41	0.71	**
U uptake [µg kg^{-1}]	$N_1P_1S_1$	Y= 0.0186 X + 1.61	0.84	***
	$N_2P_2S_2$	Y= 0.037 X - 2.03	0.77	**
N concentration [%]	$N_1P_1S_1$	Y= 0.0013 X + 2.48	0.69	**
	$N_2P_2S_2$	Y= -0.0002 X + 3.46	0.01	ns
P concentration [%]	$N_1P_1S_1$	Y= 0.0001 X + 0.16	0.58	*
	$N_2P_2S_2$	Y= 0.0002 X + 0.39	0.48	*
*, **, *** and ns: significant at p <0.05, p= <0.01, p <0.001 and not significant, respectively				

The regression coefficients for the relationships between U rate and the concentrations of S, Ca, Mg, Fe, Mn, Zn, Cu, B, and Mo are presented in the appendix (Table A.8)

The results in Table 4.20 reveal that about 90 % at low nutritional level and 77 % at high nutritional level of the variation of U concentration (Figure 4.12) and U uptake by sunflower could be explained by the U rate. Striking is furthermore that the U rate accounted for 69 % of the variation of the N and even 58 % of P concentration in the plant tissue at the low nutritional level. No relationships between U rate and biomass production at the low and high nutritional levels were found.

Figure 4.11: Influence of U rate on biomass production of sunflower in relation to low ($N_1P_1S_1$) nutritional level (photo: D. Gardiman).

Figure 4.12: Influence of the U rate on biomass production of sunflower in relation to high ($N_2P_2S_2$) nutritional level (photo: D. Gardiman).

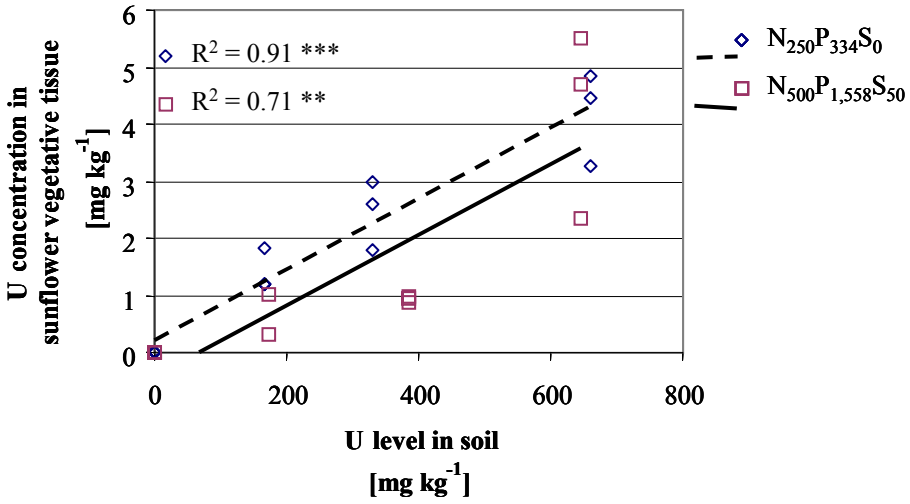


Figure 4.13: Influence of the U rate on the U concentration in vegetative tissues of sunflower.

4.1.5 Biomass production and root morphology of faba bean (*Vicia faba L.*)

Faba bean is a leguminous specie due to that no nitrogen rate was applied. Besides U rates, which were the same like for maize and sunflower (U_1: control, U_2: 170 mg kg^{-1}, U_3: 357 mg kg^{-1}, and U_4: 652 mg kg^{-1}) only two rates of P and two rates of S were tested.

The lower rate 334 mg kg^{-1} P and 0 mg kg^{-1} S were insufficient optimum for plant growth and the higher application rates of 1,558 mg kg^{-1} P and 50 mg kg^{-1} S fully marginally satisfying the nutritional demand. All three factors were factorials combined.

The sufficiency ranges for N, P and S of faba bean in the whole shoot plant sample onset flowering; BBCH code is macro stadium 6 (main branch), 61 (beginning of flowering) are summarized in the Table 4.21.

Table 4.21: Sufficiency ranges for the N, P and S supply of faba bean (BBCH-code macro stadium 6; 61)

Nutrient	Sufficiency range			Deficiency range
	[%]			
N	2.8	-	3.5	-
P	0.25	-	0.45	-
S		-		<0.31

Source: Reuter D J, 1997

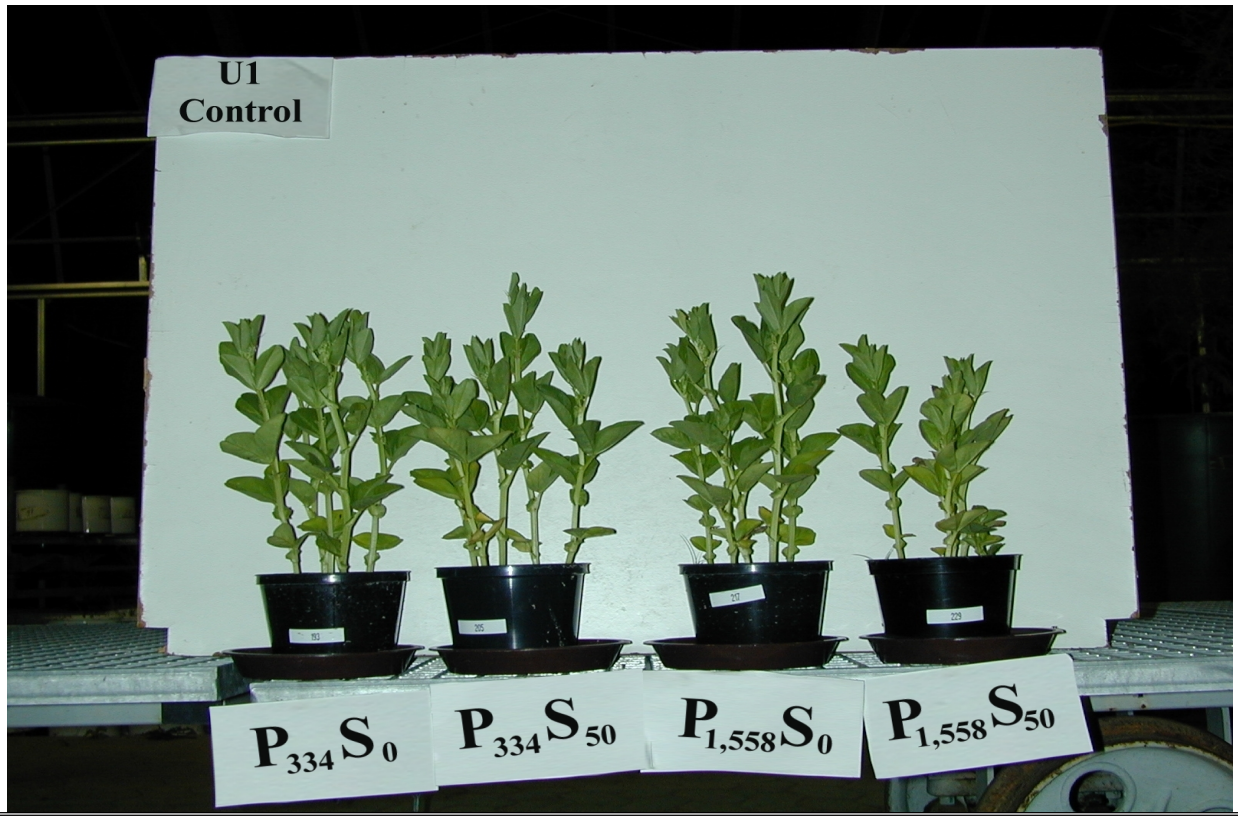
In Table 4.22, the mean values of N, P and S concentrations are shown for the low and high nutritional levels.

Table 4.22: Influence of P and S rates on the N, P and S concentrations of faba bean cultivated on a soil without U contamination (control).

Nutrient	Treatment			
	S_1P_1	S_1P_2	S_2P_1	S_2P_2
	[%]			
N	2.34*	2.07*	2.16*	1.91*
P	0.30	0.46	0.28	0.48
S	0.12*	0.11*	0.29*	0.30*

*Deficient according to data in Table 4.21
P rate: 1 = 334, 2 = 1,558,
S rate: 1 = 0, 2 = 50

The results presented in Table 4.22 reveal that no treatment combination yielded a sufficient N and S supply. From the two nutrients tested, the P rate had the distinctly strongest influence on the biomass production with a mean increase by 123% (Figure 4.13). The higher S rate led accordingly to a relatively higher crop productivity of 107%.



Biomass (g pot^{-1})	3.0	3.2	3.7	3.0

Figure 4.14: Influence of P and S rates on growth of faba bean on a soil without U contamination (control) (photo: D. Gardiman).

Root morphology

Leguminous species are capable of forming symbiotic associations with effective bacteria. This interaction leads to the formation of nodules, specialized structures in which nitrogen fixation occurs. Nodules are formed through a series of unique developmental process, which are the result of *Rhizobium* species dependent. For the host nodulated *Vicia faba* the species of rhizobia is *Rhizobium leguminosarum bv, Leguminosarum.*

A qualitative assessment of the influence of P- and S rates on root development of faba bean cultivated on a soil without U-contamination was carried out.

Figure 4.15: Influence of P and S rates at a high P rate on nodules density in faba bean cultivated on soil without U contamination (photo: D. Gardiman).

Mineral nutrients may influence N_2 fixation in legumes and non-legumes at various levels of symbiotic interactions like infection and nodule development, nodule function, and host plant growth (Marschner, 2002). The Figure 4.14 shows high amount of nodulation at P_2 rate.

Symptoms of sulfur deficiency in symbiotically grown legumes are therefore indistinguishable of nitrogen deficiency symptoms (Marschner, 2002). The Figure 4.15 shows finer roots when grown at low nutrient supply rates than when grown at high nutrient supply.

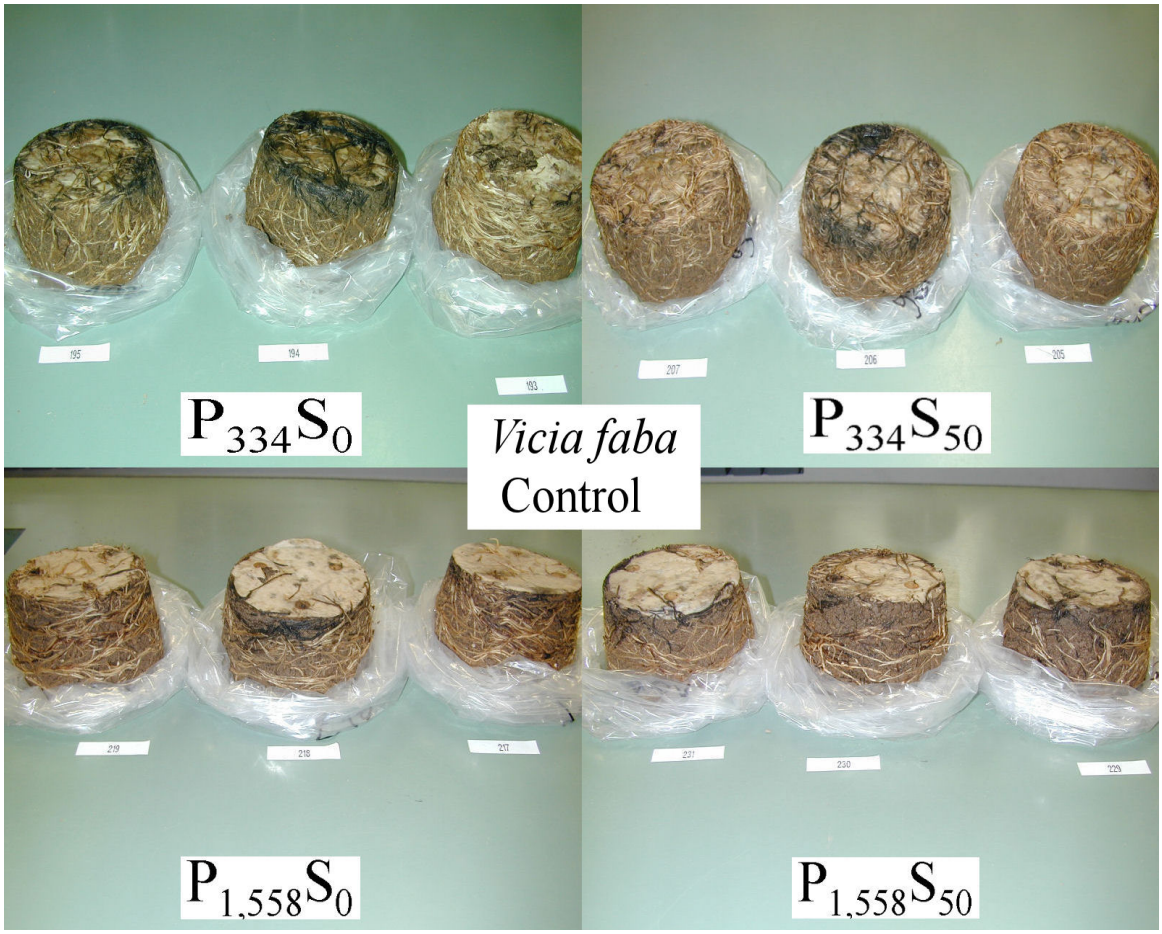


Figure 4.16: Influence of increasing P and S rates on root density of faba bean cultivated on soil without U contamination (control) (photo: D. Gardiman).

4.1.6 Influence of U contamination levels and P, N and S rates on biomass production, U concentration, U uptake and macro and micronutrients of faba bean

The U contamination levels significantly decreased the biomass production at U_2 (170 mg kg $^{-1}$) and U_4 (652 mg kg^{-1}) U rates compared to the control (Table 4.23). The P concentration was significantly higher in the U_3 (357 mg kg^{-1}) and U_4 (652 mg kg^{-1}) compared to the control. Additionally with increasing U rates the U, Mg, Cu and Mo concentrations increased, while S, Zn and B decreased (Table 4.23). Besides, the direct effect of the U rate interactions with P and S application rate obviously favored in particular a higher Fe and Mn plant uptake (Table 4.26).

Table 4.23: Influence of the U rate on biomass, U concentration, U uptake and the concentrations of macro and micronutrients in faba bean (3 way ANOVA).

Variable / Factor	Biomass	U concentration	U Uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
U rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	3.2	<LLD	0.1	2.1	0.38	0.20	1.9	0.1	236.4	152.0	65.9	10.3	26.3	2.6
2	2.6	1.8	4.7	2.3	0.38	0.16	1.8	0.2	240.4	150.7	60.5	9.7	24.2	3.0
3	2.9	1.7	4.8	2.6	0.40	0.21	1.8	0.1	185.9	149.2	57.2	15.2	23.2	3.5
4	2.5	5.4	13.5	2.7	0.40	0.20	2.0	0.2	182.9	152.2	47.4	12.3	23.3	3.2
LSD[3)] $_{5\%}$	0.6	0.6	1.9	0.2	0.02	0.02	0.3	0.01	53.8	29.5	7.3	2.4	2.7	0.5

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[3]LSD: least significant difference

The P rate significantly increased the concentration of Mo ($p<0.05$) and Fe, while it led to a significant decrease of the N, S, Mg, Mn, Zn, Cu and B concentrations (Table 4.24).

Table 4.24: Influence of the P rate on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients in faba bean (3 way ANOVA).

Variable / Factor	Biomass	U concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
P rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	2.9	2.2	5.7	2.6	0.29	0.20	1.8	0.2	195.8	184.9	64.7	13.0	26.6	2.9
2	2.7	2.3	5.8	2.3	0.49	0.19	1.9	0.1	226.9	117.1	50.8	10.8	22.0	3.2
LSD[3)] $_{5\%}$	0.4	0.5	1.3	0.2	0.02	0.01	0.2	0.0	38.0	20.8	5.1	1.7	1.9	0.3

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]P rate: [mg kg^{-1}]: 1 = 334, 2 = 1,558
[3]LSD: least significant difference

In the case of faba bean the P rate did not decrease the U concentration in plant tissue and U-plant uptake, in contrast to maize and sunflower. One reason may be due to the nitrogen fixation process. Hopkins (1995) describes that the principal product of biological nitrogen fixation is ammonia, but that for every dinitrogen molecule reduced one molecule of hydrogen is generated. When dinitrogenase is not operating optimally even more electrons may be diverted to the production of hydrogen. Thereby, acidify the root zone and thus, leaving the uranyl ion free and available to plant uptake. Thus, soil pH assumes a major role in the availability of phosphorus and less affinity to form complexes with uranium.

Sorption of U(VI) onto soil surfaces tends to increase with increasing pH (up to pH 7) and is readily reversible by decreasing the pH. Other explanation can be the phytochelators synthesis in respond to uranium contamination. Since the present work was focused on the transfer of U into the food chain, root analysis were not carried out. However, for better interpretation of the background of this results, further investigations on the root physiology in the relationship with uranium should be taken into account.

The increase of Fe and Mo concentrations could be because Fe and Mo are structural components of nitrogenase and in symbiotic bacteria, phosphorus (P) appears to activate the gene for the synthesis of nitrogenase (Schlesinger, 1997).

The S rate significantly ($p<0.05$) decreased the concentration of U, Ca, Mg and the U uptake (Table 4.25).

Table 4.25: Influence of the S rate on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients in faba bean (3 way ANOVA).

Variable / Factor	Biomass	U concentration	U uptake[1)]	N	P	S	Ca	Mg	Fe	Mn	Zn	Cu	B	Mo
				Concentrations										
S rate[2)]	**[g pot^{-1}]**	**[mg kg^{-1}]**	**[µg pot^{-1}]**	**[%]**					**[mg kg^{-1}]**					
1	2.9	2.5	6.9	2.4	0.39	0.11	2.0	0.2	225.2	157.6	58.7	12.0	25.1	3.0
2	2.7	2.0	4.6	2.5	0.38	0.28	1.8	0.1	197.6	144.4	56.8	11.8	23.4	3.1
LSD[3)] 5%	0.4	0.5	1.3	0.2	0.02	0.01	0.2	0.001	38.0	20.8	5.1	1.7	1.9	0.3

[1]U uptake was calculated as follows:

$$U_{uptake} = \frac{\sum_{i=1}^{n} U_{uptake_i}}{n}$$

[2]S rate [mg kg^{-1}]: 1 = 0, 2 = 50
[3]LSD: least significant difference

From the results of the analysis of variance of faba bean (Table 4.26), it can be concluded that it is the crop which present less interactions between all the factors tested.

Table 4.26: Statistical significance (F test) for the comparison of the influence of U, P and S rates on biomass production, U concentration, U uptake and the concentrations of macro and micronutrients in faba bean.

	Biomass	**U concentration**	**U uptake**	**N**	**P**	**S**	**Ca**	**Mg**	**Fe**	**Mn**	**Zn**	**Cu**	**B**	**Mo**
				Concentrations										
U rate	ns	***	***	***	**	**	ns	**	ns	ns	***	***	ns	**
P rate	ns	ns	ns	***	***	ns	ns	***	ns	***	***	*	***	ns
S rate	ns	**	***	ns	*	***	ns	ns	ns	ns	ns	ns	ns	ns
U rate * P rate	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	**	ns	ns
U rate * S rate	ns	*	**	ns	ns	*	ns	ns	ns	ns	*	ns	ns	ns
S rate * P rate	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
U rate * S rate * P rate	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively

Nevertheless, low (P_1S_1) and high (P_2S_2) nutritional levels were separated for regression analysis (Table 4.27).

Table 4.27: Regression significance for the relationships between U rate and nutrient content of faba bean in relation to the P and S rates.

X=U rate in soil Y=Parameter	Treatment	Regression equation	Coefficient of determination (R^2)	Significance
Biomass [g pot^{-1}]	P_1S_1	Y = -0.0008 X + 3.08	0.09	ns
	P_2S_2	Y = -0.0007 X + 2.77	0.03	ns
U concentration [mg kg^{-1}]	P_1S_1	Y = 0.0098 X - 0.31	0.84	**
	P_2S_2	Y = 0.0054 X + 0.31	0.62	*
U uptake [µg kg^{-1}]	P_1S_1	Y = 0.0227 X - 0.15	0.87	**
	P_2S_2	Y = 0.0122 X + 0.56	0.56	ns
*, **, *** and ns: significant at p <0.05, p <=0.01, p <0.001 and not significant, respectively				

The results in Table 4.27 reveal that more than 80 % of the variation of U concentration (Figure 4.17) and U uptake by faba bean could be explained by the U rate at the low (P_1S_1) nutritional level. No relationship exists between U rate and U uptake at the higher (P_2S_2) nutritional level.

The regressions significance for the relationship between U rate and the concentrations of N, P, S, Ca, Mg, Fe, Mn, Zn, Cu, B, and Mo are shown in appendix (Table A.10).

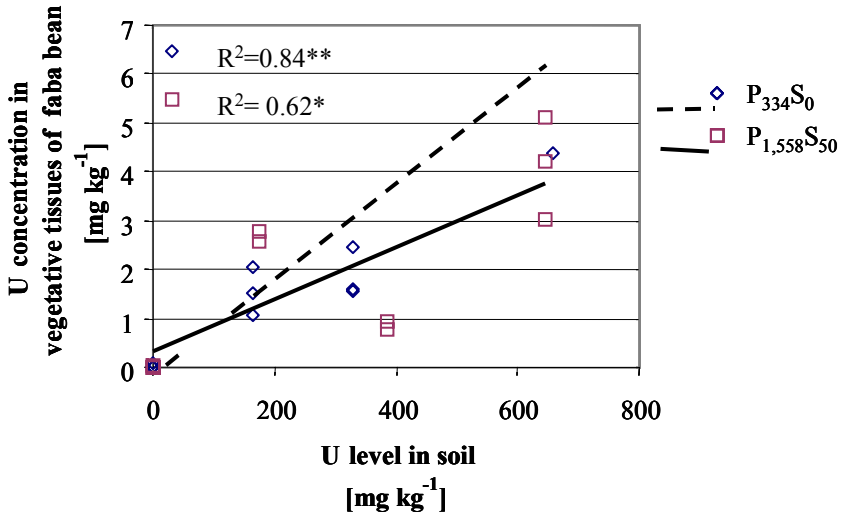


Figure 4.17: Influence of the U rate on the U concentration in vegetative tissues of faba bean in relation to the P and S rates.

4.1.7 Comparison of growth and uranium uptake of dicotyledonous, monocotyledonous and leguminous species

Biomass production

The U rate effects were modified by the effects of N, P, and S rates, which was very well demonstrated by ANOVA methods. Therefore, interactions between the factors tested were presented for maize, sunflower, and faba bean. No relationships between U contamination levels and biomass production were shown in all three crops (Table 4.28).

Table 4.28: Comparison of the regression significance for the relationships between U rates and biomass, U concentration in plant tissue and U plant uptake of maize, sunflower and faba bean in relation to the P, N, and S rates.

X = U rate in soil Y = Parameter	**Treatment**	**Maize**		**Sunflower**		**Faba bean**		
		R^2	**Sig.[1)]**	**R^2**	**Sig.**	**Treatment**	**R^2**	**Sig.**
Biomass [g pot^{-1}]	$N_1P_1S_1$	0.26	ns	0.39	ns	P_1S_1	0.09	ns
	$N_2P_2S_2$	0.15	ns	0.27	ns	P_2S_2	0.03	ns
U concentration [mg kg^{-1}]	$N_1P_1S_1$	0.65	**	0.91	***	P_1S_1	0.84	**
	$N_2P_2S_2$	0.75	**	0.71	**	P_2S_2	0.62	*
U uptake [µg kg^{-1}]	$N_1P_1S_1$	0.65	**	0.84	***	P_1S_1	0.87	**
	$N_2P_2S_2$	0.72	**	0.77	**	P_2S_2	0.56	ns

[1]Sig. Significance
R^2 Coeficient of determination
*, **, *** and ns: [1]significant at p <0.05, p <=0.01, p <0.001 and not significant, respectively

U concentration

The highest values of U concentration in the vegetative tissue at both low (P_1S_1) (Figure 4.18) and at higher nutritional level (P_2S_2) were showed for faba bean, the (Figure 4.19). The stronger influence of the nutrient supply on the U concentration in vegetative plant tissues was found for maize, which had shown values of U concentration about more than 3 time lower than for faba bean and sunflower.

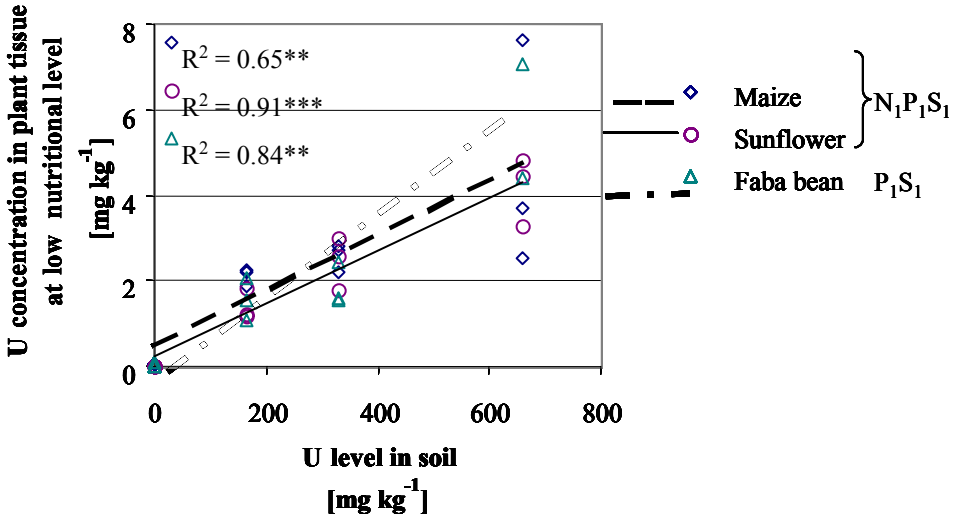


Figure 4.18: Influence of U rate on the U concentration in plant tissue of maize, sunflower and faba bean at low ($N_1P_1S_1$) nutritional level.

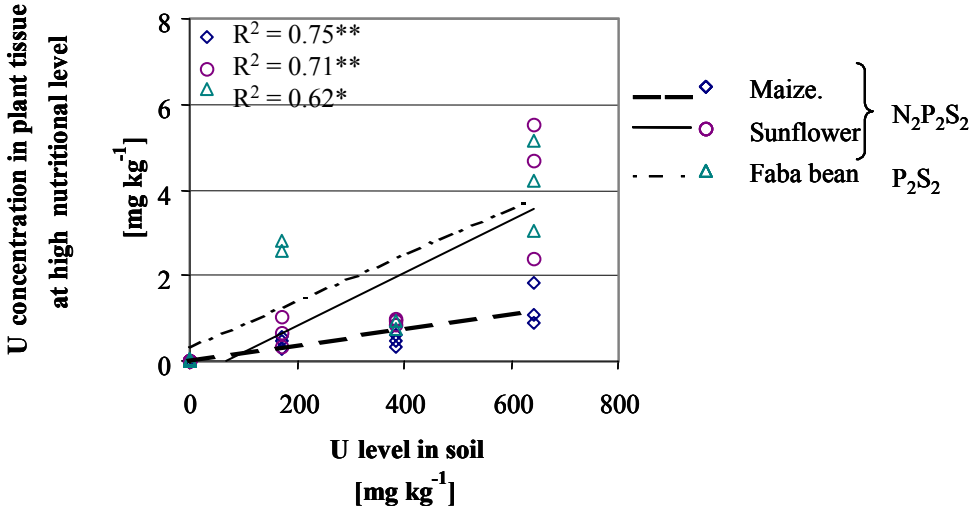


Figure 4.19: Influence of U rate on the U concentration in plant tissue of maize, sunflower, and faba bean at high nutritional level.

U uptake

The U uptake was calculated as a product between U concentration in plant tissues and biomass. A sufficient nutrient supply (N, P and S) is expecting a higher biomass production since more nutrients available lead to a high uptake. For the parameter U uptake, the maize crop showed a near 2 times higher increase in U uptake than faba bean and sunflower at the

lower nutrient level (Table 4.29; Figure 4.20). In contrast at the high nutrient level the U plant uptake was most strongly increased in case of sunflower and about 3 times higher than for faba bean (Figure 4.21).

Table 4.29: Comparison of U plant uptake of maize, sunflower, and faba bean in relationship with U contamination levels.

U plant uptake [µg pot^{-1}]			
Crop / **Factor** / **U rate**	**Maize**	**Sunflower**	**Faba bean**
1	0.10	<LLD[1)]	0.1
2	7.39	3.6	4.7
3	10.88	9.8	4.8
4	23.24	17.3	13.5
LSD[2)] $_{5\%}$	4.50	3.9	1.9

[1]<LLD: lower limit of detection (15 ng L^{-1})
[2]LSD: least significant difference

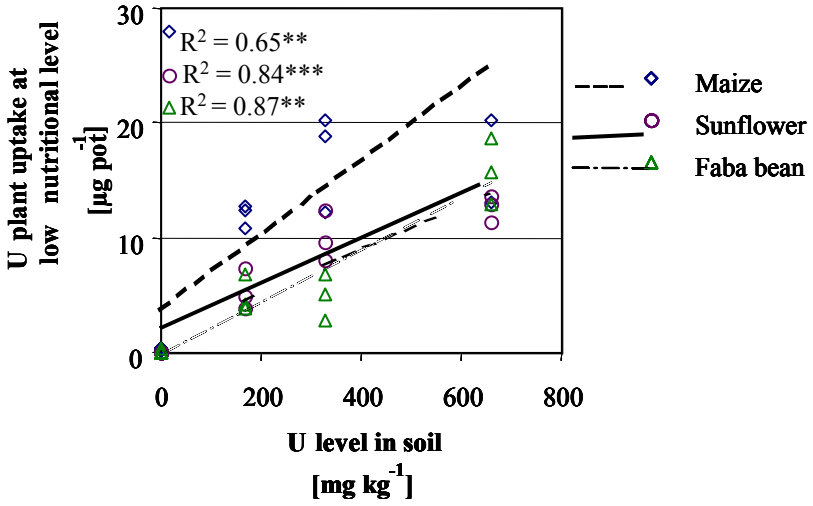


Figure 4.20: Influence of U evel on the U lant uptake by maize, sunflower and faba bean at low nutritional level.

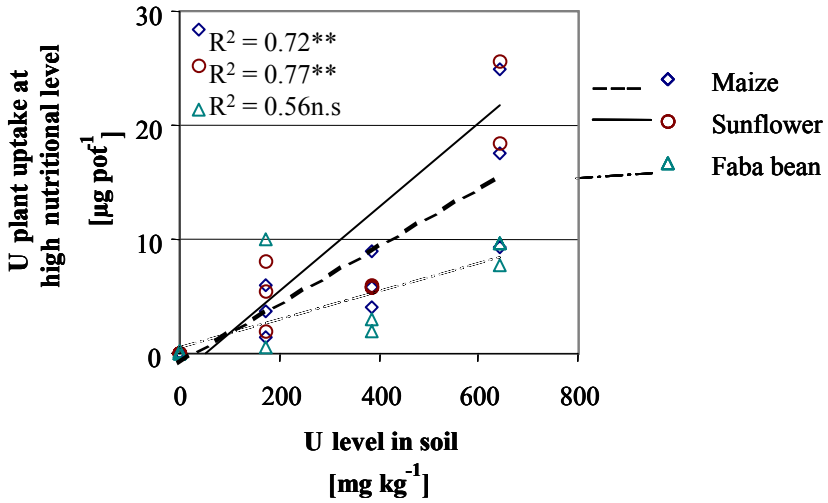


Figure 4.21: Influence of U rate on the U pant uptake by maize, sunflower and faba bean at high ($N_2P_2S_2$) nutritional level.

It was mentioned before that at the high nutritional level the U-available in soil was reduced about 25 % due to the P rate. The question arises: What happens at the same concentration of U available in soil at both situations, low and high nutritional level?

Extrapolating a determinate U plant-available concentration, which was obtained at the high nutritional level, to the situation of low nutritional level for each crop the regression equations become as follows:

Maize:

Data:

- At high (H) nutritional level:

X_H (U available in soil) = 68 mg kg^{-1}

Y_H (U uptake by plant) = **9.35 µg pot^{-1}**

- At low (L) nutritional level:

Y_L (U uptake by plant) = ?

Regression equation: $Y_L = 0.9049 \cdot X_L^{0.5869}$

Outlining:

Replacing:

X_L for X_H in :

$$Y_L = 0.9049 \cdot X_L^{0.5869}$$

$$Y_L = 0.9049 \cdot X_H^{0.5869} \rightarrow Y_L = 0.9049 \cdot 68^{0.5869}$$

$$\mathbf{Y_L = 10.8\ \mu g\ pot^{-1}}$$

Replacing in Figure 4.22:

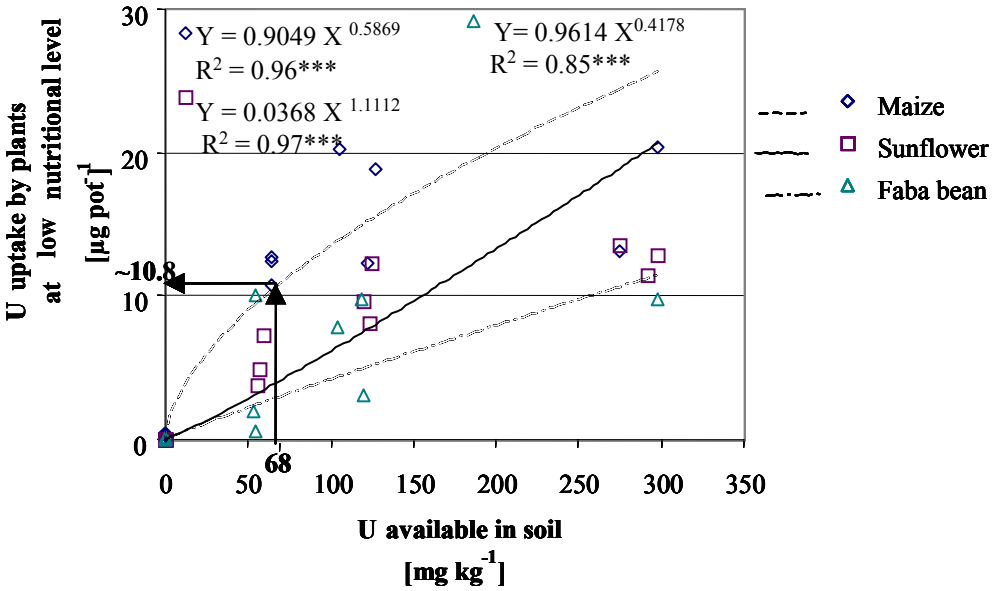


Figure 4.22: Extrapolation of U plant available in soil at high nutritional level to the low nutritional level and the U maize uptake response.

Apparently the monocotyledonous plant maize has a constant U uptake rate of U (≈ **9.35 µg pot^{-1}** in comparison with **≈10.08 µg pot^{-1}**). Extrapolation is always associated with uncertainly, which cannot be excluded if the effects of very large or very small quantities of a factor on a process are to be estimated. It should be remembered that biological systems also exhibit threshold reactions.

Sunflower:

Data:

- At high (H) nutritional level:

X_H (U available in soil) ≈68 mg kg^{-1}

Y_H (U uptake by plant) **≈26 µg pot^{-1}**

- At low (L) nutritional level:

Y_L (U uptake by plant) = ?

Regression equation: $Y_L = 0.0368 \cdot X_L^{1.1112}$

Outlining:

Replacing:

X_L for X_H in :

$Y_L = 0.0368 \cdot X_L^{1.1112}$

$Y_L = 0.0368 \cdot X_H^{1.1112} \rightarrow Y_L = 0.0368 \cdot 68^{1.1112}$

$\mathbf{Y_L = 4\ \mu g\ pot^{-1}}$

Replacing in Figure 4.22

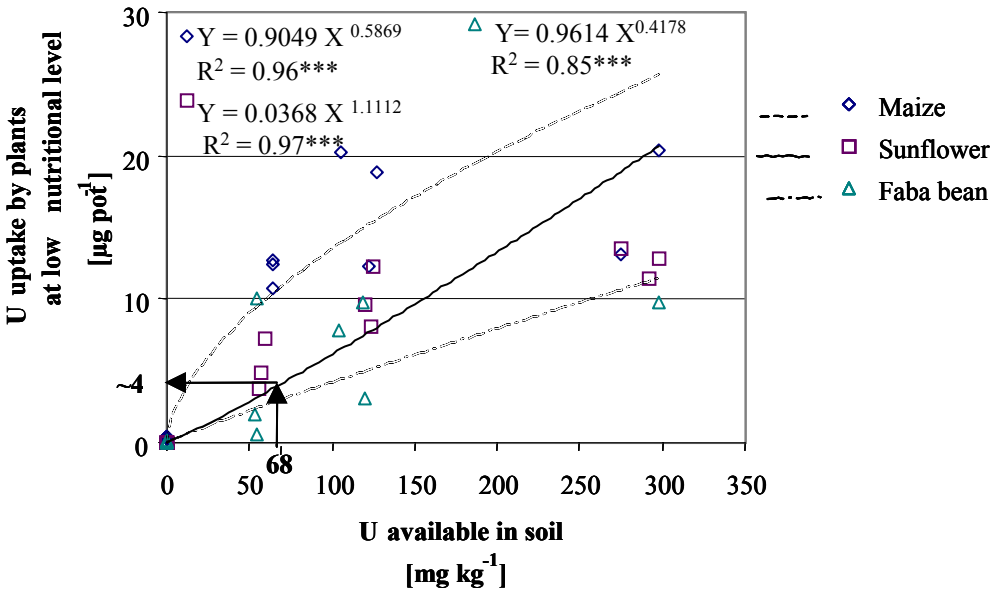


Figure 4.23: Extrapolation of U plant available in soil at high nutritional level to the low nutritional level and the U sunflower uptake response.

It can be concluded from these data (the U uptake at high nutritional level was ≈**26 µg pot^{-1}** in comparison with ≈**4 µg pot^{-1}** at low nutritional level**)** that the dicotyledonous plant sunflower has an over proportional uptake rates at higher values of U concentrations. As the cation-exchange capacity of plants increase (e.g., dicots > monocots), the ratio of cation to anion

uptake usually increases, with a corresponding lowering of rhizosphere pH (Marschner, 1986).

Faba bean:

Data:

- At high (H) nutritional level:

X_H (U available in soil) ≈69 mg kg^{-1}

Y_H (U uptake by plant) ≈**8 µg pot^{-1}**

- At low (L) nutritional level:

Y_L (U uptake by plant) = ?

Regression equation: $Y_L = 0.0755 \cdot X_L^{0.9314}$

Outlining:

Replacing:

X_L for X_H in :

$Y_L = 0.0755 \cdot X_L^{0.9314}$

$Y_L = 0.0755 \cdot X_H^{0.9314} \rightarrow Y_L = 0.0755 \cdot 69^{0.9314}$

Y_L = 4 µg pot^{-1}

Replacing in Figure 4.22

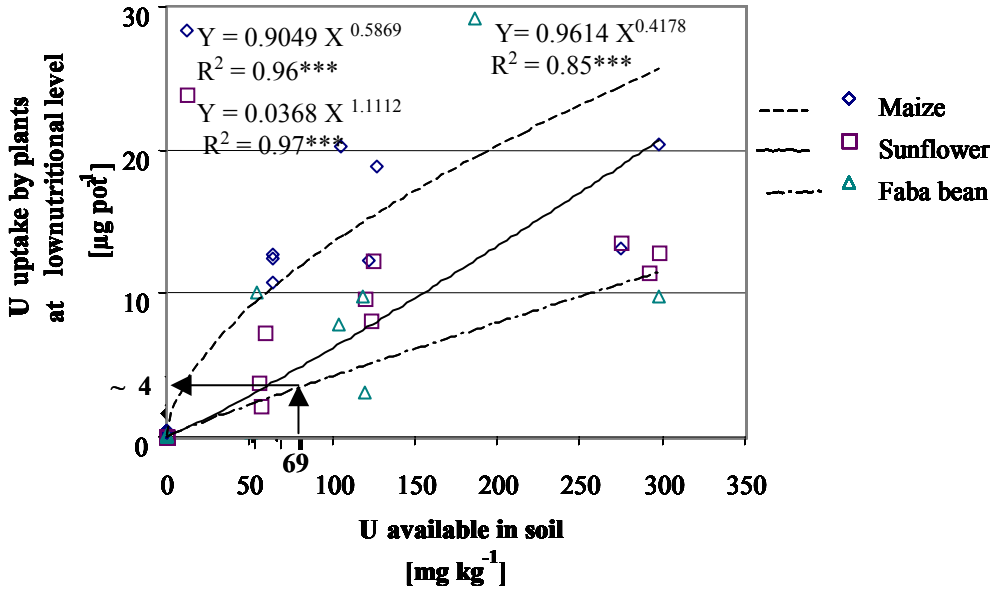


Figure 4.24: Extrapolation of U plant available in soil at high nutritional level to the low nutritional level and the U faba bean uptake response.

The U uptake by faba bean at the high nutritional level was ≈ **8 µg pot^{-1}** in comparison with ≈ **4 µg pot^{-1}** at the low nutritional level, which suggest the same conclusion that in the case of sunflower. Additionally, plant that meet their nitrogen requirement by N_2 fixation rather than nitrate uptake take up more cations than anions since uncharged N_2 enters the roots (Marschner, 1986). This results are also supported by Coughtrey and Thorne (1983), which conclude that the soil proprieties such as pH, clay mineral, Ca, K and organic matter content and soil amendments such as fertilizer application strongly affect the uptake, retention and distribution profile of radionuclides in plants.

Concentration factor:

The concentration factor (CF) describes the amount of one element expected to enter a plant from its substrate, under equilibrium conditions (Sheppard and Sheppard, 1985). It is important to distinguish Transfer Factor (TF) of Concentration Factor (CF):

$$TFi = \frac{Cip}{Cis}$$

Where:

TFi: is the transfer factor for the transport of the radionuclide from the soil (s) into the plant (p) [Bq kg^{-1}TS / Bq kg^{-1} TS]

Cip: specific activity of the radionuclide in the plant (p) [Bq kg^{-1}DM]

Cis: specific activity of the radionuclide

$$CFi \frac{Cpr}{Csr}$$

Where:

CFi: is the concentration factor for the transport of the stable isotopes from the soil (s) in vegetal products (p) [µg g^{-1} DM / µg g^{-1} DM]

Cpr: concentration ratio of the stable isotope in the plant [µg g^{-1} DM]

Csr: concentration of the plant available stable isotope in the soil [µg g^{-1}]

Assessment models normally make use of a plant/substrate concentration factor, referred as a concentration factor (CF) to estimate the transport of radionuclides and other elements of

interest through the food chain as well as in biochemical explorations for uranium (Mortverdt, 1994).

Factors such as soil characteristics, climatic conditions, type of plants, part of the plant concerned, physico chemical form of the radionuclides and the effect of the competitive species can influence the CF values (Bettencourt et al., 1988).

The CF of uranium for each treatment and for the three agricultural crops are shown in Table 4.30, which were made up on the basis of soil and plant DM (dry matter).

It can be seen that sulfur fertilization increase the uranium plant uptake but sulfur rate is correlated with more vigorous growth, which dilute the uranium concentration in plant tissue, thereby small CF were observed.

As it was mentioned before, P rate in soil influenced on U plant availability in soil this could be explain because of the precipitation of insoluble uranyl phosphate minerals. On the other hand, N ratios had not influenced significantly on the CF.

The CF values decreased as the corresponding soil concentration increased (Table 4.30) and this result correlated well with the compiles of CR made by Sheppard and Evenden (1988) (Table 4.31).

Table 4.30: Concentration factors of maize, sunflower and faba bean in relationship with the mineral nutrients P, N and S.

Treatments				U plant available in soil			U concentration in plant tissues			Concentration factor: $CFi\frac{Cpr}{Csr}$		
				Maize	Sunflower	Faba bean	Maize	Sunflower	Faba bean	Maize	Sunflower	Faba bean
P_1	N_1	S_1	U_2	64.32	57.37	54.1	2.12	1.4	1.55	0.0330	0.0244	0.0287
			U_3	118.16	122.88	114.19	2.57	2.5	1.9	0.0218	0.0200	0.0164
			U_4	288.39*	288.39*	288.39*	4.62	4.2	6.6	0.0160	0.0145	0.0229
		S_2	U_2	55.99	59.90	52.46	0.58	0.7	1.05	0.0103	0.0112	0.0199
			U_3	124.49	117.67	124.75	1.01	1.7	1.9	0.0081	0.0148	0.0154
			U_4	288.39*	288.39*	288.39*	2.65	3.1	4.5	0.0092	0.0107	0.0157
	N_2	S_1	U_2	63.68	60.13		2.33	1.2		0.0366	0.0199	
			U_3	110.69	108.57		2.65	4.25		0.0239	0.0391	
			U_4	288.39*	288.39*		5.78	6.4		0.0201	0.0223	
		S_2	U_2	60.00	60.10		0.95	0.8		0.0159	0.0134	
			U_3	127.11	113.66		1.48	4.65		0.0117	0.0409	
			U_4	288.39*	288.39*		4.03	5.3		0.0140	0.0183	
P_2	N_1	S_1	U_2	15.89	14.77	14.14	0.97	1.3	1.98	0.0610	0.0861	0.1396
			U_3	48.38	56.23	25.99	1.23	1.9	1.9	0.0254	0.0336	0.0768
			U_4	84.87	63.66	60.16	2.70	4.9	5.9	0.0319	0.0769	0.0992
		S_2	U_2	17.47	13.44	13.14	0.66	0.33	2.7	0.0379	0.0242	0.2049
			U_3	32.46	31.34	33.25	0.42	0.4	0.7	0.0129	0.0121	0.0258
			U_4	70.67	62.63	75.16	1.04	1.7	4.13	0.0147	0.0265	0.0549
	N_2	S_1	U_2	16.79	20.18		1.67	1.14		0.0995	0.0563	
			U_3	33.14	33.53		3.42	2.15		0.1032	0.0642	
			U_4	62.83	64.69		9.77	4.9		0.1556	0.0760	
		S_2	U_2	18.81	14.48		0.43	0.7		0.0228	0.0462	
			U_3	30.41	29.31		0.47	0.9		0.0155	0.0317	
			U_4	67.76	65.87		1.26	4.2		0.0186	0.0636	

(*) U available of a composite sample. Calculated in vegetative tissues. Not include roots.

Table 4.31: Compilation of uranium concentration factors by Sheppard and Evenden, (1988).

Reference	Plant	CF	Reference	Plant	CF	Reference	Plant	CF	Reference	Plant	CF
Schoenbuchner, 2002*	Grass	0.0010	Dressen et al., 1982	f	0.3000	Morishima et al., 1977	v	0,0020	Van Netten & Morley, 1982	cf	0,0050
Schoenbuchner, 2002*	Clover	0.0032	Dressen et al., 1982	f	0.0340	Morishima et al., 1977	cb	<LLD	Van Netten & Morley, 1983	r	0,0400
Schoenbuchner, 2002*	Dandelion	0.0038	Dunn, 1981	ts	0.0460	Morishima et al., 1977	r	<LLD	Walker, 1979	ts	0,0200
Adams et al., 1975	cf	<LLD	Dunn, 1981	ts	0.0460	Prister & Prister, 1970	v	0,0400	Whicker & Ibrahim, 1983	fa	0,0010
Amiro & Sheppard, 1987	a	0.0020	Dunn, 1981	ts	0.9400	Rayno et al., 1980	f	0,0300	Yamamoto et al., 1968	v	0,0010
Arkhipov et al., 1985	c	0.0200	Evans % Eriksson, 1983	c	0.0010	Rumble & Bjugstad, 1986	f	0,0100	Yang & Liao, 1983	c	<LLD
Arkhipov et al., 1985	c	0.0130	Frindik, 1986	c	0.0010	Rumble & Bjugstad, 1986	f	0,0060			
Arkhipov et al., 1985	c	0.0070	Frindik, 1986	v	0.0040	Schreckhise & Cline, 1978	f	0,0060			
Arkhipov et al., 1985	f	0.0550	Frindik, 1986	b	0.0100	Schreckhise & Cline, 1978	c	<LLD			
Arkhipov et al., 1985	c	0.0140	Garten, 1980	ts	0.0030	Sheard et al., 1986	ts	0,0550			
Baeva et al., 1975	fbt	0.0100	Garten, 1981	a	0.0040	Sheard et al., 1986	ts	0,0020			
Bondietti et al., 1979	f	0.0180	Garten et al., 1981	f	0.0100	Sheppard et al., 1984	fv	0,6000			
Bondietti et al., 1979	crb	0.0010	Gulati et al., 1980	cb	0.0300	Sheppard et al., 1984	f	0,0330			
Bufatin et al., 1986	c	0.0010	Lopatkina et al., 1970	ts	0.0020	Sheppard & Thibault, 1983	ts	0,0050			
Bufatin et al., 1986	c	0.0010	Lopatkina et al., 1970	ts	<LLD	Sheppard et al., 1985	t	0,0300			
Bufatin et al., 1986	c	<LLD	Lopatkina et al., 1970	ts	<LLD	Sheppard & Eveden, 1985	f	0,0080			
Cannon, 1952	sa	0.0280	Mahon & Mathewes, 1983	tsa	0.0070	Smith et al., 1982	c	<LLD			
CBCL. 1985	ts	<LLD	Milosevic et al., 1980	f	0.0030	Titaeva et al., 1978	f	0,0010			
Davy & Conway, 1974	f	0.0290	Moffett & Tellier, 1977	f	0.0010	Titaeva et al., 1978	f	<LLD			
Davy & Conway, 1974	ts	0.0070	Mordberg et al., 1976a	c	0.0010	Titaeva et al., 1978	t	<LLD			
Dressen & Marple, 1979	fs	0.0060	Mordberg et al., 1976b	r	0.0020	Tracy et al., 1983	v	0,0030			
Dressen & Marple, 1979	fs	0.2700	Mordberg et al., 1976b	v	0.0120	Tracy et al., 1983	r	<LLD			
Dressen et al., 1982	fs	0.0030	Mordberg et al., 1976b	b	0.0030	Tracy et al., 1983	b	0,0020			
Dressen et al., 1982	fs	0.0200	Morishima et al., 1976	v	<LLD	Van Netten & Morley, 1981	f	0,0680			

Plant species: tree parts (t); Shrubs (s); Annuals (a); Grain of cereal (c); fruit and berries (b); leafy vegetables (v); root crops (r); and forages (f)

4.2 The effect of uranium contamination on microbiological parameters

The development and biochemical activities of soil microorganisms undergo several alterations under stress conditions caused by adverse anthropogenic effects such as dissemination of chemical pollutants (Filip, 2002). Soil microorganisms, one part of the living component of the soil, usually occupy less than 1 % of the soil volume while their number and efficiency are very high. The mobilization and immobilization of inorganic nutrients and trace elements are also mainly a result of microbial activities. Special enzymes catalyze the organic matter turnover. These enzymes are produced by the organisms and act intra or extracellular. Enzymes are proteins that lower the activation energy of chemical reactions.
In the presented study the influence of the soil depths, phosphorus (P) and lime application rates in relation to the uranium contamination levels on dehydrogenase activity and microbial count of two types of soil substrates, grassland and forest soil, were determined.

4.2.1 Influence of uranium contamination, soil depth, phosphorus and liming supply on dehydrogenase activity

Dehydrogenase activity is an intracellular process that occurs in every viable microbial cell and is measured to determine overall microbiological activity of soil. Dehydrogenase (DHA) is a nonspecific assay in that it represents the activity of several different enzymes. An electron acceptor, triphenyltetrazolium chloride (TTC), is added to soil as a terminal electron acceptor. The colorless TTC is reduced to triphenylformazan (TPF) by cellular respiratory enzymes, TPF (a red colored product) is quantified spectrophotometrically. The quantity of triphenyformazan yielded in the assay is proportional to microbial respiration and in some cases to microbial biomass production.

Grassland soil

The increase of Ucontamination levels, P rate and liming application significantly increase the dehydrogenase activity compared to control. In contrast increase in the soil depth represent decrease of the dehydrogenase activity. It can be explained because the microbial counts rapidly decrease with soil depth due to the deterioration of the substrate (Table 4.32).

Table 4.32: Influence of U rate, soil depth, lime and P rate on dehydrogenase activity (TPF) on grassland soil (4 way ANOVA).

DEHYDROGENASE ACTIVITY (DHA)[1] (grassland soil)												
	U rate[2]	**TPF[3]**		**Liming[4]**	**TPF**		**P rate[5]**	**TPF**		**Soil depth[6]**	**TPF**	
	1	33.5	a	1	36.5	a	1	35.6	a	1	61.5	a
	2	41.7	b	2	41.3	b	2	42.3	b	2	16.3	b
	3	38.1	c		-			-			-	
	4	42.5	b		-			-			-	
LSD[7]$_{5\%}$		3.2			2.3			2.3			2.3	

[1]DHA: measured by [3]TPF: triphenylformazan [µg g^{-1} d^{-1}]
[2]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[4]Liming [mg kg^{-1}]: 1 = 1,177, 2 = 3,097
[5]P rate [mg kg^{-1}]: 1 = 334, 2 = 1,558
[6]Soil depth [cm]: 1 = 0 – 25 (soil substrate from top soil), 2 = 25 – 50 (soil substrate from sub soil)
[7]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different, mean at p<0.05

Forest soil

In the case of forest soil the increase of U rate significantly decrease the DHA, P and lime application significantly increase the dehydrogenase activity compared to control. In contrast, the soil depth represent decreased of the dehydrogenase activity (Table 4.33).

Table 4.33: Influence of U rate, soil depth, lime and P rate on dehydrogenase activity (TPF) in forest soil (4 way ANOVA).

DEHYDROGENASE ACTIVITY (DHA)[1] (forest soil)												
	U rate[2]	**TPF[3]**		**Liming[4]**	**TPF**		**P rate[5]**	**TPF**		**Soil depth[6]**	**TPF**	
	1	22.1	a	**1**	12.2	a	**1**	10.8	a	**1**	24.0	a
	2	15.7	b	**2**	20.1	b	**2**	21.5	b	**2**	8.3	b
	3	13.8	c					-			-	
	4	12.9	c		-			-			-	
LSD[7]$_{5\%}$		1.5			1.1			1.1			1.1	

[1]DHA: measured by [3]TPF: triphenylformazan [µg g^{-1} d^{-1}]
[2]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[4]Liming [mg kg^{-1}]: 1 = 1,177, 2 = 3,097
[5]P rate [mg kg^{-1}]: 1 = 334, 2 = 1,558
[6]Soil depth [cm]: 1 = 0 – 25 (soil substrate from top soil), 2 = 25 – 50 (soil substrate from sub soil)
[7]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different, mean at p<0.05

Table 4.34: Statistical significance (F test) for the comparison of the influence of U contaminations, soil depth, P rate and liming on dehydrogenase activity (TPF) on a grassland soil.

DEHYDROGENASE ACTIVITY (DHA) (grassland soil)	
Parameter	**Significance**
Uranium (U)	***
Liming (Ca)	***
Phosphorus (P)	***
Soil depth	***
U * Ca	ns
U * P	***
U * Soil depth	**
Ca * P	*
Ca * Soil depth	ns
P * Soil depth	**
U * Ca * P	ns
U * Ca * Soil depth	ns
U * P * Soil depth	*
Ca * P * Soil depth	***
U * Ca * P * Soil depth	ns
*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively	

The result of the analysis of variance revealed several interactions between U contamination, soil depth, P and lime supply with view to the parameter measured (TPF) (Table 4.34), which would be present also in forest soil. Therefore, low (P_1Ca_1) and high (P_2Ca_2) nutrients levels for each soil depth, and for each kinds of soil, were separated for regression analysis (Table 4.35).

Table 4.35: Regression significance for the relationships between U rate and dehydrogenase activity in relation to the soil depth, P rate and liming.

X =U rate Y =DHA[1)]	**Soil**	**Soil depth[2)]**	**Treatment**	**Regression equation**	**Coefficient of determination (R^2)**	**Significance**
TPF	**Grassland**	1	$P^{3)}{}_1Ca^{4)}{}_1$	Y = 0.0038 X + 59	0.005	ns
			P_2Ca_2	Y = 0.0304 X + 47.54	0.3	*
		2	P_1Ca_1	Y = -0.0043 X + 15	0.13	ns
			P_2Ca_2	Y = 0.0084 X + 18	0.25	*
	Forest	1	P_1Ca_1	Y = -0.023 X + 26.35	0.35	*
			P_2Ca_2	Y = -0.0011 X + 28.4	0.006	ns
		2	P_1Ca_1	Y = -0.006 X + 11.2	0.22	*
			P_2Ca_2	Y = -0.0096 X + 9.7	0.26	*
[1]DHA: measured by TPF: triphenylformazan [µg g^{-1} d^{-1}]						
[2]Soil depth [cm]: 1 = 0 – 25 (soil substrate from top soil), 2 = 25 – 50 (soil substrate from sub soil)						
[3]P rate [mg kg^{-1}]: 1 = 334, 2 = 1,558						
[4]Liming [mg kg^{-1}]: 1 = 1,177, 2 = 3,097						
*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively						

The results shown in Table 4.35 reveal that about 30% of the variation of dehydrogenase activity in grassland top and sub soil at the high (P_2Ca_2) level of nutrition could be explained by the U rate (Figure 4.25; Figure 4.26).

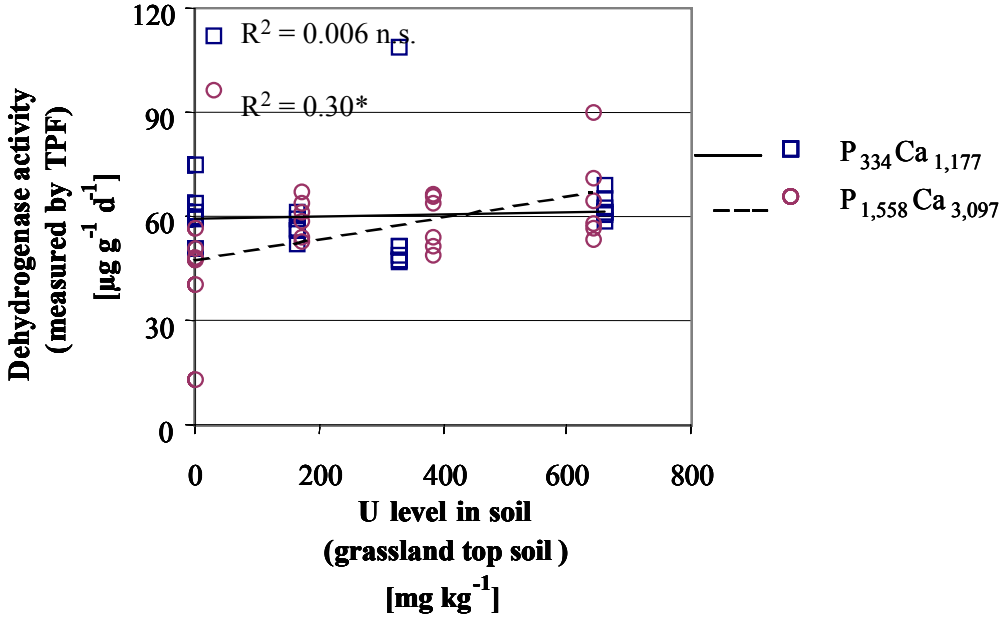


Figure 4.25: Relationship between U rate and dehydrogenase activity at low and high nutritional level, grassland substrate from top soil (0-25 cm).

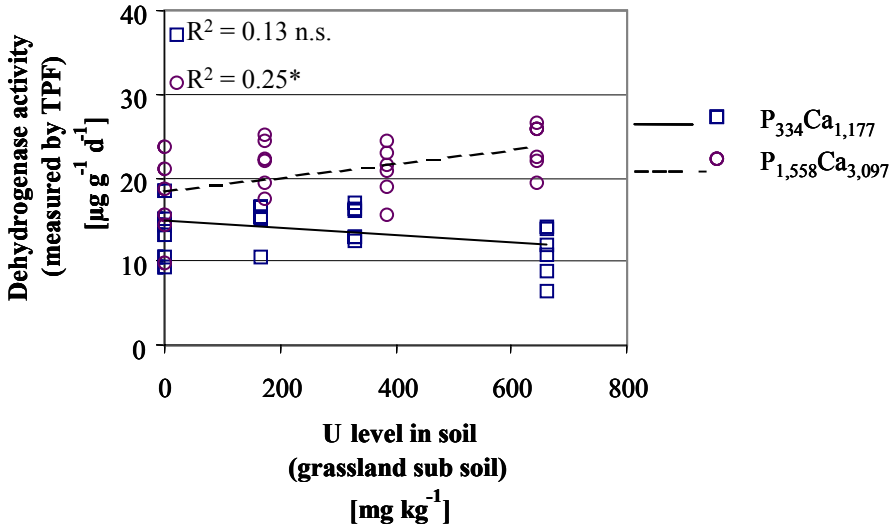


Figure 4.26: Relationship between U rate and dehydrogenase activity at low and high nutritional level, grassland substrate from sub soil (25-50 cm).

In the case of the forest top soil about 35 % of the variance of dehydrogenase activity at the low ($P_1Ca_{1,177}$) nutritional level could be explained by the U rate (Figure 4.27).

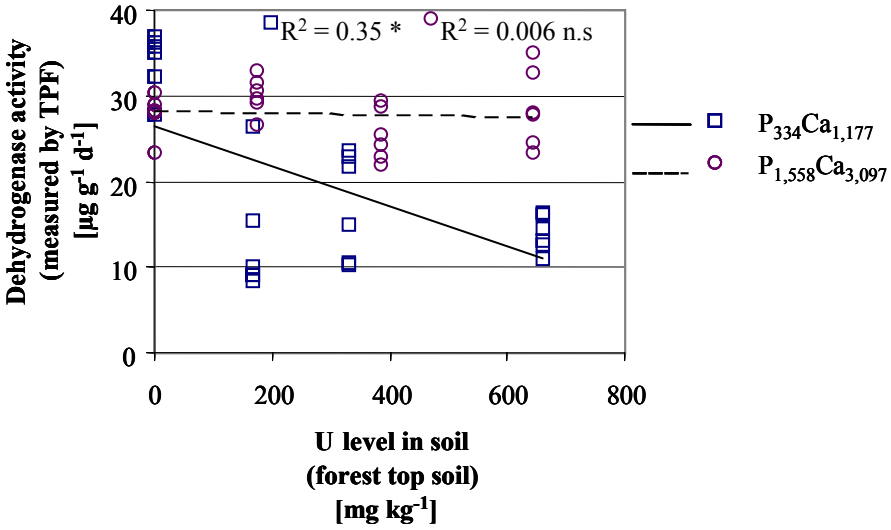


Figure 4.27: Relationship between U rate and dehydrogenase activity at low and high nutritional level, forest substrate from top soil (0-25 cm).

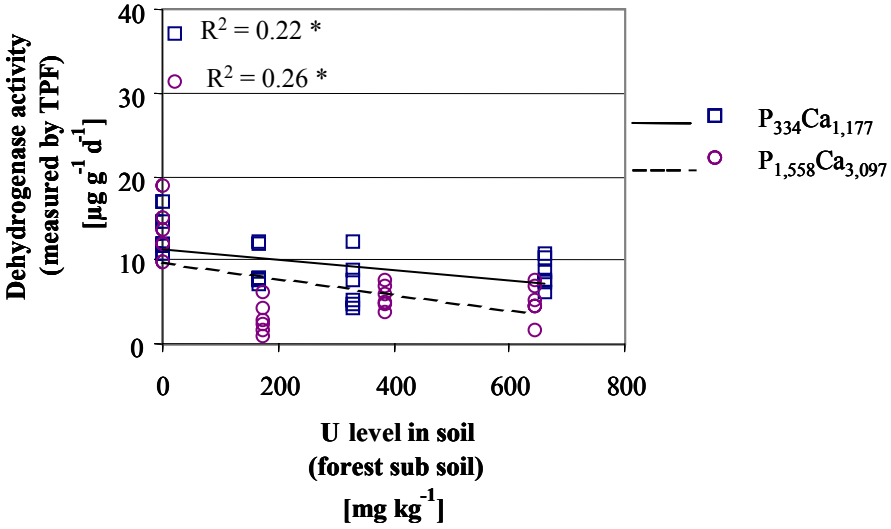


Figure 4.28: Relationship between U rate and dehydrogenase activity at low (P_1Ca_1) and high (P_2Ca_2) nutritional level, forest substrate from sub soil (0-25 cm).

Striking is furthermore that the U rate accounted about 25% of the variation of dehydrogenase activity for both, low and high nutritional level in forest sub soil (Figure 4.28).

4.2.2 Influence of uranium contamination, phosphorus and liming on the count of microorganisms

The number and activity of soil microorganisms are dependent on plant growth, soil type, soil treatment, and soil cultivation as well as on the macro and microclimate at each location. The soil microflora is composed of a number of different groups of organisms: Bacteria, fungi, algae, etc. In the present study the influence of phosphorus and liming application rate in relation to the uranium contamination on microbial count of aerobic heterotrophic bacteria, actinomycetes, and fungi in grassland and forest soil was determined.

Grassland soil

The U rate significantly decreased the actinomycetes count at the U_3 (357 mg kg^{-1}) and U_4 (652 mg kg^{-1}) compared to the control (Table 4.36).

Table 4.36: Influence of U rate on fungi, aerobic heterotrophic bacteria and actinomycetes count in grassland soil (3 way ANOVA).

U rate	Fungi	Aerobic heterotrophic bacteria	Actinomycetes
	[CFU[2] g^{-1} dry soil]		
1	4,248 a	4,621,169 a	703,125 a
2	4,003 a	2,555,506 b	596,251 a
3	2,675 b	2,825,030 b	265,618 b
4	5,206 a	2,893,130 b	313,982 b
LSD[3]$_{5\%}$	2,040	955,019	136,246

[1]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[2]CFU: colony forming units
[3]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different, mean at $p<0.05$

The aerobic heterotrophic bacteria population was significantly lower for all the U rates compared to the control. While fungi count showed significantly decrease at of U_3 (357 mg kg^{-1}) compared with the rest of the treatments (Figure 4.29).

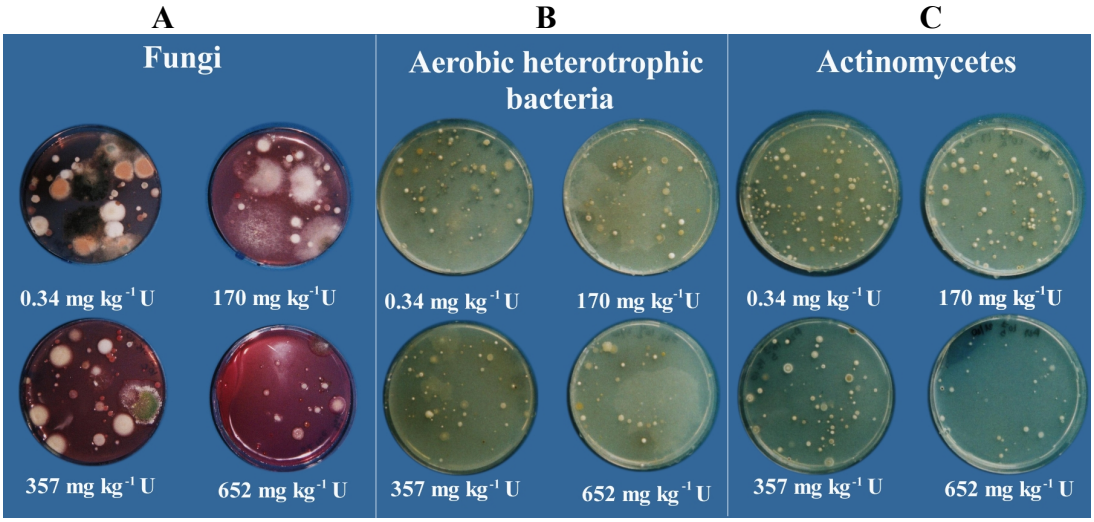


Figure 4.29: Petri dishes with cultures of: A- fungi (no significant differences), B- aerobic heterotrophic bacteria (significant differences) and C- actinomycetes (significant differences) in grassland topsoil (photos: D. Gardiman).

The higher application of lime significantly increased the count of aerobic heterotrophic bacteria and actinomycetes ($p<0.05$), while the fungi population was not influenced (Table 4.37).

Table 4.37: Influence of the liming on fungi, aerobic heterotrophic bacteria and actinomycetes count in grassland soil (3 way ANOVA).

Liming[1)]	**Fungi**		**Aerobic heterotrophic bacteria**		**Actinomycetes**	
	[CFU[2)] g^{-1} dry soil]					
1	4,265	a	2,484,117	a	357,842	a
2	3,801	a	3,963,301	b	581,646	b
LSD[3)]$_{5\%}$	1,443		675,300		96,340	

[1]Liming [mg kg^{-1}]: 1 = 1,177, 2 = 3,097
[2]CFU: colony forming units.
[3]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different mean at $p<0.05$

The application of P significantly ($p<0.05$) increased the count of all three microorganisms measured (Table 4.38).

Table 4.38: Influence of P rate on fungi, aerobic heterotrophic bacteria and actinomycetes count in grassland soil (3 way ANOVA).

P rate[1)]	Fungi	Aerobic heterotrophic bacteria	Actinomycetes
	[CFU[2)] g^{-1} dry soil]		
1	2,510 a	1,064,154 a	199,350 a
2	5,556 b	5,383,264 b	740,138 b
LSD[3)]$_{5\%}$	1,443	675,300	96,340

[1]P rate: [mg kg^{-1}]: 1 = 334, 2 = 1,558
[2]CFU: colony forming units.
[3]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different mean at $p<0.05$

Besides the direct effect of U rate, interactions with P and lime application rate obviously favored the higher amount of all the three microorganisms (Table 4.39).

Table 4.39: Statistical, F test for the comparison of the influence of U rate, P and lime application on fungi, aerobic heterotrophic bacteria and actinomycetes count in grassland soil.

Parameter	Fungi	Aerobic heterotrophic bacteria	Actinomycetes
U rate	ns	***	***
Liming	ns	***	***
P rate	***	***	***
U rate *Liming	**	***	***
U rate * P rate	***	**	***
P rate * Liming	***	***	***
U rate * P rate * Liming	ns	**	***

*, **, *** and ns: significant at $p<0.05$, $p<0.01$, $p<0.001$ and not significant, respectively

Significant interactions between the three factors were determined by the analysis of variance (Table 4.39). Next, low (P_1Ca_1) and high (P_2Ca_2) nutrients levels were separated for regression analysis (Table 4.40).

Table 4.40: Regression significance for the relationship between U rate and microorganisms count in relation to P rate and liming in grassland soil.

X=U rate Y=Parameter	Treatment	Regression equation	Coefficient of determination (R^2)	Significance
Fungi	$P^{1)}{}_1Ca^{2)}{}_1$	$Y = 3.8 X + 3,140$	0.03	ns
	P_2Ca_2	$Y = -5.8 X + 8,567$	0.08	ns
Aerobic heterotrophic bacteria	P_1Ca_1	$Y = -246.51 X + 1\cdot10^6$	0.035	ns
	P_2Ca_2	$Y = -9,329 X + 1\cdot10^7$	0.55	***
Actinomycetes	P_1Ca_1	$Y = -281.6 X + 294,578$	0.21	*
	P_2Ca_2	$Y = -2,241 X + 2\cdot10^6$	0.61	***

[1]P rate [mg kg^{-1}]: 1 = 334, 2 = 1,558
[2]Liming [mg kg^{-1}]: 1 = 1,177, 2 = 3,097
*, **, *** and ns: significant at $p<0.05$, $p<0.01$, $p<0.001$ and not significant, respectively

The results in Table 4.40 reveal that nearly 60% of the variation of aerobic heterotrophic bacteria and actinomycetes at high (P_2Ca_2) nutritional level and about 20% of the variation of actinomycetes at low (P_1Ca_1) could be explained by the U rate.

Forest soil

The U rate significantly decrease the actinomycetes count at the U_4 (652 mg kg^{-1}) treatment compared to the control (Table 4.41). The aerobic heterotrophic bacteria population was significantly higher at the U_2 (170 mg kg^{-1}) and U_3 (357 mg kg^{-1}) treatments compared to the control, while fungi count was not influenced (Figure 4.30).

Table 4.41: Influence of U rate on fungi, aerobic heterotrophic bacteria and actinomycetes count on a forest soil (3 way ANOVA).

U rate[1)]	**Fungi**		**Aerobic heterotrophic bacteria**		**Actinomycetes**	
	[CFU[2)] g^{-1} dry soil]					
1	112,119	a	518,807	a	110,624	ab
2	62,621	a	9,465,121	b	39,075	ac
3	72,534	a	3,592,914	c	157,992	b
4	70,370	a	505,972	a	29,191	c
LSD[3)]$_{5\%}$	68,906		1,704,134		75,395	

[1]U rate [mg kg^{-1}]: 1 = 0.34, 2 = 170, 3 = 357, 4 = 652
[2]CFU: colony forming units
[3]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different, mean at $p<0.05$

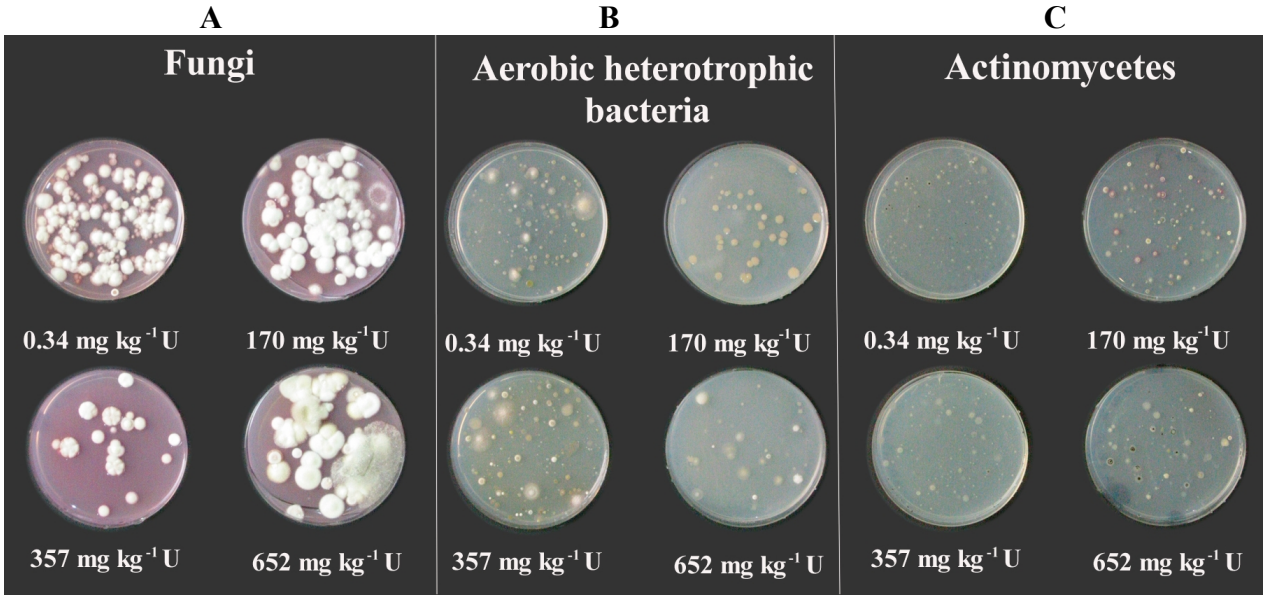


Figure 4.30: Petri dishes of: A- fungi (no significant differences), B- aerobic heterotrophic bacteria (significant differences) and C- actinomycetes (significant differences) in forest soil (photos: D. Gardiman).

The higher application of lime significantly ($p<0.05$) increased the count of aerobic heterotrophic bacteria and actinomycetes, while the fungi population was not influenced (Table 4.42).

Table 4.42: Influence of the liming on fungi, aerobic heterotrophic bacteria and actinomycetes count, forest soil (3 way ANOVA).

Liming[1)]	**Fungi**		**Aerobic heterotrophic bacteria**		**Actinomycetes**	
	[CFU[2)] g^{-1} dry soil]					
1	57,293	a	235,947	a	29,330	a
2	101,529	a	6,805,461	b	139,111	b
LSD[3)]$_{5\%}$	48,724		1,205,005		53,312	

[1]Liming [mg kg^{-1}]: 1 = 1,177, 2 = 3,097
[2]CFU: Colony forming units.
[3]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different mean at $p<0.05$

The P rate significantly ($p<0.05$) increased the count of aerobic heterotrophic bacteria and actinomycetes, while fungi significant decrease (Table 4.43).

Table 4.43: Influence of P rate on fungi, aerobic heterotrophic bacteria and actinomycetes count, forest soil (3 way ANOVA).

P rate[1)]	**Fungi**	**Aerobic heterotrophic bacteria**	**Actinomycetes**
	[CFU[2)] g^{-1} dry soil]		
1	99,080 a	165,843 a	75,986 a
2	59,742 b	6,875,564 b	92,456 b
LSD[3)]$_{5\%}$	48,724	1,205,005	53,312

[1]P rate: [mg kg^{-1}]: 1 = 334, 2 = 1,558
[2]CFU: colony forming units.
[3]LSD: least significant difference. Mean values followed by different letters in column indicate statistically different mean at $p<0.05$

Practically, interactions between all the factor tested and the microorganisms count influenced in the result obtained (Table 4.44) .

Table 4.44: Statistical significance (F test) for the comparison of the influence of U, P rates and liming on fungi, aerobic heterotrophic bacteria and actinomycetes count in forest soil.

Parameter	Fungi	Aerobic heterotrophic bacteria	Actinomycetes
U rate	***	***	**
Liming	***	***	***
P rate	***	***	ns
U rate *Liming	ns	***	*
U rate * P rate	**	***	***
P rate * Liming	***	***	ns
U rate * P rate * Liming	ns	***	*
*, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively			

Later, in order to realize the meaning of the results, low (P_1Ca_1) and high (P_2Ca_2) nutritional levels were isolated for regression analysis (Table 4.45).

Table 4.45: Regression significance for the relationship between U rate and microorganisms count in relation to P rate and liming on a forest soil.

X = U rate Y = Parameter	Treatment	Regression equation	Coefficient of determination (R^2)	Significant
Fungi	$P^{1)}{}_1Ca^{2)}{}_1$	Y = -7,436 X + 79.36	0.21	ns
	P_2Ca_2	Y = -374 X + 74.02	0.42	**
Aerobic heterotrophic bacteria	P_1Ca_1	Y = 69 X + 39.14	0.02	ns
	P_2Ca_2	Y = -149 X + $2 \cdot 10^7$	0.05	ns
Actinomycetes	P_1Ca_1	Y = 238 X + 22.29	0.14	ns
	P_2Ca_2	Y = -133 X +159.44	0.0001	ns
[1]P rate [mg kg^{-1}]: 1 = 334, 2 = 1,558 [2]Liming [mg kg^{-1}]: 1 = 1,177, 2 = 3,097 *, **, *** and ns: significant at p <0.05, p <0.01, p <0.001 and not significant, respectively				

The results in Table 4.45 reveal that about 40 % of the variation of fungi at high (P_2Ca_2) nutritional level could be explained by the U rate.

Summarizing the results from microbiological parameters (Table 4.46) it can be seen that fungi count was not influenced by U rate and liming in both grassland and forest soil. The P rate decreased the amount of fungi colonies in the forest soil, whereas in grassland soil had increased the fungi count.

Table 4.46: Summary of the influence of U, P rates and liming on dehydrogenase activity (DHA), sub and top soil, and microbial count in grassland and forest soil.

Soil	Parameter	U rate	Liming	P rate	Soil depth
Grassland	**DHA**	↑	↑	↑	↓
Forest	**DHA**	↓	↑	↑	↓
Grassland	**Fungi**	ns	ns	↑	-
	Aerobic heterotrophic bacteria	↓	↑	↑	-
	Actinomycetes	↓	↑	↑	-
Forest	**Fungi**	ns	ns	↓	-
	Aerobic heterotrophic bacteria	ns	↑	↑	-
	Actinomycetes	↓	↑	↑	-

↑: Positive significant difference; ↓: Negative significant difference. ns: No significant difference. (-): No tested

The aerobic heterotrophic bacteria and actinomycetes count decreased significantly with the U rate in grassland soil. Liming and P rate increased significantly the aerobic heterotrophic bacteria and actinomycetes counts at both type of soil (Table 4.46).

5 Discussion

The present research work proposes to reduce the uranium plant availability and uptake by plants using fertilizers (specifically phosphorus, sulfur, and nitrogen) in order to minimize the U transfer into the food chain and the contamination of the other environmental components. Thereby, investigations on the effect of uranium soil contamination on plants growth and the influence of nitrogen, phosphorus and sulfur fertilization on uranium concentration and uranium uptake by plants as well as the influence of uranium contamination on soil microbial parameters were carried out. A greenhouse pot experiment was designed with four treatments: Two rates of N, P, S and 3 rates of U contamination plus control. All four factors were factorially combined. The investigated agricultural crops have been maize (*Zea mays L.*), sunflower (*Helianthus annuus L.*), and faba bean (*Vicia faba L.*).

In the present work, attention has been focused mainly on U uptake by plants, which was calculated as a product between U concentration in plant tissues and biomass production, in relation to N, P, and S fertilizations.

The main functions in plant physiology of mineral nutrients such as N, P, and S that serve as constituents of proteins and nucleic acids are quite evident. In addition, chemical reactions between P and U in soil influence the U availability to plant and thus the U uptake.

The uptake of metallic elements by plants cell, especially in the roots, is facilitated by appropriate mechanisms for their transport and accumulation, since several heavy metals are in fact required by plants as microelements. The plant cannot, however, prevent toxic elements from entering by the same mechanisms. The toxicity of heavy metal ions is chiefly due to their interference with electron transport in respiration and photosynthesis, the inactivation of vital enzymes (like for instance: ATP ase, phosphatase, malate dehydrogenase, etc.) as a result of the lowered energy status, decrease the uptake of mineral nutrients, and reduce the growth (Larcher, 1995).

The discussion of the results of this thesis start with the description of the effects of U soil contamination on maize, sunflower, and faba bean growth (chapter 5.1). In the following chapter, the nutritional state of the tested crops is considered along with a discussion of the high and low nutritional levels in relation to the different U plant uptake for each crop (chapter 5.2). In the last chapter, the influence of uranium contamination on soil microbial parameters under P fertilization and liming is discussed (chapter 5.3).

5.1 Effect of uranium soil contamination on the plants growth

Effects of heavy metals on plants result in growth inhibition, structure damage, a decline of physiological and biochemical activities as well as of the function of plants. The effects and bioavailability of heavy metals depend on many factors, such as environmental conditions, pH, species of element, organic substances, fertilization, and plant species (Cheng, 2003).

There is a contradictory information on the phytotoxicity of soil U to plants (Sheppard et al., 1992). Although, no visible symptoms of toxicity, in all three agricultural crops tested were observed. However, levels as low as 1 mg kg^{-1} in soil, well within the normal background range, have been cited as toxic (Aery et al., 1998). Jain et al. (1998) found that root and shoot length, seedling dry weight, and chlorophyll contents started decreasing even at 1.25 µg ml^{-1} concentration of U.

In the present work, it was observed that for all the crops tested, the biomass production decreased with U rate. Nevertheless, the diminution of the growth was predominantly determined by the nutritional levels and the interactions between all the factors.

Gulati et al. (1980) reported that the highest yield of wheat was obtained at 3.0 mg U kg^{-1} and that tomato yield decreased continuously with increasing U level in soil from 1 to 6 mg kg^{-1}, which is in line with the results presented here.

Other studies have reported no toxicity at levels 100- to 1000-fold higher. For example, *Brassica napus* produced seed and high biomass yields at U levels of 10,000 mg kg^{-1} in soil (Sheppard et al., 1992).

To identify the toxic threshold of soil U, Sheppard et al. (1992) tested nine levels of U in 11 soils with 5 plant species. They found no detrimental effects below 300 mg U kg^{-1} in soil.

Plant species that are naturally high in heavy metals have developed a strategy to tolerate the heavy metals by unrestricted absorption and, as a result, accumulate high concentrations of the heavy metal in the plant tissue. Since heavy metals are damaging to most plants at relatively low concentrations, the hyperaccumulation strategy requires some mechanisms to detoxify the metals. This mechanisms include:

1. Reduced uptake,
2. Immobilization of the toxic metal in the cell walls, thus preventing contact with the protoplast as well as further transport through the apoplast,
3. Chelation in the cytoplasm to sulfur containing polypeptides (glutathione and glutamylcysteine derivates),

4. Compartmentalization and the formation of complexes with organic and inorganic acids, phenol derivates and glycosides in the vacuole, and lastly,
5. Retranslocation (Larcher, 1995) (Figure 5.1).

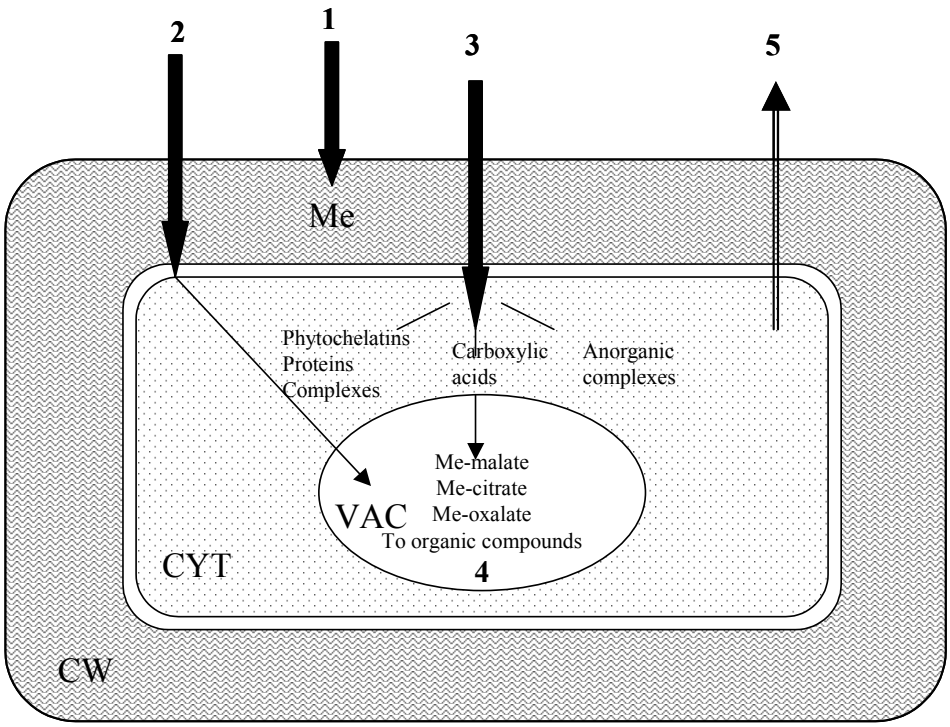


Figure 5.1: Possible mechanisms of resistance to heavy metals. 1- Immobilization of metal ions in the cell wall, especially by pectins; **2-** impeded permeation across the cell membrane; **3-** formation of chelates by metal-binding proteins and polypeptides (phytochelatins) in the cytoplasm; **4-** compartmentalization in the vacuoles; **5-** active export; **ME**: metal, **CW:** cell wall; **CYT:** cytoplasm; **VAC:** vacuole (according to Larcher, 1995).

Nevertheless, no information about U and phytochelatins are reported, so further investigation are needed in this field.

5.2 Influence of nitrogen, phosphorus and sulfur fertilization on uranium concentration and uranium plant uptake

No combination of the treatments yielded a sufficient N, P, and S nutrition in maize and faba bean, while in sunflower only $N_2P_2S_2$ was sufficient for plant growth. These situations were chosen regarding the conditions of the region affected by U contamination due to war activities. Low natural soil fertility and the restrictions for the fertilizer purchase characterize this scene.

S was the primarily limiting element in almost the treatments, thereby N effect was suppressed and yield responses to N fertilization were not that expected. The explanation is

Liebig´s "Low of the minimum", which states that growth is controlled not by the total of resources available, but by the scarcest resource.
However, the N rate increases significantly the U concentration in the plant material, U uptake and the concentrations of P, S, Ca and Fe in both maize and sunflower. According with Miller (1974) the presence of nitrogen did not directly increase the absorption process but instead increase the rate of translocation of phosphorus to the shoot, which indirectly influenced the absorption rate.
Little information is available on the accumulation of U in plants, except in the literature related to the use of native plant species in the biological exploration of metals. Furthermore, there are studies related to the mechanisms by which plants absorb and accumulate U (Boileau et al., 1985; Campbell and Rechel, 1979; Saric et al., 1995; Sheard, 1986a,b; Sheppard et al., 1984; Titaeva et al., 1979; Zafrir et al., 1992). In these studies, it was generally observed that plant species differ in U accumulation. Uranium accumulates mainly in the roots and depth of U placement and soil properties influence absorption by plants.

Recently, there have been reports of the high bioavailability of U to agricultural plants. Sunflower is very effective in recovering U from U contaminated water, mainly in the roots, with concentrations 5,000 to 10,000 times greater than that in the water (Entry et al., 1996).

Huang et al. (1997) reported that they have developed remediation technologies for the clean up of U contaminated soils and techniques to trigger U hyperaccumulation in plants. They observed that U accumulation in plant shoots increased by more than 1000 fold by use of organic acids.
Shoot U concentration of *Brassica juncea* increased from 5 mg kg^{-1} to more than 5,000 mg kg^{-1} with organic treated soil.

U plant uptake

It is possible to classify plants into three groups, according to their metal uptake characteristics:

1. Excluder: plants with restricted uptake of toxic metals or restricted translocation into the shoot over a wide range of soil metal concentrations
2. Index plants: plants those uptake and translocation reflect soil metal concentrations
3. Accumulators: plants which actively concentrate metals in their tissues

4. Hyperaccumulators: plants in which the tissue metal concentration can exceed 1,000 μg metal g^{-1} (Ross, 1994).

In the present study, the average of U uptake at the high nutritional level, was highest in sunflower, lower in maize and the lowest in faba beans. This result confirm the findings of Jovanovic et al. (2001) and also supported by Bargagli (1998) who says that herbaceous dicots accumulate grater amount of elements in the above ground biomass than do herbaceous monocots.

Toxic metal ions are thought to enter cells by means of the same processes that move essential micronutrients metal ions, such as Cu and Zn. Ross (1994) describe the ion uptake process as a process which comprises two stages: Passive uptake via apoplast fallowed for active uptake via symplast. Solute cations move passively into the root cortical cell walls, which form a hydrated free space continuum between the external bathing solution and the cortical cell membranes (apoplast). Since primary cell walls consists of a network of cellulose, hemicellulose, and glucoprotein, negative charges act as cation exchangers and anion repellers.

However, for Kabata- Pendias (2000) this two stages of ion uptake are two different processes. Nevertheless, he clarifies that when the concentration of elements pass over a threshold value for a physiological barrier, all elements are taken up passively.

Furthermore, Russel (1977) established the so called transpiration stream concentration factor (TSCF) as the ratio between element concentration in xylem sap moving from the root into the shoot and element concentration in external solution:

$$TSCF = \frac{element\ concentration\ in\ the\ transpiration\ stream}{element\ concentration\ in\ the\ solution\ outside\ the\ root}$$

When TSCF reaches values of 1, the taken up ion has to start moving to the xylem by active uptake mechanisms requiring the energy of ATP. Then a movement of the taken up ion against the direction of its decreasing concentration gradient is involved. At TSCF values smaller than 1, the ion is taken up by the root in the direction of decreasing concentration gradient and thus a passive uptake may be involved.

In a general sense, the plant can be viewed as a hydraulic conduit for water stored in the soil to travel upward and be evaporated from the leaves. This stream of water carries ions, for instance uranium, dissolved in the soil water to the plant roots. Obviously, the flow of water is

dependent on soil moisture retention and supply. It is also mechanistically linked to the size and growth rate of the plant. A greater flow of water through the plant will tend to increase the uptake of uranium but flow rate is correlated with more vigorous growth which dilutes the U concentration in the plant tissues.
Bonetto et al. (2005) investigated on the quantification of U retained during a soil passage of the pit water, and reported that plant growth decreased the leachate volumes between 30 – 65 % through evapotranspiration, which caused an increase of the U concentrations in the leachates, but reduced the total discharge of U from the columns.
Considering water requirements of each agricultural crop tested, sunflower is the crop with the highest water requirement (600 - 1000 mm), lower for maize (500 – 800 mm) and lowest for faba bean (300 – 500 mm). Contrary, the water utilization efficiency for harvested yield is highest in faba bean (80 – 90) lower in maize (0.8 – 1.6) and lowest in sunflower (0.2 to 0.5) (FAO, 1998).

In the light of the above discussion it was, therefore, concluded that sunflower showed the highest U uptake under fertilization management; not only due to the high biomass production but the increased U concentration in vegetative tissues. The explanations could be a high cation exchange capacity and/or the highest evapotranspiration rate, thus, the suction force increasing the intensity of root to shoot transport of elements.

It is beyond the scope of this work to discuss the U–concentration in grain. However, the information available reflect the same tendency that the result present here. The concentration of ^{238}U is reduced by a factor of 2 in grains obtained from the plants grown in fertilized field as compared to the grains from unfertilized fields (Pulhani, 2005). Butnik and Ischenko (1989) report about a 2.6 times reduction in uptake of ^{238}U by wheat plants due to the application of mineral and organic fertilizer. Corey et al. (1977) have also observed lowest concentration of radionuclide in the grains.

The relationship between uptake by plants and off take by harvested biomass production in the moment of make a decision: apply or not fertilizer plays a fundamental role. If the purpose of the agricultural crop is seeds and oil production (e.g sunflower) then the uranium off take would be very small. However, if the agricultural crop is looking forward “green production”, (grass for breeding animals, maize for silage, etc.) the off take could be about 100 %, since mostly of the harvest product is returning to the environment. Therefore, the decision must

take into account the needs of the population versus the potential food chain contamination and environmental risk.

The P rate decreased the U available in soil by complex compounds, however no effects were found in the case of faba bean. This result may implied the production of phytochelators which could be accumulated in the radical wall cells, nevertheless further investigation on U concentration in non green parts (roots and grain) are needed.

5.3 Influence of uranium contamination on soil microbial parameters under phosphorus fertilization and liming

Microorganisms are very important ecologically because they are the producing, consuming and transporting members of the soil ecosystem and therefore are involved in the flow of energy and in the cycling of chemical elements. The basic microbial phenomena in cycling processes in the soil environment are:

- Transport of an element into or out of a cell
- charge alteration of an element
- interaction of an element with organic compounds to become a functional part of the system
- complexing an element by organic acids and other compounds produced by microorganisms
- microbial accumulation or mobilization of an element
- microbial detoxication of poisoned soil at a site
- microbial methylation of an element (Kabata-Pendias, 2001).

The objective of this study was investigate the influence of uranium soil contamination on dehydrogenase activity and on microbial count under phosphorus fertilization and lime application. To achieve this aim the soil microbial activity was estimated by the measurement of dehydrogenase activity (DHA) according to Thalman (1968, modified). For microbial count agar spread technique was performed.

Dehydrogenase activity (DHA) and microbial count (CFU):

The result of the present research work demonstrated cleary that liming application favored the microbiological activity, aerobic heterotrophic bacteria and actinomycetes CFU. These findings are well in accordance with Bezdicek´s (2003) investigations, who found that liming

had a positive and significant effect on microbial biomass and dehydrogenase activity. Carter (1986), Baath (1994), Chagnon et al. (2000) and Stenberg et al., (2000) concluded that in response to liming treatments the pH significant increases, therefore microbial activity increases.

Wright et al. (2001) found that heterotrophic microbial activities measured under field and laboratory conditions were higher in areas impacted by P loading as compared to the unimpacted interior marsh. Microbial heterotrophic activities were higher in detritus and surface soils and decreased with depth, which support the finding of this study. With respect to the results from the influence of U rate on CFU and DHA, it can be say that with exception of fungi, all the rest of parameters tested were decreased with the U rate. The investigations of Kelly et al. (2003) displayed that the elevated levels of heavy metals in soils have had significant impacts showing decreases in the CFU of fungi, Gram positive bacteria, and actinomycetes.

Metal contamination are much more serious perturbations to soil microbial communities than are the applications of pesticides. Remediation of damage may require long periods of time, and damaged sites that return to relatively healthy status may not return to their pristine states. Remediation usually begins with ceasing the perturbation (Sims, 1990).

6 Summary

Uranium is the heaviest chemical element to be found in nature. It is a radioactive alpha emitter and a toxic heavy metal, which endangers environment and human health. In the past 50 years the quantities of uranium, which have been set free into the environment by human activities increased, and the danger of importing uranium into the food chain increased simultaneously. The big input of uranium into the environment comes from mining operations, nuclear industry, industrial, and medical wastes, the use of phosphate fertilizers in agriculture, and last but not least important depleted uranium (DU) ammunition used during the wars. The potential risk of uranium soil contaminations is a global problem since about every country is affected by one or more activities mentioned before.

The main objective of the present research work was to investigate the effect of the U contamination of a soil substrate in relation to plants nutrients and soil microbial parameters. The research strategy was based on the hypothesis that the rate of N, P and S directly may reduce U uptake and moderate the adverse effects on microbiological parameters. The investigations were conducted in pot experiments in the greenhouse under controlled water conditions. The pots (1 L capacity) contained 750 g soil substrate and were seeded with 5 seeds per pot on June 25th 2003 and harvested on August 4th 2003, before the generative stage begin. Three agricultural crops, maize (*Zea mays L.*), sunflower (*Helianthus annuus L.*), and faba bean (*Vicia faba L.*) were tested.

Several treatments have been performed using three different uranium contaminations plus control (U_1: control, U_2: 170 mg kg^{-1}, U_3: 357 mg kg^{-1}, and U_4: 652 mg kg^{-1} added as U_3O_8) combined with two different fertilizer levels and three different fertilizers (N_1: 250 mg kg^{-1}, N_2: 500 mg kg^{-1} added as NH_4NO_3 , P_1: 334 mg kg^{-1}, P_2: 1,558 mg kg^{-1} added as $CaHPO_4$, S_1: 0 mg kg^{-1}, and S_2: 50 mg kg^{-1} added as K_2SO_4). In the case of faba bean, no nitrogen fertilization had been applied. In addition, Mg and micronutrients were supplied in sufficient amounts to satisfy the demand of the plants. Each treatment combination was carried out with 3 replicates, resulting in a total of 96 pots of maize, 96 pots of sunflowers, and 48 pots of faba bean which sums up to a total of 240 pots in the experiment.
The main results of the research work presented here were:

U soil contamination levels

The U rate significantly decreased the biomass production, whereas the U concentration in plant tissues and the U plant uptake significantly increased in all the three crops compared to that control. It was observed that the concentrations of P and N increased significantly. In contrast, the Fe concentration decreased significantly with the U rate.

N fertilization

The N rate did not influence on the biomass production in both maize and sunflower crops (faba bean has not been treated with N). The nutritional state of the plants was between critical and deficient, thereby the high level of nitrogen did not improve the biomass production, mainly due to S deficiency. However, N rate affect the translocation of the elements into the plant, hence increasing significantly the uptake of U, N, P, S, and Ca by the plant.

P fertilization

The P rate significantly decreased the biomass production and the U concentration in plant tissues in sunflower, while no effects were observed in maize and faba bean on the same parameters. The U plant uptake decrease significantly by the P rate in maize and sunflower meanwhile in faba bean was not affected. This may indicate that the acidification of the root zone due to the N fixing process turns P more plant available and thus, less affinity to form complexes with uranium.

S fertilization

The S rate had a significant positive effect on the biomass production of maize and sunflower and also decreasing the U concentration in plant tissues of all the three crops. The increment of the biomass implied the dilution of the N, P, Ca, Mg, Zn, and B concentrations.

Microbial parameters

The uranium contaminated soil used for this experiment has been derived from a previous pot experiment. It consisted of two kinds of soil, a silty-loamy sand soil extracted from a grassland site and a sandy soil from a forest site. Samples from different soil depths (0-25 cm and 25-50 cm) had been taken. The soil substrates had the following treatments:

3 contamination levels of U (170, 360 and 650 mg kg^{-1} U) plus control (0.34 mg kg^{-1} U)

2 P fertilization rates given as $CaHPO_4$ (0 and 1,200 mg kg^{-1} P)
2 liming doses given as $CaCO_3$ (1,177 and 3,097 mg kg^{-1} Ca)

Dehydrogenase activity (DHA)

Grassland soil
The increase of U rate, P and lime application significantly increased the dehydrogenase activity compared to control. In contrast an increase in soil depth represents a decrease of the dehydrogenase activity.

Forest soil
The U rate decreased significantly the DHA. It was also observed that in the soil substrate from sub soil the DHA was lower than in soil substrate from top soil. P and lime application significantly increased the DHA activity compared to control.

Microorganism count

Grassland soil
Atinomycetes and aerobic heterotrophic bacteria count significantly decreased with U rate, while fungi count only at U_3 compared to control.
The higher application of lime significantly increased the count of aerobic heterotrophic bacteria and actinomycetes, while the fungi population was not influenced.
The application of P significantly increased the count of all the three microorganisms measured.
Forest soil
The U rate significantly decreased the actinomycetes count, while the aerobic heterotrophic bacteria count was significantly higher compared to the control. Fungi count was not influenced by the U rate.
The higher application of lime and the P rate increased significantly aerobic heterotrophic bacteria and actinomycetes count. Fungi count was not influenced by liming but was significantly lower by the P rate.

The present work tried to deliver a contribution to diminish the possible food contamination, which involves an important health risk. Therefore, the behavior of different plant species in relation to the uranium uptake in different situations of fertilization has been discussed.

Regarding the long half life and the toxicity of U is important to apply the precautionary principle. This concept includes risk prevention, cost effectiveness, ethical responsibilities towards maintaining the integrity of natural systems, and the fallibility of human understanding.

Interaktionen zwischen einer Uranbelastung von Böden und Düngung mit N, P S auf die Urangehalte und -aufnahme von Mais, Sonnenblumen und Bohnen sowie mikrobiologische Parameter der Böden

6 Zusammenfassung

Uran ist das schwerste in der Natur vorkommende Element. Es ist ein radioaktiver Alpha-Strahler und ein toxisches Schwermetall, welches Umwelt und Gesundheit des Menschen bedroht. In den letzten 50 Jahren stieg die Menge an Uran, die anthropogen in die Umwelt eingetragen wurde und damit auch das Risiko eines Eintrages von Uran in die Nahrungskette. Uran gelangt hauptsächlich über Uranbergbauaktivitäten, die Nuklearindustrie, medizinische Abfälle und den Gebrauch von Phosphordüngemitteln in die Umwelt. Hinzu kommt in nicht unerheblichem Maße der Einsatz von Munition mit abgereichertem Uran (DU) in Kriegsgebieten. Das Problem der Belastung von Böden mit Uran ist ein universelles, da in jedem Land eine der zuvor genannten Quellen existiert.

Das Ziel der vorliegenden Arbeit war es, den Einfluß steigender Uranbelastungen auf die Uranaufnahme von Pflanzen und mikrobiologische Parameter von Böden in Abhängigkeit von der Nährstoffversorgung des Bodens in Gefäßversuchen zu untersuchen. Hierbei wurde davon ausgegangen, daß die Höhe der N, P und S Versorgung einen direkten Einfluß auf die Uranaufnahme der Pflanzen und die mikrobiologische Aktivität der Böden ausübt und somit diese über ein gezieltes Nährstoff-Management reduziert werden kann, bzw. negative Effekte abgemildert werden können. Hierzu wurden kontrollierte Gefäßversuche unter Gewächshausbedingungen durchgeführt. Die Gefäße (1 Liter) wurden mit 750 g Boden gefüllt und die Bodenfeuchte durch entsprechende Zufuhr von Wasser konstant gehalten. In jedes Gefäß wurden 5 Samen von Mais (*Zea mays L.*), Sonnenblume (*Helianthus annuus L.*) und Ackerbohne (*Vicia faba L.*) am 25. Juni 2003 eingesät und die oberirdische Masse jeweils am 4. August 2003 in der noch vegetativen Phase geerntet.

Die Uranbelastungen der Böden enthielten die folgenden Varianten: (U_1: 0,34 (Kontrolle), U_2: 170 mg kg^{-1}, U_3: 357 mg kg^{-1}, und U_4: 652 mg kg^{-1}, zugeführt als U_3O_8). Stickstoff (N), Phosphor (P) und Schwefel (S) wurden in folgenden Stufen appliziert: N_1: 250 mg kg^{-1}, N_2: 500 mg kg^{-1} zugeführt als NH_4NO_3; P_1: 334 mg kg^{-1}, P_2: 1.558 mg kg^{-1} zugeführt als $CaHPO_4$; S_1: 0 mg kg^{-1}, S_2: 50 mg kg^{-1} zugeführt als K_2SO_4). Zu Ackerbohnen wurde kein Stickstoff gedüngt. Magnesium und Mikronährstoffe wurden in Mengen, die den Bedarf der Pflanzen decken, zugeführt. Jede Behandlung wurde in dreifacher Wiederholung angelegt, so daß

insgesamt 240 Gefäße mit jeweils 96 Gefäßen für Mais und Sonnenblumen und 48 Gefäßen für Ackerbohnen angesetzt wurden.

Die Ergebnisse der Untersuchungen zu den Haupteffekten lassen sich wie folgt zusammenfassen:

U Belastung der Böden

Mit steigender Uranbelastung nahm die Biomasseproduktion aller drei Kulturen signifikant ab, während gleichzeitig Urangehalt und Uranaufnahme signifikant stiegen. Des weiteren führte eine steigende Uranbelastung zu signifikant höheren P und N Gehalten, während die Fe Konzentrationen signifikant abnahmen.

N Düngung

Die Höhe der N Düngung hatte keinen Einfluß auf die Biomasseproduktion von Mais und Sonnenblume (zu Ackerbohnen wurde kein N gedüngt). Die N Versorgung war insgesamt als unzureichend bis marginal zu bewerten. Die höhere N Stufe führte zu keiner Steigerung der Biomasseproduktion, da auch S im Mangel war. Die Höhe der N Düngung beeinflußte jedoch die Mineralstoffaufnahme dahingehend, daß signifikant höhere U , N , P , S und Ca Gehalte bestimmt wurden.

P Düngung

Mit steigender P Zufuhr wurde eine signifikant geringere Biomasseproduktion und ein signifikant reduzierter Urangehalt in Sonnenblumen ermittelt. Dieser Effekt trat bei Mais und Ackerbohnen nicht auf. Die P Düngung führte zu einer signifikanten Abnahme der Uranaufnahme von Mais und Sonnenblumen, die von Ackerbohnen blieb hingegen unbeeinflußt. Dies deutet darauf hin, daß durch die Versauerung der Wurzelzone im Zuge N fixierender Prozesse P mobilisiert und damit pflanzenverfügbar wird, und Uran in vermindertem Maß in Komplexen gebunden wird.

S Düngung

Die S Düngung führte zu einem signifikanten Anstieg der Biomasseproduktion von Mais und Sonnenblumen. In allen drei Kulturen führte sie zu einer signifikanten Abnahme der Urangehalte im Pflanzenmaterial. Der Anstieg der Biomasseproduktion war mit einem

Verdünnungseffekt, der sich in entsprechend reduzierten N, P, Ca, Mg , Zn, und B Gehalten niederschlug, verbunden.

Mikrobiologische Parameter

Die Untersuchungen zur Bestimmung des Einflusses der Uranbelastung von Böden auf mikrobiologische Parametern erfolgte in Substraten aus einem vorherigen Experiment. Diese wurden zum einen mit einem schluffig-lehmigen Sandboden eines Grünlandstandortes, zum anderen mit einem Sandboden eines Waldstandortes durchgeführt. Des weiteren wurde jeweils Boden der Schichten 0 – 25 cm und 25 – 50 cm in die Untersuchungen einbezogen. Der Einfluß der folgenden Faktoren auf die mikrobiologischen Parameter wurde untersucht: Uranbelastung des Bodens (0,34; 170; 369 und 650 mg kg^{-1} U, P Düngung (0 und 1.200 mg kg^{-1} P als $CaHPO_4$) und Kalkung (1.177 und 3.097 mg kg^{-1} Ca als $CaCO_3$).

Dehydrogenase-Aktivität (DHA)

Grünland-Boden

Mit steigender U, P und Kalkzufuhr wurde ein Anstieg der DHA festgestellt. In Bodenmaterial aus tieferen Horizonten nahm die DHA hingegen ab.

Wald-Boden

Mit steigender Uranzufuhr und in tieferen Bodenschichten kam es zu einer signifikanten Abnahme der DHA, während die Zufuhr von P und Kalk diese signifikant erhöhte.

Anzahl Mikroorganismen

Grünland-Boden

Mit steigender Uranzufuhr nahm die Anzahl an Actinomyceten, aeroben heterotrophen Bakterien und Pilzen signifikant ab. Im Vergleich hierzu bewirkte die Kalkzufuhr einen Anstieg der Populationen an Actinomyceten und aeroben heterotrophen Bakterien. Die Behandlung zeigte keine Wirkung auf die Anzahl an Pilzen. Mit steigender P Zufuhr stieg die Anzahl aller drei Mikroorganismen signifikant an.

Wald-Boden

Mit steigender Uranzufuhr nahm die Anzahl an Actinomyceten ab, während die der aeroben heterotrophen Bakterien zunahm. Die Anzahl an Pilzen wurde durch die Höhe der Uranzufuhr nicht beeinflußt. Sowohl die Düngung mit P, als auch Kalk erhöhte die Anzahl an heterotrophen Bakterien und Actinomyceten signifikant. Die Anzahl an Pilzen blieb bei Kalkung unverändert, nahm hingegen signifikant mit der P Düngung ab.

Ziel der vorliegenden Arbeit war es, einen Beitrag zu leisten, die Belastung von Lebensmitteln mit Uran zu verringern, da diese ein erhebliches Gesundheitsrisiko darstellt. Daher wurde die Uranaufnahme verschiedener Kulturen in Abhängigkeit von der N, P und S Versorgung untersucht. Aufgrund der langen Halbwertszeit und hohen Schwermetalltoxizität von Uran ist das Vorsorgeprinzip anzuwenden. Dieses beinhaltet Risikovermeidung, Kosteneffizienz, ethische Verantwortung hinsichtlich des Erhaltes natürlicher Systeme und die Fehlbarkeit menschlichen Verständnisses.

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8 Appendix

Table A.1: Analytical data from maize for each treatment and replication

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
Control	250	0	334	0.069	6.020	0.4130	2.415	0.199	0.050	0.359	0.130	28.55	10.09	54.30	84.63	5.20	2.23
Control	250	0	334	0.044	6.370	0.281	2.262	0.181	0.055	0.424	0.139	21.80	7.44	54.83	93.61	5.73	2.45
Control	250	0	334	0.028	8.050	0.225	2.355	0.171	0.054	0.432	0.139	20.05	6.86	58.21	82.83	5.30	2.08
Control	500	0	334	0.043	6.650	0.284	3.641	0.180	0.051	0.459	0.125	24.65	6.82	48.50	70.00	4.57	1.97
Control	500	0	334	0.030	6.810	0.205	4.403	0.168	0.055	0.498	0.131	27.98	7.82	46.31	72.46	6.42	1.72
Control	500	0	334	0.045	5.080	0.229	4.533	0.207	0.054	0.499	0.143	27.90	8.90	57.89	110.83	7.25	2.04
Control	250	50	334	0.050	10.180	0.505	1.431	0.123	0.116	0.373	0.120	18.52	7.15	43.67	99.09	7.15	0.97
Control	250	50	334	0.001	9.900	0.010	1.777	0.133	0.131	0.383	0.134	20.23	7.40	34.70	82.32	4.73	0.99
Control	250	50	334	0.001	11.640	0.012	1.508	0.121	0.120	0.367	0.116	18.19	6.19	41.48	72.28	3.83	0.86
Control	500	50	334	0.001	11.450	0.011	2.930	0.131	0.164	0.492	0.134	27.78	7.87	52.26	117.86	5.25	0.91
Control	500	50	334	0.001	11.330	0.011	2.802	0.132	0.161	0.532	0.133	27.99	6.52	48.02	109.09	6.11	0.65
Control	500	50	334	0.001	12.540	0.013	2.582	0.124	0.157	0.524	0.125	23.63	7.01	50.83	124.80	5.61	0.71
Control	250	0	1,558	0.001	4.900	0.005	2.753	0.394	0.051	0.520	0.124	19.90	6.68	45.23	108.78	3.73	2.45
Control	250	0	1,558	0.001	4.950	0.005	2.725	0.381	0.046	0.475	0.118	20.60	7.02	39.94	64.68	3.98	2.37
Control	250	0	1,558	0.001	4.960	0.005	2.780	0.393	0.048	0.534	0.126	24.93	6.75	53.62	97.18	4.82	2.65
Control	500	0	1,558	0.001	4.240	0.004	5.013	0.428	0.051	0.688	0.120	23.74	6.23	31.91	372.46	4.72	2.54
Control	500	0	1,558	0.001	4.650	0.005	5.563	0.489	0.058	0.721	0.125	26.35	7.77	35.49	195.02	5.36	2.97
Control	500	0	1,558	0.001	4.980	0.005	4.827	0.438	0.052	0.678	0.134	22.25	7.08	33.34	303.90	6.55	2.62
Control	250	50	1,558	0.001	12.660	0.013	1.620	0.284	0.103	0.405	0.113	11.07	4.33	26.56	85.45	4.42	0.78
Control	250	50	1,558	0.001	12.760	0.013	1.474	0.285	0.106	0.385	0.107	13.23	5.42	25.46	87.55	4.74	0.75
Control	250	50	1,558	0.001	14.190	0.014	1.484	0.258	0.105	0.416	0.100	12.49	4.33	31.20	81.49	3.58	0.75
Control	500	50	1,558	0.001	14.540	0.015	2.425	0.381	0.151	0.518	0.119	18.97	4.60	27.88	151.69	6.39	0.80
Control	500	50	1,558	0.001	16.100	0.016	2.150	0.317	0.143	0.482	0.109	19.73	4.13	37.89	137.48	4.72	0.71
Control	500	50	1,558	0.001	15.200	0.015	2.387	0.395	0.142	0.538	0.133	17.91	5.46	31.25	152.46	6.74	0.83
166	250	0	334	1.877	6.780	12.729	2.367	0.170	0.052	0.411	0.138	22.05	9.47	61.01	150.26	4.58	2.29

Table A.1: continued

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
166	250	0	334	2.217	5.600	12.415	2.392	0.186	0.053	0.422	0.135	20.92	9.25	67.00	206.35	4.99	2.47
166	250	0	334	2.251	4.770	10.737	2.417	0.197	0.054	0.466	0.126	21.44	10.12	74.00	214.13	5.50	2.70
166	500	0	334	2.195	2.100	4.609	3.582	0.149	0.047	0.407	0.107	24.15	8.85	52.00	144.36	4.89	1.16
166	500	0	334	2.014	5.590	11.258	3.679	0.159	0.052	0.499	0.119	22.90	8.02	57.00	144.57	4.01	1.39
166	500	0	334	2.775	5.380	14.928	3.722	0.182	0.057	0.506	0.113	26.46	8.55	55.97	353.08	5.25	1.99
166	250	50	334	0.511	11.070	5.661	1.388	0.111	0.118	0.333	0.119	15.44	5.67	47.91	76.01	3.71	0.64
166	250	50	334	0.875	10.710	9.369	1.469	0.124	0.124	0.395	0.123	16.38	6.44	51.72	108.62	3.94	0.73
166	250	50	334	0.348	12.140	4.229	1.347	0.116	0.120	0.391	0.120	14.98	5.89	50.54	64.27	3.61	0.67
166	500	50	334	1.082	10.650	11.524	2.543	0.127	0.166	0.484	0.133	22.69	7.90	52.74	186.11	5.26	0.77
166	500	50	334	0.940	9.340	8.783	2.547	0.125	0.179	0.496	0.131	28.40	9.16	51.18	105.75	5.57	0.72
166	500	50	334	0.842	10.150	8.543	2.750	0.130	0.163	0.511	0.123	27.91	7.76	63.29	117.70	5.50	0.67
173	250	0	1,558	1.330	4.310	5.731	2.492	0.381	0.048	0.478	0.119	18.81	6.93	32.82	169.33	3.71	2.65
173	250	0	1,558	0.738	0.610	0.450	2.975	0.361	0.049	0.460	0.111	20.28	7.09	32.94	125.91	3.63	2.43
173	250	0	1,558	0.839	4.040	3.389	2.798	0.370	0.070	0.507	0.122	19.51	6.28	32.93	135.57	4.33	2.48
173	500	0	1,558	2.346	3.340	7.834	4.414	0.412	0.053	0.653	0.122	21.93	5.25	28.18	181.27	3.98	2.31
173	500	0	1,558	1.227	3.750	4.601	5.136	0.457	0.056	0.670	0.139	25.58	6.49	28.48	136.96	4.42	2.36
173	500	0	1,558	1.439	3.350	4.820	4.575	0.442	0.058	0.643	0.122	24.01	6.75	32.90	255.18	4.53	2.33
173	250	50	1,558	0.373	12.500	4.664	1.439	0.276	0.116	0.411	0.119	13.20	4.81	24.48	92.91	3.18	0.66
173	250	50	1,558	1.276	12.400	15.822	1.488	0.272	0.113	0.420	0.118	13.08	4.97	33.54	238.94	3.22	0.79
173	250	50	1,558	0.338	12.420	4.201	1.322	0.267	0.110	0.416	0.110	22.82	4.76	27.29	89.03	3.22	0.58
173	500	50	1,558	0.450	13.160	5.920	2.384	0.330	0.152	0.499	0.123	19.29	4.52	26.12	103.73	4.24	0.71
173	500	50	1,558	0.271	13.920	3.770	2.407	0.353	0.146	0.495	0.123	15.97	3.97	23.37	82.69	4.63	0.89
173	500	50	1,558	0.567	2.530	1.435	1.961	0.253	0.129	0.414	0.100	17.41	2.39	34.08	97.40	3.65	0.66
329	250	0	334	2.794	7.250	20.256	2.160	0.174	0.049	0.397	0.137	23.85	7.83	79.24	87.77	4.29	2.17
329	250	0	334	2.217	5.530	12.262	2.551	0.218	0.048	0.409	0.139	24.45	8.07	85.59	73.93	4.63	2.37
329	250	0	334	2.710	6.960	18.858	2.345	0.197	0.051	0.436	0.154	23.32	7.81	53.62	82.67	4.37	2.17
329	500	0	334	2.120	5.130	10.877	4.158	0.193	0.046	0.470	0.150	25.74	9.95	54.19	67.96	3.43	1.77
329	500	0	334	2.660	5.450	14.499	4.395	0.209	0.051	0.528	0.153	28.88	10.11	62.13	118.74	7.26	2.00

Table A.1: continued

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
329	250	50	334	1.357	11.250	15.270	1.675	0.119	0.120	0.357	0.103	19.44	8.86	47.08	84.18	7.71	0.87
329	250	50	334	0.943	11.460	10.805	1.643	0.111	0.112	0.380	0.122	24.05	8.38	49.40	71.48	7.92	0.86
329	250	50	334	0.722	14.560	10.507	1.339	0.107	0.092	0.321	0.104	14.16	6.23	43.74	65.28	6.14	0.78
329	500	50	334	0.919	10.600	9.745	2.922	0.150	0.162	0.538	0.151	31.02	8.90	69.56	94.06	8.58	0.96
329	500	50	334	2.001	11.990	23.997	2.369	0.125	0.152	0.435	0.130	40.98	7.26	55.16	97.33	7.47	1.02
329	500	50	334	1.528	12.110	18.507	2.457	0.121	0.134	0.456	0.109	43.40	5.53	57.33	91.84	7.28	0.84
385	250	0	1,558	1.428	3.640	5.198	2.941	0.426	0.046	0.553	0.122	23.37	7.43	45.56	115.99	6.61	3.28
385	250	0	1,558	1.148	3.420	3.926	2.966	0.394	0.049	0.539	0.120	21.80	8.63	44.34	79.13	6.31	3.12
385	250	0	1,558	1.114	3.570	3.976	2.901	0.438	0.052	0.573	0.127	27.48	8.14	46.84	91.80	6.75	3.49
385	500	0	1,558	4.625	3.300	15.263	4.763	0.372	0.049	0.645	0.114	20.02	7.21	28.45	112.68	5.81	2.98
385	500	0	1,558	1.788	3.920	7.010	4.663	0.413	0.052	0.646	0.115	20.20	6.05	30.97	82.68	6.59	2.46
385	500	0	1,558	3.850	2.600	10.010	5.332	0.523	0.064	0.755	0.128	27.22	8.69	34.50	157.28	7.30	3.05
385	250	50	1,558	0.622	9.480	5.898	1.594	0.314	0.124	0.430	0.125	34.91	6.81	33.44	83.31	6.56	1.06
385	250	50	1,558	0.398	11.280	4.491	1.438	0.301	0.117	0.439	0.120	16.87	6.52	31.98	78.43	6.61	1.09
385	250	50	1,558	0.234	11.660	2.733	1.226	0.274	0.099	0.401	0.106	11.60	4.79	39.70	85.90	6.95	0.96
385	500	50	1,558	0.345	11.640	4.020	2.925	0.463	0.169	0.605	0.148	17.25	6.50	31.84	101.15	8.41	1.16
385	500	50	1,558	0.447	12.840	5.734	2.549	0.389	0.142	0.542	0.124	14.29	5.72	26.75	84.40	7.96	0.97
385	500	50	1,558	0.619	14.460	8.950	2.269	0.401	0.135	0.502	0.110	14.70	3.96	39.09	92.17	7.27	0.98
660	250	0	334	3.686	5.510	20.312	2.405	0.212	0.046	0.358	0.129	18.45	9.46	50.98	80.72	8.34	2.31
660	250	0	334	2.546	5.170	13.160	2.426	0.214	0.047	0.396	0.112	17.13	13.29	54.96	155.42	10.43	2.28
660	250	0	334	7.632	4.730	36.099	2.605	0.214	0.047	0.470	0.131	23.07	12.30	61.89	77.24	4.12	1.94
660	500	0	334	3.377	4.940	16.683	4.269	0.217	0.051	0.548	0.120	24.58	13.86	66.34	129.35	4.65	2.28
660	500	0	334	10.279	4.490	46.153	4.148	0.220	0.055	0.492	0.135	24.80	10.10	62.98	81.25	6.16	1.93
660	500	0	334	3.692	4.850	17.906	4.802	0.246	0.053	0.349	0.107	12.23	7.99	43.41	83.15	3.85	0.87
660	250	50	334	1.887	10.740	20.271	1.254	0.128	0.121	0.342	0.127	17.06	6.47	83.52	85.49	13.43	0.79
660	250	50	334	2.074	10.510	21.795	1.640	0.126	0.113	0.364	0.132	13.62	4.95	49.18	91.77	4.33	0.70
660	250	50	334	4.002	11.330	45.341	1.503	0.124	0.113	0.519	0.158	21.57	9.57	71.99	98.78	6.58	0.68
660	500	50	334	2.668	9.540	25.451	2.813	0.147	0.167	0.574	0.162	23.71	10.42	68.47	105.59	6.95	0.60

Table A.1: continued

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
660	500	50	334	3.606	9.110	32.846	3.157	0.146	0.170	0.413	0.117	17.50	5.47	54.22	99.01	4.79	0.72
660	500	50	334	5.817	12.630	73.468	2.351	0.116	0.138	0.531	0.133	17.40	7.98	27.42	111.72	7.07	2.72
643	250	0	1,558	3.924	3.430	13.460	2.938	0.451	0.047	0.593	0.158	20.89	8.18	26.41	83.23	4.16	2.72
643	250	0	1,558	2.233	4.130	9.224	3.489	0.469	0.051	0.584	0.145	20.69	8.15	35.66	81.08	4.28	3.15
643	250	0	1,558	1.961	3.430	6.727	3.346	0.492	0.052	0.765	0.128	24.09	9.07	26.59	104.04	6.78	3.09
643	500	0	1,558	2.494	2.310	5.761	5.170	0.474	0.057	0.729	0.110	23.31	7.23	49.84	131.14	11.88	3.24
643	500	0	1,558	12.469	3.320	41.396	4.578	0.400	0.052	0.790	0.104	20.04	4.85	35.18	177.48	3.95	3.66
643	500	0	1,558	14.361	1.740	24.988	4.829	0.436	0.056	0.412	0.125	10.27	4.97	23.77	67.73	3.53	0.80
643	250	50	1,558	0.569	11.350	6.461	1.608	0.327	0.118	0.389	0.117	10.28	4.21	28.82	68.76	7.25	0.71
643	250	50	1,558	0.731	11.280	8.249	1.469	0.289	0.111	0.480	0.120	19.21	5.51	73.66	123.77	15.50	0.80
643	250	50	1,558	1.820	11.140	20.278	1.538	0.306	0.118	0.585	0.144	15.10	5.00	38.87	99.87	5.72	0.69
643	500	50	1,558	0.896	10.430	9.348	3.038	0.404	0.161	0.495	0.119	11.84	3.99	24.14	92.00	4.08	0.60
643	500	50	1,558	1.802	13.840	24.937	2.257	0.336	0.141	0.477	0.100	9.69	3.36	31.20	76.34	3.61	0.54
643	500	50	1,558	1.078	16.260	17.520	2.080	0.323	0.121	2.232	0.368	40.68	42.34	148.51	86.18	12.35	0.83

Table A.2: Regression coefficients for the relationships between U rate and nutrient content of maize in relation to the N, P, and S nutritional levels.

X = U rate in soil Y = Parameter	**Treatment**	**Regression equation**	**Coefficient of determination (R^2)**	**Significance**
Ca concentration [%]	$N_1P_1S_1$	$Y = -8 \cdot 10^{-06} X + 0.42$	0.00	ns
	$N_2P_2S_2$	$Y = 0.0009 X + 0.39$	0.19	ns
Mg concentration [%]	$N_1P_1S_1$	$Y = -2 \cdot 10^{-05} X + 0.14$	0.17	ns
	$N_2P_2S_2$	$Y = 0.0001 X + 0.10$	0.16	ns
Mn concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = -0.0028 X + 63.78$	0.00	ns
	$N_2P_2S_2$	$Y = 0.0555 X + 23.5$	0.16	ns
Zn concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = -0.0050 X + 23.54$	0.19	ns
	$N_2P_2S_2$	$Y = 0.0022 X + 17.47$	0.01	ns
Cu concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = 0.0035 X + 4.61$	0.22	ns
	$N_2P_2S_2$	$Y = 0.0028 X + 5.34$	0.07	ns
B concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = 0.0047 X + 7.96$	0.39	ns
	$N_2P_2S_2$	$Y = 0.0184 X + 2.04$	0.18	ns
Mo concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = -0.0002 X + 2.36$	0.09	ns
	$N_2P_2S_2$	$Y = -7 \cdot 10^{-05} X + 0.83$	0.01	ns

Appendix

Table A.3: Analytical data from sunflower for each treatment and replication

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
Control	250	0	334	0.001	4.270	0.004	2.541	0.189	0.067	1.802	0.240	43.67	31.13	114.88	71.94	7.80	1.66
Control	250	0	334	0.001	4.190	0.004	2.695	0.165	0.065	1.787	0.243	36.75	30.83	98.43	86.23	7.55	0.77
Control	250	0	334	0.001	4.180	0.004	2.519	0.161	0.065	1.746	0.223	34.69	30.12	111.16	80.51	7.45	0.70
Control	500	0	334	0.001	4.120	0.004	3.584	0.151	0.070	1.504	0.187	30.54	30.64	67.29	59.69	6.52	0.78
Control	500	0	334	0.001	4.320	0.004	3.622	0.146	0.064	1.483	0.177	31.80	30.35	71.72	68.95	6.68	0.69
Control	500	0	334	0.001	3.520	0.004	3.761	0.156	0.075	1.684	0.205	34.52	31.10	85.50	90.86	6.78	0.83
Control	250	50	334	0.001	7.780	0.008	1.839	0.126	0.232	1.340	0.181	33.36	19.58	195.20	110.56	6.29	0.57
Control	250	50	334	0.001	7.780	0.008	1.905	0.115	0.223	1.296	0.173	35.70	17.55	231.17	162.52	6.02	0.57
Control	250	50	334	0.001	7.380	0.007	1.864	0.124	0.247	1.459	0.187	34.81	20.72	248.28	113.24	7.76	0.72
Control	500	50	334	0.001	6.700	0.007	3.694	0.122	0.267	1.609	0.222	42.31	22.04	136.00	124.01	8.17	0.59
Control	500	50	334	0.001	7.940	0.008	3.179	0.111	0.232	1.487	0.182	36.41	18.12	213.11	142.64	7.76	0.54
Control	500	50	334	0.001	6.490	0.006	3.403	0.112	0.248	1.715	0.215	46.07	20.99	236.63	190.27	6.15	0.55
Control	250	0	1,558	0.001	3.640	0.004	3.141	0.440	0.067	2.254	0.247	31.79	33.22	47.02	72.42	9.97	0.98
Control	250	0	1,558	0.001	2.910	0.003	3.324	0.409	0.066	2.192	0.200	30.80	34.79	44.67	79.38	8.50	1.00
Control	250	0	1,558	0.001	3.420	0.003	3.390	0.445	0.065	2.355	0.232	31.67	31.05	46.97	75.29	9.68	0.89
Control	500	0	1,558	0.001	3.550	0.004	4.675	0.447	0.076	2.182	0.200	36.68	30.46	43.90	93.22	8.21	0.84
Control	500	0	1,558	0.001	3.160	0.003	4.721	0.384	0.068	1.871	0.179	31.57	26.96	31.95	71.01	7.37	0.89
Control	500	0	1,558	0.001	3.240	0.003	4.404	0.361	0.072	2.088	0.168	24.72	29.23	30.87	118.49	6.60	0.80
Control	250	50	1,558	0.001	8.740	0.009	1.854	0.300	0.217	1.453	0.144	21.35	19.88	150.03	78.80	7.89	0.45
Control	250	50	1,558	0.001	8.250	0.008	1.801	0.316	0.244	1.583	0.139	21.60	16.45	186.23	70.58	7.55	0.49
Control	250	50	1,558	0.001	8.600	0.009	1.952	0.291	0.250	1.519	0.135	20.39	14.96	156.05	73.44	6.92	0.46
Control	500	50	1,558	0.001	7.420	0.007	3.656	0.407	0.339	2.092	0.157	27.95	15.73	157.14	100.44	7.82	0.61
Control	500	50	1,558	0.001	8.110	0.008	3.851	0.407	0.282	2.039	0.177	30.69	15.11	131.31	105.58	6.58	0.63
Control	500	50	1,558	0.001	6.900	0.007	3.614	0.352	0.296	2.039	0.171	21.57	14.54	129.41	86.61	6.84	0.57
329	250	0	334	1.176	3.240	3.810	2.779	0.189	0.076	1.823	0.258	53.15	33.67	124.07	73.88	6.84	0.71
166	250	0	334	1.824	3.980	7.259	2.608	0.165	0.068	1.594	0.228	29.17	30.78	107.36	80.18	5.62	0.73
166	250	0	334	1.197	4.100	4.909	2.700	0.195	0.073	1.815	0.209	37.01	36.30	161.65	66.43	8.87	0.74
166	500	0	334	0.748	3.190	2.388	4.549	0.171	0.081	1.761	0.159	41.72	34.75	116.04	51.56	8.05	0.89

Table A.3: continued

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
166	500	0	334	2.202	3.080	6.783	4.130	0.188	0.079	1.710	0.157	33.47	34.57	71.76	65.47	7.64	0.92
166	500	0	334	0.635	3.380	2.145	4.083	0.151	0.068	1.642	0.181	29.90	30.47	81.95	37.37	6.85	0.59
166	250	50	334	0.836	6.620	5.532	2.127	0.119	0.209	1.355	0.198	28.87	20.27	150.83	53.19	6.28	0.52
166	250	50	334	0.524	6.310	3.308	2.034	0.125	0.220	1.343	0.188	29.89	19.54	205.46	50.14	6.05	0.45
166	250	50	334	0.652	7.050	4.600	2.013	0.115	0.222	1.370	0.181	31.06	18.52	233.89	59.09	5.40	0.53
166	500	50	334	0.896	4.800	4.301	3.756	0.118	0.249	1.453	0.208	31.60	22.29	98.79	62.72	6.33	0.52
166	500	50	334	0.915	5.180	4.740	3.978	0.129	0.282	1.622	0.238	37.01	23.38	110.95	62.76	7.43	0.50
166	500	50	334	0.597	4.750	2.833	3.938	0.115	0.254	1.525	0.194	35.73	22.32	136.98	52.10	7.21	0.44
173	250	0	1,558	1.522	2.020	3.075	3.143	0.332	0.068	2.029	0.213	31.94	31.29	46.50	61.74	5.75	1.03
173	250	0	1,558	1.241	1.960	2.433	3.728	0.441	0.081	2.408	0.285	38.19	39.07	57.97	65.50	8.95	1.05
173	250	0	1,558	1.053	2.080	2.190	3.375	0.382	0.070	2.149	0.211	30.07	36.37	31.25	57.36	8.06	0.96
173	500	0	1,558	1.370	1.570	2.151	4.982	0.518	0.080	2.486	0.235	36.24	35.74	37.66	72.43	7.43	1.29
173	500	0	1,558	1.311	1.680	2.202	5.328	0.488	0.079	2.367	0.240	39.06	40.76	26.96	98.25	7.98	1.39
173	500	0	1,558	0.723	2.250	1.627	4.736	0.385	0.076	2.318	0.215	26.70	33.24	23.79	51.88	5.68	1.17
173	250	50	1,558	0.359	6.720	2.411	1.814	0.343	0.243	1.561	0.185	23.46	17.03	96.19	48.19	5.32	0.60
173	250	50	1,558	0.296	7.920	2.341	1.663	0.324	0.213	1.493	0.168	25.04	14.21	123.69	79.23	4.60	0.53
173	250	50	1,558	0.322	2.980	0.958	1.561	0.280	0.196	1.465	0.155	18.10	13.70	126.89	45.93	4.15	0.55
173	500	50	1,558	0.308	6.100	1.881	3.892	0.509	0.318	2.323	0.234	29.05	18.68	45.95	63.71	7.23	0.84
173	500	50	1,558	1.022	7.960	8.139	2.811	0.359	0.247	1.783	0.184	21.33	14.21	199.26	69.55	5.18	0.60
173	500	50	1,558	0.675	8.070	5.445	2.848	0.380	0.250	1.957	0.175	20.75	13.85	221.15	71.83	5.37	0.68
329	250	0	334	2.987	3.210	9.589	2.982	0.214	0.072	1.532	0.217	63.59	25.28	130.28	73.61	9.52	1.53
329	250	0	334	1.791	4.510	8.079	2.377	0.167	0.094	1.788	0.241	62.53	27.24	239.65	70.93	18.46	1.60
329	250	0	334	2.587	4.770	12.342	2.644	0.190	0.084	1.737	0.216	30.95	30.82	79.27	152.34	13.91	1.37
329	500	0	334	7.683	3.270	25.124	4.337	0.168	0.077	1.905	0.220	38.71	33.11	107.26	92.31	15.90	1.40
329	500	0	334	3.213	2.880	9.253	4.521	0.175	0.079	1.686	0.190	31.51	30.44	117.07	65.77	15.36	1.25
329	500	0	334	1.843	3.610	6.652	4.050	0.153	0.072	1.127	0.175	24.49	19.32	143.18	71.79	9.67	1.02
329	250	50	334	1.739	6.650	11.566	2.139	0.120	0.209	1.112	0.159	24.30	17.62	167.40	73.57	12.85	0.92
329	250	50	334	2.322	6.960	16.163	1.864	0.121	0.225	1.216	0.164	26.55	17.36	202.05	72.31	15.36	1.07

Table A.3: continued

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
329	250	50	334	1.147	7.460	8.560	1.751	0.116	0.204	1.498	0.202	37.87	20.27	171.94	83.67	15.02	0.89
329	500	50	334	1.653	6.850	11.324	3.372	0.116	0.249	1.479	0.186	33.79	18.46	167.07	68.39	9.20	0.88
329	500	50	334	1.348	6.220	8.386	3.492	0.115	0.239	1.611	0.196	29.68	20.27	145.90	76.17	13.63	0.65
329	500	50	334	10.934	5.310	58.059	3.766	0.109	0.275	2.346	0.272	41.82	38.45	35.08	80.19	11.94	1.70
385	250	0	1,558	1.797	2.390	4.295	3.905	0.495	0.074	2.290	0.247	45.79	35.19	36.50	66.70	10.26	1.13
385	250	0	1,558	1.836	1.620	2.974	3.575	0.420	0.070	2.406	0.280	37.30	35.05	42.36	82.85	19.18	1.21
385	250	0	1,558	2.033	1.900	3.862	3.470	0.491	0.068	2.132	0.218	28.96	35.85	30.49	89.59	8.70	1.20
385	500	0	1,558	1.973	1.810	3.571	4.667	0.414	0.072	2.121	0.211	31.04	34.83	29.26	116.02	15.40	1.20
385	500	0	1,558	2.579	2.160	5.571	4.950	0.467	0.069	2.294	0.233	26.87	32.17	33.60	96.81	12.99	1.32
385	500	0	1,558	1.904	1.880	3.580	4.901	0.428	0.074	1.629	0.195	22.42	17.84	81.21	63.02	17.91	0.93
385	250	50	1,558	0.418	6.680	2.794	1.681	0.412	0.262	1.514	0.181	22.48	16.05	126.99	55.95	14.37	0.76
385	250	50	1,558	0.427	6.400	2.736	1.653	0.362	0.222	1.535	0.163	19.17	14.66	128.95	48.48	12.12	0.85
385	250	50	1,558	0.290	7.300	2.117	1.654	0.336	0.218	2.027	0.180	32.75	21.58	119.73	94.69	22.51	1.02
385	500	50	1,558	0.996	5.780	5.757	3.228	0.451	0.302	2.040	0.177	28.49	17.40	153.44	89.90	13.66	0.91
385	500	50	1,558	0.931	6.410	5.965	3.345	0.486	0.307	1.924	0.167	21.79	16.03	132.90	85.57	6.57	0.84
385	500	50	1,558	0.861	6.670	5.742	3.069	0.413	0.287	1.921	0.146	69.94	27.97	166.68	165.46	18.11	3.17
660	250	0	334	4.459	2.880	12.841	3.629	0.290	0.081	1.869	0.291	32.49	35.09	137.32	74.56	13.90	0.84
660	250	0	334	4.831	2.800	13.528	3.380	0.225	0.070	1.995	0.294	32.53	33.94	119.11	105.56	8.80	0.73
660	250	0	334	3.273	3.480	11.390	3.239	0.212	0.070	1.636	0.225	28.69	34.57	85.90	85.89	8.16	0.78
660	500	0	334	7.677	2.290	17.579	4.650	0.183	0.075	1.613	0.225	25.07	32.78	84.39	66.36	6.11	0.65
660	500	0	334	5.589	3.440	19.225	4.435	0.162	0.073	1.709	0.199	25.05	34.92	77.84	68.80	9.00	0.78
660	500	0	334	6.035	2.320	14.001	4.703	0.170	0.074	1.326	0.195	26.80	21.13	184.61	72.12	8.74	0.64
660	250	50	334	4.054	5.920	24.002	2.449	0.140	0.253	1.225	0.173	23.64	17.61	155.02	63.94	7.93	0.59
660	250	50	334	2.862	7.000	20.034	2.099	0.130	0.218	1.221	0.161	19.17	16.03	157.49	58.44	5.46	0.59
660	250	50	334	2.353	7.630	17.953	1.952	0.116	0.201	1.590	0.219	31.11	20.95	135.56	74.91	13.65	0.56
660	500	50	334	2.316	5.700	13.201	3.476	0.115	0.273	1.363	0.191	25.06	18.09	120.22	82.68	6.72	0.56
660	500	50	334	8.773	6.350	55.708	3.445	0.117	0.233	1.558	0.192	24.82	18.58	151.70	61.80	6.28	0.59
660	500	50	334	4.731	6.110	28.904	3.229	0.120	0.257	2.647	0.342	39.71	41.14	35.70	86.05	11.22	1.11

Table A.3: continued

U rate in soil	N rate	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
							Concentrations										
----------[mg kg^{-1}]----------					[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
643	250	0	1,558	4.707	1.630	7.672	4.197	0.543	0.077	2.352	0.280	37.13	40.47	33.68	80.77	9.51	1.08
643	250	0	1,558	5.056	2.290	11.578	3.910	0.466	0.073	2.564	0.300	32.25	34.85	57.60	123.85	11.39	1.23
643	250	0	1,558	4.930	1.990	9.810	3.343	0.432	0.079	2.488	0.265	33.22	45.40	32.76	135.45	8.09	1.54
643	500	0	1,558	6.464	1.600	10.343	5.247	0.537	0.087	1.868	0.206	24.78	25.20	24.64	86.38	7.23	0.91
643	500	0	1,558	3.190	3.170	10.113	4.174	0.428	0.076	3.004	0.295	25.80	36.52	45.99	100.40	6.94	1.73
643	500	0	1,558	5.098	1.620	8.258	5.713	0.524	0.094	1.659	0.186	19.62	21.22	127.99	105.71	9.48	0.79
643	250	50	1,558	2.345	5.800	13.598	2.408	0.396	0.258	1.593	0.172	21.90	16.02	123.25	67.63	7.56	0.63
643	250	50	1,558	1.351	6.130	8.282	2.036	0.382	0.246	1.419	0.146	15.92	14.26	93.66	63.46	10.10	0.60
643	250	50	1,558	1.291	7.610	9.821	1.846	0.289	0.219	2.181	0.192	26.44	19.21	94.19	101.14	8.22	0.68
643	500	50	1,558	4.694	5.460	25.629	3.961	0.516	0.334	2.123	0.183	39.82	18.07	116.03	116.92	7.79	0.82
643	500	50	1,558	5.516	5.830	32.156	3.620	0.519	0.324	1.954	0.159	19.89	14.29	226.68	80.71	6.38	0.65
643	500	50	1,558	2.366	7.800	18.453	2.929	0.450	0.265	1.812	0.153	45.93	22.14	183.78	182.47	12.28	3.68

Table A.4: Regression significance for the relationships between U rate and nutrient content of sunflower in relation to the N, P and S nutritional level.

X =U rate Y =Parameter	**Treatment**	**Regression equation**	**Coefficient of determination (R^2)**	**Significance**
S concentration [%]	$N_1P_1S_1$	$Y = 1 \cdot 10^{-05} X + 0.07$	0.13	ns
	$N_2P_2S_2$	$Y = 2 \cdot 10^{-05} X + 0.29$	0.02	ns
Ca concentration [%]	$N_1P_1S_1$	$Y = 0.8785 X + 17349$	0.03	ns
	$N_2P_2S_2$	$Y = -1.5425 X + 20469$	0.07	ns
Mg concentration [%]	$N_1P_1S_1$	$Y = 0.5338 X + 2250.6$	0.24	ns
	$N_2P_2S_2$	$Y = -0.2289 X + 1804.2$	0.07	ns
Fe concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = 0.0206 X + 79.21$	0.05	ns
	$N_2P_2S_2$	$Y = 0.0653 X + 81.92$	0.19	ns
Mn concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = 0.0045 X + 124.45$	0.00	ns
	$N_2P_2S_2$	$Y = 0.0491 X + 140.54$	0.06	ns
Zn concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y -0.0095 X + 43.17$	0.04	ns
	$N_2P_2S_2$	$Y = 0.0191 X + 25.68$	0.11	ns
Cu concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = 0.0055 X + 8.14$	0.14	ns
	$N_2P_2S_2$	$Y = 0.0053 X + 7.06$	0.11	ns
B concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = 0.004 X + 30.49$	0.10	ns
	$N_2P_2S_2$	$Y = 0.0062 X + 15.47$	0.14	ns
Mo concentration [mg kg^{-1}]	$N_1P_1S_1$	$Y = -0.0002 X + 1.06$	0.01	ns
	$N_2P_2S_2$	$Y = 0.002 X + 0.57$	0.22	ns

Table A.5: Analytical data from faba bean for each treatment and replication

U rate in soil	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn	Fe	Cu	Mo
						Concentrations										
----------[mg kg^{-1}]----------				[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
Control	0	334	0.077	3.920	0.30	2.413	0.287	0.119	2.021	0.164	70.05	27.04	179.01	184.48	10.27	2.17
Control	0	334	0.001	2.450	0.00	2.239	0.279	0.111	1.578	0.156	62.90	29.01	201.69	339.78	8.80	1.87
Control	0	334	0.001	2.620	0.00	2.379	0.319	0.123	1.892	0.182	73.68	30.95	209.07	331.85	9.29	2.38
Control	50	334	0.053	3.830	0.20	2.098	0.283	0.269	1.647	0.156	78.01	26.38	175.90	145.27	10.02	2.76
Control	50	334	0.001	3.730	0.00	2.076	0.273	0.272	1.735	0.161	79.11	27.11	161.43	177.40	10.08	1.93
Control	50	334	0.001	3.490	0.00	2.297	0.283	0.320	1.897	0.179	90.86	28.43	203.95	204.17	11.58	2.45
Control	0	1,558	0.053	4.050	0.22	2.219	0.512	0.105	2.078	0.120	54.23	21.84	96.92	224.32	9.91	3.11
Control	0	1,558	0.001	2.650	0.00	1.774	0.376	0.100	2.045	0.125	52.07	23.44	154.56	283.04	9.39	2.72
Control	0	1,558	0.001	2.880	0.00	2.223	0.485	0.124	2.213	0.129	62.39	25.88	103.86	261.77	11.55	2.69
Control	50	1,558	0.048	2.360	0.11	1.610	0.495	0.292	2.099	0.113	53.29	26.69	120.37	252.68	9.86	3.61
Control	50	1,558	0.001	3.280	0.00	1.940	0.452	0.308	1.973	0.134	54.69	24.99	100.43	203.88	11.37	2.96
Control	50	1,558	0.001	3.470	0.00	2.180	0.507	0.314	1.899	0.127	58.96	24.24	116.67	227.55	11.90	2.72
166	0	334	2.047	3.340	6.84	2.472	0.291	0.121	1.774	0.171	60.43	25.18	157.20	155.45	9.93	2.25
166	0	334	1.521	2.760	4.20	2.787	0.302	0.120	1.854	0.183	57.86	28.08	139.41	178.61	9.64	3.33
166	0	334	1.068	3.530	3.77	2.740	0.271	0.112	1.755	0.174	63.94	24.18	164.37	142.75	9.36	2.94
166	50	334	1.270	2.940	3.73	2.513	0.262	0.255	1.664	0.170	71.33	28.95	172.64	157.38	10.00	3.04
166	50	334	0.855	2.710	2.32	2.202	0.257	0.234	1.548	0.171	71.68	27.05	264.44	185.22	10.32	2.57
166	50	334	1.013	2.350	2.38	3.029	0.272	0.270	1.777	0.174	72.54	29.33	156.17	197.77	11.70	2.75
173	0	1,558	1.774	2.240	3.97	1.874	0.477	0.092	2.221	0.149	60.56	24.04	182.11	317.13	8.23	2.44
173	0	1,558	2.173	3.360	7.30	1.860	0.499	0.086	1.886	0.141	53.19	20.92	123.30	242.39	7.91	2.51
173	50	1,558	2.592	0.210	0.54	2.580	0.465	0.135	1.605	0.139	50.84	19.99	85.40	382.79	11.60	4.96
173	50	1,558	2.794	3.590	10.03	1.933	0.476	0.262	1.818	0.127	54.09	19.83	112.26	302.92	9.19	2.50
329	0	334	1.548	1.780	2.76	2.163	0.298	0.119	1.868	0.148	62.94	26.08	211.58	242.05	12.85	3.52
329	0	334	1.609	3.160	5.08	2.517	0.285	0.115	2.005	0.155	66.39	30.10	200.05	179.38	19.54	2.82
329	0	334	2.439	2.780	6.78	2.251	0.304	0.127	1.709	0.156	90.20	26.55	194.83	175.62	23.34	3.42
329	50	334	1.476	2.680	3.96	3.162	0.308	0.294	1.814	0.145	58.65	24.21	166.90	173.18	21.95	3.23
329	50	334	1.868	3.140	5.87	3.177	0.279	0.287	1.926	0.166	70.17	26.51	241.00	194.29	21.37	3.48
329	50	334	2.427	2.640	6.41	2.915	0.293	0.320	2.375	0.132	52.84	21.87	129.94	132.44	15.92	3.86

Table A.5: continued

U rate in soil	S rate	P rate in soil	U concentration	Biomass	U uptake	N	P	S	Ca	Mg	Zn	B	Mn.	Fe	Cu	Mo
						Concentrations										
----------[mg kg^{-1}]----------				[g pot^{-1}]	[µg pot^{-1}]	----------[%]----------					----------[mg kg^{-1}]----------					
385	0	1,558	1.831	3.340	6.12	3.061	0.536	0.132	2.051	0.132	56.52	22.80	100.49	158.87	12.48	3.48
385	0	1,558	1.621	3.130	5.08	2.435	0.491	0.098	2.166	0.148	60.86	27.81	187.92	485.21	19.45	5.04
385	0	1,558	2.531	3.000	7.59	2.409	0.533	0.096	2.247	0.121	56.46	22.95	113.71	162.27	11.85	3.55
385	50	1,558	0.767	2.600	1.99	2.185	0.484	0.333	2.114	0.124	56.46	24.23	105.89	132.12	11.41	3.73
385	50	1,558	0.952	3.180	3.03	2.613	0.464	0.306	0.336	0.123	17.99	9.16	56.47	86.12	4.68	2.43
660	0	334	4.399	3.580	15.75	2.981	0.279	0.113	1.456	0.159	39.49	22.41	194.34	213.24	12.21	2.94
660	0	334	8.290	1.550	12.85	2.369	0.297	0.110	2.024	0.186	47.15	24.22	200.73	192.57	9.50	3.86
660	0	334	7.068	2.640	18.66	3.210	0.327	0.122	1.920	0.185	60.43	29.14	199.00	184.58	13.60	3.13
660	50	334	4.944	2.500	12.36	2.958	0.316	0.311	1.813	0.180	53.60	27.44	200.78	174.10	13.74	3.00
660	50	334	3.919	2.700	10.58	2.907	0.281	0.276	1.760	0.175	51.55	25.54	210.50	186.55	11.96	2.49
660	50	334	4.702	2.550	11.99	2.831	0.302	0.267	2.333	0.146	45.90	21.56	103.14	251.63	14.60	3.66
643	0	1,558	7.586	3.000	22.76	2.480	0.503	0.110	1.993	0.144	43.87	20.37	116.59	169.79	10.55	3.59
643	0	1,558	5.525	2.870	15.86	2.713	0.496	0.111	1.992	0.128	41.71	20.43	94.09	144.87	9.89	3.26
643	0	1,558	4.786	3.100	14.84	2.476	0.517	0.098	2.400	0.152	53.51	27.50	105.46	153.96	19.24	3.19
643	50	1,558	4.225	1.840	7.77	2.589	0.507	0.317	1.962	0.134	44.18	22.62	133.55	168.69	9.72	3.13
643	50	1,558	5.123	1.900	9.73	2.610	0.476	0.269	1.928	0.121	43.69	17.89	134.29	179.99	12.02	2.99
643	50	1,558	3.031	3.210	9.73	2.352	0.457	0.287	0.000	0.000						

Table A.6: Regression significance for the relationships between U rate and nutrient content of faba bean in relation to the P and S rates.

X =U rate in soil Y =Parameter	Treatment	Regression equation	Coefficient of determination (R^2)	Significance
N concentration [%]	P_1S_1	Y = 0.0006 X + 2.36	0.24	ns
	P_2S_2	Y = 0.0009 X + 1.98	0.49	ns
P concentration [%]	P_1S_1	Y = 0.1331 X + 2911.6	0.04	ns
	P_2S_2	Y = -0.0421 X + 4794.9	0.00	ns
S concentration [%]	P_1S_1	Y = -0.035 X + 1188.2	0.03	ns
	P_2S_2	Y = 0.2444 X + 2746.9	0.01	ns
Ca concentration [%]	P_1S_1	Y = -0.2378 X + 18282	0.00	ns
	P_2S_2	Y = -2.9836 X + 18280	0.02	ns
Mg concentration [%]	P_1S_1	Y = 0.0772 X + 1660.9	0.02	ns
	P_2S_2	Y = 0.0072 X + 1266.9	0.00	ns
Fe concentration [mg kg^{-1}]	P_1S_1	Y = -0.0876 X + 235.33	0.12	ns
	P_2S_2	Y = -0.1662 X + 259.64	0.23	ns
Mn concentration [mg kg^{-1}]	P_1S_1	Y = 0.0253 X + 180.29	0.08	ns
	P_2S_2	Y = 0.021 X + 101.65	0.05	ns
Zn concentration [mg kg^{-1}]	P_1S_1	Y = -0.0261 X + 70.48	0.28	ns
	P_2S_2	Y = -0.0232 X + 54.44	0.23	ns
Cu concentration [mg kg^{-1}]	P_1S_1	Y = 0.0051 X + 10.9	0.08	ns
	P_2S_2	Y = -0.0013 X + 10.55	0.02	ns
B concentration [mg kg^{-1}]	P_1S_1	Y = -0.0044 X + 28.19	0.18	ns
	P_2S_2	Y = -0.0089 X + 23.44	0.19	ns
Mo concentration [mg kg^{-1}]	P_1S_1	Y = 0.0017 X + 2.41	0.47	ns
	P_2S_2	Y = -0.0003 X + 3.3	0.01	ns

Table A.7: Data of leaf weight and leaf area of sunflower for each treatment and replication

U rate in soil	N rate	P rate in soil	S rate	Leaf weight	Leaf area
[mg kg^{-1}]				[g]	[cm2]
Control	250	334	0	0.41	143.3
Control	250	334	0	0.33	102.77
Control	250	334	0	0.41	123.88
Control	500	334	0	0.35	126
Control	500	334	0	0.32	111.06
Control	500	334	0	0.34	115.83
Control	250	334	50	0.81	259.61
Control	250	334	50	0.85	252.23
Control	250	334	50	0.91	266.94
Control	500	334	50	0.95	279.97
Control	500	334	50	0.97	265.92
Control	500	334	50	0.96	249
Control	250	1,558	0	0.34	116.89
Control	250	1,558	0	0.3	109.22
Control	250	1,558	0	0.33	108.18
Control	500	1,558	0	0.38	133.27
Control	500	1,558	0	0.34	112.69
Control	500	1,558	0	0.32	101.8
Control	250	1,558	50	0.87	281.05
Control	250	1,558	50	0.82	283.95
Control	250	1,558	50	0.95	290.19
Control	500	1,558	50	1.11	361.38
Control	500	1,558	50	0.92	316.06
Control	500	1,558	50	1.1	356.11
166	250	334	0	0.36	137.35
166	250	334	0	0.34	133.21
166	250	334	0	0.44	161.27
166	500	334	0	0.36	139.58
166	500	334	0	0.33	122.51
166	500	334	0	0.36	119.53
166	250	334	50	0.69	216.49
166	250	334	50	0.75	250.66
166	250	334	50	0.81	238.47
166	500	334	50	0.74	242.29
166	500	334	50	0.72	258.39
166	500	334	50	0.71	207.49
173	250	1,558	0	0.24	88.28
173	250	1,558	0	0.26	103.3
173	250	1,558	0	0.24	82.41
173	500	1,558	0	0.25	99.67
173	500	1,558	0	0.26	111.91
173	500	1,558	0	0.23	84.33
173	250	1,558	50	0.8	276.77
173	250	1,558	50	0.89	305.35
173	250	1,558	50	0.92	301.52
173	500	1,558	50	0.93	352.61
173	500	1,558	50	1.08	350.73
173	500	1,558	50	1.22	405.27
329	250	334	0	0.38	142.75

Table A.7: continued

U rate in soil	N rate	P rate in soil	S rate	Leaf weight	Leaf area
----------[mg kg^{-1}]----------				[g]	[cm2]
329	250	334	0	0.44	157.55
329	250	334	0	0.5	169.01
329	500	334	0	0.34	125.99
329	500	334	0	0.35	151.11
329	500	334	0	0.38	128.95
329	250	334	50	0.8	251.2
329	250	334	50	0.9	272.53
329	250	334	50	0.99	251.21
329	500	334	50	0.91	260.58
329	500	334	50	0.95	256.14
329	500	334	50	0.82	206.21
385	250	1,558	0	0.28	112.23
385	250	1,558	0	0.31	108.85
385	250	1,558	0	0.34	111.22
385	500	1,558	0	0.24	83.96
385	500	1,558	0	0.27	97.16
385	500	1,558	0	0.19	68.01
385	250	1,558	50	0.85	295.17
385	250	1,558	50	0.73	258.64
385	250	1,558	50	0.92	305.02
385	500	1,558	50	1.03	382.33
385	500	1,558	50	0.99	375.13
385	500	1,558	50	1.01	315.83
660	250	334	0	0.45	154.81
660	250	334	0	0.45	144.78
660	250	334	0	0.44	132.2
660	500	334	0	0.39	144.22
660	500	334	0	0.37	121.42
660	500	334	0	0.34	113.85
660	250	334	50	0.79	272.54
660	250	334	50	0.84	268.64
660	250	334	50	0.94	272.08
660	500	334	50	0.8	223.93
660	500	334	50	0.9	262.17
660	500	334	50	0.96	265.24
643	250	1,558	0	0.31	106.58
643	250	1,558	0	0.3	117.34
643	250	1,558	0	0.35	127.4
643	500	1,558	0	0.25	96.28
643	500	1,558	0	0.45	178.64
643	500	1,558	0	0.35	121.65
643	250	1,558	50	0.89	345.56
643	250	1,558	50	0.97	357.94
643	250	1,558	50	0.92	320.97
643	500	1,558	50	0.78	307.68
643	500	1,558	50	0.93	373.52
643	500	1,558	50	1.14	402.26

Table A.8: Data from microbial parameter: dehydrogenase activity (DHA) from each treatment and replication

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Grassland	0-25cm	Control	334	1,177	75.26
Grassland	0-25cm	Control	334	1,177	61.44
Grassland	0-25cm	Control	334	1,177	59.43
Grassland	0-25cm	Control	334	1,177	59.76
Grassland	0-25cm	Control	334	1,177	50.63
Grassland	0-25cm	Control	334	1,177	64.10
Grassland	0-25cm	166	334	1,177	56.51
Grassland	0-25cm	166	334	1,177	52.06
Grassland	0-25cm	166	334	1,177	56.00
Grassland	0-25cm	166	334	1,177	59.41
Grassland	0-25cm	166	334	1,177	61.12
Grassland	0-25cm	166	334	1,177	56.25
Grassland	0-25cm	329	334	1,177	46.81
Grassland	0-25cm	329	334	1,177	47.69
Grassland	0-25cm	329	334	1,177	51.46
Grassland	0-25cm	329	334	1,177	49.22
Grassland	0-25cm	329	334	1,177	109.24
Grassland	0-25cm	329	334	1,177	51.76
Grassland	0-25cm	660	334	1,177	61.03
Grassland	0-25cm	660	334	1,177	60.37
Grassland	0-25cm	660	334	1,177	68.88
Grassland	0-25cm	660	334	1,177	58.38
Grassland	0-25cm	660	334	1,177	65.43
Grassland	0-25cm	660	334	1,177	62.88
Grassland	0-25cm	Control	1,558	1,177	47.78
Grassland	0-25cm	Control	1,558	1,177	39.25
Grassland	0-25cm	Control	1,558	1,177	48.19
Grassland	0-25cm	Control	1,558	1,177	51.96
Grassland	0-25cm	Control	1,558	1,177	65.38
Grassland	0-25cm	Control	1,558	1,177	55.38
Grassland	0-25cm	173	1,558	1,177	73.87
Grassland	0-25cm	173	1,558	1,177	85.94
Grassland	0-25cm	173	1,558	1,177	83.54
Grassland	0-25cm	173	1,558	1,177	68.28
Grassland	0-25cm	173	1,558	1,177	69.74
Grassland	0-25cm	173	1,558	1,177	73.59
Grassland	0-25cm	385	1,558	1,177	61.25
Grassland	0-25cm	385	1,558	1,177	65.15
Grassland	0-25cm	385	1,558	1,177	73.35
Grassland	0-25cm	385	1,558	1,177	64.10
Grassland	0-25cm	385	1,558	1,177	65.79
Grassland	0-25cm	385	1,558	1,177	77.97
Grassland	0-25cm	644	1,558	1,177	78.19
Grassland	0-25cm	644	1,558	1,177	85.38
Grassland	0-25cm	644	1,558	1,177	96.90
Grassland	0-25cm	644	1,558	1,177	69.69
Grassland	0-25cm	644	1,558	1,177	88.06

Table A.8: continued

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Grassland	0-25cm	644	1,558	1,177	84.76
Grassland	0-25cm	Control	334	3,097	61.10
Grassland	0-25cm	Control	334	3,097	56.82
Grassland	0-25cm	Control	334	3,097	55.13
Grassland	0-25cm	Control	334	3,097	57.81
Grassland	0-25cm	Control	334	3,097	59.10
Grassland	0-25cm	Control	334	3,097	52.72
Grassland	0-25cm	166	334	3,097	58.78
Grassland	0-25cm	166	334	3,097	48.35
Grassland	0-25cm	166	334	3,097	53.93
Grassland	0-25cm	166	334	3,097	64.59
Grassland	0-25cm	166	334	3,097	90.46
Grassland	0-25cm	166	334	3,097	72.74
Grassland	0-25cm	329	334	3,097	53.13
Grassland	0-25cm	329	334	3,097	48.81
Grassland	0-25cm	329	334	3,097	49.44
Grassland	0-25cm	329	334	3,097	66.09
Grassland	0-25cm	329	334	3,097	53.29
Grassland	0-25cm	329	334	3,097	58.34
Grassland	0-25cm	660	334	3,097	58.51
Grassland	0-25cm	660	334	3,097	79.09
Grassland	0-25cm	660	334	3,097	68.41
Grassland	0-25cm	660	334	3,097	57.00
Grassland	0-25cm	660	334	3,097	53.44
Grassland	0-25cm	660	334	3,097	51.91
Grassland	0-25cm	Control	1,558	3,097	50.56
Grassland	0-25cm	Control	1,558	3,097	48.04
Grassland	0-25cm	Control	1,558	3,097	47.54
Grassland	0-25cm	Control	1,558	3,097	13.24
Grassland	0-25cm	Control	1,558	3,097	56.56
Grassland	0-25cm	Control	1,558	3,097	40.22
Grassland	0-25cm	173	1,558	3,097	64.07
Grassland	0-25cm	173	1,558	3,097	67.38
Grassland	0-25cm	173	1,558	3,097	61.44
Grassland	0-25cm	173	1,558	3,097	54.46
Grassland	0-25cm	173	1,558	3,097	52.96
Grassland	0-25cm	173	1,558	3,097	58.88
Grassland	0-25cm	385	1,558	3,097	63.82
Grassland	0-25cm	385	1,558	3,097	66.63
Grassland	0-25cm	385	1,558	3,097	65.76
Grassland	0-25cm	385	1,558	3,097	54.15
Grassland	0-25cm	385	1,558	3,097	51.41
Grassland	0-25cm	385	1,558	3,097	48.87
Grassland	0-25cm	644	1,558	3,097	58.07
Grassland	0-25cm	644	1,558	3,097	64.85
Grassland	0-25cm	644	1,558	3,097	89.71
Grassland	0-25cm	644	1,558	3,097	53.53
Grassland	0-25cm	644	1,558	3,097	56.91

Table A.8: continued

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Grassland	0-25cm	644	1,558	3,097	71.04
Grassland	25-50cm	Control	334	1,177	13.24
Grassland	25-50cm	Control	334	1,177	15.01
Grassland	25-50cm	Control	334	1,177	14.32
Grassland	25-50cm	Control	334	1,177	10.43
Grassland	25-50cm	Control	334	1,177	18.43
Grassland	25-50cm	Control	334	1,177	9.32
Grassland	25-50cm	166	334	1,177	15.34
Grassland	25-50cm	166	334	1,177	15.35
Grassland	25-50cm	166	334	1,177	15.18
Grassland	25-50cm	166	334	1,177	10.51
Grassland	25-50cm	166	334	1,177	16.51
Grassland	25-50cm	166	334	1,177	16.62
Grassland	25-50cm	329	334	1,177	13.01
Grassland	25-50cm	329	334	1,177	12.93
Grassland	25-50cm	329	334	1,177	12.35
Grassland	25-50cm	329	334	1,177	16.04
Grassland	25-50cm	329	334	1,177	17.04
Grassland	25-50cm	329	334	1,177	16.38
Grassland	25-50cm	660	334	1,177	8.87
Grassland	25-50cm	660	334	1,177	6.50
Grassland	25-50cm	660	334	1,177	10.74
Grassland	25-50cm	660	334	1,177	14.15
Grassland	25-50cm	660	334	1,177	13.91
Grassland	25-50cm	660	334	1,177	12.09
Grassland	25-50cm	Control	1,558	1,177	12.07
Grassland	25-50cm	Control	1,558	1,177	17.13
Grassland	25-50cm	Control	1,558	1,177	24.76
Grassland	25-50cm	Control	1,558	1,177	9.71
Grassland	25-50cm	Control	1,558	1,177	23.47
Grassland	25-50cm	Control	1,558	1,177	14.06
Grassland	25-50cm	173	1,558	1,177	23.94
Grassland	25-50cm	173	1,558	1,177	29.24
Grassland	25-50cm	173	1,558	1,177	23.62
Grassland	25-50cm	173	1,558	1,177	23.69
Grassland	25-50cm	173	1,558	1,177	23.32
Grassland	25-50cm	173	1,558	1,177	21.38
Grassland	25-50cm	385	1,558	1,177	22.76
Grassland	25-50cm	385	1,558	1,177	19.22
Grassland	25-50cm	385	1,558	1,177	21.40
Grassland	25-50cm	385	1,558	1,177	20.37
Grassland	25-50cm	385	1,558	1,177	20.68
Grassland	25-50cm	385	1,558	1,177	22.76
Grassland	25-50cm	644	1,558	1,177	21.32
Grassland	25-50cm	644	1,558	1,177	20.43
Grassland	25-50cm	644	1,558	1,177	30.47
Grassland	25-50cm	644	1,558	1,177	26.99
Grassland	25-50cm	644	1,558	1,177	30.94

Table A.8: continued

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Grassland	25-50cm	644	1,558	1,177	22.56
Grassland	25-50cm	Control	334	3,097	7.07
Grassland	25-50cm	Control	334	3,097	6.15
Grassland	25-50cm	Control	334	3,097	5.25
Grassland	25-50cm	Control	334	3,097	9.63
Grassland	25-50cm	Control	334	3,097	8.68
Grassland	25-50cm	Control	334	3,097	7.29
Grassland	25-50cm	166	334	3,097	15.18
Grassland	25-50cm	166	334	3,097	16.19
Grassland	25-50cm	166	334	3,097	13.22
Grassland	25-50cm	166	334	3,097	14.69
Grassland	25-50cm	166	334	3,097	16.62
Grassland	25-50cm	166	334	3,097	16.21
Grassland	25-50cm	329	334	3,097	5.96
Grassland	25-50cm	329	334	3,097	9.75
Grassland	25-50cm	329	334	3,097	6.01
Grassland	25-50cm	329	334	3,097	8.66
Grassland	25-50cm	329	334	3,097	8.99
Grassland	25-50cm	329	334	3,097	7.69
Grassland	25-50cm	660	334	3,097	5.93
Grassland	25-50cm	660	334	3,097	4.66
Grassland	25-50cm	660	334	3,097	4.59
Grassland	25-50cm	660	334	3,097	8.75
Grassland	25-50cm	660	334	3,097	4.72
Grassland	25-50cm	660	334	3,097	5.88
Grassland	25-50cm	Control	1,558	3,097	23.69
Grassland	25-50cm	Control	1,558	3,097	18.79
Grassland	25-50cm	Control	1,558	3,097	21.04
Grassland	25-50cm	Control	1,558	3,097	14.35
Grassland	25-50cm	Control	1,558	3,097	15.51
Grassland	25-50cm	Control	1,558	3,097	9.82
Grassland	25-50cm	173	1,558	3,097	22.24
Grassland	25-50cm	173	1,558	3,097	17.57
Grassland	25-50cm	173	1,558	3,097	22.07
Grassland	25-50cm	173	1,558	3,097	25.09
Grassland	25-50cm	173	1,558	3,097	24.41
Grassland	25-50cm	173	1,558	3,097	19.38
Grassland	25-50cm	385	1,558	3,097	23.07
Grassland	25-50cm	385	1,558	3,097	19.01
Grassland	25-50cm	385	1,558	3,097	24.40
Grassland	25-50cm	385	1,558	3,097	20.81
Grassland	25-50cm	385	1,558	3,097	15.68
Grassland	25-50cm	385	1,558	3,097	21.68
Grassland	25-50cm	644	1,558	3,097	22.03
Grassland	25-50cm	644	1,558	3,097	19.50
Grassland	25-50cm	644	1,558	3,097	25.85
Grassland	25-50cm	644	1,558	3,097	26.49
Grassland	25-50cm	644	1,558	3,097	25.90

Table A.8: continued

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Grassland	25-50cm	644	1,558	3,097	22.43
Forest	0-25cm	Control	334	1,177	32.29
Forest	0-25cm	Control	334	1,177	27.84
Forest	0-25cm	Control	334	1,177	37.00
Forest	0-25cm	Control	334	1,177	35.12
Forest	0-25cm	Control	334	1,177	35.88
Forest	0-25cm	Control	334	1,177	36.15
Forest	0-25cm	166	334	1,177	15.44
Forest	0-25cm	166	334	1,177	9.13
Forest	0-25cm	166	334	1,177	26.51
Forest	0-25cm	166	334	1,177	9.15
Forest	0-25cm	166	334	1,177	10.10
Forest	0-25cm	166	334	1,177	8.41
Forest	0-25cm	329	334	1,177	22.84
Forest	0-25cm	329	334	1,177	23.59
Forest	0-25cm	329	334	1,177	21.66
Forest	0-25cm	329	334	1,177	15.01
Forest	0-25cm	329	334	1,177	10.22
Forest	0-25cm	329	334	1,177	10.50
Forest	0-25cm	660	334	1,177	16.10
Forest	0-25cm	660	334	1,177	16.38
Forest	0-25cm	660	334	1,177	12.72
Forest	0-25cm	660	334	1,177	11.10
Forest	0-25cm	660	334	1,177	13.16
Forest	0-25cm	660	334	1,177	14.62
Forest	0-25cm	Control	1,558	1,177	52.46
Forest	0-25cm	Control	1,558	1,177	42.78
Forest	0-25cm	Control	1,558	1,177	39.18
Forest	0-25cm	Control	1,558	1,177	43.49
Forest	0-25cm	Control	1,558	1,177	55.96
Forest	0-25cm	Control	1,558	1,177	42.07
Forest	0-25cm	173	1,558	1,177	39.71
Forest	0-25cm	173	1,558	1,177	42.18
Forest	0-25cm	173	1,558	1,177	36.62
Forest	0-25cm	173	1,558	1,177	48.13
Forest	0-25cm	173	1,558	1,177	43.00
Forest	0-25cm	173	1,558	1,177	42.69
Forest	0-25cm	385	1,558	1,177	35.60
Forest	0-25cm	385	1,558	1,177	43.15
Forest	0-25cm	385	1,558	1,177	41.72
Forest	0-25cm	385	1,558	1,177	34.47
Forest	0-25cm	385	1,558	1,177	40.51
Forest	0-25cm	385	1,558	1,177	36.28
Forest	0-25cm	644	1,558	1,177	35.07
Forest	0-25cm	644	1,558	1,177	35.49
Forest	0-25cm	644	1,558	1,177	35.81
Forest	0-25cm	644	1,558	1,177	29.63
Forest	0-25cm	644	1,558	1,177	30.35

Table A.8: continued

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Forest	0-25cm	644	1,558	1,177	30.97
Forest	0-25cm	Control	334	3,097	8.19
Forest	0-25cm	Control	334	3,097	7.04
Forest	0-25cm	Control	334	3,097	10.65
Forest	0-25cm	Control	334	3,097	9.06
Forest	0-25cm	Control	334	3,097	5.56
Forest	0-25cm	Control	334	3,097	7.01
Forest	0-25cm	166	334	3,097	23.97
Forest	0-25cm	166	334	3,097	24.06
Forest	0-25cm	166	334	3,097	22.56
Forest	0-25cm	166	334	3,097	11.76
Forest	0-25cm	166	334	3,097	10.00
Forest	0-25cm	166	334	3,097	10.71
Forest	0-25cm	329	334	3,097	9.96
Forest	0-25cm	329	334	3,097	9.49
Forest	0-25cm	329	334	3,097	11.22
Forest	0-25cm	329	334	3,097	3.34
Forest	0-25cm	329	334	3,097	2.15
Forest	0-25cm	329	334	3,097	2.10
Forest	0-25cm	660	334	3,097	3.25
Forest	0-25cm	660	334	3,097	4.54
Forest	0-25cm	660	334	3,097	4.41
Forest	0-25cm	660	334	3,097	1.87
Forest	0-25cm	660	334	3,097	2.31
Forest	0-25cm	660	334	3,097	1.50
Forest	0-25cm	Control	1,558	3,097	23.41
Forest	0-25cm	Control	1,558	3,097	28.12
Forest	0-25cm	Control	1,558	3,097	28.28
Forest	0-25cm	Control	1,558	3,097	28.99
Forest	0-25cm	Control	1,558	3,097	30.50
Forest	0-25cm	Control	1,558	3,097	28.16
Forest	0-25cm	173	1,558	3,097	30.75
Forest	0-25cm	173	1,558	3,097	29.35
Forest	0-25cm	173	1,558	3,097	32.87
Forest	0-25cm	173	1,558	3,097	31.69
Forest	0-25cm	173	1,558	3,097	29.72
Forest	0-25cm	173	1,558	3,097	26.78
Forest	0-25cm	385	1,558	3,097	22.10
Forest	0-25cm	385	1,558	3,097	23.00
Forest	0-25cm	385	1,558	3,097	24.25
Forest	0-25cm	385	1,558	3,097	29.51
Forest	0-25cm	385	1,558	3,097	28.79
Forest	0-25cm	385	1,558	3,097	25.38
Forest	0-25cm	644	1,558	3,097	35.18
Forest	0-25cm	644	1,558	3,097	32.65
Forest	0-25cm	644	1,558	3,097	27.88
Forest	0-25cm	644	1,558	3,097	23.49
Forest	0-25cm	644	1,558	3,097	27.96

Table A.8: continued

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Forest	25-50cm	Control	334	1,177	14.71
Forest	25-50cm	Control	334	1,177	11.62
Forest	25-50cm	Control	334	1,177	11.74
Forest	25-50cm	Control	334	1,177	11.93
Forest	25-50cm	Control	334	1,177	10.85
Forest	25-50cm	Control	334	1,177	17.07
Forest	25-50cm	166	334	1,177	12.09
Forest	25-50cm	166	334	1,177	12.10
Forest	25-50cm	166	334	1,177	7.88
Forest	25-50cm	166	334	1,177	7.25
Forest	25-50cm	166	334	1,177	7.94
Forest	25-50cm	166	334	1,177	7.60
Forest	25-50cm	329	334	1,177	5.38
Forest	25-50cm	329	334	1,177	4.75
Forest	25-50cm	329	334	1,177	4.37
Forest	25-50cm	329	334	1,177	8.76
Forest	25-50cm	329	334	1,177	7.71
Forest	25-50cm	329	334	1,177	12.31
Forest	25-50cm	660	334	1,177	10.31
Forest	25-50cm	660	334	1,177	8.69
Forest	25-50cm	660	334	1,177	10.71
Forest	25-50cm	660	334	1,177	7.31
Forest	25-50cm	660	334	1,177	7.78
Forest	25-50cm	660	334	1,177	6.21
Forest	25-50cm	Control	1,558	1,177	36.37
Forest	25-50cm	Control	1,558	1,177	25.66
Forest	25-50cm	Control	1,558	1,177	27.76
Forest	25-50cm	Control	1,558	1,177	14.71
Forest	25-50cm	Control	1,558	1,177	28.26
Forest	25-50cm	Control	1,558	1,177	16.01
Forest	25-50cm	173	1,558	1,177	10.90
Forest	25-50cm	173	1,558	1,177	11.53
Forest	25-50cm	173	1,558	1,177	6.34
Forest	25-50cm	173	1,558	1,177	4.34
Forest	25-50cm	173	1,558	1,177	5.91
Forest	25-50cm	173	1,558	1,177	1.62
Forest	25-50cm	385	1,558	1,177	6.29
Forest	25-50cm	385	1,558	1,177	3.79
Forest	25-50cm	385	1,558	1,177	4.94
Forest	25-50cm	385	1,558	1,177	10.29
Forest	25-50cm	385	1,558	1,177	3.43
Forest	25-50cm	385	1,558	1,177	6.10
Forest	25-50cm	644	1,558	1,177	11.69
Forest	25-50cm	644	1,558	1,177	11.65
Forest	25-50cm	644	1,558	1,177	14.28
Forest	25-50cm	644	1,558	1,177	2.22
Forest	25-50cm	644	1,558	1,177	3.97
Forest	25-50cm	644	1,558	1,177	3.13

Table A.8: continued

Soil type	Depth	U rate in soil	P rate in soil	Liming (Ca)	DHA (measured by TPF)
		[mg kg^{-1}]			[µg g^{-1} d^{-1}]
Forest	0-25cm	644	1,558	3,097	24.46
Forest	25-50cm	Control	334	3,097	12.38
Forest	25-50cm	Control	334	3,097	10.25
Forest	25-50cm	Control	334	3,097	7.38
Forest	25-50cm	Control	334	3,097	8.31
Forest	25-50cm	Control	334	3,097	6.28
Forest	25-50cm	Control	334	3,097	13.75
Forest	25-50cm	166	334	3,097	3.07
Forest	25-50cm	166	334	3,097	3.12
Forest	25-50cm	166	334	3,097	4.75
Forest	25-50cm	166	334	3,097	4.46
Forest	25-50cm	166	334	3,097	6.87
Forest	25-50cm	166	334	3,097	4.38
Forest	25-50cm	329	334	3,097	4.16
Forest	25-50cm	329	334	3,097	4.81
Forest	25-50cm	329	334	3,097	4.76
Forest	25-50cm	329	334	3,097	4.07
Forest	25-50cm	329	334	3,097	3.35
Forest	25-50cm	329	334	3,097	3.65
Forest	25-50cm	660	334	3,097	4.78
Forest	25-50cm	660	334	3,097	3.53
Forest	25-50cm	660	334	3,097	1.84
Forest	25-50cm	660	334	3,097	2.59
Forest	25-50cm	660	334	3,097	4.51
Forest	25-50cm	660	334	3,097	2.53
Forest	25-50cm	Control	1,558	3,097	9.91
Forest	25-50cm	Control	1,558	3,097	13.57
Forest	25-50cm	Control	1,558	3,097	11.90
Forest	25-50cm	Control	1,558	3,097	11.97
Forest	25-50cm	Control	1,558	3,097	15.13
Forest	25-50cm	Control	1,558	3,097	18.91
Forest	25-50cm	173	1,558	3,097	2.28
Forest	25-50cm	173	1,558	3,097	0.91
Forest	25-50cm	173	1,558	3,097	1.56
Forest	25-50cm	173	1,558	3,097	2.94
Forest	25-50cm	173	1,558	3,097	6.22
Forest	25-50cm	173	1,558	3,097	4.37
Forest	25-50cm	385	1,558	3,097	4.79
Forest	25-50cm	385	1,558	3,097	5.01
Forest	25-50cm	385	1,558	3,097	6.88
Forest	25-50cm	385	1,558	3,097	7.57
Forest	25-50cm	385	1,558	3,097	5.93
Forest	25-50cm	385	1,558	3,097	3.90
Forest	25-50cm	644	1,558	3,097	4.54
Forest	25-50cm	644	1,558	3,097	1.76
Forest	25-50cm	644	1,558	3,097	4.53
Forest	25-50cm	644	1,558	3,097	7.72
Forest	25-50cm	644	1,558	3,097	7.00

Table A.9: Data from microbial parameter: Microbial count for each treatment and replication

Soil-Type	U rate in soil	P rate in soil	Liming (Ca)	Fungi	Heterotrophic bacteria	Actinomycetes
	----------[mg kg^{-1}]----------			----------[CFU]----------		
Forest	25-50cm	644	1,558	3,097	5.16	
Grassland	Control	334	3,097	202.70	1,824,262.69	364,852.54
Grassland	Control	334	3,097	0.00	1,560,758.08	263,504.61
Grassland	Control	334	3,097	0.00	1,489,814.53	385,122,12
Grassland	Control	334	3,097	0.00	1,202,020.20	288,888.89
Grassland	Control	334	3,097	0.00	1,080,808.08	222,222.22
Grassland	Control	334	3,097	101.01	1,030,303.03	235,353.54
Grassland	Control	334	1,177	1,938.78	1,346,938.78	663,265.31
Grassland	Control	334	1,177	510,20	1,204,081.63	602,040.82
Grassland	Control	334	1,177	612.24	1,377,551.02	500,000.00
Grassland	Control	334	1,177	618.56	1,515,463.92	202,061.86
Grassland	Control	334	1,177	927.84	1,381,443.30	204,123.71
Grassland	Control	334	1,177	721.65	1,494,845.36	209,278.35
Grassland	Control	1,558	3,097	8,107.83	9,121,313.47	1,662,106.01
Grassland	Control	1,558	3,097	13,175.23	8,817,269.69	1,773,588.73
Grassland	Control	1,558	3,097	17,229.15	8,715,921.76	1,793,858.32
Grassland	Control	1,558	3,097	10,204.08	14,489,795.92	1,540,816.33
Grassland	Control	1,558	3,097	14,285.71	11,326,530.61	1,877,551.02
Grassland	Control	1,558	3,097	13,265.31	10,102,040.82	1,704,081.63
Grassland	Control	1,558	1,177	4,141.41	10,101,010.10	555,555.56
Grassland	Control	1,558	1,177	4,949.49	8,080,808.08	616,161.62
Grassland	Control	1,558	1,177	1,616.16	7,878,787.88	545,454.55
Grassland	Control	1,558	1,177	2,033.97	1,952,608.56	233,906.23
Grassland	Control	1,558	1,177	4,678.12	2,186,514.80	255,262.89
Grassland	Control	1,558	1,177	2,644.16	1,627,173.80	175,938.17
Forest	Control	334	3,097	272,397.09	1,644,471.35	162,429.38
Forest	Control	334	3,097	320,181.78	1,240,920.10	156,992.36
Forest	Control	334	3,097	309,565.58	1,624,293.79	141,368.28
Forest	Control	334	3,097	71,630.35	60,532.69	413,640.03
Forest	Control	334	3,097	63,003.51	64,568.20	278,868.00
Forest	Control	334	3,097	72,231.97	80,710.25	22,7014.76
Forest	Control	334	1,177	99,003.23	46,470.91	26,266.16
Forest	Control	334	1,177	85,582.89	69,706.36	28,871.34
Forest	Control	334	1,177	95,921.78	51,522.09	24,754.01
Forest	Control	334	1,177	188,337.19	52,371.84	21,150.17
Forest	Control	334	1,177	117,836.64	58,414.74	12,085.81
Forest	Control	334	1,177	113,808.04	20,143.02	24,171.62
Forest	Control	1,558	3,097	70,621.47	847,372.14	103903.96
Forest	Control	1,558	3,097	98,860.08	907,898.72	163,421.77
Forest	Control	1,558	3,097	84,737.21	837,284.37	140,219.91
Forest	Control	1,558	3,097	78,692.49	948,345.44	132,163.03
Forest	Control	1,558	3,097	78,692.49	1,069,410.82	146,287.33
Forest	Control	1,558	3,097	70,621.47	1,079,499.60	164,447.13
Forest	Control	1,558	1,177	72,845.00	192,229.87	50,586.81
Forest	Control	1,558	1,177	77,903.68	232,699.31	38,445.97
Forest	Control	1,558	1,177	61,715.90	242,816.67	46,539.86
Forest	Control	1,558	1,177	69,624.30	343,076.25	37,334.77

Table A.9: continued

Soil-Type	U rate in soil	P rate in soil	Liming (Ca)	Fungi	Heterotrophic bacteria	Actinomycetes
	----------[mg kg^{-1}]----------			----------[CFU]----------		
Forest	Control	1,558	1,177	68,615.25	332,985.77	64,579.06
Forest	Control	1,558	1,177	48,434.29	403,619.12	49,443.34
Grassland	166	334	3,097	2,299.57	1,212,501.31	114,978.57
Grassland	166	334	3,097	627.16	1,526,079.23	135,883.77
Grassland	166	334	3,097	1,045.26	1,191,596.11	103,480.71
Grassland	166	334	3,097	996.35	1,328,462.30	138,381.49
Grassland	166	334	3,097	553.53	1,472,379.05	110,705.19
Grassland	166	334	3,097	1,328.46	1,173,475.04	143,916.75
Grassland	166	334	1,177	888.89	544,444.44	142,222,22
Grassland	166	334	1,177	555.56	511,111.11	147,777.78
Grassland	166	334	1,177	333.33	466,666.67	148,888.89
Grassland	166	334	1,177	25,183.40	613,161.06	168,619.29
Grassland	166	334	1,177	9,854.37	744,552.72	167,524.36
Grassland	166	334	1,177	4,379.72	646,008.98	159,859.85
Grassland	173	1,558	3,097	3,402.06	7,731,958.76	2,185,567.01
Grassland	173	1,558	3,097	3,298.97	8,247,422.68	1,536,082.47
Grassland	173	1,558	3,097	3,711.34	8,659,793.81	2,092,783.51
Grassland	173	1,558	3,097	4,225.20	7,394,105.84	485,898.38
Grassland	173	1,558	3,097	3,274.53	1,267,561.00	338,016.27
Grassland	173	1,558	3,097	4,753.35	8,239,146.51	1,605,577.27
Grassland	173	1,558	1,177	3,214.40	1,692,917.60	900,032.14
Grassland	173	1,558	1,177	3,535.84	1,789,349.62	846,458.80
Grassland	173	1,558	1,177	2,357.23	1,703,632.27	0.00
Grassland	173	1,558	1,177	6,373.63	923,076.92	1,010,989.01
Grassland	173	1,558	1,177	6,263.74	1,340,659.34	879,120,88
Grassland	173	1,558	1,177	3,626.37	912,087.91	747,252.75
Forest	166	334	3,097	119,971.77	119,971.77	85,694.12
Forest	166	334	3,097	96,783.95	96,783.95	100,816.61
Forest	166	334	3,097	107,873.78	107,873.78	111,906.44
Forest	166	334	3,097	108,662.84	108,662.84	98,601.47
Forest	166	334	3,097	95,583.06	95,583.06	78,478.72
Forest	166	334	3,097	101,619.88	101,619.88	192,172.25
Forest	166	334	1,177	31,250.32	31,250.32	19,153.42
Forest	166	334	1,177	18,145.34	18,145.34	31,250.32
Forest	166	334	1,177	33,266.46	33,266.46	40,322.99
Forest	166	334	1,177	14,096.32	14,096.32	54,371.54
Forest	166	334	1,177	34,233.93	34,233.93	37,254.57
Forest	166	334	1,177	26,178.89	26,178.89	30,206.41
Forest	173	1,558	3,097	61,715.90	29,340,348.04	9,105.63
Forest	173	1,558	3,097	62,727.64	28,328,611.90	3,035.21
Forest	173	1,558	3,097	67,786.32	27,316,875.76	8,093.89
Forest	173	1,558	3,097	55,497.63	39,352,864.01	8,072.38
Forest	173	1,558	3,097	60,542.87	42,380,007.40	13,117.62
Forest	173	1,558	3,097	54,488.58	57,515,724.33	7,063.33
Forest	173	1,558	1,177	61,576.77	474,443.96	2,018.91
Forest	173	1,558	1,177	60,567.31	504,727.62	1,009.46
Forest	173	1,558	1,177	66,624.05	444,160.30	3,028.37
Forest	173	1,558	1,177	50,527.17	212,214.10	1,010.54

Table A.9: continued

Soil-Type	U rate in soil	P rate in soil	Liming (Ca)	Fungi	Heterotrophic bacteria	Actinomycetes
	----------[mg kg^{-1}]----------			----------[CFU]----------		
Forest	173	1,558	1,177	55,579.88	303,163.00	2,021.09
Forest	173	1,558	1,177	57,600.97	202,108.67	0.00
Grassland	329	334	3,097	1,204.42	821,197.85	177,378.74
Grassland	329	334	3,097	437.97	635,059.67	225,555.68
Grassland	329	334	3,097	985.44	875,944.38	197,087.48
Grassland	329	334	3,097	978.26	1,021,739.13	201,086.96
Grassland	329	334	3,097	1,413.04	967,391.30	193,478.26
Grassland	329	334	3,097	1,413.04	923,913.04	170,652.17
Grassland	329	334	1,177	1,090.87	687,247.74	205,083.45
Grassland	329	334	1,177	3,272.61	578,160.79	184,356.93
Grassland	329	334	1,177	2,181.74	665,430.35	166,903.02
Grassland	329	334	1,177	6,716.67	929,139.15	73,883.35
Grassland	329	334	1,177	5,597.22	1,063,472.52	85,077.80
Grassland	329	334	1,177	6,716.67	1,007,500.28	97,391.69
Grassland	385	1,558	3,097	3,505.15	5,567,010.31	309,278.35
Grassland	385	1,558	3,097	3,814.43	6,082,474.23	329,896.91
Grassland	385	1,558	3,097	2,680.41	5,979,381.44	443,298.97
Grassland	385	1,558	3,097	3,195.88	5,876,288.66	443,298.97
Grassland	385	1,558	3,097	2,680.41	2,989,690.72	412,371.13
Grassland	385	1,558	3,097	1,546.39	7,422,680.41	484,536.08
Grassland	385	1,558	1,177	3,402.06	6,804,123.71	371,134.02
Grassland	385	1,558	1,177	2,680.41	6,288,659.79	340,206.19
Grassland	385	1,558	1,177	2,680.41	7,731,958.76	309,278.35
Grassland	385	1,558	1,177	1,500.05	1,017,893.50	289,296.05
Grassland	385	1,558	1,177	1,500.05	857,173.47	460,730.74
Grassland	385	1,558	1,177	3,000.11	1,007,178.83	203,578.70
Forest	329	334	3,097	120,992.14	120,992.14	87,719.30
Forest	329	334	3,097	124,016.94	124,016.94	107,884.65
Forest	329	334	3,097	98,810.24	98,810.24	122,000.40
Forest	329	334	3,097	222,356.37	222,356.37	85,521.68
Forest	329	334	3,097	228,393.20	228,393.20	65,398.93
Forest	329	334	3,097	157,963.58	157,963.58	27,165.71
Forest	329	334	1,177	44,286.39	44,286.39	24,156.21
Forest	329	334	1,177	43,279.88	43,279.88	15,097.63
Forest	329	334	1,177	33,214.79	33,214.79	19,123.67
Forest	329	334	1,177	38,226.81	38,226.81	14,083.56
Forest	329	334	1,177	34,202.94	34,202.94	9,053.72
Forest	329	334	1,177	54,322.31	54,322.31	10,059.69
Forest	385	1,558	3,097	62,698.04	16,888,019.96	1,071,934.20
Forest	385	1,558	3,097	51,574.19	11,326,097.22	788,781.77
Forest	385	1,558	3,097	77,866.92	12,944,111.10	910,132.81
Forest	385	1,558	3,097	56,476.28	14,018,220.32	11,093.56
Forest	385	1,558	3,097	56,476.28	14,421,622.35	18,153.09
Forest	385	1,558	3,097	35,297.68	12,606,313.24	16,136.08
Forest	385	1,558	1,177	22,995.46	534,543.62	134,140.19
Forest	385	1,558	1,177	16,137.17	544,629.35	148,260.21
Forest	385	1,558	1,177	22,894.60	443,772.06	99,848.71
Forest	385	1,558	1,177	50,486.35	434,182.63	3,029.18

Table A.9: continued

Soil-Type	U rate in soil	P rate in soil	Liming (Ca)	Fungi	Heterotrophic bacteria	Actinomycetes
	----------[mg kg^{-1}]----------			----------[CFU]----------		
Forest	385	1,558	1,177	48,466.90	484,668.98	1,009.73
Forest	385	1,558	1,177	39,379.35	383,696.27	2,019.45
Grassland	660	334	3,097	1,267.56	1,140,804.90	61,265.45
Grassland	660	334	3,097	950.67	1,288,687.02	79,222.56
Grassland	660	334	3,097	845.04	897,855.71	86,616.67
Grassland	660	334	3,097	535.73	910,746.81	178,934.96
Grassland	660	334	3,097	535.73	1,232,186.86	186,435.23
Grassland	660	334	3,097	1,071.47	1,339,333.55	185,363.76
Grassland	660	334	1,177	7,664.51	832,147.16	193,802.69
Grassland	660	334	1,177	1,094.93	1,083,981.17	218,986.09
Grassland	660	334	1,177	7,664.51	843,096.46	200,372.28
Grassland	660	334	1,177	5,494.51	1,142,857.14	125,274.73
Grassland	660	334	1,177	2,197.80	1,153,846.15	118,681.32
Grassland	660	334	1,177	5,494.51	1,098,901.10	132,967.03
Grassland	644	1,558	3,097	11,340.21	2,731,958.76	350,515.46
Grassland	644	1,558	3,097	13,402.06	2,752,577.32	371,134.02
Grassland	644	1,558	3,097	15,463.92	2,886,597.94	360,824.74
Grassland	644	1,558	3,097	2,650.27	6,042,616.35	413,442.17
Grassland	644	1,558	3,097	1,908.19	5,618,573.09	360,436.76
Grassland	644	1,558	3,097	2,544.26	4,028,410.90	593,660.55
Grassland	644	1,558	1,177	12,044.24	6,460,089.78	558,414.54
Grassland	644	1,558	1,177	12,044.24	5,912,624.55	514,617.32
Grassland	644	1,558	1,177	14,234.10	6,569,582.83	722,654.11
Grassland	644	1,558	1,177	1,642.40	5,365,159.31	525,566.63
Grassland	644	1,558	1,177	1,532.90	3,722,763.60	492,718.71
Grassland	644	1,558	1,177	1,313.92	4,379,721.89	503,668.02
Forest	660	334	3,097	157,289.78	157,289.78	77,636.62
Forest	660	334	3,097	130,066.55	130,066.55	83,686.23
Forest	660	334	3,097	116,959.06	116,959.06	60,496.07
Forest	660	334	3,097	80,672.27	80,672.27	53,445.38
Forest	660	334	3,097	91,764.71	91,764.71	67,563.03
Forest	660	334	3,097	97,815.13	97,815.13	60,504.20
Forest	660	334	1,177	47,332.91	47,332.91	27,191.25
Forest	660	334	1,177	48,339.99	48,339.99	56,396.66
Forest	660	334	1,177	48,339.99	48,339.99	55,389.57
Forest	660	334	1,177	47,351.98	47,351.98	13,097.36
Forest	660	334	1,177	54,404.41	54,404.41	45,337.01
Forest	660	334	1,177	38,284.58	38,284.58	61,456.83
Forest	644	1,558	3,097	45,472.92	1,455,133.39	3,031.53
Forest	644	1,558	3,097	62,651.58	1,212,611.16	2,021.02
Forest	644	1,558	3,097	50,525.46	1,354,082.46	3,031.53
Forest	644	1,558	3,097	44,484.89	1,122,232.33	2,022.04
Forest	644	1,558	3,097	53,584.07	1,172,783.34	3,033.06
Forest	644	1,558	3,097	64,705.29	1,243,554.75	2,022.04
Forest	644	1,558	1,177	43,386.14	454,040.96	1,008.98
Forest	644	1,558	1,177	78,700.43	443,951.17	0.00
Forest	644	1,558	1,177	80,718.39	524,669.56	3,026.94
Forest	644	1,558	1,177	38,377.38	878,639.96	8,079.45

Table A.9: continued

Soil-Type	U rate in soil	P rate in soil	Liming (Ca)	Fungi	Heterotrophic bacteria	Actinomycetes
	----------[mg kg^{-1}]----------			----------[CFU]----------		
Forest	644	1,558	1,177	87,864.00	706,951.69	6,059.59
Forest	644	1,558	1,177	79,784.55	616,057.90	5,049.65

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