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SWITCH Deliverable Summary Sheet

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Deliverable reference: 4.1.6 Plant growth tests, addressing the fate and behaviour of pharmaceutical residues in plant tissues and soils
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Audience This document is presenting scientific results conducted within SWITCH and is prepared in form of a journal article for the scientific community working in this area.
Purpose This report shows first results on the behaviour of plants towards pharmaceuticals. It is a scientific contribution to solving this problem. The deliverable reports on research on the individual and combined behaviour of carbamazepine and ibuprofen in a greenhouse experiment using rye grass fertilised with spiked urine by GC/MS analysis.
Background Urine is looked at as an alternative fertiliser for agriculture. However, its usage includes the risk of spreading pharmaceutical residues to fields. Little is known on the fate of pharmaceuticals regarding their accumulation in soils, transfer to groundwater, and incorporation by plants. These effects cannot be ignored as fairly high concentrations of pharmaceuticals are expected in urine. The uptake of pharmaceuticals in plants and the effects they exaggerate on plant physiology and development were of major interest when crops are fertilised with urine. In this specific research carbamazepine could be detected in soil as well as in roots and aerial plant parts.
Potential Impact It can be assumed that ibuprofen (neither detected in soil nor in plant roots) is not incorporated by plants due to its fast biodegradation in soil while carbamazepine which is present in soil for longer periods due to its recalcitrance. Thus, CZ remains available for plants for a much longer period and is thus transferred to the plants, especially to the aerial plant parts in the case of rye grass. Hence, fertilization with urine containing such substances needs further evaluation regarding the contained risks.
Recommendations No evaluation of potential toxic effects of pharmaceuticals ingested by humans with crops is possible at the moment with respect to the findings of this research. However, there are concerns and as long as the concerns are not allayed, it is recommended not to use urine of people under medication for fertilisation of food crops. It might be a solution that people under medication use a separate toilet.

Fertilisation of rye grass using urine spiked with carbamazepine, ibuprofen, and 17α-ethinylestradiol – a greenhouse experiment

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Abstract

Urine is looked at as an alternative fertiliser for agriculture. However, its usage includes the risk of spreading pharmaceutical residues to fields. The individual and combined behaviour of carbamazepine and ibuprofen, and 17α-ethinylestradiol was investigated in a greenhouse experiment using rye grass fertilised with spiked urine by GC/MS analysis. Only carbamazepine could be detected in soil as well as in roots and aerial plant parts. Approximately 50 % of carbamazepine originally contained in the urine was recovered in soil samples taken after 3 months. Additionally, 30 % of the carbamazepine was found in aerial plant parts and 0.2 % in roots. The uptake investigated at two different concentration levels was non-linear. However, ibuprofen was neither detected in the soil nor in any plant parts after 3 months. It is assumed that this is a consequence of the biodegradation of ibuprofen.

1 Introduction

Urine, also called yellowwater, is discussed as an alternative fertiliser for agriculture as it contains relatively high concentrations of the macronutrients nitrogen, phosphorus, and potassium *(1-4)*. But this usage of urine includes the risk of transfer of pharmaceutical residues to agricultural fields *(5,6)*. Little is known on the fate of pharmaceuticals regarding their accumulation in soils, transfer to groundwater, and incorporation by plants. However, these effects cannot be excluded as fairly high concentrations of pharmaceuticals are expected in urine (*5*). The uptake of pharmaceuticals in plants and the effects they exaggerate on plant physiology and development were of major interest when crops are fertilised with urine. Literature states that the uptake of organic compounds by plants is correlated with the molecular weight of the organics (*7*). It is assumed that a molecular weight of >1000 makes the absorption by cellular membranes impossible (*8*). Moreover, the octanol-water partition coefficient is looked at as a characteristic of the organics strongly affecting their uptake. Briggs et al. (*9,10*) have detected that uptake into shoots was most efficient for chemicals with log K_{OW} = 2. Constrastingly, K_{OW} is reported to be of lower importance according to Trapp (*11*) and Topp et al. (*7*) who found predictions of the membrane permeability of very lipophobic compounds by their K_{OW} to be very weak (*11*). An ion-trap mechanism, i.e. a process with the chemical being neutral outside and dissociated inside the cell (*11,12*), is responsible for the incorporation of organics by plants. Additionally, literature shows that pharmaceuticals can affect plant growth when dosed in sufficient concentrations (*13,14*) and can be taken up by plants *(15,16)*. The question is, whether concentrations applied by urine fertilisation to fields are causing any adverse effects towards plants. Another question to be clarified was whether pharmaceuticals are taken up by the investigated plants, and in which concentrations by which plant parts.

2 Experimental Section

2.1 Selection of pharmaceuticals

The number of pharmaceuticals selected for these investigations were limited due to the decision that not only the effects of single substances but also of combinations of several substances should be tested. With respect to conditions such as space requirements etc., it was decided to investigate the effects of three pharmaceuticals as well as various combinations of them at two different concentration levels.

The selected pharmaceuticals had to fulfil several requirements. First, they should have been detected in German groundwater. Additionally, they should differ with respect to log K_{OW}, molecular weight and their expected concentrations in “Average German Urine” (AGU (*5*); see also Supporting Information).

Consequently, three pharmaceuticals were selected: carbamazepine (CZ, CAS-N°. 298-46-4), ibuprofen (IBU, CAS-N°. 15687-27-1), and 17α-ethinylestradiol (EE2, CAS-N°. 57-63-6) (for their characteristics see Supporting Information). All three pharmaceuticals were purchased from Sigma-Aldrich: IBU, minimum 98 % GC; EE2, minimum 96 % HPLC, and CZ (no information on purity given by provider).

2.2 Preparation of spiked yellowwater

The urine used as fertiliser in the plant experiments was spiked with the average concentration of the three pharmaceuticals calculated for AGU further on referred to as "natural" (n) concentration level and at a higher level referred to as "artificial" (a): the ten-fold concentration in case of CZ and IBU and a 40-fold concentration for EE2 in order to ensure analytical detection. Twofold pharmaceutical concentrations compared to AGU were added as pots obtained only half of the liquid planned to add. Nevertheless, the amount of urine guaranteed an optimal supply for the growth period of three months.
Beside the single pharmaceuticals, all combinations possible with the three pharmaceuticals were tested at the natural and artificial concentration level (see Supporting Information). Each pharmaceutical in urine mixture was applied to plants in triplicate.

Urine was collected from healthy males in bottles in the two weeks prior to application of urine (week 25) spiked with the pharmaceuticals designated as "UPmix". It was decided to use male urine to keep the hormone level as low as possible. None of the donors were under any medication. Concentrations of macronutrients and TOC as well as average conductivity and pH in the mixed urine were in the usual range (see Supporting Information).

Preparation and application of UPmix was carried out in three steps. First, pharmaceutical concentrates were prepared in the laboratory. The required concentration for the three repetitions was achieved by dissolving the solid pharmaceutical in 500 µl methanol. The pharmaceutical-methanol (PM) solution was stored in the fridge at 5°C and transported in a cooling box. In the second step the PM solution was added to 750 ml of urine. The vial containing the PM solution was rinsed with 100 µl distilled water which was added to the UPmix as well. In the case of blanks, the same procedure was executed, but adding 500 µl pure methanol without any pharmaceuticals. Afterwards, the UPmix was stirred with a glass rod, divided into three equal portions of 250 ml and applied to the pots. After each application, the full equipment was thoroughly rinsed with distilled water before the next UPmix was prepared.

2.3 Application of pharmaceuticals

The plant experiments were accomplished at the Institute of Plant Nutrition of the University of Bonn from June to September 2007 in the greenhouse of the institute. A number of 64 "Kick-Brauckmann-pots" (height: 26 cm, diameter: 20 cm (*17*)) were filled with 9 kg air-dried soil (type: Meckenheimer Krume; luvisol: 16 % clay, 77 % silt, 7 % sand (*18*)). The

pots contained a bottomless inner pot in a planter with drainage for leachate. The drainage was closed and all the water remained within the pot. Pots were connected to a micro-

irrigation system (drop irrigation; Blunat) and adjusted to keep the soil moisture at 80 %. Soil moisture was controlled from time to time by weighing the pots. In week 23, seeds of ryegrass (*Lolium perenne*) were seeded in rows into the pots (0.95 g seeds per pot). Germination and development of seedlings was regular.
After establishment and initial growth for a period of 2 weeks, the seedlings were treated with different UPmix as direct application on seeds may lead to delay of germination (*19*). 250 ml liquid were added to each pot.

To keep pots with equal pharmaceutical additions as far away as possible from each other, the experiment was parted into three series: pot N^{o}. 1-20, No. 21-40, and N^{o}. 41-60. Pots N^{o}. 61-64 were additional ones. In each section the same UPmix was only once applied. The experimental setup contained 2 blanks (pots only treated with urine and methanol) in each series as well as 4 pots completely untreated (no pharmaceuticals, no urine). The following morning plants of pots treated with UPmix did not show any differences to the untreated ones.
Plants were cut seven times until harvest, the last time being September 9, 2007. Fresh and dry weight of the aerial plant parts cut was determined on each occasion.

2.4 Final harvest and sample preparation

The experiment was terminated on September 17, 2007. After the last cutting of the grass, soil samples from top to bottom of each pot were taken using a mechanical soil corer. Approximately 150 g DM were taken from each pot resulting in 5 to 8 holes per pot. The samples were dried at 40°C until a constant weight was achieved, and then ground to small pieces by mechanical pressure exaggerated by a wooden cylinder (specifically used for soil samples). The low drying temperature was used to prevent pharmaceuticals from eventual destruction. After this procedure, soil was sieved through a certified sieve with a pore size of 2 mm. During sieving, grit, gravel and plant parts were removed manually from the sieve. The fine fractions passing the sieve were collected on a foil. After each sieving, the four endings were lifted; the sample was moved mechanically to the centre and divided into two final samples. Subsequently to processing of each soil sample all tools were cleaned carefully.

After soil samples were taken all pots were completely emptied and the root soil mixture was cut into several parts. From this mixture roots were collected manually for further analysis. Roots as well as plant parts close to soil, leftovers from cuttings, were washed with tap water and distilled water in sieves of pore sizes of 0.25 and 0.49 mm, separated from each other and air dried. Aerial plant parts were dried at 40°C for approx. 3 d until constant weight.

2.5 Analytical determination of pharmaceuticals

All pharmaceutical analyses were performed in the central laboratory of Hamburg University of Technology.

Soil

Prior to analyses soil samples were dried once more at 50°C and ground. Samples of 10 g soil were shaken with 50 ml methanol for 2 hours. Subsequently, the suspension was filtered over a paper filter. The extract was concentrated to 1 ml in a rotary evaporator and stored in 2 ml vials until further processing.
For silylation, 800 µl of the concentrated extract were pipetted into a compression-proved vial, evaporated to dryness by a gentle stream of nitrogen, and silylated with 200 µl MSHFBA (N-methyl-N-trimethylsilylhepta-fluorobutyramide) at 70°C for 1 hour. The residue was dissolved in a small volume of acetonitrile which was transferred to a graduated flask adjusted to a final volume of 1 ml.
The silylated extracts were analysed by GC/MS. For GC/MS conditions and m/z see Supporting Information. Each sample was analysed in duplicate. For recovery rates (derived from pharmaceutical-spiked blank soil analysed as described), LOD, and LOQ see Table 1.

Table 1: Recovery rates, limits of detection and quantification of the three investigated pharmaceuticals in soil.

Pharmaceutical	Recovery rate (%)	Limit of detection (µg kg^{-1} DM)*	Limit of quantification (µg kg^{-1} DM)*
CZ	90 - 120	0.2	0.6
IBU	30 - 60	1	2
EE2	50 - 60	1	2

* Lowest recovery rate considered.

Roots and aerial plant parts

Roots were cut with a cutting machine into fine parts and further ground in a coffee grinder. As only small amounts of root material were available (1.5-4 g DM), the entire material from one plant was used to prepare one sample extract.
The ground material was shaken for 2 h in a buffer solution of HCl and KCl (6.5 ml 0.2 M HCl and 25 ml 0.2 M KCl) at pH 2. Solid parts were filtered off by a fluted paper filter and the extract was subdued to solid-phase extraction with abselut Nexus cartridges (500 mg/12 ml, VARIAN). After washing the cartridges with rinsing water of the extraction bottles and subsequently with little H_2O of analytical grade, the analytes were eluted with 5 ml methanol and the eluate was concentrated to a volume of 1 or 2 ml (roots) and 2 ml (aerial plant parts).

The solutions were analysed by GC/MS as described for soil samples. The identification of each substance was verified by spiking one part of each sample extract with the respective pharmaceutical standards as the matrix of the extracts was very complex. For recovery rates, LOD, and LOQ see Table 2. Out of the three substances only carbamazepine could be detected in the extracts, however as its decomposition product iminostilbene (CAS-N^{o}. 256-96-2).

Table 2: Recovery rates, limit of detection and quantification of carbamazepine** and ibuprofen in roots and aerial plant parts.

Pharmaceutical	Recovery rate (%)	Limit of detection (µg kg^{-1} DM)*	Limit of quantification (µg kg^{-1} DM)*
Roots			
CZ**	56 - 61	10	20
IBU	67 - 98	20	30
Aerial plant parts			
CZ**	15 - 20	20	75
IBU	Not determined		

* Lowest recovery rate considered. ** Detected in form of iminostilbene.

EE2 was not detected. The reason being that uptake rates of the investigated plants were much lower than expected from results reported by Schneider (*18*). So, even the artificial concentration for EE2 was selected as too low.

2.6 Statistical evaluation of experimental results

Results of the pot experiments on rye grass were statistically evaluated with SPSS 15. A one-way ANOVA was accomplished as a one-way descriptive method in cooperation with a Student-Newman-Keuls procedure. α was set to be 0.05 to determine a significant difference between various treatments.

3 Results & Discussion

3.1 Pharmaceutical concentrations determined in soil

IBU and EE2 could not be detected in any soil sample after a three months growth period, although the pots exposed to the "artificial" IBU level contained an initial pharmaceutical concentration of 490 µg IBU kg^{-1} DM soil after addition of the UPmix. This concentration is 490 times larger than the limit of detection of IBU in soil. Initial EE2 concentration theoretically only amounted to 0.053 µg kg^{-1} DM (artificial level) in soil and thus was below its determined limit of quantification in soil. Contrasting to IBU and EE2, CZ was detected in all pots except in one of the two duplicate analyses of the pot exposed to 3n (58 µg CZ l^{-1}

urine). In this case however CZ was detected in traces which were above LOD. CZ is known to be persistent (*20-22*), and additionally its concentration was higher than that of EE2.

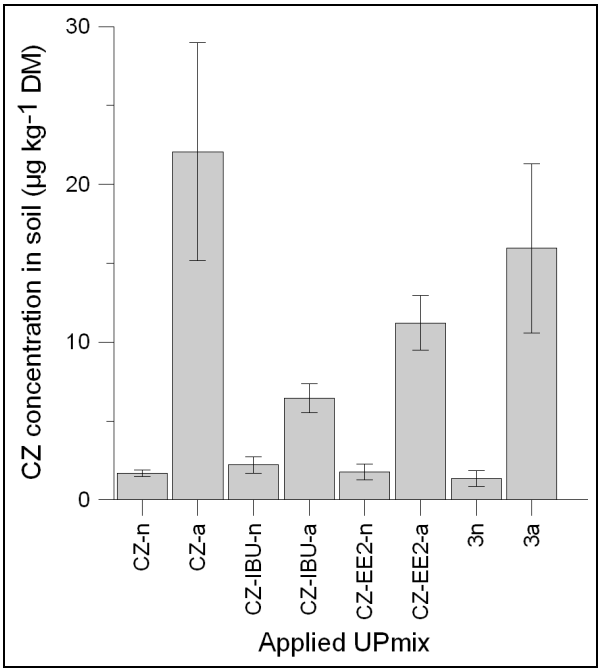

Figure 1: Measured mean concentrations of carbamazepine in soil samples at day 92. By n "natural", and by a "artificial" concentrations applied are indicated. Error bars show standard deviations of triplicate fertilisation experiment.

Concentrations found in soil samples after 92 d (Figure 1) correlate clearly with the applied initial concentrations of 3.2 µg kg^{-1} DM ("natural concentration", n) and 32 µg kg^{-1} DM ("artificial concentration", a). On average, 49 % of the applied CZ concentrations were recovered 3 months after application varying between 20 % (in CZ-IBU-n) and 69 % (CZ-IBU-a). Additionally, variations between the duplicate analyses for one pot were low. Variations observed between pots with the same treatment were higher, however, especially for exposures to CZ-a and 3a (standard deviation of 13.8 and 10.7 µg kg^{-1} DM soil respectively; Figure 1). Therefore results presented in Figure 1 are only based on two pots. In the case of 3a, concentrations of CZ in one pot were significantly lower with only 4.4 µg kg^{-1} DM, resulting in the large standard deviation.

Via one-way ANOVA the null hypothesis could be clearly rejected: concentrations of CZ applied with the UPmix had clear consequences for concentrations measured in soil. The probability that differences between groups are of coincidental origin was 0.7 %. This result is additionally supported by the Student-Newman-Keuls test partitioning samples into two sub groups confirming that differences were significant.

These findings are concurrent with literature on soils irrigated with treated wastewater. Kinney et al. (*20*) report detection and even accumulation of CZ in the upper 30 cm layer of soils irrigated with treated wastewater containing approx. 70 ng l^{-1} of CZ. Concentrations decreased with depth. Additionally, CZ was detected in groundwater wells 12-15 m below fields irrigated with treated wastewater exhibiting 200 ng l^{-1} of CZ, whereas IBU and EE2 were not detected in groundwater below the respective areas (*21*). This is an additional indicator for the higher stability and mobility of CZ compared to IBU and EE2 (*23-25*).

3.2 Effects of the investigated pharmaceuticals on rye grass

Indirect effects on aerial plant parts

The growth of aerial plant matter was identified for the entire 3 months experimental period. The factor of fresh weight to dry weight was only slightly varying between 6.01 (Control 2) and 7.03 (CZ-IBU-n). Therefore, the results are presented and discussed only in terms of dry weight.

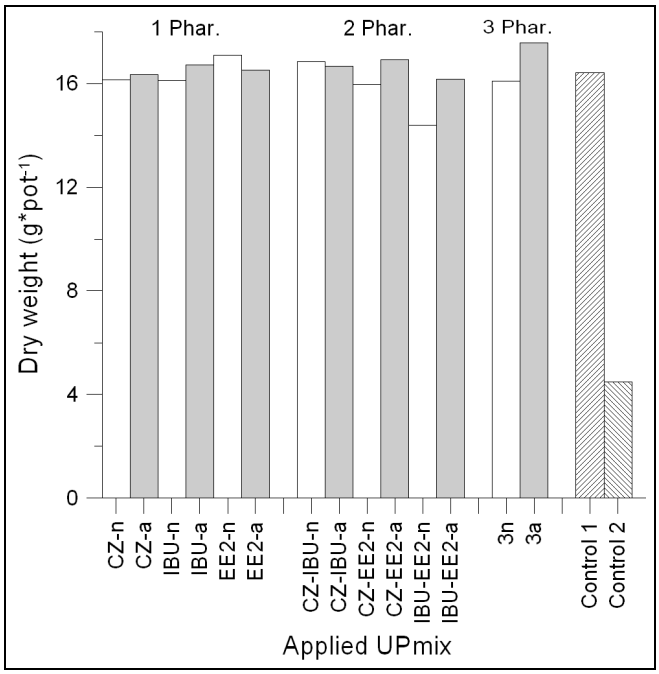

Figure 2: Overall dry weight of aerial plant parts of rye grass determined during the full growth period. n = natural concentration (white bars), a = artificial concentration (grey bars). "Control 1" indicates plants treated with MeOH and urine, "Control 2" did not receive any application beside water; "3" is the designation for the combination of CZ, IBU, and EE2.

No visual effects were observed except for Control 2 which received only irrigation water without nutrients and thus showed only about 25 % of the biomass production compared to the fertilised grass (Figure 2). Aerial plant parts of the non-fertilised Control 2 were smaller and thinner. The lack of fertilisation led to a large weight reduction. The overall fresh as well as dry matter of all plants fertilised with yellowwater did not show any effect of the added pharmaceuticals (Figure 2; for fresh weight see Supporting Information).

Statistical analysis confirmed these findings. One-way ANOVA, $P < 0.05$, showed a significant difference of the unfertilised control group for fresh and dry matter. Apart from this result, no significant differences were observed and the null hypothesis cannot be rejected which says that pharmaceutical applications at the tested concentrations do not affect the synthesis of plant matter. Moreover, no relationship was detected between the amount of harvested plant matter and the added doses of the particular pharmaceuticals applied disregarding the specific pharmaceuticals contained in the UPmix. As well, pharmaceuticals did not have any effect on the course of the production of aerial plant parts during the growth period (for details see Supporting Information).

Uptake of carbamazepine and ibuprofen into roots and aerial plant parts

Due to the matrix of the plant material extract, detection of CZ and IBU were difficult. Only in those plants exposed to artificial concentrations, CZ could be quantified in the roots. Similarly, CZ could be quantified only in the aerial parts of plants exposed to artificial concentration levels. CZ concentrations in the aerial parts of plants exposed to the natural CZ level (58 µg l^{-1} in AGU) were similar to limit of quantification (75 µg kg^{-1} DM). IBU was not determined in aerial plant parts due to the problematic matrix.

CZ concentrations in roots were between 131 µg kg^{-1} DM (one sample of CZ-EE2-a) and 426 µg kg^{-1} DM (one sample of CZ-a) with a mean concentration of 225 µg kg^{-1} DM while a tenfold concentration was reached in aerial plant parts (mean concentration: 4950 µg kg^{-1} DM; span: 2600 µg kg^{-1} DM (CZ-EE2-a) to 6950 µg kg^{-1} DM (CZ-a)). Instead of measuring CZ concentrations in roots, in two pots (exposing CZ-IBU-a and 3a) the plant crowns were extracted and analysed as amount of root material was insufficient. As concentrations in the two samples of stocks were within the range of the two analyses of roots of the same series (CZ-IBU-a: 243 µg kg^{-1} DM in the crown, 202 and 321 µg kg^{-1} DM in the roots; 3a: 131 µg kg^{-1} DM, 175 and 184 µg kg^{-1} DM), for these two pots the concentration in crown was assumed to be close to mean concentrations in roots (Figure 3).

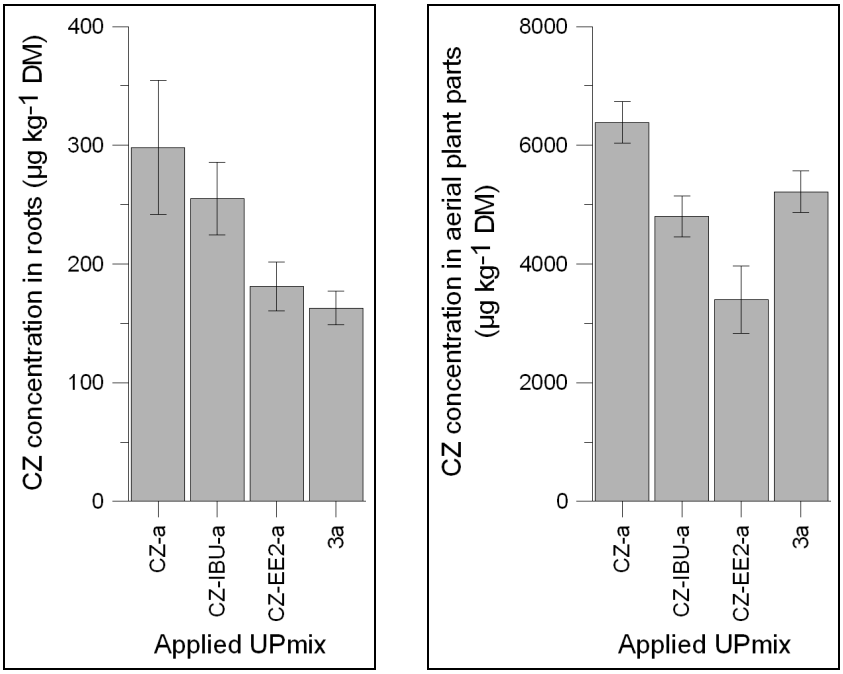

Figure 3: Mean concentrations of carbamazepine measured in roots and aerial plant parts of rye grass after 92 days growth period. Error bars show standard deviations for the three equally exposed pots.

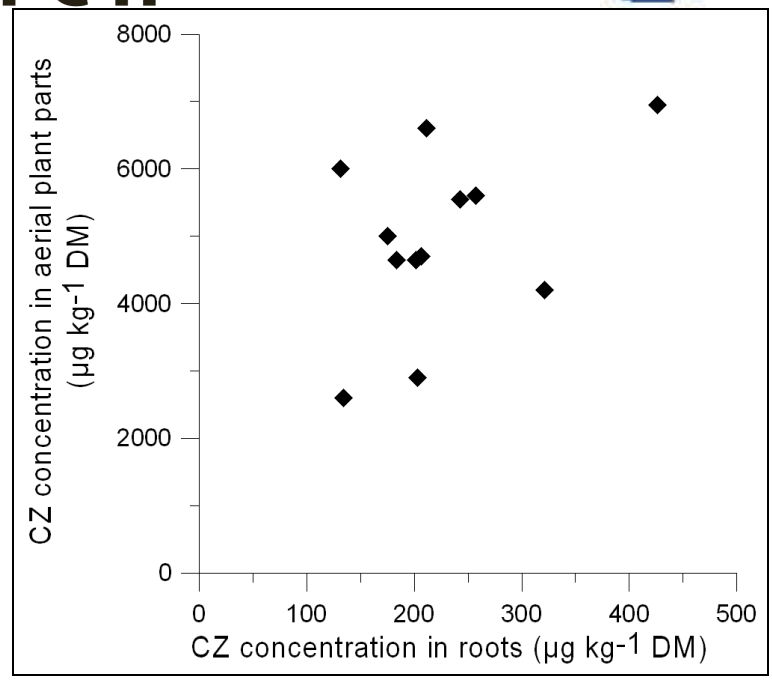

Figure 4: Comparison between incorporation of carbamazepine (applied at artificial level) into aerial plant parts and roots of rye grass.

Statistically relevant correlations between the uptake of CZ into roots and into aerial plant parts could not be determined (Figure 4). This might be a consequence of considerable coefficients of variation in the difficult matrix of plant extracts. Only when comparing the mean concentrations of the three pots of one UPmix, a weak correlation was observed. In general, CZ concentrations in aerial plant parts were one order of magnitude larger then in roots. An average of 0.21 % (between 0.12 and 0.40 %) of the total amount of CZ applied to each pot (under artificial conditions 290 µg per pot) was found in the roots of rye grass, but 30 % (between 15 and 42 %) in the aerial plant parts.

It can be assumed that IBU (neither detected in soil nor in plant roots) is not incorporated by plants due to its fast biodegradation in soil while CZ which is present in soil for longer periods due to its recalcitrance. Thus, CZ remains available for plants for a much longer period and is thus transferred to the plants, especially to the aerial plant parts in the case of rye grass. It has to be pointed out that the uptake rates of the aerial plant parts of natural and artificial concentrations were non-linear. CZ concentrations in the aerial plant parts exposed to the natural CZ level were in the range of the limit of quantification (75 µg kg^{-1} DM) and those exposed to artificial CZ levels showed an average concentration of 4950 µg kg^{-1} DM. While CZ concentrations measured in soil and roots reflected the order of one magnitude (10 fold) which was chosen for the two application regimes (“natural” and “artificial”).

This research did not consider potential degradation of pharmaceuticals in the rye grass during the growth period. Moreover, this study does not allow for an evaluation of potential toxic effects of pharmaceuticals ingested by humans with crops.

4 References

(1) Larsen, T.; Gujer, W.: Separate management of anthropogenic nutrient solutions (human urine). *Water Sci. Technol.* **1996**, *34 (3-4)*, 87-94.

(2) Muskolus, A. Anthropogenic plant nutrients as fertiliser. PhD Thesis, Institut für Pflanzenbauwissenschaften, Humboldt-Universität zu Berlin, Berlin, Germany, 2008.

(3) Otterpohl, R.: Options for alternative types of sewerage and treatment systems directed to improvement of the overall performance. *Water Sci. Technol.* **2002**, *45 (3)*, 149-158.

(4) Vinnerås, B.; Jönsson, H.: The performance and potential of faecal separation and urine diversion to recycle plant nutrients in household wastewater. *Bioresour. Technol.* **2002**, *84 (3)*, 275-282.

(5) Winker, M.; Tettenborn, F.; Faika, D.; Gulyas, H.; Otterpohl, R.: Comparison of analytical and theoretical pharmaceutical concentrations in human urine in Germany. *Water Res.* **2008**, *42 (14)*, 3633-3640.

(6) Lienert, J.; Güdel, K.; Escher, B.: Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. *Environ. Sci. Technol.* **2007**, *41 (12)*, 4471-4478.

(7) Topp, E.; Scheunert, I.; Attar, A.; Korte, F.: Factors affecting the uptake of ^{14}C-labeled organic chemicals by plants from soil. *Ecotoxicol. Environ. Saf.* **1986**, *11 (2)*, 219-228.

(8) Sanderson, H.; Brain, R.; Johnson, D.; Wilson, C.; Solomon, K.: Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular, and sex hormones. *Toxicology* **2004**, *203 (1-3)*, 27-40.

(9) Briggs, G.; Rigitano, R.; Bromilow, R.: Relationship between lipophilicity and root uptake and translocation of non-ionised chemicals by barley. *Pestic. Sci.* **1982**, *13 (5)*, 495-504.

(10) Briggs, G.; Bromilow, R.; Evans, A.; Williams, M.: Relationship between lipophilicity and the distribution of non-ionised chemicals in barley shoots following uptake by the roots. *Pestic. Sci.* **1983**, *14*, 429-500.

(11) Trapp, S.: Modelling uptake into roots and subsequent translocation of neutral and ionisable organic compounds. *Pest Manag. Sci.* **2000**, *56 (9)*, 767-778.

(12) Briggs, G.; Rigitano, R.; Bromilow, R.: Physico-chemical factors affecting uptake by roots and translocation to shoots of weak acids in barley. *Pestic. Sci.* **1987**, *19 (2)*, 101-112.

(13) Jjemba, P.: The effect of chloroquine, quinacrine, and metronidazole on both soybean plants and soil microbiota. *Chemosphere* **2002**, *46 (7)*, 1019-1025.

(14) Kumar, K.; Gupta, S.; Chander, Y.; Singh, A.: Antibiotic use in agriculture and its impact on the terrestrial environment. *Adv. Agron.* **2005**, *87*, 1-54.

(15) Dolliver, H.; Kumar, K.; Gupta, S.: Sulfamethazine uptake by plants from manure-amended soil. *J. Environ. Qual.* **2007**, *36 (4)*, 1224-1230.

(16) Boxall, A.; Johnson, A.; Smith, E.; Sinclair, C.; Stutt, E.; Levy, L.: Uptake of veterinary medicines from soils into plants. *J. Agric. Food Chem.* **2006**, *54 (6)*, 2288-2297.

(17) Kick, H.; Große-Brauckmann, E.: Über die Konstruktion eines Vegetationsgefäßes aus Kunststoff. *J. Plant Nutr. Soil Sci.* **1961**, *95 (1)*, 52-55.

(18) Schneider, R. Pharmaka im Urin: Abbau und Versickerung vs. Pflanzenaufnahme. In Proceedings of *Nährstofftrennung und -verwertung in der Abwassertechnik am Beispiel der "Lambertsmühle"*; Bonn, Germany, 2005.

(19) Simons, J.; Clemens, J. Urin-/Pharmaka-Keimtest. In Proceedings of *Nährstofftrennung und -verwertung in der Abwassertechnik am Beispiel der "Lambertsmühle"*; Bonn, Germany, 2005.

(20) Kinney, C.; Furlong, E.; Werner, S.; Cahill, J.: Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. *Environ. Toxicol. Chem.* **2006**, *25 (2)*, 317-326.

(21) Ternes, T.; Bonerz, M.; Herrmann, N.; Teiser, B.; Andersen, H.: Irrigation of treated wastewater in Braunschweig, Germany: An option to remove pharmaceuticals and musk fragrances. *Chemosphere* **2007**, *66 (5)*, 894-904.

(22) Winker, M.; Faika, D.; Gulyas, H.; Otterpohl, R.: A comparison of human pharmaceutical concentrations in raw municipal wastewater with yellowwater. *Sci. Total Environ.* **2008**, *399 (1-3)*, 96-104.

(23) Quintana, J.; Weiss, S.; Reemtsma, T.: Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor. *Water Res.* **2005**, *39 (12)*, 2654-2664.

(24) Ying, G.; Kookana, R.: Sorption and degradation of estrogen-like-endocrine disrupting chemicals in soil. *Environ. Toxicol. Chem.* **2005**, *24 (10)*, 2640-2645.

(25) Richter, O.; Kullmer, C.; Kreuzig, R.: Metabolic fate modeling of selected human pharmaceuticals in soils. *Clean* **2007**, *35 (5)*, 495-503.