# Plant Uptake of some Pharmaceuticals Commonly Present in Sewage Sludge Compost

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***Abstract*—The application of sewage sludge compost as a fertilizer can be a source of the contamination of food plants by pharmaceutical products. In this study the uptake of ciprofloxacin, ofloxacin, norfloxacin, sulfadimethoxine and sulfamethoxazole from soil into potato was demonstrated. The concentrations of the studied pharmaceuticals were of considerable magnitude in the plant samples, if compared to their concentrations in soil.**

***Keywords - sewage sludge; pharmaceutical; plant uptake; compost***

## I. INTRODUCTION

Sewage sludge is the residue from the treatment of domestic and industrial wastewater. It contains organic matter and nutrients that are useful for plants [1]. The contents of nitrogen, phosphorus and organic matter are up to 10 times higher in sewage sludge and its compost, if compared to common agricultural soils [2]. Still, its usage as a fertilizer is restricted due to a large number of toxic pollutants found in this matter. Composting or aerobic biological treatment of organic wastes is an ancestral way to reduce wastes and to reuse organic matter. Among the range of existing organic wastes, sewage sludge composting enables the production of quality compost that may be used as a soil conditioner or as an organic fertilizer [3], since its organic matter content can vary from 50% to 70% of the total solids content [4]. Compost is a relatively stable material similar to humus. It is fine textured and has low moisture. The quality of compost depends on its environmental compatibility and the correspondence with market necessities. These features involve the absence of toxic inorganic and organic substances that may enter the food chain through the plant uptake [5].

Among these, pharmaceuticals are frequently present in sewage sludge. Their quantities rise from year to year. In spite of the fact that very low drug levels in the environment can have undesirable ecological and health effects, until now the problems related to the presence of pharmaceuticals in sewage sludge and its compost have received little attention [6]. Over the past decade, the scientific community has become increasingly interested in the impacts of pharmaceutical contaminants on the environment and human health. A large proportion of pharmaceuticals are introduced into the environment via sewage treatment plants. The presence and possible accumulation of these substances in sewage sludge are known, but little information is available on biodegradation of these pollutants. Current national and international regulations governing the application of sewage sludge ignore the presence and fate of pharmaceuticals. It has been shown that the degradation of pharmaceuticals may take place during bio-solid composting [7]. It is considered to be very useful to study the degradation of pharmaceuticals if diverse sewage sludge treatment technologies are to be applied. Still, no systematic work concerning biodegradation of pharmaceuticals during sewage sludge composting has been published. The utilization of untreated raw sewage sludge in agriculture is prohibited.

Medical substances have many of the necessary properties to bio-accumulate and provoke changes in ecosystems. There exist no trigger values for drug residues in sewage compost in the European Union. Plants unlike animals have no excretion. Therefore, drug residues may accumulate into plants. As a result, concentrations of drug residues in food plants may exceed the maximum residue levels (MRL) for meat and milk. No limits have been set for drug residues in plant products at present time. Various treatment technologies including composting and methane fermentation are known. Although the usage of such compost as a fertilizer widely takes place in Eastern Europe, the presence of drug residues in this matter has been ignored.

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The possible degradation of drug residues as a result of sludge composting needs to be studied.

Humans may be exposed to residues of drugs in the environment by a number of routes including the consumption of crops that have accumulated substances from fertilized soils [8]. Experiments at elevated concentrations of pharmaceuticals in soil have shown that they are able to accumulate in plants [9]. The methodology for the simultaneous determination of the studied pharmaceuticals from sewage sludge is described in detail in [10]. There is a need to determine the significance of uptake into plants from soil under "real" conditions as a potential route for pharmaceuticals. Therefore, the current study was conducted to investigate the potential for some widely used pharmaceuticals – fluoroquinolones and sulfonamides (ciprofloxacin $C_{17}H_{18}FN_3O_3$, norfloxacin $C_{16}H_{18}FN_3O_3$ and ofloxacin $C_{18}H_{20}FN_3O_4$, sulfadimethoxine $C_{12}H_{14}N_4O_4S$ and sulfamethoxazole $C_{10}H_{11}N_3O_3S$) – to be taken up by food plants (namely potatoes in this study) from soil fertilized with sewage sludge or its compost.

## II. Experimental

### A. Chemicals and Equipment

Pharmaceuticals were purchased from Riedel-de-Haën (Seelze, Germany) - three fluoroquinolones: ciprofloxacin (CIP, purity 99.8%), norfloxacin (NOR, purity 99.9%) and ofloxacin (OFL, purity 99.3%); two sulfonamides: sulfadimethoxine (SDM, purity 99.4%) and sulfamethoxazole (SMX, purity 99.9%). Hydrophilic-lipophilic balanced (HLB) cartridges (Oasis HLB (60 µm), 500 mg/6 mL) by Waters (Milford, MA, USA). Acetonitrile and methanol were obtained from J. T. Baker (Deventer, The Netherlands), acetic acid and ammonia from Riedel-de-Haën, 1,1,1,3,3,3-hexafluoro-2-propanol from Sigma (St. Louis, MO, USA). All solvents were of reagent grade or higher quality.

### B. Liquid Extraction

250 mg of dried potato sample was extracted with 10 mL of 1:1 (v/v) mixture of acetonitrile and 1% acetic acid, then homogenized with laboratory homogenizer DIAX 900 (Heidolph Instruments, Germany) 25 000 rpm, sonicated (5'), vortexed (1') and centrifuged at 8000 rpm. The supernatant was then separated and dried by nitrogen stream. Approximately 15 mL of 1 % acetic acid was added to the 1 mL of evaporation residue.

### C. Solid-Phase Extraction

The extract collected by liquid extraction was cleaned up by solid phase extraction (SPE). Antibiotics - CIP, NOR, OFL, SDM and SMX - were extracted using HLB cartridges. For SPE procedure the vacuum manifold, supplied by Agilent Technologies, was used. HLB cartridges were preconditioned with 20 mL of methanol and 10 mL of Milli-Q water. The sample was loaded at a rate of 6 mL/min. After extraction, the compounds were eluted from cartridges using 12 mL of methanol. The SPE extracts were concentrated in polypropylene vials in $N_2$ stream. Residue was dissolved in 1 mL of 10 % methanol with buffer solution (5 mM 1,1,1,3,3,3-hexafluoro-2-propanol, pH adjusted to 9.0 with $NH_4OH$).

### D. Liquid Chromatography - Mass Spectrometry

The SPE extracts were analyzed by liquid chromatography–mass spectrometry (LC–MS) (Agilent Series 1100 LC-MSD Trap XCT (Santa-Clara, CA, USA)) equipped with a binary pump, a degasser, an auto-sampler and a column thermostat. Antibiotics were chromatographed using a Waters XBridge C18 column (150 mm × 3 mm, 3.5 µm) equipped with a Waters Guard Cartridge 4.6 mm × 20 mm. Electrospray interface (ESI) was used in positive ion mode for ionization. Selected reaction monitoring was used. Full $MS^2$ were recorded and the following transitions were applied for quantification: OFL m/z 362→ 261, 318, NOR m/z 320→302, 276, CIP m/z 332→288, 314, SMX m/z 254→108, 188, SDM m/z 311→108, 156, 218, 245. For instrument control and data analysis software: Agilent ChemStation for LC Rev. A. 10.02; MSD Trap Control version 5.2 and Data Analysis for LC-MSD Trap 3.2 were used. Gradient elution was carried out with methanol and hexafluoroisopropanol (HFIP) buffer solution (5 mM 1,1,1,3,3,3-hexafluoro-2-propanol, pH adjusted to 9.0 with $NH_4OH$). The linear gradient started at 10% methanol and was raised to 100% within 50 min, after that methanol concentration was 100% for 5 min, then lowered to 10% in 5 min and kept in 10% for 5 min. The eluent flow rate was 0.3 mL/min, the column temperature was set to 30 °C and the injection volume was 10 µL.

### E. Plant Samples

Aqueous solutions of the studied pharmaceuticals were mixed with soil. The final concentration of each pharmaceutical was 10 mg/kg and 1 mg/kg (dry weight). To assure better dissolution of the studied pharmaceuticals fluoroquinolones were dissolved in 2 ml of 0.1 mM ammonium acetate buffer solution with pH=2.8 and sulfonamides were dissolved in 2 ml of 0.3 M NaOH. The potatoes were planted into the pots and cultivated in the presence of the five pharmaceuticals during 120 days from planting. Then the potatoes were collected, washed, dried and milled. The milled potatoes were hold in hermetical plastic bags at –80 °C. The samples were dried at 45 °C before analysis.

## III. Results and Discussion

The selection of pharmaceuticals was made according to their stability in soil and their potential accumulation into plants. Typical chromatograms for SMX, SDM, NOR, CIP and OFL obtained in the case of potato sample are shown in Fig. 1. Recoveries for the detected pharmaceuticals varied from 80 to 94%. The average concentration values showing the accumulation of these pharmaceuticals into potatoes are presented in table 1. The variation of the concentrations detected from three parallel samples was within ±35%, which is fully acceptable in the case of biological objects. The upper numbers in each row show the concentrations of pharmaceuticals in potatoes cultivated in loamy soil, the

concentrations presented below these correspond to potato samples obtained from plants cultivated in sandy soil.

TABLE I. CONCENTRATIONS OF PHARMACEUTICALS IN POTATO SAMPLES

| Pharmaceuticals | In Potato Samples, µg/kg | | | | |
|---|---|---|---|---|---|
| In Soil, µg/kg | *CIP* | *NOR* | *OFL* | *SMD* | *SMX* |
| 10,000 | 140<br>80 | 230<br>100 | 150<br>100 | 1,500<br>170 | 3,900<br>390 |
| 1,000 | n.d.<br>n.d. | n.d.<br>n.d | 33<br>36 | 37<br>40 | n.d.<br>n.d. |

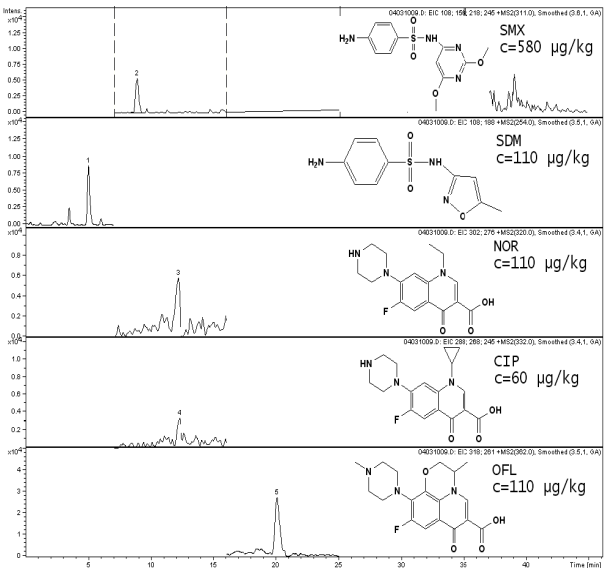


Figure 1. Chromatograms for SMX, SDM, NOR, CIP and OFL in potato samples.

Sulfonamides are among the most commonly used antibiotics in veterinary medicine and to a lesser extent in human medicine [11]. They are both fairly water-soluble and polar [12]. The low adsorption of sulfonamides on soil particles is known [13] and due to this phenomenon they are "free" to migrate into plants. An opposite behavior is characteristic to fluoroquinolones. It has been shown that more than 90% of applied ciprofloxacin and ofloxacin is adsorbed on different soils [13]. For this reason no significant migration of fluoroquinolones from soil into plants takes place. The amounts of fluoroquinolones going into potato do not depend much on soil type (table 1). In loamy soil the molecules of sulfonamides attach to clay particles [11], reducing their uptake by plants.

Figure 2. Potatoes, 30th day after planting. Pots on the left – the concentrations of SMX, SDM, NOR, CIP and OFL (each) in sandy soil were 10,000 µg/kg; pots on the right – the concentrations of the pharmaceuticals were 1,000 µg/kg (each).

Figure 2 clearly shows that the growth rate of a potato considerably depends on the concentration of pharmaceuticals in the soil. In this experiment all five pharmaceuticals were cumulatively present in soil. On the left the pots were filled with soil having the 10,000 µg/kg concentrations of each pharmaceutical. In pots on the right the relevant concentrations were 10 times lower (1,000 µg/kg).

Soils and sediments are the ultimate sinks for different pollutants [14]. Recently the uptake of ciprofloxacin, norfloxacin, ofloxacin, sulfadimethoxine and sulfamethoxazole was demonstrated in lettuce [15]. The results were obtained at relatively high concentrations (10 mg/kg) of pharmaceuticals in soil. The uptake of fluoroquinolones and sulfonamides by plants like lettuce did not seem to be a major human health risk, as the detected levels of the studied pharmaceuticals in lettuce leaves were relatively low if compared to their soil concentrations. Still in the case of CIP and OFL the possibility exists that even under "mild" conditions their concentrations in lettuce leaves may reach undesirable levels. The concentrations of sulfonamides in lettuce roots were of the same magnitude if compared to the eatable part of potato (3,700 µg/kg in the case of both SMD and SMX). Once released into the environment, sulfonamides become very mobile, and as a consequence they may affect many non-target organisms. As there are usually several pollutants of the same family or the same type that coexist in soil, synergic or antagonistic effects should be considered [16].

The CIP, NOR and OFL concentrations in lettuce roots – 560, 500 and 1,200 µg/kg, respectively [15] – were lower than the concentrations of sulfonamides, but they still exceeded the concentrations that were detected from potato samples. Therefore, further studies are needed to determine the uptake of different pharmaceuticals and other organic pollutants from different types of soils by various crop plants.

## IV. Conclusions

Only a relatively small number of investigations have been published on the mobility and bioavailability of pharmaceuticals. In the current study uptake of ciprofloxacin, norfloxacin, ofloxacin, sulfadimethoxine and sulfamethoxazole was demonstrated in the case of potato. The uptake of fluoroquinolones and especially sulfonamides by plants like potato might pose health risk, as the detected levels of the studied pharmaceuticals were of considerable magnitude, if compared to their soil concentrations. The low adsorption of sulfonamides on soil may cause contamination of food plants. Further studies should be conducted to determine the uptake of different types of pharmaceuticals and other organic pollutants by various crop plants, and sorption kinetics at the soil-root interface has to be studied.

## Acknowledgment

M.L. thanks Mr. Avo Toomsoo and Mr. Tõnu Tõnutare for fruitful discussions, Dr. Alar Astover and Mr. Hendrik Peterson for their assistance in carrying out experiments. This work was funded by Estonian Environmental Investment Centre, Estonian Science Foundation (grant no 7127) and Estonian research target project SF 0180058s07.

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