

## Environmental Exposure Assessment of Fluoroquinolone Antibacterial Agents from Sewage to Soil

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The behavior of fluoroquinolone antibacterial agents (FQs) during mechanical–biological wastewater treatment was studied by mass flow analysis. In addition, the fate of FQs in agricultural soils after sludge application was investigated. Concentrations of FQs in filtered wastewater (raw sewage, primary, secondary, and tertiary effluents) were determined using solid-phase extraction with mixed phase cation exchange disk cartridges and reversed-phase liquid chromatography with fluorescence detection. FQs in suspended solids, sewage sludge (raw, excess, and anaerobically digested sludge), and sludge-treated soils were determined as described for the aqueous samples but preceded by accelerated solvent extraction. Wastewater treatment resulted in a reduction of the FQ mass flow of 88–92%, mainly due to sorption on sewage sludge. A sludge–wastewater partition coefficient ( $\log K_d \sim 4$ ) was calculated in the activated sludge reactors with a hydraulic residence time of about 8 h. No significant removal of FQs occurred under methanogenic conditions of the sludge digesters. These results suggest sewage sludge as the main reservoir of FQ residues and outline the importance of sludge management strategies to determine whether most of the human-excreted FQs enter the environment. Field experiments of sludge-application to agricultural land confirmed the long-term persistence of trace amounts of FQs in sludge-treated soils and indicated a limited mobility of FQs into the subsoil.

### Introduction

Municipal wastewater treatment plants play an important role in the life cycle of human-use pharmaceuticals because they act as point sources to the aquatic environment. If sorption to sewage sludge is the major removal pathway from the wastewater stream, the application of sewage sludge as soil fertilizer represents an additional entry route for human-use pharmaceuticals into the environment. Hence, it is important to understand the behavior of pharmaceuticals as they pass through wastewater treatment plants. Although it

is well-known that the discharge of treated wastewater effluents to surface water accounts as the major entry pathway to the environment for most human-use pharmaceuticals (1–3), their behavior during wastewater treatment is still mostly unknown. Some studies have investigated the fate of pharmaceuticals in laboratory-scale sewage treatment plants (4, 5) or have involved laboratory testing on biodegradability (1, 4, 5). Some data are also available for full-scale wastewater treatment plants on the overall removal of some pharmaceuticals from the aqueous phase (3, 6–8), but there is still a lack of detailed studies on the efficiency of single treatment processes on the elimination of pharmaceuticals. Quantitative data on the occurrence of pharmaceuticals in sewage sludge are also scarce.

Fluoroquinolone antibacterial agents (FQs) are among the few pharmaceuticals that have been measured in both wastewater effluents (6) and in sewage sludges (9, 10). The most consumed FQs in Switzerland, ciprofloxacin (CIP) and norfloxacin (NOR), were determined at the ng/L level in Swiss wastewater effluents (6) and at the low mg/kg level in sewage sludges (9, 10). Because FQs are excreted by the human body in urine (CIP 45–62%, NOR 20–40%) and feces (CIP 15–25%, NOR 28%) (11, 12), the analysis of both liquid and solid sewage matrices is necessary to determine the entry route of FQs to the environment. During wastewater treatment, FQs are substantially removed from the aqueous phase (6). Based on laboratory studies (13–15), sorption to sewage sludge appears to be the main removal process, yet confirmation of such predictions for full-scale wastewater treatment is needed.

The sludge management strategy ultimately implemented will determine the final fate of FQ residues sorbed to sewage sludge. Although controversial (16), the application of sewage sludge as fertilizer to agricultural land is still widely used in several countries. In Switzerland for instance, 42% of the sewage sludge produced in 1999 (about 88 000 tons) were applied to agricultural land (17). As protection against potential adverse effects of sewage sludge constituents, a revision of the European Union Directive 86/278/EEC is planned to limit the amount of organic micropollutants released to the environment by sewage sludge disposal (18). However, pharmaceuticals are not included in the directives. A more rigorous decision has been taken in Switzerland, where the disposal of sewage sludge into agriculturally used areas is forbidden since May 2003. Nonetheless, as long as such a disposal practice occurs elsewhere, better knowledge on the fate and effects of chemicals in sludge-treated soils is needed. FQs have already been identified as persistent pollutants in sludge treated soils (9) and sediments (19), so their behavior in the terrestrial environment is of special interest.

The first part of this study reports on field measurements in which the analytical methods for the determination of FQs in aqueous (20) and solid (9) sewage matrices are jointly used to perform a mass balance of FQs in a municipal wastewater treatment plant. In the second part, a field investigation was conducted to gain insight on the fate of FQs in sludge-treated soils. Finally, the obtained field data are compared with the literature on FQs sorption and biodegradation to assess the physicochemical and biological processes that may affect the environmental behavior of FQs.

### Experimental Section

**Description of the Wastewater Treatment Plant.** The largest municipal wastewater treatment plant (WWTP) in Switzer-

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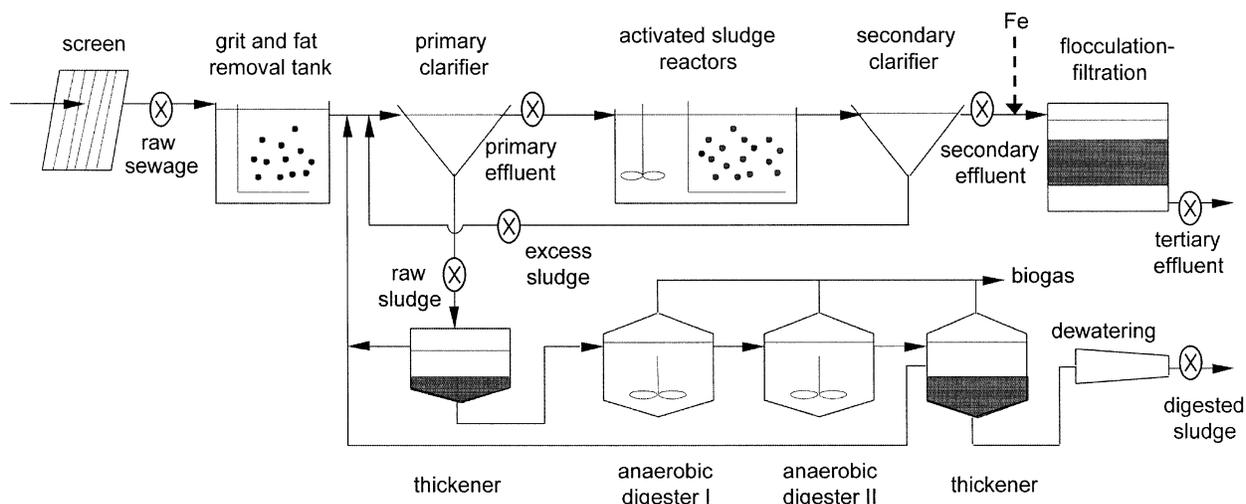


FIGURE 1. Sampling locations (indicated with a cross) at the Zurich-Werdhölzli wastewater treatment plant in Switzerland.

land, Zurich-Werdhölzli WWTP (Figure 1), was chosen to determine the mass balance of FQs. This WWTP serves a residential population of around 275 000 (1998), which due to the commuters to Zurich can increase to 450 000 persons on business days. If we consider in addition the industrial wastewater, the load increases to about 600 000 person equivalents. The sewage entering the WWTP first is treated mechanically using a screen and a combined grit and fat removal tank followed by primary clarification (residence time 2–3 h). The primary effluent flows through the activated sludge systems with an influent to return sludge ratio of about 2:1. The wastewater in the activated sludge reactors (pre-denitrification with a 72%-aerated volume) and the secondary clarifiers have a combined residence time of approximately 20 h. The total solid retention time is about 11 days, which means full nitrification throughout the year with about 60% nitrogen removal due to denitrification and biomass incorporation. Additionally, the facility has a flocculation-filtration step. The primary sludge from mechanical treatment and the excess sludge from biological treatment and filtration are mixed and settled in the primary clarifier. The raw sludge is thickened during 2–4 days. The supernatant is decanted, while the thickened sludge is stabilized in a two-stage anaerobic digestion process. The total sludge residence time is approximately 30 days.

Samples of 24-h flow proportional composites of raw sewage, primary, secondary, and tertiary effluents were collected over 7 days (October 4–10, 2000) by means of automated samplers. In addition, on July 8, 2002 a 24-h flow proportional raw sewage sample was taken for the analysis of suspended solids. After collection in dark amber bottles, all samples were immediately transported to the laboratory. There the samples were filtered through 0.45  $\mu\text{m}$ -cellulose nitrate filters (Sartorius GmbH), and conductivity and pH were determined. Water samples were then preserved by acidification to pH 3.0 and stored in the dark at 4 °C until analysis. Suspended solids retained on the filter after filtering 500 mL of raw sewage were immediately analyzed. Suspended solids concentration was estimated to be between 150 and 300 mg/L in raw sewage, 50–100 mg/L in primary effluent, 5–15 mg/L in secondary effluent, and 1–5 mg/L in tertiary effluent. Conductivity of water samples ranged from 0.77 to 0.93 mS/cm and from 0.59 to 0.70 mS/cm in the primary and tertiary effluents, respectively. Sample pH ranged from 7.5 to 8.4. Water temperature at the sampling stations was between 18 and 20 °C. For the study period, the flow of wastewater through the plant varied from 122 000 to 215 000 m<sup>3</sup>/day.

Grab samples of raw sludge and excess sludge were collected in polypropylene bottles over a 4–5-day period (October 4, 5, 8–10, 2000) and a single raw sludge sample was taken on July 8, 2002. Anaerobically digested sludges were sampled after the anaerobic digesters on October 4, 2000 and July 8 and 15, 2002. All the sludge samples were immediately dried at 60 °C for 72 h, finely ground, and stored in amber bottles at room temperature. During the study period, the daily average of dry sludge matter was as follows: 40 tons of raw sludge, 20 tons of excess sludge, and 25 tons of anaerobically digested sludge.

#### Description of the Sludge-Treated Experimental Field.

In October 1999, an experimental field was set up on free land in Reckenholz in the region of Zurich, Switzerland (21), in which anaerobically digested sludge from the municipal WWTP in Uster was added to the field at 50 t/ha (10 times the amount allowed in Switzerland every third year, i.e., 5 t/ha/3 years). FQ concentrations in anaerobically digested sludge from the Uster WWTP showed similar FQ concentrations (e.g.,  $2.8 \pm 0.3$  mg/kg CIP and  $2.7 \pm 0.2$  kg/d NOR, July 8, 2002) as anaerobically digested sludge from the Zurich-Werdhölzli WWTP. Soil samples were collected using a steel cylinder after 5 and 21 months of sludge-application. Soil physicochemical characteristics are shown in Table 1. Each sample consisted of four aliquots of randomly chosen soil from the same depth of the study plot: 0–2.5 cm, 2.5–5 cm, 5–7.5 cm, 7.5–10 cm, 10–15 cm and 15–20 cm. Samples were dried at 40 °C and passed through a 0.2 mm sieve.

**Analytical Methods.** Filtered wastewater samples were analyzed for FQs by solid-phase extraction using mixed-phase cation-exchange disk cartridges and reversed-phase liquid-chromatography with fluorescence detection as described in ref 20. The precision of the method for CIP and NOR in wastewater effluents, as indicated by the relative standard deviation, was between 5 and 8%. Recoveries were always higher than 90%. The quantification limit was 90 ng/L for raw sewage and primary effluents and 30 ng/L for secondary and tertiary effluents.

Suspended solids, sewage sludge, and sludge-treated soil samples were extracted using accelerated solid extraction followed by solid-phase extraction and reversed-phase liquid-chromatography with fluorescence detection as described in ref 9. The precision of the method for FQs in sewage sludge and sludge-treated soil samples was between 8 and 11%. Overall recoveries were always > 80%. For sewage sludge and for sludge-treated soil limits of detection were 0.12 and 0.05 mg/kg and limits of quantification 0.45 mg/kg and 0.18

**TABLE 1. Physicochemical Soil Characteristics of the Experimental Plot**

|   | soil depth (cm) |       |       |        |       |       |
|---|-----------------|-------|-------|--------|-------|-------|
|   | 0–2.5           | 2.5–5 | 5–7.5 | 7.5–10 | 10–15 | 15–20 |
| soil composition (%)                    |                 |       |       |        |       |       |
| sand                                    | 54              | 58    | 58    | 58     | 59    | 61    |
| silt                                    | 21              | 19    | 20    | 20     | 20    | 20    |
| clay                                    | 18              | 18    | 18    | 18     | 18    | 17    |
| organic matter content                  | 4               | 3     | 3     | 2      | 2     | 1     |
| pH                                      | 6.7             | 7.1   | 6.9   | 7.0    | 7.6   | 7.8   |
| cation exchange capacity (mequiv/100 g) | 28              | 22    | 21    | 19     | 18    | 16    |

**TABLE 2. Ciprofloxacin and Norfloxacin Concentrations in Filtered Wastewater Effluents and Sewage Sludges**

| sampling date                     | sewage discharge, <sup>b</sup><br><i>Q</i> (m <sup>3</sup> /d) | filtered wastewater effluents <sup>a</sup> (ng/L) |                  |                    |                   | sewage sludges <sup>a</sup> (mg/kg) |            |                 |           |
|-----------------------------------|--|---|------------------|--------------------|-------------------|-------------------------------------|------------|-----------------|-----------|
|                                   |  | raw sewage  | primary effluent | secondary effluent | tertiary effluent | excess sludge                       | raw sludge | digested sludge |           |
| <b>Ciprofloxacin</b>              |  |   |                  |                    |                   |                                     |            |                 |           |
| Wed                               | 04-Oct-2000  | 144 955   | 571 ± 29         | 431 ± 28           | 109 ± 7           | 76 ± 7                              | 2.4 ± 0.2  | 1.7 ± 0.1       | 2.5 ± 0.2 |
| Thu                               | 05-Oct-2000  | 137 920   | 511 ± 51         | 387 ± 2            | 109 ± 6           | 87 ± 3                              | 2.6 ± 0.1  | 1.9 ± 0.1       |           |
| Fri                               | 06-Oct-2000  | 198 720   | 329 ± 5          | 272 ± 25           | 94 ± 6            | 68 ± 3                              |            |                 |           |
| Sat                               | 07-Oct-2000  | 136 293   | 469 ± 29         | 417 ± 36           | 92 ± 6            | 65 ± 4                              |            |                 |           |
| Sun                               | 08-Oct-2000  | 121 599   | 351 ± 10         | 308 ± 11           | 90 ± 1            | 73 ± 6                              | 2.3 ± 0.2  | 2.7 ± 0.2       |           |
| Mon                               | 09-Oct-2000  | 214 754   | 315 ± 10         | 279 ± 12           | 79 ± 3            | 61 ± 2                              | 2.5 ± 0.1  | 2.6 ± 0.2       |           |
| Tue                               | 10-Oct-2000  | 154 910   | 433 ± 1          | 276 ± 25           | 99 ± 9            | 73 ± 2                              |            | 2.0 ± 0.2       |           |
| Mon                               | 08-Jul-2002  | 143 760   | 525 ± 35         |                    |                   |                                     |            | 2.0 ± 0.1       | 3.5 ± 0.3 |
| Mon                               | 15-Jul-2002  |   |                  |                    |                   |                                     |            |                 | 3.3 ± 0.1 |
| av <sup>c</sup> ± SD <sup>d</sup> | 156 610  | 427 ± 69  | 331 ± 53         | 95 ± 15            | 71 ± 11           | 2.5 ± 0.1                           | 2.2 ± 0.4  | 3.1 ± 0.4       |           |
| <b>Norfloxacin</b>                |  |   |                  |                    |                   |                                     |            |                 |           |
| Wed                               | 04-Oct-2000  | 144 955   | 494 ± 14         | 489 ± 43           | 70 ± 5            | 54 ± 5                              | 2.4 ± 0.2  | 1.7 ± 0.1       | 2.3 ± 0.2 |
| Thu                               | 05-Oct-2000  | 137 920   | 515 ± 8          | 428 ± 5            | 78 ± 4            | 64 ± 3                              | 2.5 ± 0.2  | 2.0 ± 0.1       |           |
| Fri                               | 06-Oct-2000  | 198 720   | 382 ± 12         | 327 ± 12           | 68 ± 2            | 50 ± 1                              |            |                 |           |
| Sat                               | 07-Oct-2000  | 136 293   | 484 ± 29         | 473 ± 6            | 68 ± 5            | 53 ± 4                              |            |                 |           |
| Sun                               | 08-Oct-2000  | 121 599   | 429 ± 39         | 339 ± 6            | 65 ± 1            | 49 ± 3                              | 2.7 ± 0.2  | 2.3 ± 0.1       |           |
| Mon                               | 09-Oct-2000  | 214 754   | 343 ± 14         | 327 ± 3            | 67 ± 6            | 39 ± 1                              | 2.6 ± 0.1  | 2.3 ± 0.2       |           |
| Tue                               | 10-Oct-2000  | 154 910   | 433 ± 14         | 348 ± 18           | 70 ± 6            | 52 ± 2                              |            | 2.0 ± 0.2       |           |
| Mon                               | 08-Jul-2002  | 143 760   | 435 ± 21         |                    |                   |                                     |            | 2.0 ± 0.1       | 3.3 ± 0.1 |
| Mon                               | 15-Jul-2002  |   |                  |                    |                   |                                     |            |                 | 3.1 ± 0.1 |
| av <sup>c</sup> ± SD <sup>d</sup> | 156 610  | 431 ± 45  | 383 ± 61         | 69 ± 15            | 51 ± 7            | 2.6 ± 0.1                           | 2.1 ± 0.2  | 2.9 ± 0.4       |           |

<sup>a</sup> Mean and standard deviation of duplicate measurements. <sup>b</sup> The relative error of daily flow measurements is estimated to be around 10%. <sup>c</sup> Average concentration normalized to *Q*, calculated as  $\sum(Q_i \times \text{conc.})/\sum Q_i$ . <sup>d</sup> Standard deviation of weekly variation.

mg/kg, respectively. Similar validation parameters were assumed for suspended solids.

## Results and Discussion

**Concentrations and Mass Flows in Wastewater Effluents and Sewage Sludges.** Concentrations of the most used FQs in Switzerland, ciprofloxacin (CIP) and norfloxacin (NOR), in filtered wastewater effluents and sewage sludges of Zurich-Werdhölzli WWTP are given in Table 2. Over the sampling period, FQ concentrations are comparable and within the range of those previously reported (6, 9). Concentrations of individual FQs varied in filtered raw sewage from 315 to 571 ng/L and in filtered primary effluents from 272 to 489 ng/L. In samples of filtered secondary effluents, FQ concentrations ranged from 65 to 109 ng/L and in filtered tertiary effluents from 39 to 87 ng/L. In addition, FQs were measured in suspended solids (SS) of raw sewage at concentrations of 180 ng/L CIP and 200 ng/L NOR. These concentrations are calculated based on measured sorbed concentration of 0.9 mg CIP/kg SS and 1.0 mg NOR/kg SS, assuming an average suspended solids concentration of 200 mg SS/L of raw sewage (estimated range 150–300 mg SS/L). For excess, raw and anaerobically digested sludge the concentration of individual FQs ranged from 2.3 to 2.7 mg/kg, 1.7 to 2.7 mg/kg, and 2.3 to 3.5 mg/kg, respectively.

Mass flows of individual FQs were determined for 24-h composite wastewater effluents and grab samples of sewage

sludge (Figure 2). Table 3 gives the average mass flow of each FQ at the various treatment stages, and its relative contribution to the total FQ-input entering the WWTP. A similar contribution of each FQ to the total FQ mass flow entering the treatment plant as well as during the treatment process was observed. The mass flow of FQs entering the Zurich-Werdhölzli WWTP as dissolved fraction in filtered raw sewage ranged from 43 to 83 g/d ( $m_{\text{CIP}} = 67 \pm 12$  g/d;  $m_{\text{NOR}} = 68 \pm 8$  g/d), and it was  $26 \pm 2$  g/d for CIP and  $29 \pm 3$  g/d for NOR sorbed to suspended solids of raw sewage. Therefore, of the total FQ-inputs entering the WWTP as raw sewage, about one-third is associated to suspended solids. For filtered primary effluents, the mass flow varied from 41 to 71 g/d ( $m_{\text{CIP}} = 52 \pm 9$  g/d;  $m_{\text{NOR}} = 61 \pm 10$  g/d). Moreover, assuming a suspended solids concentration in primary effluents of about 30% of the one in raw sewage (estimated range 50–100 mg SS/L), a mass flow of 8 g/d CIP and 9 g/d NOR are expected to occur sorbed to suspended solids of primary effluent. For filtered secondary and tertiary effluents, the mass flow of individual FQs varied from 8 to 19 g/d ( $m_{\text{CIP}} = 15 \pm 3$  g/d;  $m_{\text{NOR}} = 11 \pm 2$  g/d) and from 6 to 13 g/d ( $m_{\text{CIP}} = 11 \pm 2$  g/d;  $m_{\text{NOR}} = 8 \pm 1$  g/d), respectively. For such effluents the suspended solids concentration in relation to raw sewage can be assumed to be less than 5% per secondary effluent (estimated range 5–15 mg SS/L) and around 1% for tertiary effluents (estimated range 1–5 mg SS/L). Thus, the amount of FQs sorbed to suspended solids of such effluents

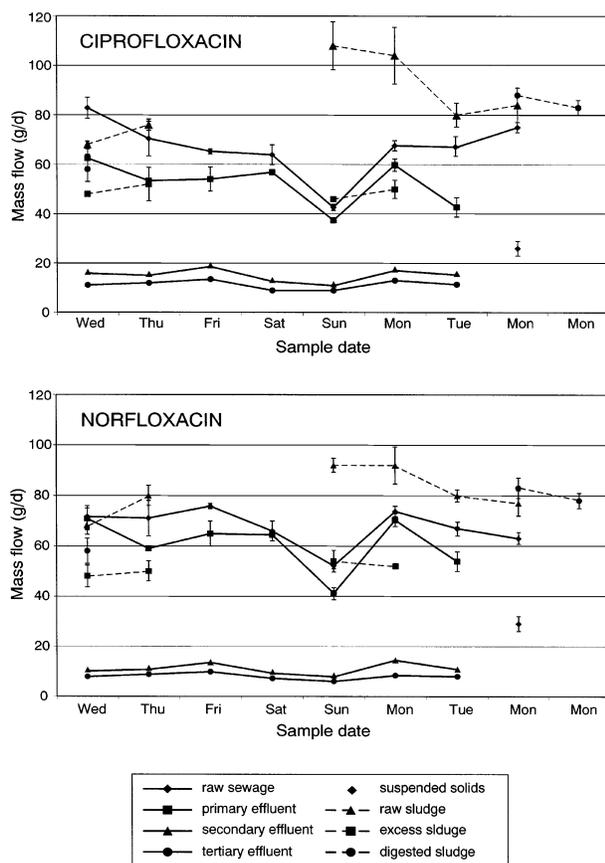


FIGURE 2. Mass flows of ciprofloxacin and norfloxacin during the study period in filtered wastewater (raw sewage, primary effluent, secondary effluent, and tertiary effluent), suspended solids of raw sewage, and sewage sludge (excess sludge, raw sludge, and anaerobically digested sludge).

can be neglected, and so only the filtered fraction contributes to the total FQ mass flow occurring in secondary and tertiary effluents.

The mass flow of FQs associated with excess sludge varied from 46 to 54 g/d with an average of  $m_{\text{CIP}} = 49 \pm 2$  g/d and  $m_{\text{NOR}} = 51 \pm 2$  g/d. For raw sludge, the mass flow ranged from 68 to 108 g/d ( $m_{\text{CIP}} = 87 \pm 16$  g/d;  $m_{\text{NOR}} = 82 \pm 9$  g/d). The amount of FQs associated with anaerobically digested sludge varied from 58 to 88 g/d ( $m_{\text{CIP}} = 78 \pm 11$  g/d;  $m_{\text{NOR}} = 73 \pm 11$  g/d) and was used to determine the mass flow of FQs exported from the wastewater treatment plant with the digested sludge.

For filtered raw sewage and primary effluent, the mass flow of FQs fluctuated considerably in comparison to those of filtered secondary and tertiary effluents (Figure 2). This difference is due to the mixing effect and the long residence time during wastewater treatment that contributes to a better homogenization of FQs. Remarkable, however, is the lower mass flow of FQs entering the Zurich-Werdhölzli WWTP on Sunday, which corresponds to only 52–67% on the mass flow of other days. This trend correlates well with the person-traffic movement to Zurich City (the residential population is 58% of the total population during business days). The lower variability observed for FQ mass flows in excess sludge compared to raw sludge is due to the complete mixing of the excess sludge in the activated sludge reactors and the 11 days solid retention time. The high mass flow observed for raw sludge can be attributed to the fact that it accounts not only for the sorbed fraction removed with the settled particles of the raw sewage but also for the amount of FQs associated with the excess sludge that also settles in the primary clarifier.

It should be noted that the percentage of FQs excreted as conjugates, which may be hydrolyzed and reverted to the parent compound during wastewater treatment, is minor (<2% of the administered dose (11)). The results here presented give support to previous assumptions that regarded FQs excreted in urine as the only source of FQs to aqueous pollution (6).

**Behavior during Wastewater Treatment.** The similarity between the composition of raw sewage (filtered wastewater and suspended solids) (49% CIP, 51% NOR) and that of the human consumption pattern in Switzerland (48% CIP, 42% NOR) suggests that little alteration of the FQ distribution occurs during transport to the wastewater treatment plant. Likewise, about one-third of FQs are excreted in feces (11), which compares well to the percentage of FQs found associated with suspended solids of raw sewage. Within the wastewater treatment plant, the behavior and degree of removal for FQs will depend on their physicochemical properties. Based on the mass flows presented in Figure 2 and Table 3, Figure 3 shows the magnitude of FQ mass transfer (% relative to single FQ-input) in the Zurich-Werdhölzli WWTP that indicates the importance of each stage of treatment on the fate of FQs during wastewater treatment. During mechanical treatment, FQs are removed from the total input mass flow (raw sewage = dissolved in filtered raw sewage + sorbed in suspended solids of raw sewage) up to  $35 \pm 10\%$  of CIP and  $28 \pm 11\%$  of NOR. The FQs associated to suspended solids are here deposited, and in addition probably dissolved FQs might also sorb onto the recirculated excess sludge, that is added at the inlet of the grit removal tank. The main removal of FQs occurs, however, during biological treatment ( $49 \pm 9\%$  CIP,  $61 \pm 9\%$  NOR). Finally, an additional  $4 \pm 1\%$  of CIP and  $3 \pm 2\%$  of NOR is removed in the flocculation-filtration step, probably due to sorption of FQs to small particles and precipitates originating from the iron added to the filter inlet, which are removed during filtration. The combined removal during wastewater treatment is  $88 \pm 2\%$  for CIP and  $92 \pm 1\%$  for NOR. Because the percentage of FQs associated with excess sludge ( $53 \pm 2\%$  CIP,  $53 \pm 3\%$  NOR) roughly corresponds to the FQ removal during activated sludge treatment and flocculation-filtration ( $53 \pm 9\%$  CIP,  $64 \pm 9\%$  NOR), the major process relevant for removing FQs is sorption to sewage sludge. Likewise, the overall removal of FQs is within the range of recovered FQs in the raw sludge ( $94 \pm 19\%$  CIP,  $85 \pm 10\%$  NOR). This finding confirms that the observed removal of FQs from the wastewater stream can be attributed to sorption processes and not to biodegradation during activated sludge treatment. Thus, biodegradation appears to be of minor importance in the elimination of FQs during wastewater treatment. This argumentation is supported by the low biodegradability of FQs shown during laboratory experiments (13, 14). Although FQs are degraded by direct sunlight-photolysis (22), this process appears to be of minor significance, probably because sunlight cannot penetrate deeper water layers as high turbidity is generated by suspended solids.

FQs seem quite stable under methanogenic (anaerobic) conditions in the sludge digesters. A difference of only about 10% was observed between the FQ mass flow entering the digesters as raw sludge and the anaerobically digested sludge, which can be attributed to the average character of the digested sludge (~30 days residence time in the digester). In addition, anaerobically degradable compounds are commonly eliminated by a considerable extent (23), whereas FQs are still largely found in anaerobically digested sludge ( $83 \pm 14\%$  CIP and  $75 \pm 14\%$  NOR).

**Sludge-Water Partitioning.** The tendency of a chemical to sorb and accumulate to solids can be assessed by the octanol-water partition coefficient ( $K_{\text{ow}}$ ). This approach,

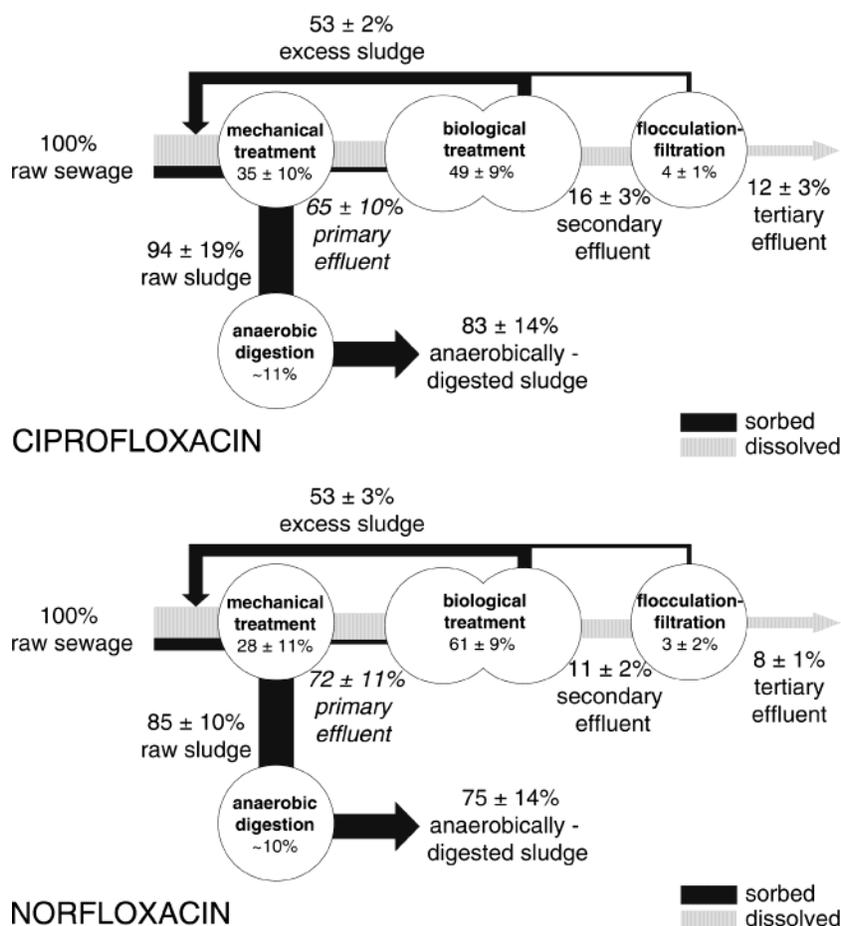


FIGURE 3. Relative mass transfer and removal (in %) of ciprofloxacin and norfloxacin through mechanical treatment, biological treatment, flocculation-filtration, and anaerobic digestion in Zurich-Werhölzli WWTP (100% equal to single FQ-input mass flow entering the WWTP). Estimated values are given in cursive (see text for details).

TABLE 3. Average Mass Flows<sup>a</sup> (g/d) of FQs during Wastewater Treatment in Zurich-Werhölzli WWTP for the Study Period

|  | mass flow (g/d)            |                           |                |
|--|----------------------------|---------------------------|----------------|
|  | ciprofloxacin              | norfloxacin               | total FQs      |
| raw sewage                                 | 93 ± 12 (49%) <sup>a</sup> | 97 ± 9 (51%) <sup>a</sup> | 190 ± 9 (100%) |
| filtered raw sewage                        | 67 ± 12 (35%)              | 68 ± 8 (36%)              | 135 ± 7 (71%)  |
| suspended solids <sup>b</sup>              | 26 ± 2 (14%)               | 29 ± 3 (15%)              | 55 ± 5 (29%)   |
| primary effluent                           | 60 ± 9 (31%)               | 70 ± 10 (37%)             | 130 ± 18 (68%) |
| filtered primary effluent                  | 52 ± 9 (27%)               | 61 ± 10 (32%)             | 113 ± 18 (59%) |
| suspended solids <sup>c</sup>              | 8                          | 9                         | 17             |
| secondary effluent (filtered) <sup>d</sup> | 15 ± 3 (8%)                | 11 ± 2 (6%)               | 26 ± 5 (14%)   |
| tertiary effluent (filtered) <sup>d</sup>  | 11 ± 2 (6%)                | 8 ± 1 (4%)                | 19 ± 3 (10%)   |
| excess sludge                              | 49 ± 2 (26%)               | 51 ± 2 (27%)              | 100 ± 3 (53%)  |
| raw sludge                                 | 87 ± 16 (46%)              | 82 ± 9 (43%)              | 169 ± 2 (89%)  |
| digested sludge                            | 78 ± 11 (41%)              | 73 ± 11 (38%)             | 151 ± 10 (79%) |

<sup>a</sup> Percentage of each FQ relative to the total FQ-input mass flow entering the wastewater treatment plant (raw sewage = FQs dissolved in filtered raw sewage + FQs sorbed in suspended solids). <sup>b</sup> Measured mass flow of duplicate analysis; assuming a suspended solids concentration of about 200 mg SS/L of raw sewage. <sup>c</sup> Estimated mass flow assuming a suspended solids concentration in the primary effluent of about 30% of the one in raw sewage. <sup>d</sup> Assuming a suspended solids concentration in secondary and tertiary effluents of about 5% and 1% respectively of the one in raw sewage, respectively, therefore the mass flow of FQs sorbed to suspended solids of such effluents can be neglected. <sup>e</sup> Estimated mass flows are given in cursive (see text for details).

however, only applies to uncharged molecules, since  $K_{ow}$  values can only be used to describe hydrophobic interactions. In this way, uncharged chemicals with  $\log K_{ow} < 2.5$  are assumed to show a low sorption potential (24). Because of their zwitterionic character ( $pK_{aCOOH} = 5.9-6.4$ ,  $pK_{aNH_2} = 7.7-10.2$ ) and despite their negative  $K_{ow}$ , FQs exhibit high sorption properties as inferred from the high  $K_d$  values obtained in other studies with various solids [see Table 4 (14, 15, 25-32)]. This can be attributed to the particular sorption mechanism of FQs, which seems to occur mainly by the

effects of electrostatic interactions, although hydrophobic forces are apparently also involved (25, 26, 33). Therefore, to evaluate the sorption properties of FQs, experimental solid-water partition coefficients ( $K_d$ ) with a strict control over pH conditions are necessary, as suggested by some authors (27, 34).

Assuming equilibrium between the fraction of FQs sorbed on activated sludge and the fraction of FQs dissolved in wastewater in the activated sludge reactors (~8 h hydraulic residence time), we calculated an experimental sludge-

TABLE 4. Physicochemical Constants and Solid–Water Partition Coefficients (L/kg) of Selected FQs

|               | acidity                             |                                     | octanol–water partition coefficient       | solid–water partition coefficients  |                                     |                                     |
|---------------|-------------------------------------|-------------------------------------|---|-------------------------------------|-------------------------------------|-------------------------------------|
|               | p <i>K</i> <sub>aCOOH</sub>         | p <i>K</i> <sub>aNH2</sub>          | log <i>K</i> <sub>ow</sub>                | log <i>K</i> <sub>d</sub> sludge    | log <i>K</i> <sub>d</sub> DOM       | log <i>K</i> <sub>d</sub> soil      |
| ciprofloxacin | 5.9 <sup>a</sup> – 6.1 <sup>b</sup> | 8.7 <sup>b</sup> – 8.9 <sup>a</sup> | (–1.1) <sup>f</sup> – (–0.9) <sup>g</sup> | 4.3 <sup>j</sup>                    | 2.4 <sup>n</sup>                    | 2.6 <sup>p</sup>                    |
| norfloxacin   | 6.3 <sup>b</sup> – 6.4 <sup>a</sup> | 8.4 <sup>b</sup> – 8.6 <sup>a</sup> | (–1.0) <sup>f</sup> – (–1.0) <sup>g</sup> | 4.2 <sup>j</sup>                    | 2.7 <sup>n</sup>                    | nd                                  |
| trovafloxacin | nd                                  | nd                                  | nd  | 3.5 <sup>k</sup> – 3.8 <sup>l</sup> | nd                                  | nd                                  |
| gemifloxacin  | 6.4 <sup>c</sup>                    | 9.0 <sup>c</sup>                    | 0.0 <sup>h</sup> – 0.2 <sup>i</sup>       | 4.1 <sup>m</sup>                    | nd                                  | nd                                  |
| sarafloxacin  | 6.2 <sup>d</sup>                    | 10.2 <sup>d</sup>                   | nd  | nd                                  | 4.3 <sup>o</sup> – 4.7 <sup>o</sup> | nd                                  |
| enrofloxacin  | 6.2 <sup>d</sup> – 6.3 <sup>e</sup> | 8.0 <sup>d</sup> – 7.7 <sup>e</sup> | (–1.6) <sup>d</sup> – (2.1) <sup>e</sup>  | nd                                  | nd                                  | 2.7 <sup>p</sup> – 3.7 <sup>r</sup> |

wastewater partition coefficient (*K*<sub>d,sludge–wastewater</sub> in L/kg) for FQs according to eq 1

$$K_{d,sludge-wastewater} = C_{sludge}/C_{diss} \quad (1)$$

where *C*<sub>sludge</sub> corresponds to the FQ concentration in activated sludge (μg/kg) and *C*<sub>diss</sub> to the average dissolved concentration in the activated sludge reactors (μg/L), calculated with eq 2

$$C_{diss} = [(C_{diss,prim\ effluent} + R \cdot C_{diss,sec\ effluent}) / (1+R) + C_{diss,sec\ effluent}] / 2 \quad (2)$$

with *R* = recycle flow/inlet flow ≈ 2.

Using this approach, log *K*<sub>d,sludge–wastewater</sub> values of 4.2 and 4.3 L/kg were calculated for CIP and NOR, respectively, at a pH between 7.5 and 8.4. These values are similar to sludge–water sorption coefficients obtained from batch experiments for other FQs such as trovafloxacin (14) and gemifloxacin (15) (see Table 4). In ref 14 trovafloxacin sorption to sludge seemed to depend on equilibrium time (test for 8 and 96 h) and not on the solid concentration. The sludge–wastewater partition coefficients reported here fall within the log *K*<sub>d</sub> range reported for FQs and dissolved organic matter or soils (see Table 4).

For suspended solids in raw sewage, a log *K*<sub>d</sub> of about 3.4 for both CIP and NOR was calculated based on measured concentrations of FQs in filtered raw sewage and in suspended solids of raw sewage and accounting for an average of suspended solids concentration in the raw sewage of 200 mg/L. This value is significantly lower than the *K*<sub>d</sub> values for activated sludge, probably due to the high fat content of the suspended solids in the raw sewage, which effect on sorption based on electrostatic interaction is minor.

**Fate in Sludge-Treated Soils.** The widespread practice of applying sewage sludge as fertilizer to agricultural land emphasizes the need to assess the fate of FQs in sludge-treated soils. With that aim, measured environmental concentrations (MECs) were compared to predicted environmental concentrations (PECs) and related with literature data on sorption and biodegradation.

To estimate the exposure of FQs in Swiss agricultural soils, we considered the concentration range obtained for FQs in sewage sludge, as obtained in this and previous studies (1–4 mg/kg) (9, 10). Given the current practice of applying sewage sludge to agricultural soils, at a maximum allowable rate of 5 tons/ha sludge dry matter to farmland during a period of 3 years, the expected loading rate of CIP and NOR to agricultural soils is 5–20 g/ha every 3 years. Assuming an even distribution of FQ residues to 5 cm depth and a soil density of 1.5 g/cm<sup>3</sup> (35), an amount of 0.07–0.30 mg/kg for each FQ in soil was calculated. This predicted environmental

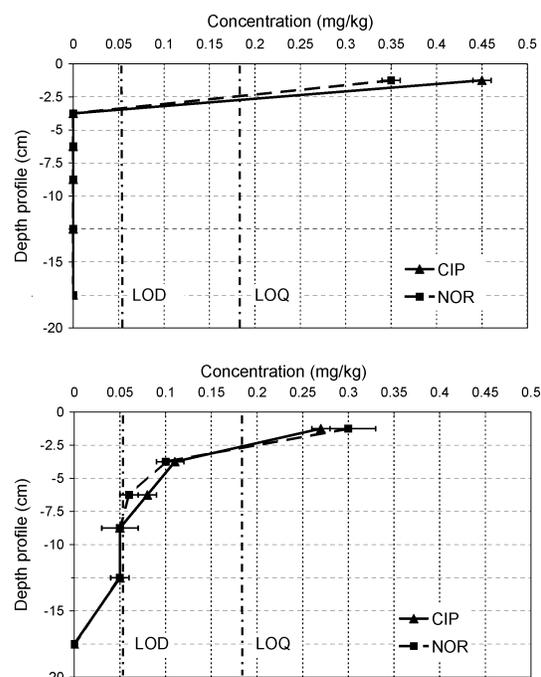


FIGURE 4. Soil profile of ciprofloxacin and norfloxacin concentrations after 5 months (a) and 21 months (b) following sludge-application of 50 t/ha, corresponding to 10 times the amount normally applied in Switzerland. Each point corresponds to an averaged concentration at the depth ranges: 0–2.5 cm, 2.5–5 cm, 5–7.5 cm, 7.5–10 cm, 10–15 cm, 15–20 cm. LOD: limit of detection, LOQ: limit of quantification.

concentration (PEC) is above the so-called “phase I trigger value” of 0.01 mg/kg recommended for pharmaceutical residues in Europe by the European Commission (35, 36), implying the need of assessing the occurrence and behavior of FQs in sludge-treated soils.

Our experimental field study aimed at investigating the behavior and fate of FQs after application of sewage sludge under severe conditions (application rate 10 times the allowed amount in Switzerland every third year), rather than simulating normal sludge-disposal practices. Figure 4 shows concentration versus depth profile of FQs measured in the sludge-treated soil of the experimental site, 5 and 21 months after sludge disposal. The FQs demonstrated some level of persistence over the study period (21 months) with residual soil concentrations in the μg/kg range. During the initial period, FQs seemed to accumulate in the topsoil, and thereafter only a limited mobility to the subsoil was observed (measurements below the limit of quantification but above

the limit of detection). These results are in agreement with the expected immobility of FQs given by the high sorption coefficients for solids (see log  $K_{d,soils}$  Table 4). The slight mobility observed could be due to dissolved organic matter (DOM) facilitated transport in soils (37, 38) (see log  $K_{d,DOM}$  Table 4).

The MECs obtained for the experimental plot were compared with estimated PECs, taking into account the sludge application rate to the experimental field (50 t/ha), and the depth range where FQs were determined. For the first period (up to 5 months), FQs were considered evenly distributed in the top 2.5 cm, and a PEC range of 1.40–6.00 mg/kg for each FQ was calculated. For the second phase (up to 21 months), a PEC range of 0.18–0.75 mg/kg was predicted if FQs were evenly distributed in the 20 cm topsoil. The MECs obtained in the top 2.5 cm were  $0.45 \pm 0.10$  mg/kg for CIP and  $0.35 \pm 0.10$  mg/kg for NOR, whereas the MEC ranged between 0.05 and 0.30 mg/kg from 0 to 20 cm depth (Figure 4). The fact that the MECs were always significantly lower than the PECs suggests that within the study period (0–5 months) FQs underwent partial disappearance either due to bio- or phototransformation. No data on phototransformation of FQs in soils are available in the literature; however, biotransformation half-lives between 3 and 5 months (39) and mineralization rates up to 27% within 8 weeks (40, 41) have been reported for several groups of soil microorganisms.

Therefore, biodegradation (or phototransformation) could have taken place in an initial phase, followed by long-term persistence in the soil. The observed persistence could either be from (1) incorporation of FQs into soil particles or a more strong sorption, making them less bioavailable to microorganisms in soil, or (2) simply because FQ concentrations have reached the biodegradable concentration threshold. Similar behavior has commonly been reported for pesticides and other micropollutants (42).

The same persistence and limited mobility was observed for FQs when sludge was applied at 5-fold the common amount in Switzerland (data not shown). Therefore, assuming that 1-fold the allowed amount results in the same behavior, concentrations of FQs would still be around the European "phase I trigger value" of 0.01 mg/kg (35, 36).

In conclusion, our results suggest that if any biodegradation (or phototransformation) of FQs occurs in soils, it is not complete, and residual FQs persist in agricultural soils. Therefore, the possibility of a continuous increase of FQ concentration with each addition of sludge cannot be excluded, although the limited mobility of FQs to the subsoil seems to rule out any threat to groundwater due to leaching.

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