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Assessing the fate of antibiotic contaminants in metal contaminated soils four years after cessation of long-term waste water irrigation

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ABSTRACT

Spreading of urban wastewater on agricultural land may lead to concomitant input of organic and inorganic pollutants. Such multiple pollution sites offer unique opportunities to study the fate of both heavy metals and pharmaceuticals. We examined the occurrence and fate of selected antibiotics in sandy-textured soils, sampled four years after cessation of 100 years irrigation with urban wastewater from the Paris agglomeration. Previous studies on heavy metal contamination of these soils guided our sampling strategy. Six antibiotics were studied, including quinolones, with a strong affinity for organic and mineral soil components, and sulfonamides, a group of more mobile molecules. Bulk samples were collected from surface horizons in different irrigation fields, but also in subsurface horizons in two selected profiles. In surface horizons, three quinolones (oxolinic acid, nalidixic acid, and flumequine) were present in eight samples out of nine. Their contents varied spatially, but were well-correlated one to another. Their distributions showed great similarities regarding spatial distribution of total organic carbon and heavy metal contents, consistent with a common origin by wastewater irrigation. Highest concentrations were observed for sampling sites close to irrigation water outlets, reaching $22 \mu\text{g kg}^{-1}$ for nalidixic acid. Within soil profiles, the two antibiotic groups demonstrated an opposite behavior: quinolones, found only in surface horizons; sulfamethoxazole, detected in clay-rich subsurface horizons, concomitant with Zn accumulation. Such distribution patterns are consistent with chemical adsorption properties of the two antibiotic groups: immobilization of quinolones in the surface horizons ascribed to strong affinity for organic matter (OM), migration of sulfamethoxazole due to a lower affinity for OM and its interception and retention in electronegative charged clay-rich horizons. Our work suggests that antibiotics may represent a durable contamination of soils, and risks for groundwater contamination, depending on the physicochemical characteristics both of the organic molecules and of soil constituents.

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1. Introduction

The elimination of antibiotics present in wastewater by sewage treatments is often incomplete. Low removal rates after wastewater treatments were reported for some compounds such as sulfamethoxazole, trimethoprim or some macrolides, ascribed to their low degradation and potential sorption on soil constituents (McArdell et al. 2003; Paxeus 2004; Lindberg et al. 2005). The discharge of treated wastewater into surface waters was recognized as a major source of antibiotics contamination (Miao et al. 2004; Siemens et al. 2008) and, consequently, cautious management of urban wastewater is essential.

Spreading of urban wastewater on soil surfaces is widely considered as an alternative way to decrease such an organic contamination discharged by wastewater (Ternes et al. 2007). The purpose of such a proceeding is to complete in a natural way the purification of wastewater by using the specific filtering and purifying characteristics of coarse textured soils, before their recharge to the groundwater. Such land management, however, may lead to a diffuse organic or inorganic contamination of soils depending on the quality of wastewater. Frequently, mineral contamination relates to the introduction and accumulation of heavy metals in soils. Although organic contaminants generally are more degradable, the fate of their long-term input in soils is not well known. Yet, such active compounds are designed to act upon soil microorganisms at low concentrations and, therefore, represent serious environmental risk (Westergaard et al. 2001; Schauss et al. 2009). Furthermore, heavy metals and antibiotic are suspected to act together in bacterial

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resistance selection or maintaining (Baker-Austin et al. 2006; Matyar et al. 2008), and many questions remain about long-term impacts on living organism by sub-inhibitory exposure in real field situations of multi organic and inorganic soil contamination.

Such concern is particularly important for antibiotics exhibiting high adsorbing properties, and for which accumulation in soil on the surface of either mineral or organic soil constituents has been assessed (Chang et al. 2009; Zhang et al. 2009). On the other hand, migration to deeper horizons of more mobile compounds represents additional risk regarding contamination of groundwater resources (Kinney et al. 2006; Ternes et al. 2007; Heberer et al. 2008; Siemens et al. 2010).

Moreover, some antibiotic compounds were shown to display relatively long half-lives, reaching several months in soils (Marengo et al. 1997), while others undergo a rapid biodegradation occurring within a few days (Burkhardt and Stamm 2007; Kotzerke et al. 2008). The sorption of antibiotics on soil constituents was shown to play a significant role in such persistence, related to a reduced biodegradability (Ingerslev and Halling-Sørensen 2001). It has been shown that the fate and impact of such compounds depend to a large extent on the nature of the molecules (Kinney et al. 2006; Ternes et al. 2007; Carrasquillo et al. 2008; Siemens et al. 2010) and on the nature and characteristics of the main soil constituents (Vasudevan et al. 2009; Zhang et al. 2009). However, in situ research on the distribution and accumulation of antibiotics at the field scale remains scarce, thus restricting sustainable land management regarding past or future spreading of wastewater.

Therefore, in this work we aimed at 1) investigating antibiotic contamination in agricultural soils irrigated for more than 100 years with untreated urban wastewater of the Paris area, previously studied for consequent heavy metal contamination; 2) assessing in situ the importance of the nature of the antibiotic molecules on their fate in soil profiles and 3) comparing the in situ fate of non-biodegradable trace elements to that of a priori biodegradable antibiotic molecules. We focused on two antibiotic groups exhibiting contrasting properties: i) the quinolones, known for their strong adsorption properties on soil constituents, and ii) the sulfonamides, with more mobile characteristics. Since metals and antibiotics were mentioned to be detected together (Lee et al. 2009; Oyetibo et al. 2010), we hypothesized that some inorganic and organic pollutants added to soils by wastewater irrigation might display similar distribution patterns, according to their physicochemical characteristics. Our sampling strategy of surface and subsurface horizons was based on previous field survey on heavy metal contamination (Lamy et al. 2006; Bourennane et al. 2006).

2. Materials and methods

2.1. Site presentation

The study site (Fig. 1) is located ≈ 65 km downstream of the center of Paris and approximately 6 km from the Seine Aval wastewater treatment plant, treating most of the Paris city and close suburbs wastewater (van Oort et al. 2008), estimated at about 300×10^6 m³/day at the end of the 1990s.

Since the 1890s, and for more than one century, raw wastewater has been transported to different irrigation fields, totalizing more than 5000 ha at the beginning of the 20th century, and still ~ 2000 ha in 2000 (Védry et al. 2001). Irrigation was done by flooding, with soils being immersed for one or more days, several times a year. The soils of these fields were chosen because of their coarse texture, allowing high water infiltration rates. Such irrigation practices enhanced agricultural use for market garden of the low fertile soils by strongly increasing crop yields, recognized as soon as 1895 (Risler 1897).

The actual irrigation perimeter, approximately 1200 ha, is located at the extremity of the general emissary of the Paris conurbation sewer network, ≈ 20 km NE of Paris. The irrigated perimeter is divided in five irrigation sectors, delivering irrigation water to about 60 irrigation fields, each field being bordered by a series of water outlets. The irrigation by gravitational flooding of wastewater is favored by annual plowing parallel to topography. During the 1990s the soils of this area still received annual rates of about 2000 mm of urban wastewater (Védry et al. 2001). Several reports stressed the strong accumulation of heavy metals leading to the stop of food crop production in 2000, while ongoing irrigation with wastewater clarified by flocculation was recommended for maize growth assigned to animal breeding. On the studied irrigation fields, irrigation was stopped during 2005.

2.2. Sampling

Based on the hypothesis that multi-pollution added in the soils by wastewater would lead to comparable distribution patterns, we used previous studies on distribution of concentrations of organic carbon and heavy metals in the surface horizons of the irrigated site (Bourennane et al. 2006) to select one characteristic irrigated parcel. In this parcel, on September 21st 2008, we collected nine bulk samples (P1–P9) in the middle of the plow layer, between 15 and 25 cm depth, following a 1 km wide topographical transect (Fig. 1). Sampling sites were selected in upslope (P9), middle or down slope positions (P1), and at various distances of wastewater irrigation

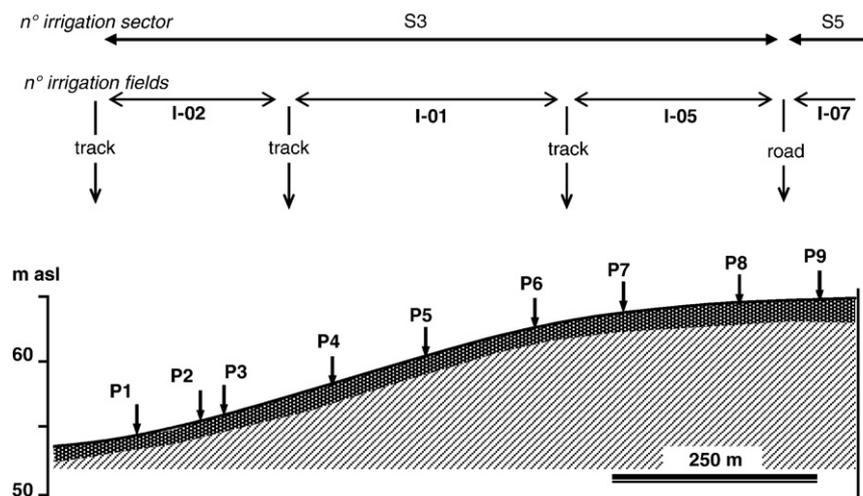


Fig. 1. Schematic presentation of the sampling locations P1 to P9, with respect to the irrigation fields and wastewater distribution sectors along a topographical transect.

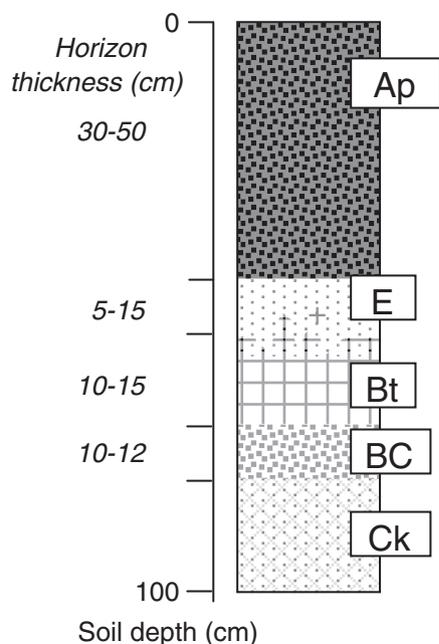


Fig. 2. Characteristic sequence of soil horizons observed in Luvisols of the irrigated plain. (Modified after van Oort et al., 2008).

outlets. This sampling scheme included two different irrigation sectors of the irrigation perimeter and crossed four different fields (Fig. 1).

Surface soil samples were separated in 5 cm thick slices, and stored frozen (-20°C) to prevent from degradation of organic compounds, prior to analysis performed within a few weeks. In addition, two sites (P3 and P9) were sampled until about 1.5 m, at different depths, following the occurrence and thickness of characteristic soil horizons.

2.3. Soil composition and metal pollutant distribution

The soils of the area were described as Luvisols developed in sandy residual formations resulting from decarbonation of different facies of Lutetian limestone. Irrigated soils generally demonstrate a sandy-textured topsoil with an approximately 30 to 50 cm thick dark-brown to black colored plow layer (Ap) and a more or less well-developed light colored eluvial (E) horizon (Fig. 2), overlying a reddish-brown illuvial clay-iron-enriched (Bt) horizon, at the contact of the partially weathered white-olive-green calcareous parent material (Lamy et al. 2006). The thickness and distinctness of soil horizons is variable (Table 1) depending on microtopography and position with regard to major irrigation water outlets.

The remarkable dark color of the Ap horizon is due to the accumulation of fine, amorphous organic matter (OM) forming coatings around the

quartz grains (van Oort et al. 2008), leading to significant high organic carbon contents (Table 1) for such sandy agricultural soils. In these coatings, numerous very fine, secondary calcite crystals occur, precipitating after evaporation of the waste water. Such coatings were highlighted to represent one of the main soil fractions involved in metal retention (van Oort et al. 2008), explaining the strong accumulation of heavy metals in the surface horizon. The surface horizons contain low clay contents (8–9% in Ap, <5% in E) that contrast with about 20% of $2\ \mu\text{m}$ fraction found in the Bt horizon (Table 1). The E and Bt horizon do not show any effervescence with 10% HCl, in contrast to the Ap (secondary carbonates) and the calcareous BC and Ck horizons. The mineralogical composition also significantly contrasts between the surface Ap and E horizons (Dère et al., 2006) dominated by Al-interlayered, non-swelling clay minerals, in contrast to the Bt, BC and Ck horizons displaying a predominant composition of swelling smectite (van Oort et al. 2008). Such a contrasting mineralogical clay composition is consistent with fairly low CEC values observed in the surface horizon, despite its high organic carbon content, whilst the Bt horizon demonstrates the highest CEC values (Table 1).

Within the irrigation fields, a great variability of metal concentrations, particularly well-correlated with the soil's total organic carbon contents was demonstrated (Bourennane et al., 2006) to a large extent explained by the geometry and distance of soil samples with respect to the irrigation outlets. At the scale of the soil profile, a consistent migration of more mobile Zn towards depth and its accumulation in the Bt horizon (Table 1) due to interception by clay-iron complexes, was clearly demonstrated by using major and trace element correlations (Lamy et al. 2006) and micro-spectroscopic analytical techniques on thin sections (Lamy et al. 2006; van Oort et al. 2008). Less mobile elements such as Pb were found predominantly immobilized in the OM-rich surface horizons.

2.4. Antibiotic analysis

A first selection of 17 compounds was made based on their use in human or veterinary medicine for monitoring in the Seine River (Tamtam et al. 2008), including quinolones (oxolinic acid, nalidixic acid, flumequine, pipemidic acid), fluoroquinolones (enrofloxacin, norfloxacin, ciprofloxacin, lomefloxacin, ofloxacin, sarafloxacin, difloxacin, danofloxacin, and enoxacin), sulfonamides (sulfamethoxazole, sulfamethazine), diaminopyrimidine (trimethoprim) and nitroimidazole (ornidazole). An optimization of soil extraction of the different compounds was performed. Soxhane extraction tests were made at various pH, from pH 2 to pH 10, in order to determine for each compound the optimal conditions for highest recovery. No single extraction pH gave sufficient recoveries for all the compounds from different groups, as the highest recoveries for quinolones were obtained at low extraction pH, with very low recoveries for fluoroquinolones and vice-versa at a high extraction pH. Further to that, a protocol involving two successive soxhane extractions was selected, including a first extraction at acidic pH 2 and then a second one at alkaline pH 10. With this protocol it was possible to obtain satisfactory recoveries for the few following compounds that were included in our work: sulfamethoxazole (sulfonamides), diaminopyrimidine (trimethoprim), nalidixic acid, oxolinic acid, pipemidic acid, flumequine (quinolones),

Table 1
Selected physicochemical data of soils irrigated with waste water. Mean values \pm SE from analyses of bulk horizons of three soil profiles, close to P3, P5, and P6; nd: not determined.

Horizon	Thickness (cm)	Clay		Sand	Org. C	CaCO ₃	pH	C/N	CEC (cmol ⁺ kg ⁻¹)	Bulk density (Mg m ⁻³)	Zn (mg kg ⁻¹)	Pb
		(g kg ⁻¹)	(g kg ⁻¹)									
Ap	39 ± 7	86 ± 5	767 ± 14	34.5 ± 6.5	38 ± 8.5	7.1 ± 0.3	15.4 ± 3.1	10.2 ± 2.1	1.40 ± 0.14	1055 ± 74	536 ± 90	
E	15 ± 5	47 ± 12	870 ± 23	1.8 ± 0.6	<1	7.4 ± 0.1	7.1 ± 0.4	3.4 ± 0.6	1.71 ± 0.03	222 ± 37	7.7 ± 0.3	
Bt	13 ± 6	199 ± 16	719 ± 17	2.6 ± 0.2	<1	7.5 ± 0.1	nd	13.3 ± 0.6	1.65 ± 0.06	402 ± 72	11.0 ± 0.7	
BC	11 ± 1	110 ± 18	748 ± 72	1.1 ± 0.6	299 ± 139	8.5 ± 0.2	nd	4.8 ± 1.4	1.61 ± 0.14	125 ± 57	5.7 ± 1.4	
Ck	>25	108 ± 10	716 ± 86	2.0 ± 1.0	409 ± 172	8.7 ± 0.2	nd	3.8 ± 1.5	nd	155 ± 131	4.2 ± 1.1	

Table 2
IUPAC names and physicochemical properties of the studied compounds.

Group compound	IUPAC Name	Solubility (g L ⁻¹)	pKa (1–2)	Log K _{ow}	K _{oc} (L/kg)	Half-life in sediment (d)
Sulfonamides						
Sulfamethoxazole	4-Amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide	0.61 ^a	6 ^b	0.89 ^b	607 ^c	dnf
Quinolones						
Nalidixic acid	1-Ethyl-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid	0.33 ^d	5.9 ^e	dnf	dnf	dnf
Oxolinic acid	1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid	0.004 ^f	6.9 ^f	0.68 ^f	7400–31,600 ^f	>300 ^g
Flumequine	7-Fluoro-12-methyl-4-oxo-1-azatricyclo[7.3.1.0 ^{5,13}]trideca-2,5,7,9(13)-tetraene-3-carboxylic acid	0.07 ^f	6.4 ^f	1.7 ^f	2750–24,500 ^f	>300 ^g
Fluoroquinolones						
Norfloxacin	1-Ethyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid	178 ^h	6.26–8.85 ^h	–1.03 ^h	7800–15,800 ⁱ	dnf

dnf: data not found.

^a Maskaoui et al. (2007).^b Gros et al. (2006).^c Drillia et al. (2005).^d Hari et al. (2005).^e Robberson et al. (2006).^f Tolls (2001).^g Hektoen et al. (1995).^h Vieno et al. (2006).ⁱ Zhang et al. (2009).

norfloxacin (fluoroquinolones). Physicochemical properties of the selected compounds are presented in Table 2.

Standard compounds of quinolones (oxolinic acid, nalidixic acid, flumequine), fluoroquinolones (norfloxacin) and sulfonamides (sulfamethoxazole) were obtained from Sigma-Aldrich (Steinheim, Germany) and diaminopyrimidine trimethoprim was purchased from VWR (France). All pharmaceuticals were of analytical grade (purity > 95%).

Antibiotics were extracted from 2 g of wet soil using microwave assisted Soxhlet extraction (Soxwave 100, Prolabo, France). A 50 mL mixture of methanol/H₃PO₄ aqueous solution (pH 2, 1 M) (90:10) was used as extraction solvent. Soil samples were extracted for 15 min (45 W). Then, samples were filtered (0.45 µm, Millipore acetate filters) and solid re-extracted using 50 mL of a methanol/NaOH (5%, pH 10) mixture (90:10), in the same conditions (15 min, 45 W). The two obtained extracts were pooled and evaporated with rotative evaporator (Büchi R-144 and R-205) at 42 °C, 280 bars for 30 min, and finally diluted in 100 mL ultra pure water (Millipore, France). Samples were then adjusted to pH 7 with few drops of orthophosphoric acid (50%), extracted and purified by Solid Phase Extraction prior to analysis by Liquid Chromatography tandem Mass Spectrometry (UPLC–MS/MS, Waters™) using operating analysis parameters described by Tamtam et al. (2009).

2.5. Method validation and quality control

Method was validated prior to the study on Seine River sediments spiked at 100 µg kg⁻¹ (triplicates), and left during 24 h for sorption and solvent evaporation. Some recovery tests on soils gave very similar results than on sediments, as reported in literature (Prat et al. 2006; Vazquez-Roig et al. 2010), thus a new validation based on soil samples was not necessary. Detection limits and quantification limits were calculated based respectively on 3 and 10 times the Signal/Noise (S/N) ratio obtained from the chromatograms of spiked sediments extracts (100 µg kg⁻¹). Due to strong matrix effects observed for the fluoroquinolone norfloxacin using standard addition, the high S/N ratio did not allow a detection limit lower than 12 µg kg⁻¹. Recoveries for each compound and method limits are given in Table 3. These detection and quantification limits are in the same range of limits reported in literature, which are often in the low µg kg⁻¹ range (Prat et al. 2006; Vazquez-Roig et al. 2010), except for norfloxacin

quantification limit, which was higher in this study than reported by Vazquez-Roig et al. (2010), close to 5 µg kg⁻¹.

2.6. Total organic carbon analysis

Total organic carbon contents were determined on air-dried, homogenized and quartered subsamples of the soils, crushed and sieved to <200 µm. Analyses were performed at the INRA – Soil Analysis Laboratory of Arras (France), according international standard methods (ISO no. 1-1380). All results are expressed on an oven-dry 110 °C basis.

3. Results and discussion

3.1. Spatial variability of the antibiotic distribution in the surface horizons

Contents in antibiotics are given Table 4 for the three antibiotics finally found in the soil surface samples. No antibiotic from the sulfonamide group was detected in the surface soil samples. The only antibiotics determined were from the quinolone group: nalidixic acid was always found above the detection limit except in the upslope sample, while oxolinic acid and flumequine were found in lower contents and often just detected but not quantified.

The highest concentrations were observed in the P3 and P6 samples with contents around 5–6 µg kg⁻¹, 21–22 µg kg⁻¹ and 6–7 µg kg⁻¹ for oxolinic acid, nalidixic acid and flumequine, respectively. The antibiotics detected in the surface soil samples were found

Table 3
Recoveries, relative standard deviation (RSD), detection limits (DL) and quantification limits (QL) for the six antibiotics of the study.

	Quantification ion (parent > daughter)	Recovery (%) ± RSD	DL (µg kg ⁻¹)	QL (µg kg ⁻¹)
Trimethoprim	291.1 > 261.1	51 ± 24	0.4	1.4
Sulfamethoxazole	253.9 > 155.9	58 ± 11	1.0	2.9
Oxolinic acid	262 > 244.1	63 ± 7	0.4	1.2
Nalidixic acid	233 > 215.1	85 ± 23	0.3	1.0
Flumequine	262 > 244.1	78 ± 17	0.7	2.2
Norfloxacin	320 > 276.1	42 ± 31	12.2	40.8

Table 4

Concentrations of the four most often found antibiotics in the surface soil samples or in the soil profiles and corresponding contents in total organic carbon in the samples.

	Depth (cm)	Soil horizon	Sulfamethoxazole ($\mu\text{g kg}^{-1}$)	Oxolinic acid ($\mu\text{g kg}^{-1}$)	Nalidixic acid ($\mu\text{g kg}^{-1}$)	Flumequine ($\mu\text{g kg}^{-1}$)	TOC (g kg^{-1})
P1	15	Ap	<ld	<1.2*	3.5	<2.2*	nd
P2	15	Ap	<ld	2.2	9.0	2.7	16.4
P3	15	Ap	<ld	4.8	20.8	6.9	38.9
	20	Ap	<ld	4.8	18.4	6.7	26.9
	40	E	<ld	<ld	3.8	<ld	1.5
	60	Bt	<ld	<ld	1.1	<ld	1.3
	75	Bt	2.5	<ld	<1*	<ld	2.8
	90	Bt	<ld	<ld	<1*	<ld	0.7
	100	BC	<ld	<ld	<1*	<ld	1.6
	115	BC	<ld	<ld	<ld	<ld	2.7
	130	Ck	<ld	<ld	<ld	<ld	0.9
P4	15	Ap	<ld	<1.2*	8.0	2.2	17.1
P5	15	Ap	<ld	1.2	6.2	<2.2*	14.5
P6	15	Ap	<ld	5.9	22.1	6.3	nd
P7	15	Ap	<ld	1.3	7.7	3.0	13.0
P8	15	Ap	<ld	1.2	8.1	2.9	15.9
P9	20	Ap	<ld	<ld	<ld	<ld	12.8
	35	E	<ld	<1.2*	4.7	<2.2*	1.2
	50	Bt	<ld	<ld	<ld	<ld	2.1
	70	Bt	<ld	<ld	<ld	<ld	1.3
	100	Bt	<ld	<ld	<ld	<ld	1.8
	120	BC	<2*	<ld	<ld	<ld	2.0
	150	Ck	<ld	<ld	<ld	<ld	1.4

(<ld) lower than the detection limit; (*) detected, but under the quantification limit; (nd) not determined.

well-related one to each other (Fig. 3), consistent with a common input into soils.

Furthermore, the antibiotic concentrations were also found related to the organic carbon contents in the surface horizons (Table 4), with correlation coefficients ≥ 0.9 for the three quinolones.

Both at the small region scale and at the scale of the studied irrigation fields, well-correlated distributions between organic carbon

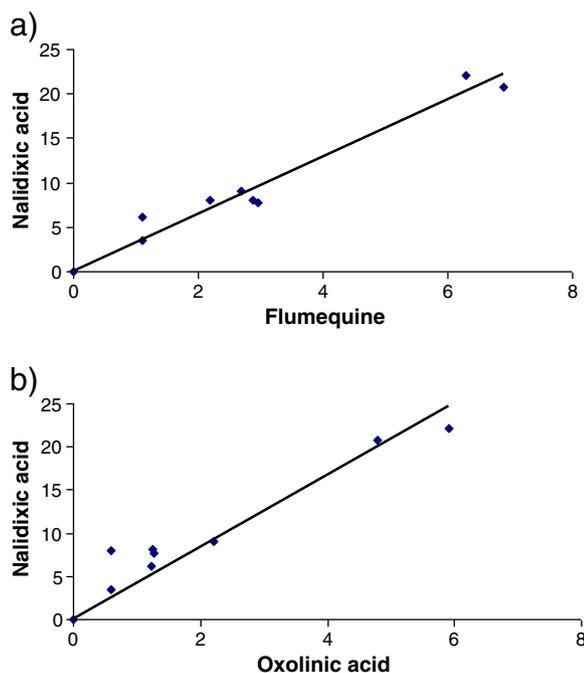


Fig. 3. Relation between antibiotic concentrations (in $\mu\text{g kg}^{-1}$) in the surface soil samples : (a) nalidixic acid vs. flumequine concentrations: $y=3.21x$ ($r^2=0.96$) and (b) nalidixic acid vs. oxolinic acid concentrations: $y=4.18x$ ($r^2=0.88$). When measured concentrations were between LD and LQ, concentrations were assimilated to half of the LQ.

and heavy metal contents were reported (Baize et al. 2002; Bourennane et al. 2006). These authors assumed that such correlation between heavy metals and organic carbon represented the signature of a common origin of pollution from waste water irrigation, which is also consistent with the presence of antibiotics.

As no direct veterinary waste inputs in the field were identified and considering the cessation of irrigation practices on the studied irrigation fields since 2005, our results suggest that the antibiotic contamination aged of several years. In broad literature, only few work deals with the persistence of antibiotics in terrestrial environments (Diaz-Cruz et al. 2003; Kinney et al. 2006), especially for quinolones. Quinolones antibiotics are either used in veterinary or human medicine, except for oxolinic acid, which is limited since 2006 only to veterinary use and therefore unlikely to occur in urban wastewater currently applied on the site. Unfortunately, antibiotic consumption data in France, allowing to estimate field concentration for each of the compounds, and to provide insight on the fate of the molecules over the years, are unavailable for the public. Nevertheless, in the light of the progressive abandon of first generation quinolone nalidixic acid in favor of more efficient compounds such as fluoroquinolones, we speculate for a hypothesis of either a progressive accumulation of nalidixic acid over the years, or a residual contamination from times where this compound was more widely used (in the 1960s).

Thus, our results are among the first to suggest that long-term wastewater irrigation on agricultural soil can induce long-term antibiotic contamination in soils. Persistence of several months was reported for some pharmaceuticals in similar conditions of sewage-irrigated soils (Kinney et al. 2006).

Surprisingly, norfloxacin, a widely used fluoroquinolone, on the market since the late 80s, was not detected in our soil samples, although norfloxacin concentrations as high as 150 ng L^{-1} were reported in the river water downstream of the WWTP discharge providing water for the irrigation field (Tamtam et al. 2008). A first explanation may be our relatively high detection limits for norfloxacin ($12 \mu\text{g kg}^{-1}$): lower concentration of norfloxacin in soils will not be detected, although such concentrations may be of the same order than those of the other compounds. Second, formation of non-extractable residues (NER) in soils, notably for sulfonamides was reported (Heise et al. 2006). This may also be the case for other groups such as quinolones and fluoroquinolones, but not reported yet. In that case, only sorption investigation in controlled condition of the fate of norfloxacin can make it possible to estimate levels of this compound in soil.

Spatial distribution of concentrations of antibiotics was found to depend on the geometry, functioning and proximity of the irrigation network, as was already reported for heavy metal pollution (Lamy et al. 2004; van Oort et al. 2008; Bourennane et al. 2006). Sampling locations close to major irrigation water outlets corresponded to the highest concentrations, either for antibiotics (this work) or for heavy metals. The highest antibiotic contamination levels were measured for the P3 and P6 samples, where heavy metal concentrations were also found the highest, i.e. ~ 1050 and $\sim 1230 \text{ mg kg}^{-1}$, for Zn in P3 and P6 respectively, compared to values of some hundreds mg Zn kg^{-1} observed for the other sampling sites, at greater distance from the water outlets.

3.2. Antibiotic distribution vs. depth in soil profiles

The contents in antibiotics determined at different soil depths in the two soil profiles (P3, P9) are presented in Table 4 as well as the total organic carbon contents. In addition, antibiotic concentrations are plotted vs. soil depth in Fig. 4, together with total Zn concentrations determined in the bulk of the characteristic soil horizons.

In the P3 profile (Fig. 4a), the highest concentrations of quinolones were observed in the plow (Ap) horizon. In the underlying E horizon,

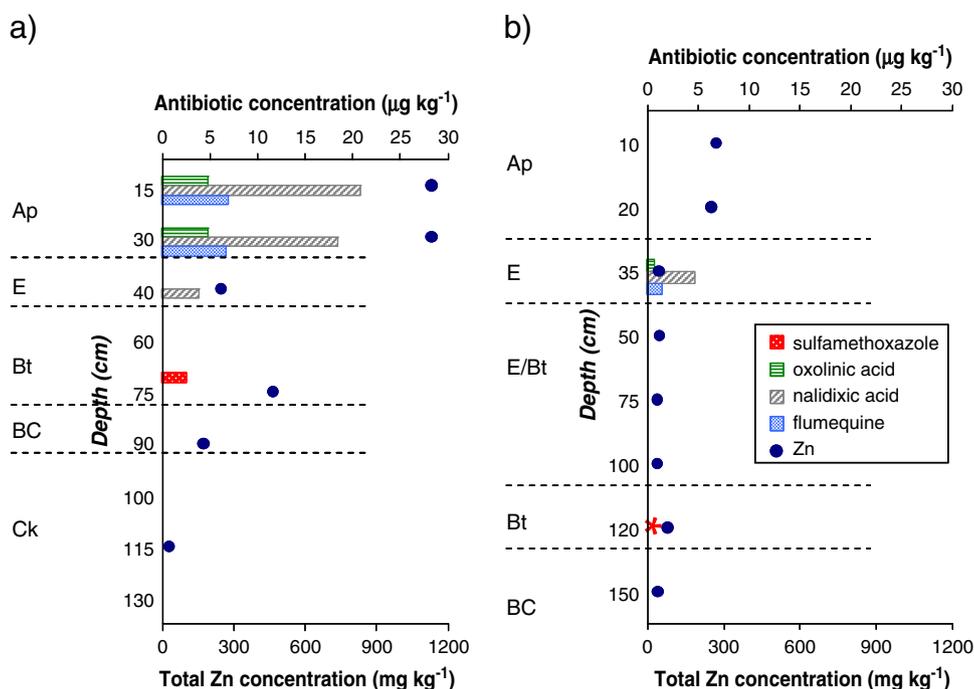


Fig. 4. Vertical distribution of antibiotic and total zinc concentrations in horizons of the soil profiles P3 (a) and P9 (b). Succession of horizons (Ap, E, Bt, BC, and Ck) are shown in dotted lines. Red star marks sulfamethoxazole detection under quantification limits.

oxolinic acid and flumequine were not detected, and the concentration of nalidixic acid decreased to values $<5 \mu\text{g kg}^{-1}$. At greater depth, no antibiotics of the quinolone group were detected. For the P9 profile (Fig. 4b), a different vertical distribution was observed for quinolones: oxolinic and nalidixic acid and flumequine were absent in the Ap horizon, but these compounds were detected in the underlying E horizon, with concentration levels fairly similar to those found in the E horizon of the P3 profile. By contrast, sulfamethoxazole was poorly detectable in samples from the plow horizons, but its presence was detected in the Bt horizons, at ~ 75 -cm depth for the P3 profile, and at ~ 120 -cm depth for the P9 profile. Surprisingly, sulfamethoxazole concentrations at such depths were higher than in samples from the surface horizons, which were generally $<1 \mu\text{g kg}^{-1}$ (Table 4). As outlined before, the low detection of sulfamethoxazole in the surface horizon may be due to the formation of NER via covalent bonding with organic matter, but its detection in the Bt horizons indicates that these compounds partially migrate to depth.

In the two studied soil profiles, the distribution of antibiotics demonstrated an opposite behavior between the two groups: quinolones were found mainly in the surface horizons, where the OM contents are highest, whereas sulfamethoxazole occurred only in deeper Bt horizons. Such a predominant immobilization of antibiotics at the soil's surface was also reported for fluoroquinolones norfloxacin and ciprofloxacin, which accumulated at concentrations of around $400 \mu\text{g kg}^{-1}$ in soils amended with sludge (Golet et al. 2003). The latter compounds were not detected in our study, but are likely to exhibit similar behavior than quinolones. These results suggest an OM-mediated location for quinolones, limiting their downward migration. The physicochemical characteristics of quinolones explain such strong sorption properties towards soil constituents (Tolls 2001; Golet et al. 2003): surface adsorption to mineral constituents, complex formation with metal ions and electrostatic interactions (Nowara et al. 1997; Tolls 2001; Diaz-Cruz et al. 2003), although interaction with other pollutants such as surfactants was shown to modify nalidixic acid sorption behavior (Hari et al. 2005). The non-detection of quinolones in the plow layer of the P9 profile may be related to low organic carbon and heavy metal contents at this

sampling site. Located at the upper slope position (Fig. 1), P9 makes part of another irrigation sector than the other sampling sites. Different irrigation management with respect to the amounts and quality of the waste water during the last years may be invoked to explain the various patterns of quinolones in surface samples. Nevertheless, the presence of oxolinic and nalidixic acid and flumequine in the E horizon of the P9 profile (Fig. 4b) indicates that such quinolone compounds have been added once in the plow layer.

By contrast, the detection of sulfamethoxazole at depth in clay-rich Bt horizons is in agreement with reported ability to migrate in soils like other sulfonamides (Blackwell et al. 2007; Burkhardt and Stamm 2007). These Bt horizons also intercept dissolved Zn out of the percolating soil solution by adsorption of Zn^{2+} on the negative charges of clay-iron complexes occurring as coatings (Dère et al. 2006; van Oort et al. 2006; van Oort et al. 2008), leading to a more or less marked increase in Zn concentrations. Such a concurrent presence of Zn and sulfamethoxazole in clay-rich B horizons suggests a common interception mechanism of electrostatic adsorption. The evidence for sulfamethoxazole migration is consistent with reported K_{oc} of several sulphonamides, varying from 97 to 323 L kg^{-1} , characteristic for mobile molecules (Thiele-Bruhn 2003). Such mobility was also invoked to explain the poor removal of sulfamethoxazole after wastewater treatment, as reported in numerous studies (Gobel et al. 2004; Lindberg et al. 2005; Batt et al. 2007). Nevertheless, significant but yet undetectable amounts of sulfonamides may be present in the surface horizons, according to different studies pointing the formation of NER for sulfonamides (Heise et al. 2006; Kahle and Stamm 2007), notably due to covalent bonding. If so, their distribution pattern in the Luvisol would be rather similar to that of Zn, with a main presence in the Ap horizon and a large interception in the Bt horizon (Table 4). Since sulfamethoxazole was detected in the clay-rich horizons of both soils profiles, we hypothesize that a non-negligible part of this compound was subject to downward leaching. In addition, our findings on accumulation of sulfamethoxazole to depths higher than 1 m corroborate with other studies reporting detection of sulfamethoxazole in groundwater (Lindsey et al. 2001; Sacher et al. 2001; Ternes et al. 2007).

4. Conclusion

This study assessed soil contamination by antibiotics from long-term wastewater irrigation. Among different antibiotics screened, four compounds were detected with different rates in soil samples: oxolinic acid, nalidixic acid, flumequine and sulfamethoxazole. Relations between concentrations of antibiotics, organic carbon, and metal pollutants (Zn), were found consistent with a single and same source of contamination.

Since the input of wastewater in these soils was decreasing since the 2000s, and stopped in 2005 (four years before our sampling campaign) the detection of significant amounts of antibiotics in the soil surface horizons highlights their persistence in soils. Concentration profiles of antibiotics vs. depth displayed a general pattern of higher quinolone contamination in the OM-rich plow layer, but low amounts of such compounds were still observed in the underlying, sandy-textured E horizons. The predominant presence of quinolones in the surface horizons is consistent with the strong adsorption properties of these molecules with large amounts of accumulated OM, likely to limit their downward migration. At the opposite, sulfamethoxazole was detected in clay-rich horizons, at great depth (75, 120 cm) at the same depths where Zn accumulated, suggesting similar immobilization mechanisms by electrostatic adsorption.

In the view of distribution patterns, according to their nature and reactivity, the occurrence of antibiotics and of heavy metal contaminants, both added to the soils by wastewater irrigation showed similarities: either a predominant accumulation with OM in the surface horizon (like quinolones and the heavy metals Pb, Cu), or a migration to depth and interception by clay-iron complexes (like sulfamethoxazole and the heavy metals Zn, Cd).

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