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Degradation of Gemfibrozil and Naproxen in a river water ecosystem

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ABSTRACT

Pharmaceuticals are nowadays generally recognized to be environmental micropollutants owing to their ubiquitous occurrence in water bodies at concentrations ranging from ng to μg/L. Since they are molecules designed to be biologically active at very low concentrations, their presence is a source of concern for both human and ecosystem health and the ecological effects on receiving ecosystems remain largely unknown.
Incomplete removal during biological wastewater treatments is the main source of surface water contamination. Some of the molecules detected are reported to be persistent in surface water while others, although they are not intrinsically persistent (being rapidly degraded), are being continuously introduced into the aquatic ecosystem, so that they can be considered pseudo-persistent compounds. Degradation of a chemical in the aquatic ecosystem depends on a variety of factors, including the compound's properties, environmental factors and above all the presence of a natural microbial community able to degrade it via metabolic and/or co-metabolic pathways.
Naproxen, a non-steroidal anti-inflammatory drug and Gemfibrozil, a fibrate drug used as a lipid regulator, have been found in several natural EU and Italian surface waters, including the River Tiber (Rome). In this context, the present work aims to evaluate if the natural bacterial community of the Tiber was able to degrade Naproxen and Gemfibrozil. Moreover the effects of these chemicals on the bacterial community structure in terms of live bacterial abundances and composition were also assessed.
For this purpose, different river water microcosms were set up (in the presence/absence of the natural microbial community) and treated with 100 μg/L of Naproxen or Gemfibrozil in order to evaluate the disappearance time of 50% of the initial concentrations (DT_{50}).
The overall results show that Gemfibrozil ($DT_{50} > 70$ days) was more persistent than Naproxen ($DT_{50} = 27$ days) and that the autochthonous microbial community had a key role in their degradation. The fact that Naproxen was found in river samples analyzed in a greater concentration was therefore due not to its persistence, but to its pseudo-persistence linked to the spread in its use among the population.

1. Introduction

Pharmaceutical occurrence in ecosystems is an emerging issue as they have been detected in soil, water and groundwater [1]. Thousands of different active compounds are currently in use in high quantities to treat or to prevent diseases [2–4]. Following therapeutic administration, a great percentage of pharmaceuticals is excreted unaltered or as an active metabolite, and enters sewage treatment systems [5]. The latter represent important points for pharmaceutical environmental pollution control, but they are currently unable to remove drugs effectively [6,7]. Consequently, many pharmaceuticals are continuously reaching surface waters [7,8] and even molecules, which are not intrinsically persistent, can be detected and can therefore be considered pseudo-persistent molecules [9].

Pharmaceuticals are designed to be biologically active at very low concentrations with different modes of action [10]. There is a lack of information on their detrimental effects on ecosystems especially from the period before the problem started being considered. There are only some limited data on single-species acute laboratory bioassays [11,12]. Despite being microcontaminants (detected at concentrations ranging from ng/L to μg/L) they can also have sub-lethal or chronic toxic effects [2,13,14] on non-target organisms [15,16], including microbial communities [17–19].

The European Medicine Agency (EMEA) issued a guideline [20] aimed at estimating the potential environmental risks of pharmaceuticals using a tiered approach. If in phase I the predicted environmental concentration of a compound in surface water is more than 10 ng/L, a phase II quantitative risk assessment on its environmental fate and effects is performed [21].

In Europe, among the most frequently detected pharmaceuticals in the aquatic environment, including drinking water, are the acidic pharmaceuticals Gemfibrozil and Naproxen [1,2,22].

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Although data on their environmental fate are quite scarce, Gemfibrozil and Naproxen are considered high priority pharmaceuticals on the basis of their consumption, physico-chemical properties, toxicity, water occurrence and persistence [23–25]. They have been included in the Current Contaminant Candidate List published by US EPA [26] aimed at identifying and listing unregulated contaminants which may require a national drinking water regulation in the future.

Naproxen is a non-steroidal anti-inflammatory drug used for mild to moderate pain relief and in the treatment of osteoarthritis and rheumatoid arthritis. Gemfibrozil is a blood lipid regulator, used to treat moderate to severe hypertriglyceridemia, clinically prescribed since the early 1980s in patients at high risk of coronary heart disease [27,28].

Recent investigations on EU and Italian waters report Naproxen and Gemfibrozil to occur, both in wastewater treatment plants and in surface waters, at concentrations from ten to five hundred-fold greater than the threshold value suggested by EMEA [1,8,29,30].

Degradation of a chemical in the aquatic ecosystem depends on a variety of factors, such as compound properties and environmental factors and above all the presence of a natural microbial community able to degrade it via metabolic and/or co-metabolic pathways [31,32]. Microorganisms are important degraders of organic matter and different organic contaminants, including pharmaceuticals, and make degradation and mineralization products accessible as nutrients to other organisms in the food web [33]. Microbial communities are therefore clearly vital for maintaining ecosystem functioning. Changes within microbial functional groups are correlated to changes in ecosystem processes [34–36]. Consequently they are essential in the overall processes that contribute to the quality state of natural ecosystems.

In this study, we assessed the capacity of the autochthonous bacterial community of the River Tiber to degrade Naproxen and Gemfibrozil in microbiologically active and sterile water microcosms. The degradation of 50% of the initial concentration (DT_{50}) of the two pharmaceuticals was evaluated in microbiologically active microcosms versus microcosms with previously sterilized river water. The concentration of the pharmaceuticals was measured immediately after the treatment and at fixed intervals until a reduction greater than 50% was reached for each compound.

Moreover the effects of these pharmaceuticals on the natural microbial community structure of the river water ecosystem were also assessed. At different times bacterial abundance, cell viability and bacterial composition were assessed and compared to those of microbiological controls (non-treated water samples).

2. Materials and methods

2.1. River water collection and characteristics

Water samples were collected from the River Tiber in a stretch inside the city of Rome, 382 km from its source and downstream from the Magliana wastewater treatment plant. Some parameters (pH, O_2, temperature) were analyzed on site and others were examined in the laboratory.

The samples were collected manually by immersing 1 L sterile glass bottles approximately 10 cm below the water surface and were transported to the laboratory within 2 h in a refrigerated (4 °C) bag. Some subsamples were fixed or treated immediately for the initial chemical and microbiological determinations, other ones were kept at 20 °C in the dark overnight prior to use for the microcosm set-up.

The ion content (HCO_3^-, F^-, Cl^-, NO_2^-, NO_3^-, SO_4^{2-}, PO_4^{3-}, expressed as mg/L) of river water samples was determined by using a Ion Chromatograph (Dionex DX-120). Aliquots of water samples were acidified and then analyzed for dissolved organic carbon content (DOC mg/L) by high temperature catalytic oxidation (HTCO) using a Shimadzu TOC-5000A analyzer with a detection limit of 0.050 mg/L.

Dissolved oxygen was measured by an Oxi 538 microprocessor oximeter and Cellox 325 probe equipped with StirrOx G integrated stir. The pH was determined with a PHM290 model Radiometer Analytical pHmeter.

Gemfibrozil and Naproxen were searched for in the river water samples using solid phase extraction (SPE) and HPLC with fluorescence detection, as described in Section 2.4.

2.2. Chemicals

Gemfibrozil, 2,2-Dimethyl-5-(2,5-dimethylphenoxy)pentanoic acid, and Naproxen (+)-(S)-2-(6-methoxynaphthalen-2-yl)propanoic acid were purchased from Sigma Aldrich (Steinheim, Germany) at ≥98% purity. Individual stock solutions (500 mg/L) were prepared by dissolving the adequate quantity of each standard in acetonitrile and were kept at −20 °C. The working standard solutions (50 mg/L) were achieved by dilution of stock solutions with acetonitrile and stored at 4 °C.

Acetonitrile of HPLC-grade was purchased from VWR (Radnor, PA, USA). Acetic acid (glacial) was supplied by Carlo Erba (Milan, Italy). Water for chromatography was purified (18 MΩ/cm quality) by a Milli-Q system (Millipore, Bedford, MA, USA).

2.3. Microcosm set-up for degradation studies

An experimental set consisting of 64 destructive closed microcoms of 100 mL capacity each was set up for both Gemfibrozil and Naproxen (128 microcosms in total). Aliquots from working standard solutions were spiked to treated microcosms to a final concentration of 100 μg/L of the selected pharmaceutical. Spiking was performed in a sterile cabinet and, once acetonitrile was completely evaporated at room temperature in order to eliminate any additional carbon source, 50 mL of the river water (natural or previously sterilized, see later) was added to each microcosm. In particular, some microcosms (16 replicates) were filled with natural river water (Microbiologically Active Water, MAW) and treated with the pharmaceutical (Naproxen or Gemfibrozil) as previously described. Some microcosms (16 replicates) were set up with previously sterilized river water (120 °C, 20 min) and then treated with the pharmaceutical (Sterile). Other 16 replicates were not treated and filled only with river water (Controls), in order to compare the effects of the pharmaceuticals on the natural bacterial community. Finally, pH and dissolved oxygen concentration were measured at each sampling time in 16 other treated microcosms sacrificed exclusively for this purpose.

All microcosms were incubated at 20 °C on an orbital shaker (125 rpm) in the dark.

At selected times two destructive replicate microcosms were collected for each condition (MAW, Sterile, Control). Two sub-samples from each single microcosm were then used for each different (chemical or microbiological) analysis in order to have four independent values for each condition. The samplings were performed 3 h after the treatment and at various times until the disappearance of at least 50% of the parent compound. All operations were conducted under sterile conditions.

2.4. Chemical analysis

The concentrations of Gemfibrozil and Naproxene in the river water samples were determined by a SPE pre-concentration and purification procedure using polymeric Strata-X extraction cartridges, followed by a RP-HPLC (Varian 9012) with fluorescence detection (Perkin Elmer LS4) analytical step, using an Alltech LC18 column (Alltima C18, 5 μm, 250×4.6 mm i.d), preceded by a guard column (4×3 mm) of the same packing material. The elution profile, at a constant flow rate of 1.0 mL/min in isocratic mode, utilized a mobile phase with acetonitrile:water (acidified to pH = 3.6 with acetic acid in order to prevent the hydrolysis of the pharmaceuticals) in a ratio 70:30 (v/v) ratio. Excitation–emission wavelengths were set as follows: $\lambda_{exc} = 230$ nm; $\lambda_{emiss} = 302$ nm for Gemfibrozil and $\lambda_{exc} = 230$ nm;

λ_{emiss} = 420 nm for Naproxen. The concentration of the two pharmaceuticals from each treated microcosm in the degradation studies, was determined by direct injection of samples into the HPLC with fluorescence detection, set up at the same analytical condition, described above. Aliquots of 50 μL of sub-sample were injected in duplicate and in these optimized analytical conditions, the chromatographic run was 10 min for the Gemfibrozil (tr = 8.54 min) and 6 min for the Naproxen (tr = 4.02 min) analysis.

2.5. Microbial analysis: total bacterial number and viability

The total bacterial number (No. bacteria/mL) was determined by direct count, in four replicates of formaldehyde-fixed subsamples (2 mL each) using 4′-6-diamidino-2-phenylindole (DAPI) as a DNA fluorescence agent [37,38]. Cell viability (% live cells/live + dead) was assessed in four non-fixed replicates (2 mL each) using two fluorescent dyes, SYBR Green II and propidium iodide (Sigma-Aldrich Germany) in order to distinguish between viable (green) and dead (red) cells under a fluorescence microscope (Leica DM 4000B Leica Microsystems GmbH, Wetzlar, Germany), as reported in a previous work [39]. We calculated the live bacterial abundance (No. live bacteria/mL) from the total bacterial number, obtained by DAPI counts, multiplied by cell viability.

2.6. Microbial analysis: bacterial community composition by Fluorescence In Situ Hybridization

The phylogenetic composition of the bacterioplancton was analyzed by Fluorescence In Situ Hybridization (FISH), using Cy3-labeled commercially synthesized oligonucleotide probes (MWG AG Biotech, Germany).

Four replicates of formaldehyde-fixed subsamples (2 mL each) were filtered through a 0.2-μm polycarbonate membrane using a gentle vacuum (<0.2 bar), and the filters were stored at −20 °C for successive FISH analysis. FISH was performed according to the previously published protocol [40,41].

The averages of the number of cells binding each of the probes were calculated as a proportion of the total DAPI positive cells from 10 to 20 randomly selected fields on each filter section (corresponding to 500–1000 stained cells). The slides were mounted with a drop of Vecta-Shield (Vector Laboratories, USA) and the preparations were examined and counted on a Leica DM 4000B epifluorescence microscope at ×1000 magnification.

The probes used and the corresponding bacterial groups were: ARCH915 (*Archaea* domain), EUB338I-III (*Bacteria* domain), ALF1b (*Alpha-Proteobacteria*), BET42a (*Beta-Proteobacteria*), GAM42a (*Gamma-Proteobacteria*), PLA46 and PLA886 (*Planctomycetes*), CF319a (*Cytophaga-Flaviobacterium* cluster phylum CFB), HGC69A (Gram-positive with a high DNA G + C content) and LGC354A (*Firmicutes*, Gram-positive bacteria with a low G + C content). Further details of these probes are available on http://www.microbialecology.net/probebase [42,43].

3. Results

3.1. River water characteristics

The main physico-chemical properties of the river water at the sampling point are reported in Table 1. It is possible to see that both Gemfibrozil and Naproxen were found in the river water samples at a concentration greater than the threshold of 10 ng/L suggested by EMEA for performing an environmental risk assessment. That is to say that the occurrence of such chemicals does not exclude the possibility of harmful effects for this receiving ecosystem.

Table 1
Physico-chemical properties of the river water at the sampling point.

Temperature (°C)	12.9
pH	7.7
DO (mg/L)	11.4
DOC (mg/L)	2.0
F^- (mg/L)	0.43
Cl^- (mg/L)	115.44
NO_2^- (mg/L)	0.85
NO_3^- (mg/L)	3.33
SO_4^{2-} (mg/L)	153.14
PO_4^{3-} (mg/L)	0.53
Gemfibrozil (ng/L)	65 ± 13
Naproxen (ng/L)	264 ± 5
Total bacterial number (No. bacteria/mL)	$1.31\ 10^6$
Cell viability (%)	27

3.2. pH, oxygen and DOC measurements in the microcosms

The pH and oxygen values were monitored at each sampling in the microcosms and both values did not vary significantly (*t* test not significant) either in the Naproxen or in the Gemfibrozil experiment. The pH remained at a value of about 8.00 ± 0.08 in both the experiments and a slight decrease of oxygen from the initial concentration of 11 mg/L to 8 mg/L was observed 10 days after in the case of Naproxen and 30 days in that of Gemfibrozil. The oxygen concentration was therefore always close to a saturation level. Finally, the dissolved organic carbon (DOC) measured at the end of the experiments also did not show any significant variation compared to its initial value of 2 mg/L.

3.3. Naproxen degradation experiment

The decrease (expressed in residual percentage) of the Naproxen concentration in the microbiologically active (MAW) and sterile water (Sterile) over a period of 44 days is shown in Fig. 1.

The half-life of the parent compound was about 27 days in the MAW, while in the Sterile condition no significant decrease in concentration was observed during the experimental time. The degradation starts after a lag phase of about 20 days, then the parent compound concentration decreases quickly until its complete disappearance at day 44.

3.3.1. Live bacterial abundance

The live bacterial abundance (No. live bacteria/mL) obtained from the DAPI counts (No. bacteria/mL), multiplied by viability (% live cells/live + dead) in treated (Naproxen) and untreated (Control) microcosms at 0.125 (3 h), 20 and 43 days is reported in Fig. 2.

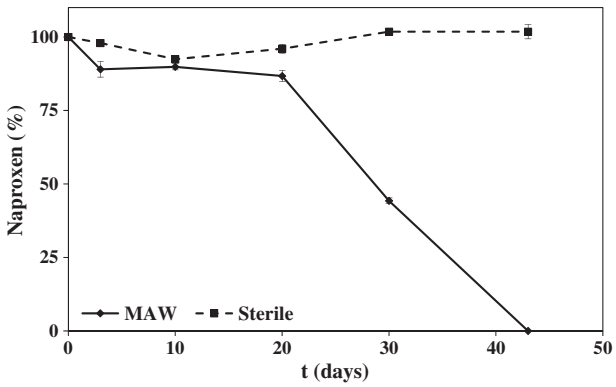

Fig. 1. Residual percentages of Naproxen in river water microbiologically active microcosms (MAW) and in Sterile ones vs time. The vertical bars represent the standard errors.

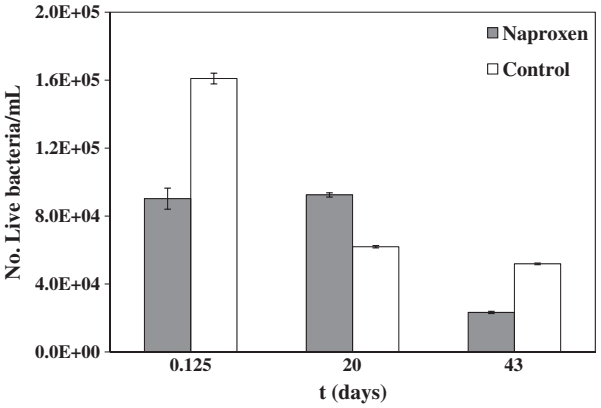

Fig. 2. Number of live bacteria (No. live bacteria/mL water) vs time in the microcosms treated with Naproxen (Naproxen) and in the control ones (Control). The vertical bars represent the standard errors.

Three hours after treatment with the pharmaceutical the live bacterial abundance was significantly lower (*t* test, $p<0.01$) than in the Control microcosms. On the contrary, at day 20 which corresponds to the start of Naproxen degradation, the number of live bacteria was greater (*t* test, $p<0.01$) in the treated microcosms. However, at the end of the experiment (43 days) the live bacterial abundance, although decreased in both conditions, was again significantly lower in the treated microcosms.

3.3.2. Phylogenetic analysis of bacterial community composition by FISH

The use of 16S rRNA gene-targeted oligonucleotide probes made it possible to determine the structure of the autochthonous bacterial community of the river water at the sampling point. The natural river water was composed mainly of the *Bacteria* domain (more than 60% of the DAPI positive cells), and no positive cells to the *Archaea* domain probe were detected (Fig. 3). The *Beta-Proteobacteria* sub-class was the most abundant group and the percentage of Gram-positive bacteria (HGC- and LGC-content) was lower than 1% (data not reported).

The comparison of the autochthonous bacterial community analyzed at the sampling point with the same in the microcosms 20 days after the treatment with Naproxen shows changes in the abundance and shifts in the dominance of several groups (Fig. 4). At day 20, close to the Naproxen DT_{50}, a significant increase in *Alpha-* and *Gamma-Proteobacteria* groups was observed.

3.4. Gemfibrozil degradation experiment

The decrease (expressed in residual percentage) of the Gemfibrozil concentration in the microbiologically active (MAW) and sterile water (Sterile) over a period of 143 days is shown in Fig. 5.

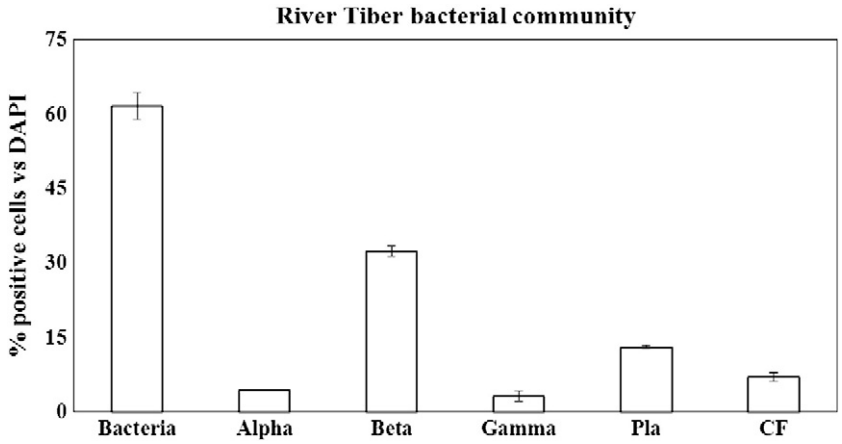

Fig. 3. Bacterial community structure detected by Fluorescence In Situ Hybridization in the River Tiber samples. The vertical bars represent the standard errors. Bacteria: *Bacteria* domain; Alpha: *Alpha-Proteobacteria*; Beta: *Beta-Proteobacteria*; Gamma: *Gamma-Proteobacteria*; Pla: *Planctomycetes*; CF: Cytophaga-Flaviobacterium cluster phylum CFB.

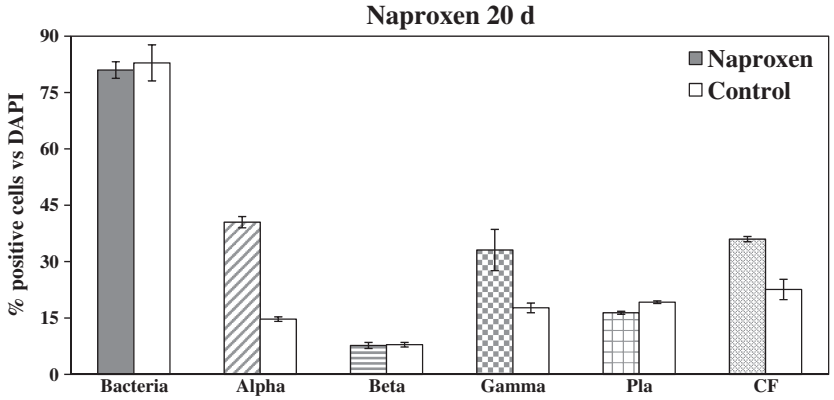

Fig. 4. Bacterial community structure detected by Fluorescence In Situ Hybridization 20 days after the treatment with Naproxen in the treated microcosms and in the control ones (Control). Bacteria: *Bacteria* domain; Alpha: *Alpha-Proteobacteria*; Beta: *Beta-Proteobacteria*; Gamma: *Gamma-Proteobacteria*; Pla: *Planctomycetes*; CF: Cytophaga-Flaviobacterium cluster phylum CFB. The vertical bars represent the standard errors.

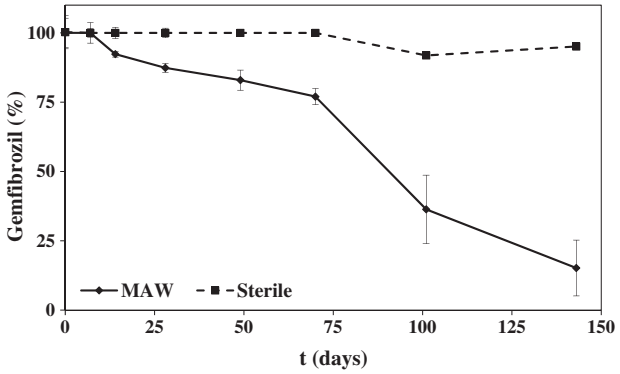

Fig. 5. Residual percentages of Gemfibrozil in river water microbiologically active microcosms (MAW) and in Sterile ones vs time. The vertical bars represent the standard errors.

The half-life of the parent compound was more than 70 days in the MAW. After a lag phase of 7 days, a relatively slow degradation is observable (just 15% degraded in the first 70 days); then a quick disappearance of 50% of the parent compound occurs in the following few days. However, at the end of the experiment (143 days) 15% of the initial concentration of Gemfibrozil (about 15.0 μg/L) is still present. On the contrary, in the Sterile condition, no decrease in concentration was observed (Fig. 5).

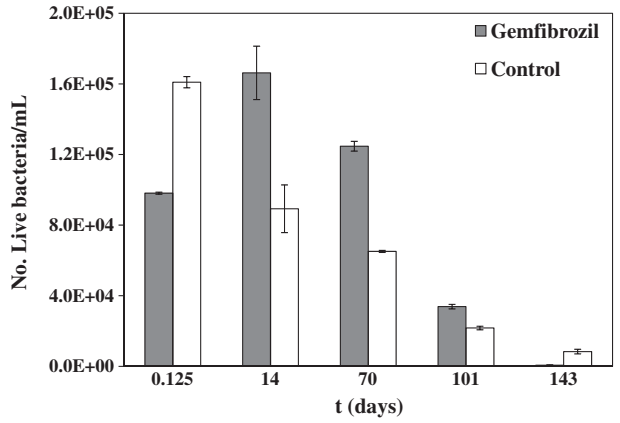

Fig. 6. Number of live bacteria (No. live bacteria/mL water) detected vs in the microcosms treated with Gemfibrozil (Gemfibrozil) and in the control ones (Control). The vertical bars represent standard errors.

3.4.1. Live bacterial abundance

Similarly to the Naproxen experiment, 3 h after the pharmaceutical treatment the live bacterial abundance (No. live bacteria/mL) was significantly lower than in the Control microcosms (*t* test, $p<0.01$). However, an increase in live bacterial abundance (*t* test, $p<0.01$) was observed at 14 and 70 days in the treated microcosms, while in the Control ones there was a generally decreasing trend (Fig. 6). Finally, at the end of the experiment (143 days) the live bacterial abundance was again significantly lower in the treated microcosms.

3.4.2. Phylogenetic analysis of bacterial community composition by FISH

The comparison of the natural bacterial community (Fig. 3) with the same in the microcosms 70 days after the Gemfibrozil treatment shows changes in the abundance and shifts in the dominance of some groups in this case too (Fig. 7). At day 70, close to the halving, a significant increase in the *Gamma-Proteobacteria* group was observed.

4. Discussion

The decrease in the concentrations of the pharmaceuticals observed in the microbiologically active water shows the role of the microbial community in their degradation. Moreover, Naproxen was shown to be less persistent than Gemfibrozil (Figs. 1 and 5).

Data from literature on the environmental fate of these pharmaceuticals are quite scarce so far. Photolytic degradation is reported to be the main possible abiotic degradation process for both the pharmaceuticals, although it is not able to degrade them thoroughly [9,44]. However, we can exclude this abiotic process in our experiments because we performed on purpose in the dark in order to highlight as clearly as possible the real activity of the natural microbial community in the degradation of the pharmaceuticals.

Naproxen was found to be not-readily biodegradable when probability of biodegradation models Biowin1 and Biowin2 [45,46] were used, while other authors [9], extrapolating monitoring data, calculated a half-life of 1–5 days and a field study reports a half-life in surface water ranging from 10 to 27 days. The biodegradation of Naproxen was found in aerobic soil (half-life from 17 to 69 days) by some microbial consortia, including two microfungi of the *Cunninghamella* and *Aspergillus* genera, which were able to co-metabolize this anti-inflammatory drug [47,48].

Gemfibrozil was reported to be not biodegradable [49], but the fungus *Cunninghamella elegans* ATCC 9245, previously tested for its ability to efficiently degrade another pharmaceutical [50], was able to degrade it in a liquid culture by hydroxylation processes [51]. Finally, a recent study reports Gemfibrozil to be a persistent compound with half-lives ranging from 119 to 288 days in surface water [52].

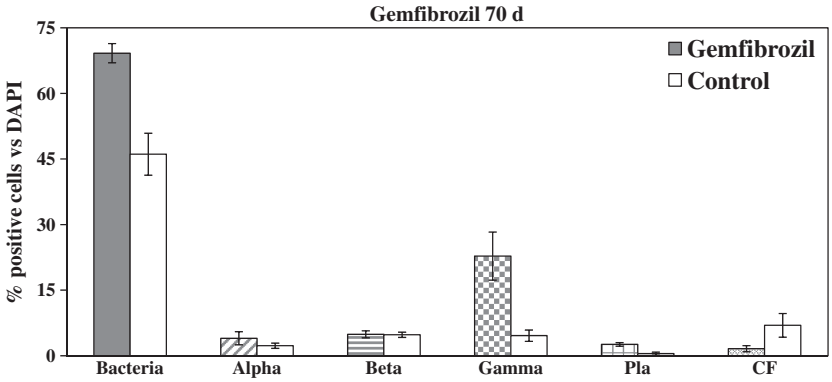

Fig. 7. Bacterial community structure detected by Fluorescence In Situ Hybridization 70 days after the treatment with Gemfibrozil in the treated microcosms and in the control ones (Control). Bacteria: *Bacteria* domain; Alpha: *Alpha-Proteobacteria*; Beta: *Beta-Proteobacteria*; Gamma: *Gamma-Proteobacteria*; Pla: *Planctomycetes*; CF: Cytophaga-Flaviobacterium cluster phylum CFB.

The decrease in the parent compounds does not necessarily imply their mineralization and does not exclude the formation of transformation products with higher persistence and toxicity characteristics, as found for Naproxen [53].

The live bacterial abundance trends in both experiments seem to be in line with the degradation process. In fact 3 h after the treatments, a significant decrease in the live bacterial abundance was observed (47% in the case of Naproxen and 36% in that of Gemfibrozil compared to control microcosms) which can be ascribed to an initial acute toxic effect from the pharmaceuticals on the overall bacterial community. In fact the concentrations used in the experiments were similar to the EC50 (Effective Concentration) and NOEC (No-Observed Effect Concentration) found in some ecotoxicological tests [1] and to the concentration with direct effects on ammonia oxidizing bacteria (AOB) from a wastewater treatment plant [22].

The pharmaceuticals acted as a selective force on the natural microbial community which presumably favored the increase of bacterial populations able to degrade them. In fact, close to the compound DT_{50}s (27 and 70 days for Naproxen and Gemfibrozil, respectively), the live bacterial abundance is higher in the treated microcosms. Moreover, comparing the initial analysis of the natural bacterial community with that close to the compound DT_{50}s, changes in the abundance and a shift in dominance of some groups were observed. In the case of Naproxen we found a dominance of *Alpha-* and *Gamma-Proteobacteria*. The latter bacterial group was also the dominant one in the case of Gemfibrozil. These results suggest the involvement of these groups in the biodegradation of the two pharmaceuticals.

At the end of both experiments we observed a collapse in live bacterial abundances in all conditions (including controls), but it was particularly strong in the treated microcosms. This result could be due to a possible toxic effect from the transformation products, as found for the metabolites of several pesticides [54]. Of course this hypothesis needs to be verified. Further experiments are in progress in order to better investigate the degradation pathways, the metabolite formation and particular bacterial strains involved in their (metabolic and/or co-metabolic) transformations.

5. Conclusions

The overall results show that the natural microbial community of the River Tiber had a significant role in Gemfibrozil and Naproxen degradation, as shown by the degradation results in the sterile vs microbiologically active water microcosms. The degradation capability of the microbial community was presumably acquired by the chronic exposure of the river ecosystem to Gemfibrozil and Naproxen owing to their use among the human population. Gemfibrozil was more persistent than Naproxen (half-lives of 70 days and 27 days, respectively). The fact that Naproxen was found in the River Tiber samples in a greater concentration than Gemfibrozil is therefore due not to its persistence, but to its pseudo-persistence linked to its spreading use among the human population.

The phylogenetic analysis results show that the natural microbial community structure was affected by the pharmaceuticals and the increase in *Alpha-* and *Gamma-Proteobacteria* in the case of Naproxen and *Gamma-Proteobacteria* in the case of Gemfibrozil can be ascribed to their involvement in the pharmaceutical degradation.

However, the homeostatic response shown by the degrading bacterial populations does not exclude the possibility of detrimental effects from the pharmaceuticals for specific populations involved in key ecosystem activities.

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References

[1] L.H. Santos, A.N. Araújo, A. Fachini, A. Pena, C. Delerue-Matos, M.C. Montenegro, Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment, J. Hazard. Mater. 175 (2010) 45–95.
[2] K. Kümmerer, The presence of pharmaceuticals in the environment due to human use — present knowledge and future challenges, J. Environ. Manag. 90 (2009) 2354–2366.
[3] P. Bottoni, S. Caroli, A. Barra Caracciolo, Pharmaceuticals as priority water contaminants, Toxicol. Environ. Chem. 92 (2010) 549–565.
[4] M.P. Sammartino, F. Bellanti, M. Castrucci, D. Ruiu, G. Visco, T. Zoccarato, Ecopharmacology: deliberated or casual dispersion of pharmaceutical principles, phytosanitary, personal healthcare and veterinary products in environment needs a multivariate analysis or expert systems for the control, the measure and the remediation, Microchem. J. 88 (2008) 201–209.
[5] C. Carlsson, A.K. Johansson, G. Alvan, K. Bergman, T. Kuhler, Are pharmaceuticals potent environmental pollutants? Part II: environmental risk assessments of selected pharmaceutical excipients, Sci. Total. Environ. 364 (2006) 88–95.
[6] E. Zuccato, S. Castiglioni, R. Fanelli, Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment, J. Hazard. Mater. 122 (2005) 205–209.
[7] K.J. Ottmar, L.M. Colosi, J.A. Smith, Development and application of a model to estimate wastewater treatment plant prescription pharmaceutical influent loadings and concentrations, Bull. Environ. Contam. Toxicol. 84 (2010) 507–512.
[8] R. Loos, B.M. Gawlik, G. Locoro, E. Rimaviciute, S. Contini, G. Bidoglio, EU-wide survey of polar organic persistent pollutants in European river waters, Environ. Pollut. 157 (2009) 561–568.
[9] M. Radke, H. Ulrich, C. Wurm, U. Kunkel, Dynamics and attenuation of acidic pharmaceuticals along a river stretch, Environ. Sci. Technol. 44 (2010) 2968–2974.
[10] H.K. Allen, J. Donato, H.H. Wang, K.A. Cloud-Hansen, J. Davies, J. Handelsman, Call of the wild: antibiotic resistance genes in natural environments, Nat. Rev. Microbiol. 8 (2010) 251–259.
[11] H. Schmitt, T. Boucard, J. Garric, J. Jensen, J. Parrott, A. Péry, J. Römbke, J.O. Straub, T.H. Hutchinson, P. Sánchez-Argüello, A. Wennmalm, K. Duis, Recommendations on the environmental risk assessment of pharmaceuticals: effect characterization, Integr. Environ. Assess. Manag. 6 (2010) 588–602.
[12] B. Quinn, W. Schmidt, K. O'Rourke, R. Hernan, Effects of the pharmaceuticals Gemfibrozil and Diclofenac on biomarker expression in the zebra mussel (*Dreissena polymorpha*) and their comparison with standardised toxicity tests, Chemosphere 84 (2011) 657–663.
[13] A.B.A. Boxall, The environmental side effects of medication, EMBO Rep. 5 (2004) 1110–1116.
[14] WHO, Pharmaceuticals in Drinking-Water, World Health Organization, 2011. http://www.who.int/water_sanitation_health/publications/2011/pharmaceuticals/en/ (Accessed April 2012).
[15] F. Pomati, C. Orlandi, M. Clerici, F. Luciani, E. Zuccato, Effects and interactions in an environmentally relevant mixture of pharmaceuticals, Toxicol. Sci. 102 (2008) 129–137.
[16] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli, The challenge of micropollutants in aquatic systems, Science 313 (2006) 1072–1077.
[17] S.M. Richards, C.J. Wilson, D.J. Johnson, D.M. Castle, M. Lam, S.A. Mabury, P.K. Sibley, K.R. Solomon, Effects of pharmaceutical mixtures in aquatic microcosms, Environ. Toxicol. Chem. 23 (2004) 1035–1042.
[18] S.N. Dokianakis, M.E. Kornaros, G. Lyberatos, On the effect of pharmaceuticals on bacterial nitrite oxidation, Water Sci. Technol. 50 (2004) 341–346.
[19] B. Kraigher, T. Kosjek, E. Heath, B. Kompare, I. Mandic-Mulec, Influence of pharmaceutical residues on the structure of activated sludge bacterial communities in wastewater treatment bioreactors, Water Res. 42 (2008) 4578–4588.
[20] Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency Pre-Authorisation Evaluation of Medicines for Human Use (Doc. Ref. EMEA/CHMP/SWP/4447/00), Guideline on The Environmental Risk Assessment of Medicinal Products for Human Use, 2006. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003978.pdf (Accessed April 2012).
[21] V. Christen, S. Hickmann, B. Rechenberg, K. Fent, Highly active human pharmaceuticals in aquatic systems: a concept for their identification based on their mode of action, Aquat. Toxicol. 96 (2010) 167–181.
[22] S. Wang, C.K. Gunsch, Effects of selected pharmaceutically active compounds on the ammonia oxidizing bacterium *Nitrosomonas europae*, Chemosphere 82 (2011) 565–572.
[23] P. de Voogt, M.L. Janex-Habibi, F. Sacher, L. Puijker, M. Mons, Development of a common priority list of pharmaceuticals relevant for the water cycle, Water Sci. Technol. 59 (2009) 39–46.
[24] M. Cleuvers, Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid, Ecotoxicol. Environ. Saf. 59 (2004) 309–315.
[25] C.A. Jackevicius, J.V. Tu, J.S. Ross, D.T. Ko, D. Carreon, H.M. Krumholz, Use of fibrates in the United States and Canada, JAMA 305 (2011) 1217–1224.
[26] US EPA, Fact Sheet: Final Third Drinking Water, Contaminant Candidate List (CCL 3), Office of Water (4607M) EPA EPA 815F09001, United State Environmental Protection Agency, 2009. 8 pp. www.epa.gov/safewater/ccl/index.html (Accessed April 2012).
[27] J.L. Zurita, G. Repetto, Á. Jos, M. Salguero, M. López-Artíguez, A.M. Cameán, Toxicological effects of the lipid regulator Gemfibrozil in four aquatic systems, Aquat. Toxicol. 81 (2007) 106–115.
[28] Gruppo di Lavoro OsMed, L'uso dei farmaci in Italia, Rapporto nazionale anno 2009, Il Pensiero Scientifico Editore, Roma, 2010. http://www.epicentro.iss.it/farmaci/pdf/OsMed/Osmed2009.pdf (Accessed April 2012).

[29] R. Andreozzi, M. Raffaele, P. Nicklas, Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment, Chemosphere 50 (2003) 1319–1330.
[30] E. Zuccato, S. Castiglioni, R. Fanelli, G. Reitano, R. Bagnati, C. Chiabrando, F. Pomati, C. Rossetti, D. Calamari, Pharmaceuticals in the environment in Italy: causes, occurrence, effects and control, Environ. Sci. Pollut. Res. Int. 13 (2006) 15–21.
[31] M. Gros, M. Petrović, D. Barceló, Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro River basin (northeast Spain), Environ. Toxicol. Chem. 26 (2007) 1553–1562.
[32] K.M. Onesios, J.T. Yu, E.J. Bouwer, Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review, Biodegradation 20 (2009) 441–466.
[33] M. Megharaj, B. Ramakrishnan, K. Venkateswarlu, N. Sethunathan, R. Naidu, Bioremediation approaches for organic pollutants: a critical perspective, Environ. Int. 37 (2011) 1362–1375.
[34] H.E. Reed, J.B.H. Martiny, Testing the functional significance of microbial composition in natural communities, FEMS Microbiol. Ecol. 62 (2007) 161–170.
[35] A.I. Rodarte-Morales, G. Feijoo, M.T. Moreira, J.M. Lema, Biotransformation of three pharmaceutical active compounds by the fungus *Phanerochaete chrysosporium* in a fed batch stirred reactor under air and oxygen supply, Biodegradation 23 (2011) 145–156.
[36] M. Lahti, A. Oikari, Microbial transformation of pharmaceuticals Naproxen, bisoprolol, and diclofenac in aerobic and anaerobic environments, Arch. Environ. Contam. Toxicol. 61 (2011) 202–210.
[37] A. Barra Caracciolo, P. Grenni, C. Cupo, S. Rossetti, In situ analysis of native microbial communities in complex samples with high particulate loads, FEMS Microbiol. Lett. 253 (2005) 55–58.
[38] A. Barra Caracciolo, G. Giuliano, P. Grenni, C. Cremisini, R. Ciccoli, C. Ubaldi, Effect of urea on degradation of terbuthylazine in soil, Environ. Toxicol. Chem. 24 (2005) 1035–1040.
[39] P. Grenni, A. Barra Caracciolo, M.S. Rodríguez-Cruz, M.J. Sánchez-Martín, Changes in the microbial activity in a soil amended with oak and pine residues and treated with linuron herbicide, Appl. Soil Ecol. 41 (2009) 2–7.
[40] J. Pernthaler, F.O. Glockner, W. Schonhuber, R. Amann, Fluorescence in situ hybridization with rRNA-targeted oligonucleotide probes, Methods Microbiol. 30 (2001) 207–226.
[41] A. Barra Caracciolo, P. Bottoni, P. Grenni, Fluorescence In Situ Hybridization in soil and water ecosystems: an useful method for studying the effect of xenobiotics on bacterial community structure, Toxicol. Environ. Chem. 92 (2010) 567–579.
[42] A. Loy, M. Horn, M. Wagner, probeBase: an online resource for rRNA-targeted oligonucleotide probes, Nucleic Acids Res. 31 (2003) 514–516.
[43] A. Loy, F. Maixner, W. Wagner, M. Horn, probeBase — an online resource for rRNA-targeted oligonucleotide probes: new features 2007, Nucleic Acids Res. 35 (2007) D800–D8004.
[44] E. Felis, D. Marciocha, J. Surmacz-Gorska, K. Miksch, Photochemical degradation of Naproxen in the aquatic environment, Water Sci. Technol. 55 (2007) 281–286.
[45] R.S. Boethling, D.G. Lynch, J.S. Jaworska, J.L. Tunkel, G.C. Thom, S. Webb, Using biowin, bayes, and batteries to predict ready biodegradability, Environ. Toxicol. Chem. 23 (2004) 911–920.
[46] J.O. Straub, K.M. Stewart, Deterministic and probabilistic acute-based environmental risk assessment for Naproxen for Western Europe, Environ. Toxicol. Chem. 26 (2007) 795–806.
[47] K. Lin, J. Gan, Sorption and degradation of wastewater-associated non-steroidal anti-inflammatory drugs and antibiotics in soils, Chemosphere 83 (2011) 240–246.
[48] E. Topp, J.G. Hendel, D.R. Lapen, R. Chapman, Fate of the nonsteroidal anti-inflammatory drug Naproxen in agricultural soil receiving liquid municipal biosolids, Environ. Toxicol. Chem. 27 (2008) 2005–2010.
[49] M. Stumpf, T.A. Ternes, R.D. Wilken, S.V. Rodrigues, W. Baumann, Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil, Sci. Total. Environ. 225 (1999) 135–141.
[50] S.I. Kang, S.Y. Kang, H.G. Hur, Identification of fungal metabolites of anticonvulsant drug carbamazepine, Appl. Microbiol. Biotechnol. 79 (2008) 663–669.
[51] S.I. Kang, S.Y. Kang, R.A. Kanaly, E. Lee, Y. Lim, H.G. Hur, Rapid oxidation of ring methyl groups is the primary mechanism of biotransformation of gemfibrozil by the fungus *Cunninghamella elegans*, Arch. Microbiol. 191 (2009) 509–517.
[52] L. Araujo, N. Villa, N. Camargo, M. Bustos, T. García, A. de Jesus Prieto, Persistence of gemfibrozil, naproxen and mefenamic acid in natural waters, Environ. Chem. Lett. 9 (2011) 13–18.
[53] M. Isidori, M. Lavorgna, A. Nardelli, A. Parrella, L. Previtera, M. Rubino, Ecotoxicity of Naproxen and its phototransformation products, Sci. Total. Environ. 348 (2005) 93–101.
[54] L. Fava, M.A. Orrù, A. Crobe, A. Barra Caracciolo, P. Bottoni, E. Funari, Pesticide metabolites as contaminants of groundwater resources: assessment of the leaching potential of endosulfan sulfate, 2,6-dichlorobenzoic acid, 3,4-dichloroaniline, 2,4-dichlorophenol and 4-chloro-2-methylphenol, Microchem. J. 79 (2005) 207–211.