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The Challenge Posed by Emerging Environmental Contaminants: An Assessment of the Effectiveness of Phenoxyethanol Biological Removal from Groundwater through Mesocosm Experiments

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Abstract: The occurrence of emerging pollutants (EPs) such as pharmaceuticals and personal care products (PPCPs) has raised serious concerns about the possible adverse effects on ecosystem integrity and human health. Wastewater treatment facilities appear to be the main sources of PPCPs released in aquatic environments. This research examines the effectiveness of groundwater microbial community activities to remove phenoxyethanol (Phy-Et), currently exploited as a preservative in many cosmetic formulations at a maximum concentration of 1% but which has shown, at higher levels of exposure, adverse systemic effects on animals. Mesocosm experiments were carried out for 28 days using two different concentrations of the substance (5.2 mg/L and 27.4 mg/L). The main results obtained through chemical and microbiological investigations revealed a significant Phy-Et reduction (≈100% when added at a concentration of 5.2 mg/L and ≈84% when added at a concentration of 27.4 mg/L), demonstrating that some autochthonous microorganisms in the analyzed samples played a "key role" in removing this compound, despite its proven antimicrobial activity. Nevertheless, the decrease in the "natural attenuation" efficacy (≈16%) when using higher concentrations of the chemical suggests the existence of a "dose-dependent effect" of Phy-Et on the process of biodegradation. Biomolecular investigations carried out through next-generation sequencing (NGS) revealed (i) the presence of a significant fraction of hidden microbial diversity to unravel, (ii) variations of the composition and species abundance of the groundwater microbial communities induced by Phy-Et, and (iii) a biodiversity reduction trend correlated to the increase of Phy-Et concentrations. Overall, the preliminary information obtained from the experiments carried out at the laboratory scale appears encouraging, although it reflects only partially the complexity of the phenomena that occur in natural environments and influences their "auto-purification capability". Accordingly, this research paves the way for more in-depth investigations to develop appropriate tools and protocols to evaluate the occurrence and fate of Phy-Et in nature and assess the impact of its release and the effects of long-term exposure (even at low concentrations) on ecosystems and health.

Keywords: emerging environmental contaminants; phenoxyethanol; mesocosms; natural attenuation; groundwater microbial communities

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1. Introduction

Environmental pollution threatens human and planetary health, jeopardizing modern societies' sustainability [1]. In 2015, diseases attributable to pollution caused 9 million premature deaths, accounting for 16% of all deaths worldwide, a number three times higher compared to deaths for AIDS, tuberculosis, and malaria combined and 15 times higher compared to deaths for all wars and other forms of violence [2]. Unfortunately, estimates updated to 2019 still confirmed this number of deaths per year [1]. Overall, ambient air pollution and toxic chemical pollution, which are consequences of industrialization and urbanization, have caused many deaths, rising by 7% since 2015 and over 66% since 2000 [1]. Among the various categories of pollutants, there has been a growing interest in the so-called emerging pollutants (EPs) in the last decades. This category includes all the substances that could be pollutants that are not currently covered by controls but that could be covered in the future; this will depend on future ecotoxicity studies and concentrations found [3]. More than 700 compounds grouped in 20 classes of EPs are present in the NORMAN database (www.norman-network.net) [4] and include surfactants, antibiotics and other pharmaceuticals, steroid hormones and other endocrine-disrupting compounds (EDCs), fire retardants, sunscreens, disinfection by-products, new pesticides and pesticide metabolites, naturally occurring algal toxins, etc. [5,6]. In addition, another group of pollutants denoted as "contaminants of emerging concern" (CECs) chemical substances used for long periods in various anthropic sectors and released into the environment in the absence of control, and by-products of these, which are now detected by analysis of soils, surface waters, and groundwater. The term "contaminants of emerging concern" is usually used when very little information is available about the magnitude and frequency of risks posed by this category of substances in the environment and human health [7–9].

Contamination caused by urbanization, fast population growth, agricultural activities, and industrial development has drastically affected water resource quality [10]. The discovery of numerous new compounds in drinking, ground, and surface water has alarmed the public, mainly when human health-based guidelines are unavailable [11,12]. One of the largest sources of EPs is represented by wastewater treatment plants (WWTPs) [13] since wastewater treatments are not designed to treat these compounds and, thus, high amounts are released into the environment through effluents [14].

The main aim of this research was to investigate the effectiveness of biological removal of (Phy-Et) from groundwater through mesocosm experiments to assess the contribution of autochthonous microbial communities in the natural attenuation process of this chemical substance.

Phy-Et is an aromatic glycol ether that occurs naturally in green tea or can be produced in the laboratory due to its commercial importance [15] It is widely found in a large range of leave-on and rinse-off cosmetic products such as moisturizers, hand disinfectants, soaps, serums, sunscreen creams, mascaras, eyeliners, eye balms, and perfumes due to its pure chemical form, pleasant smell, and colorless appearance [16,17]. Other uses include shampoos, shaving creams, ultrasound gels, insect repellents, antiseptics, solvents, anesthetics, cellulose acetate solvents, dyes, and ink and ink manufacture [15]. This compound has a large spectrum of antimicrobial activity and is effective against Gram-negative and Gram-positive bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, and yeasts, such as *Candida* [15,18,19], although it has only a weak inhibitory effect on resident skin flora [20].

The European Scientific Committee on Consumer Safety considers Phy-Et safe for all consumers (including children of all ages) when used as a preservative in cosmetic products up to a concentration of 1%. However, the French National Agency for the Safety of Medicines and Health Products (ANSM) advised against using Phy-Et as a preservative in cosmetic products intended for application to the nappy area of infants and children under three years [20]. Nevertheless, toxicological studies revealed adverse systemic effects in animals at levels of exposure many magnitudes higher (around 200-fold higher) than those to which consumers are exposed [20]. Accordingly, in light of the vast increase in personal care product consumption and their significant release in aquatic ecosystems, it is important to assess the fate of Phy-Et in the environment and the microorganisms' ability to degrade.

2. Materials and Methods

2.1. Study Area

Groundwater samples for mesocosm experiments have been collected from a piezometer (Pz3) located within the alluvial Parma aquifer (northern Italy; Figure 1). Parma is the second town by population of the Emilia Romagna region and represents a critical contamination source of surface waters and groundwater from leaks in the urban sewerage system [21]. The area is characterized by large industrial districts and intensive agriculture practices both in urban and suburban areas [21]. The piezometer Pz3 is very close to a pipeline of a wastewater treatment plant called "Parma Ovest", in whose waters has been ascertained a continuous and constant leak from the local sewers demonstrated by the detection of fecal coliforms and enterococci [21]. Accordingly, for assessing the biological removal of Phy-Et, groundwater microbial communities have most likely been shaped by a selective pressure caused by frequent contact with sewage and, thus, emerging environmental contaminants.

The aquifer system of the Parma urban area is represented by an alternation of fine-grained (clays and silts) and coarse-grained bodies (gravels and sands) belonging to the alluvial geological units (or synthems) that filled the Po River basin during the Pleistocene–Holocene period (Figure 1). Specifically, the sedimentation processes in this area primarily result from the sedimentary dynamics originating from Apennine streams [22–25]. Groundwater in this aquifer system exhibits a predominant southwest-to-northeast flow at the basin scale, from the Apennine to the central Po Plain and consequently toward the Adriatic Sea [25]. The hydraulic conductivity of the studied aquifer is frequently variable in the three dimensions due to the wide heterogeneity of the geological medium. Zanini et al. [26] calculated a hydraulic conductivity varying from 1.2×10^{-5} to 4.9×10^{-5} m/s (mean 2.3×10^{-5} m/s; median 1.7×10^{-5} m/s) in coarse-grained horizons and from 9.3×10^{-9} to 1.3×10^{-7} m/s (mean 1.6×10^{-7} m/s; median 9.7×10^{-8} m/s) in fine-grained layers.

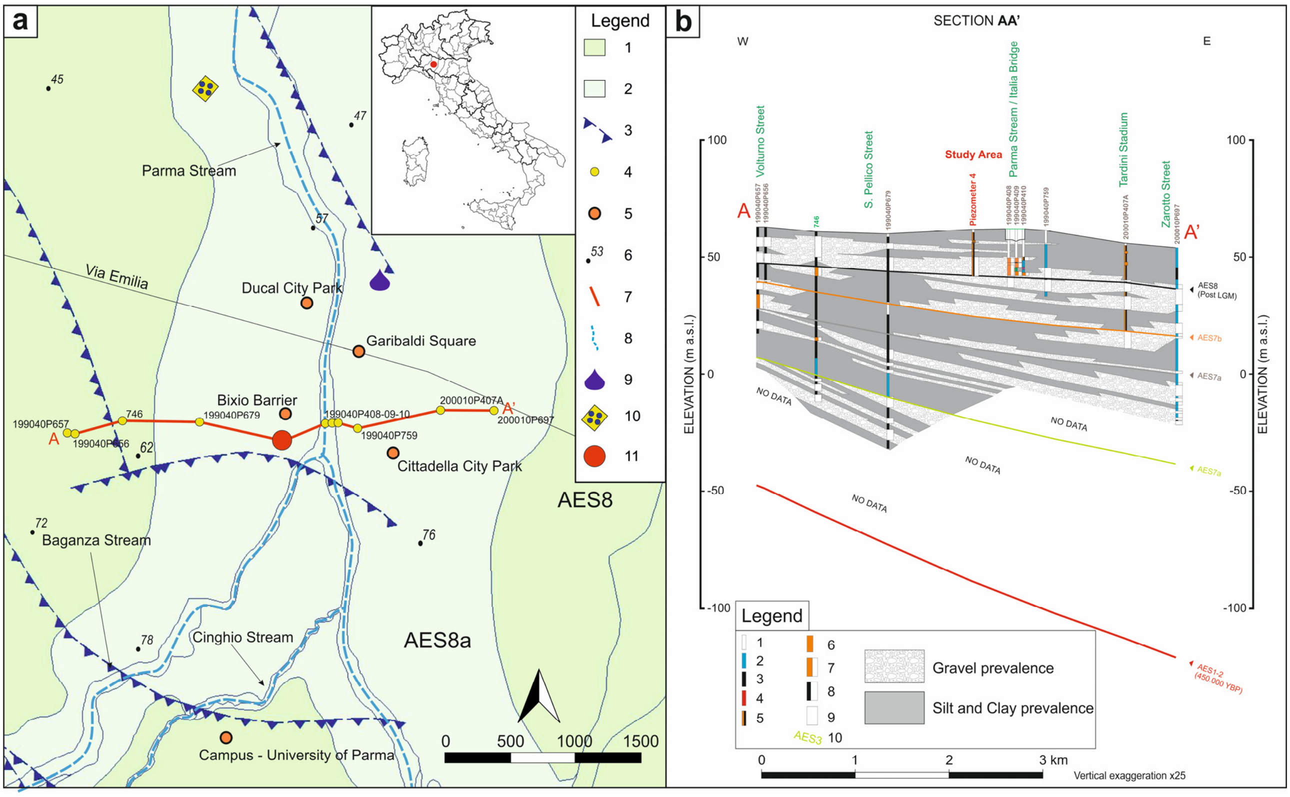

Figure 1. (**a**) Geological map and section at basin scale (from Ducci et al., 2022, modified [21]); (1) AES8: Emiliano–Romagnolo superiore synthem—Ravenna subsynthem; (2) AES8a: Emiliano–Romagnolo

superiore synthem—Modena subsynthem; (3) main thrust; (4) borehole; (5) toponym; (6) quoted point; (7) geological section's trace; (8) stream; (9) meteorological station; (10) wastewaters treatment plant "Parma Ovest"; (11) study area; (from Ducci et al., 2022, modified [21]). (**b**) Geological section at basin scale (the trace is shown in (**a**)): (1) fill material, (2) grey/light blue clay and silt; (3) yellow/brown clay and silt; (4) red clay and silt; (5) silt; (6) sand; (7) gravel and sand; (8) gravel and clay/silt; (9) gravel; (10) Code of Geological Unit (sensu Di Dio et al., 2005 [27]): AES1: Emiliano–Romagnolo superiore synthem—Monterlinzana subsynthem; AES2: Emiliano–Romagnolo superiore synthem—Maiatico subsynthem; AES7a: Emiliano–Romagnolo superiore synthem—Villa Verucchio subsynthem—Niviano unit; AES7b: Emiliano–Romagnolo superiore synthem—Villa Verucchio subsynthem—Vignola unit; AES8: Emiliano–Romagnolo synthem—Ravenna subsynthem.

The presence of samples derived from 30 core borings made it possible to reconstruct the local stratigraphy at the site scale [21]. Excluding the anthropogenic material (gravel and bricks) that fill the entire study area about 1 m thick, founded succession can be summarized as follows: (i) the first layer consists of silt and clay with a thickness ranging from 5 to 7 m; (ii) the second layer is primarily composed of gravel and sand, with a thickness varying between 1 and 5 m. Particularly, it exhibits a clear separation into two distinct elements as it extends northward, near Pz3; (iii) the third layer comprises silt and clay, with a thickness ranging from 2 to 3 m; and (iv) the fourth layer represents the shallowest alluvial aquifer at the test site and is composed of gravel and sand (Figure 2). These sedimentary bodies result from ancient alluvial dynamics associated with the paleo-river systems of the Parma and Baganza streams.

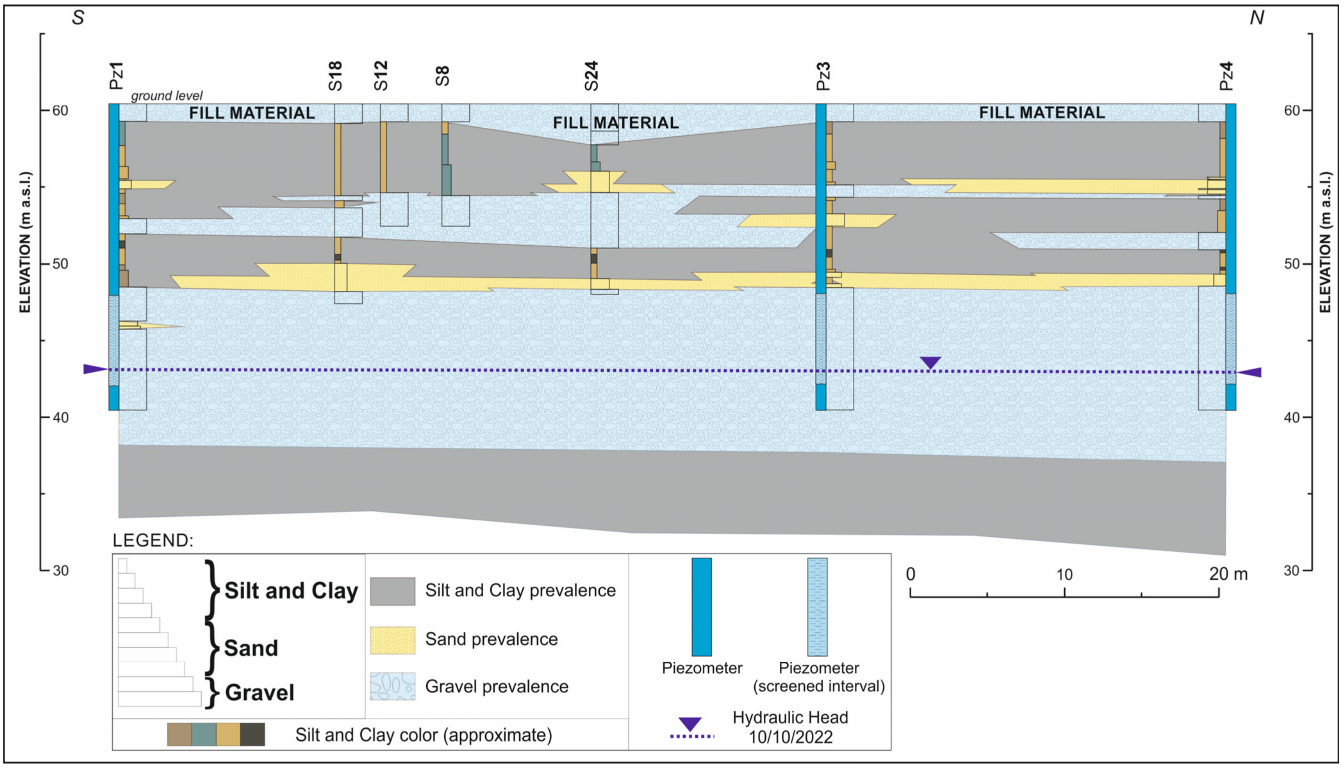

Figure 2. Geological section at site scale (from Ducci et al., 2022, modified [21]).

The groundwater flow net reconstructed in the study area is characterized by seasonal variations during the hydrologic year, even though the overall flow is always directed from South to North (Figure 3).

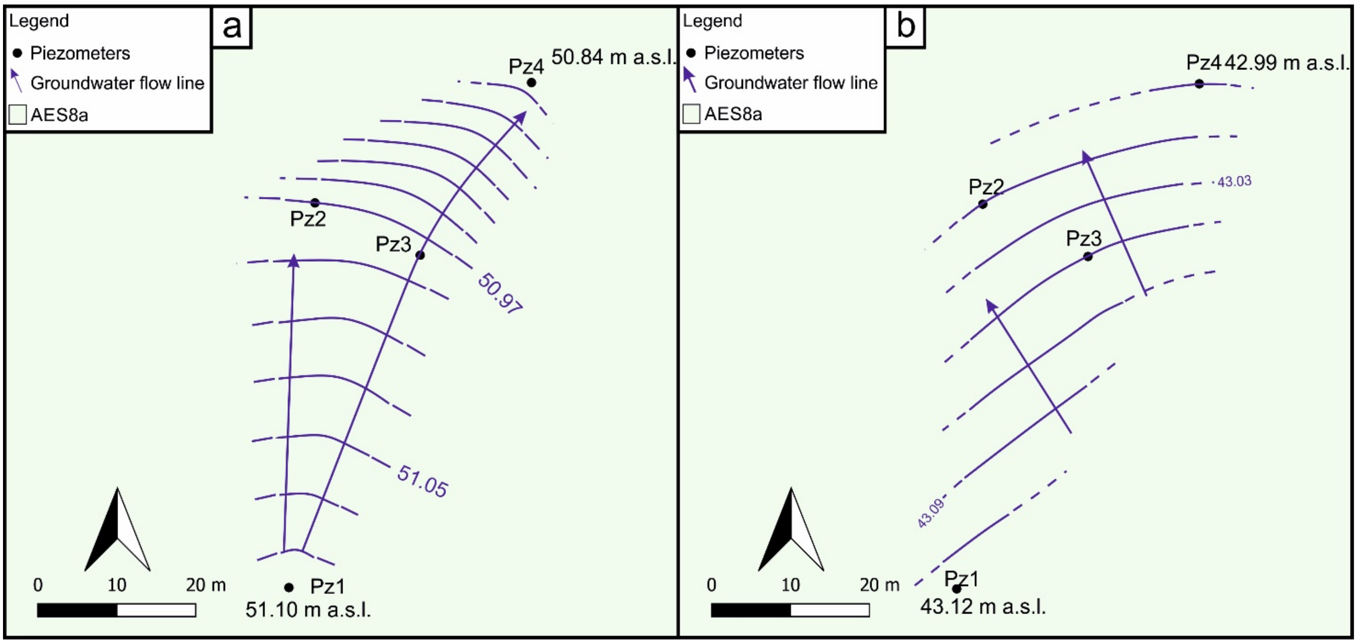

Figure 3. (**a**) Groundwater flow net in May 2022; (**b**) groundwater flow net in October 2022 (from Ducci et al., 2022, modified [21]).

2.2. Groundwater Sampling and Mesocosm Setup

Groundwater samples were collected from the piezometer Pz3, immediately below the hydraulic head, with a sterile bailer in October 2022.

The groundwater temperature has been monitored monthly at the piezometer Pz3 from March 2022 to October 2022 using the multiparameter HANNA probe (model HI9828, HANNA Instruments, Villafranca Padovana, Italy).

In detail, for mesocosm experiments, 16 L of water was sampled. In addition, another 2 L of water was used to carry out biomolecular and chemical analyses aimed at characterizing autochthonous microbial communities and determining the "background contamination" by Phy-Et (Figure 4a).

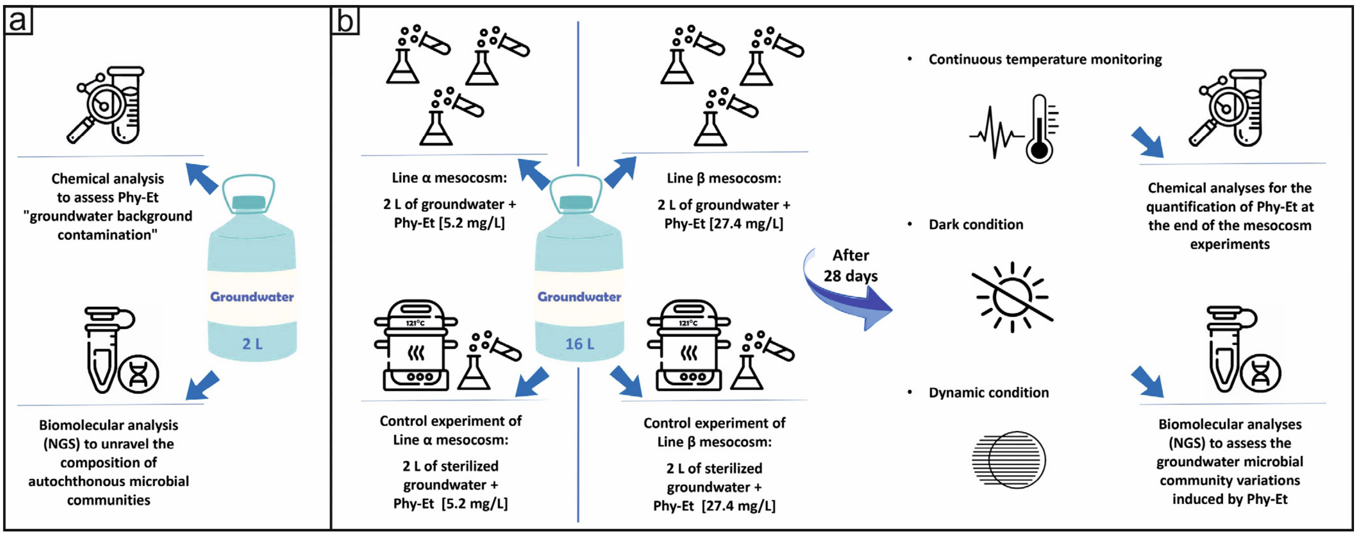

Figure 4. (**a**) Groundwater microbial community characterization and assessment of Phy-Et background contamination; (**b**) experimental protocol setup of mesocosms.

Mesocosms were prepared by transferring 2 L of groundwater in sterile glass Erlenmeyer flasks and by adding Phy-Et (BiOrigins, Fordingbridge, United Kingdom) at two different concentrations: 5.2 mg/L and 27.4 mg/L. The experiments carried out with Phy-Et at a concentration of 5.2 mg/L will be henceforth also indicated as Line α, whereas the ones carried out with Phy-Et at a concentration of 27.4 mg/L will be reported as Line β. For each Line, three different mesocosms were set up (Figure 4b). The Phy-Et concentrations arbitrarily chosen for the experiments were more than 20 and 100 times higher than the instrumental detection limit for this compound (0.2 mg/L).

Afterward, the mesocosms were sealed and incubated at room temperature for 28 days, in the dark, with agitation (140 rpm), using the OrbitalShaker—OHAUS® (Model: SHHD6825DG, S/N: 210331001, Nänikon, Zürich, Switzerland).

In addition, the experimental plan has included two controls, consisting of sterilized groundwater samples added with Phy-Et at the concentrations of 5.2 mg/L and 27.4 mg/L, to highlight a possible removal of this compound from groundwater not attributable to the microbial activities (Figure 4b).

2.3. Temperature Monitoring and Chemical Analyses for Phy-Et Detection

For the entire duration of the mesocosm experiments (28 days), the temperature was monitored every 5 min through an immersion thermometer with a data logger (Elitech model: GSP-6 Serial No. EFG218100242, London, UK).

Chemical analyses for Phy-Et detection were performed both on the original groundwater and samples collected at the end of the mesocosm experiments. A total of 50 mL of water was poured into amber glass bottles and transported to the laboratory in a refrigerated box. The analyses were performed at Abich S.r.l., an Italian company with UNI EN ISO 9001:2015 [28] and UNI CEI EN ISO 13485:2016 [29] accreditations. According to an accredited in-house method, they were based on the ultra-high-performance liquid chromatographic-diode array detection method (UHPLC/DAD) (Rif. HPLC n.0018).

2.4. Next-Generation Sequencing (NGS) for Bacterial Community Analyses

Next-generation sequencing (NGS) analyses were carried out firstly to characterize autochthonous groundwater microbial communities in groundwater samples collected at the piezometer (Pz3) and then to assess those variations in their composition after the addition of Phy-Et to the mesocosms at two different concentrations (5.2 mg/L and 27.4 mg/L).

Groundwater samples (1.95 L) were filtered through sterile mixed esters of cellulose filters (S-Pak™ Membrane Filters, 47 mm diameter, 0.22 μm pore size, Millipore Corporation, Billerica, MA, USA) within 24 h from the collection. Bacterial DNA extraction from filters was performed using the commercial kit FastDNA SPIN Kit for soil (MP Biomedicals, LLC, Solon, OH, USA) and FastPrep® Instrument (MP Biomedicals, LLC, Solon, OH, USA). After the extraction, DNA integrity and quantity were evaluated using electrophoresis in 0.8% agarose gel containing 1 μg/mL of Gel-Red™ (Biotium, Inc., Fremont, CA, USA). Next-generation sequencing (NGS) technologies generated the bacterial community profiles in the samples at the Genprobio Srl Laboratory following the protocol reported by Ducci et al. [21].

The 16S rRNA gene sequences obtained in this study were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive under the accession number PRJNA977861.

3. Results

3.1. Microbiological and Physico-Chemical Characterization of Groundwater

Autochthonous bacterial communities of groundwater collected from the piezometer Pz3 were characterized by a predominance of *Proteobacteria* (relative abundance 64.14%), followed by *Bacteroidetes* (relative abundance 8.34%), *Chloroflexi* (relative abundance 5.23%), *Verrucomicrobia* (relative abundance 3.91%), *Actinobacteria* (relative abundance 3.38%), *Aci-*

dobacteria (relative abundance 3.07%), and other phyla, which were detected with relative abundance values lower than 3% (Figure 5). At the genus level, the five major taxa were *Methylotenera*, *Pseudomonas*, unclassified microorganisms of the *Burkholderiaceae* family, *Massilia*, and *Cavicella*, which were retrieved at percentages of 8.71%, 7.27%, 4.36%, 2.18%, and 2.00%, respectively (Figure 5).

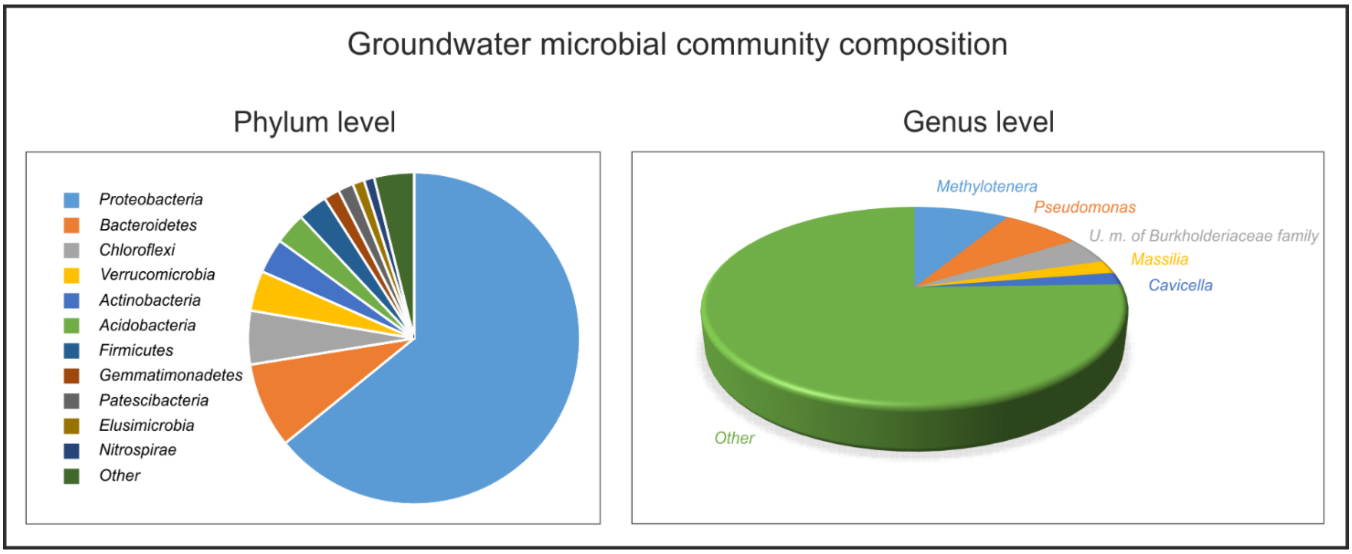

Figure 5. Microbial community composition at phylum (**on the left**) and genus (**on the right**) levels of groundwater collected from the piezometer Pz3. Taxa with relative abundance values below 1% and 2% for phylum and genus levels, respectively, are labeled "Other". U. m. refers to unclassified microorganisms.

Chemical analyses for determining Phy-Et revealed that its concentration was lower than the instrumental detection limit (0.2 mg/L). Groundwater temperature during the observation period ranged from 13.2 °C to 16.1 °C, with an average of 14.4 °C.

3.2. Results of Physico-Chemical and Microbiological Analyses for the Mesocosm Experiments

At the end of the 28-day experimental period, water samples were collected from the mesocosms in which Phy-Et was initially added at two different concentrations and were subjected to chemical analyses to assess whether there had been a reduction.

The same analyses were also performed on the two controls, consisting of sterilized groundwater added with Phy-Et, to detect any losses of this compound from the systems, not due to biodegradation processes.

The results obtained clearly showed the removal of the compound of about 100% in Line α mesocosms (from 5.2 mg/L to below the detection limit [0.2 mg/L]). On the other hand, in the Line β mesocosms, a reduction of about 84% was detected (from 27.4 mg/L to concentrations ranging between 3.21 $\pm$ 0.01 mg/L and 5.24 $\pm$ 0.02 mg/L). No significant variations in the concentration of the chemical were recorded in the controls, evidence which demonstrates that microorganisms were the "key player" in the degradation process.

The continuous monitoring of water temperature in the mesocosms allowed the detection of fluctuations varying from a minimum of 17.9 °C to a maximum of 24.2 °C, with an average of 21.0 °C.

On the whole, the results of the biomolecular investigations carried out to unravel the composition of bacterial communities exposed to the two concentrations of Phy-Et in the mesocosm experiments revealed, at the phylum level, the predominance of *Proteobacteria*, followed by *Bacteroidetes*, *Verrucomicrobia*, *Acidobacteria*, *Actinobacteria*, and *Patescibacteria* (Figure 6).

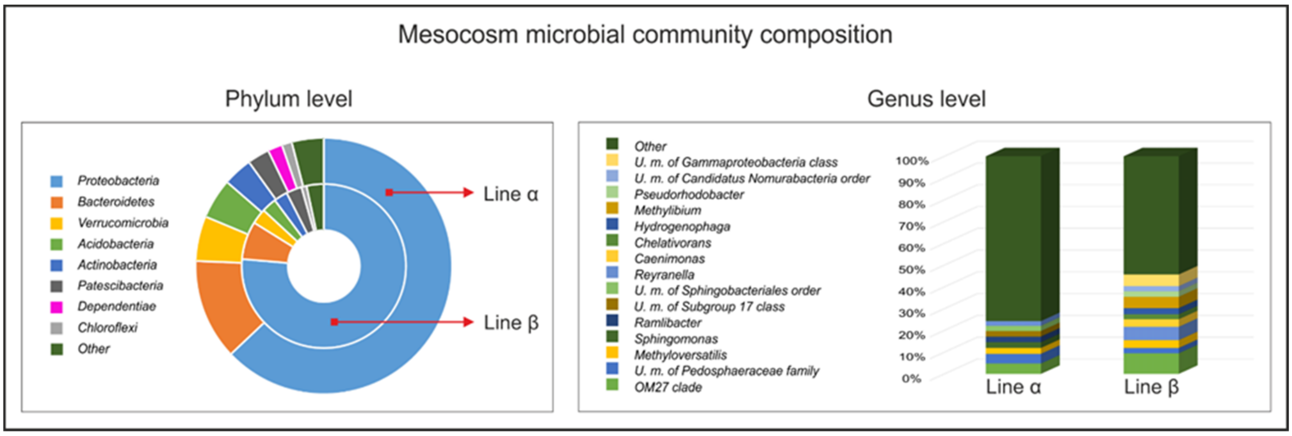

Figure 6. Mesocosm microbial community composition at phylum (**on the left**) and genus (**on the right**) levels of Lines α and β. Taxa with relative abundance values below 1% and 2% for phylum and genus levels, respectively, are labeled "Other". U. m. refers to unclassified microorganisms. The relative abundance of each taxon has been calculated as the mean of values of the triplicate experiments.

However, differences emerged when comparing the percentages of these taxa with the relative abundance of the same phyla retrieved in the original groundwater sample. For example, the abundance of *Proteobacteria* went from 64.14% to 62.92% (on average) in Line α mesocosms and 76.36% (on average) in Line β mesocosms. Moreover, the same goes for the other phyla, as reported in Table 1.

Table 1. Variations of the relative abundance average values of the main phyla in groundwater samples.

Taxonomy	Pz3	Line α	Line β
Proteobacteria	64.14%	62.92%	76.36%
Bacteroidetes	8.34%	12.71%	7.51%
Chloroflexi	5.23%	1.27%	1.02%
Verrucomicrobia	3.91%	5.68%	3.03%
Actinobacteria	3.38%	3.69%	2.70%
Acidobacteria	3.07%	5.08%	2.92%
Firmicutes	2.90%	0.22%	0.32%
Gemmatimonadetes	1.53%	0.65%	0.23%
Patescibacteria	1.49%	2.78%	3.08%

In addition, the phylum *Chloroflexi*, which was among the major three phyla in the original groundwater sample (5.23%), showed a decrease in the mesocosms to percentages of 1.27% and 1.02% when Phy-Et was added at 5.2 mg/L and 27.4 mg/L, respectively.

When analyzing the mesocosm microbial communities at the genus taxonomic level (Figure 6), the top six taxa retrieved in the Line α experiments were: microorganisms of the clade *OM27* (average relative abundance 4.77%), unclassified microorganisms of *Pedosphaeraceae* family (average relative abundance 4.46%), *Methyloversatilis* (average relative abundance 2.72%), *Sphingomonas* (average relative abundance 2.64%), *Ramlibacter* (average relative abundance 2.61%), and unclassified microorganisms of Subgroup 17 class (*Acidobacteria* phylum; average relative abundance 2.51%). On the other hand, the top six genera found in the Line β mesocosms were microorganisms of the clade *OM27* (average relative abundance 9.49%), *Reyranella* (average relative abundance 6.14%), unclassified microorganisms of *Gammaproteobacteria* class (average relative abundance 5.35%), *Methylibium* (average relative abundance 5.18%), *Methyloversatilis* (average relative abundance 3.57%), and *Caenimonas* (average relative abundance 3.47%).

These results suggest that Phy-Et has modified the composition and species abundance of the groundwater microbial communities, most likely inhibiting the growth of cells sensitive to its action and favoring the survival of those tolerant or able to metabolize it.

In addition, another important aspect that emerged is the presence of diverse unclassified microorganisms at the genus level, accounting on average for 41.58% and 29.14% in Line α Line β mesocosms, respectively. This observation demonstrates that a large portion of microbial diversity in the analyzed systems remained hidden.

The rarefaction analysis, a measure used to estimate the alpha diversity in samples and gauge whether sequencing efforts captured the microbial diversity (Supplementary Materials Figure S1), revealed a biodiversity reduction trend correlated to the increase of Phy-Et concentrations.

4. Discussion

On a global scale, pharmaceuticals and personal care products (PPCPs) have risen in the last decade due to advances in research and development, increased world population, and accessibility to healthcare [30,31]. Given their known or suspected adverse ecological or human health effects, PPCPs are considered two of the most abundant classes of emerging contaminants in the environment [32,33]. Among the most dramatic effects on living organisms are the feminization of fishes [10,34], alterations in the reproduction and development of some fish species caused by very low concentrations of environmental estrogens [35], and increased antibiotic resistance of pathogenic microorganisms [36]. Their presence in aquatic and terrestrial ecosystems is associated with their occurrence at influents and effluents of wastewater treatment plants [33], whose removal capacity strongly depends on the features of the pollutant and the technologies used for wastewater treatments.

In this research, the authors have focused on Phy-Et, a compound present as a preservative in many cosmetics. According to the currently available safety data, this substance is considered safe when used at a concentration of up to 1%, is a rare sensitizer, and is one of the most well-tolerated preservatives [20]. Nevertheless, animal studies revealed different adverse systemic effects at higher levels of exposure than consumers when using Phy-Et-containing products [20]. In addition, other authors who analyzed the toxic effects of this compound in an eukaryotic model organism, *Allium cepa*, claimed that, in the light of their data, "it should be ensured that the use of Phy-Et should be limited, if not preferred, or if it is absolutely necessary to use it in doses that do not have toxic effects on the organisms" [15]. Accordingly, the main aim of this work was to analyze the capacity of microbial communities to remove Phy-Et (supplied at two different concentrations) from groundwater through mesocosm experiments designed to represent and simulate, on a laboratory scale, the conditions of shallow groundwater flowing in the daily heterothermic zone, characterized by significant and frequent temperature fluctuations closely tied to atmospheric conditions. Additionally, in the piezometer Pz3, the recorded temperature values ranged from 13.2 °C to 16.1 °C, with an average value of 14.4 °C whereas the temperature of the mesocosms ranged from a minimum of 17.9 °C to a maximum of 24.2 °C, with an average value of 21.0 °C. Even though the water temperatures in the lab-scale experiments were higher than those measured in the field during the observation period, it is worth noting that many of the genera detected in the mesocosms include species characterized by a wide range of growth temperatures. Accordingly, the authors are convinced that the observed microbial community variations, induced by the presence of Phy-Et, nevertheless provide an accurate representation of the events that would occur in nature.

However, in a wider context, similar temperatures to those measured in the mesocosms have also been found in other hydrogeological systems [37], with values reaching 24.0 °C during summer. In a future perspective, further studies will be carried out with the use of a refrigerated thermostat (e.g., [38]) to control the temperatures to which these systems are exposed, to subject the autochthonous microbial communities to the same fluctuations observed during the hydrological year in the field.

In the frame time considered, autochthonous microorganisms (i) were able to reduce by ≈100% and ≈84% the amount of Phy-Et present in the systems with higher removal effectiveness when the concentration of the contaminant was lower (5.2 mg/L), and (ii) represented the major contributors to the natural attenuation process. Additionally, these particularly encouraging data also suggest the existence of a "dose-dependent effect" of Phy-Et on the process of biodegradation, given the observed decrease of the "natural attenuation efficacy" (≈16%) with the increasing contaminant concentrations. The analysis of bacterial communities through NGS technologies revealed drastic changes in their composition determined by the presence of Phy-Et, evidenced by a reduction in biodiversity linked to increasing Phy-Et concentrations. It is known that Phy-Et exerts its antimicrobial activity by uncoupling oxidative phosphorylation from respiration and by competitively inhibiting malate dehydrogenase [39]. Moreover, it also acts as a bactericidal agent by increasing the permeability of the cell membrane to potassium ions and exerts a direct inhibitory effect on microbial DNA and RNA synthesis [39].

Therefore, it is not surprising that the addition of the contaminant has shaped microbial communities so that the more sensitive microorganisms were under-represented in the mesocosm experiments compared to the original groundwater.

On the other hand, the presence of microorganisms of the clade *OM27* as dominant components of the "contaminated" samples is noteworthy. *OM27* clade is a cluster of unculturable bacteria, phylogenetically related to the predatory deltaproteobacterial genus *Bdellovibrio* [40,41], which have a geographically wide distribution [40] but of which very little is known [42]. Their potential predatory "attitude" could confer an advantage under the selective pressure of Phy-Et. Conversely, the biodegradation potentials of some members belonging to the bacterial genera such as, for example, *Methyloversatilis*, *Methylibium*, *Ramlibacter*, *Reyranella*, and *Sphingomonas*, are known, and this could explain why they were found as the main representatives in the mesocosm experiments.

Interestingly, there is a large portion of microbial biodiversity that remains hidden, as demonstrated by the relatively high percentages of unclassified microorganisms at the genus level, and that should be uncovered through the development of appropriate research tools, given the importance of acquiring knowledge on the main processes underlying the bacterial biodegradation of contaminants in nature.

Several thousands of PPCPs are produced globally every year, and their release into the environment remains an unavoidable by-product of a modernized lifestyle [43–46]. PPCPs may enter the environment as components of human or animal waste after incomplete absorption and excretion from the body or may result from medical, industrial, agricultural, or household waste emissions [47–50]. Trace amounts of PPCP-related compounds have been found in waste, aquatic ecosystems, or finished drinking water [48,51–58], suggesting widespread contamination and highlighting the importance of monitoring these compounds once they are released into the environment.

This research has demonstrated that microorganisms have a high potential for removing the largely used preservative Phy-Et from groundwater, at least in an artificial laboratory system. However, knowledge about the extent of their degradative capabilities in natural contexts still remains scarce. Accordingly, this topic should be further deepened, for this and many other contaminants of emerging concern, through the improvement of tools and protocols to evaluate their occurrence and fate in nature (for example, by promoting the execution of several large-scale monitoring campaigns in different environmental contexts and developing of different analytical methods for CEC detection, together with extensive analyses aimed at unraveling microbe potentiality for biodegradation, based on traditional and molecular microbiological methods) and the assessment of the impact of their release and the effects of long-term exposure (even at low concentrations) on ecosystems and health.

5. Conclusions

In light of the extensive global production of pharmaceuticals and personal care products (PPCPs), their inevitable release into the environment represents a salient implication of contemporary lifestyles. Consequently, persistent endeavors are required to evaluate these compounds' occurrence and subsequent fate in the natural environment and understand the potential long-term effects on ecosystems and human health.

This research's focal point revolved around examining Phy-Et, a common preservative in cosmetic products. Although considered safe at concentrations up to 1% based on existing data, studies have raised concerns about its adverse systemic effects at higher exposure levels.

This work sought to understand the potential of microbial communities in removing Phy-Et from groundwater through specifically designed mesocosm experiments, which aimed to simulate shallow groundwater conditions.

While this research has demonstrated encouraging results, including significant reductions in Phy-Et concentrations and the pivotal role played by autochthonous microorganisms, it has also revealed the existence of a dose-dependent effect. The composition of bacterial communities was notably altered by Phy-Et, which determined a decrease in biodiversity. These findings underscore the significance of Phy-Et's impact on microbial ecosystems.

In addition, the presence of both unculturable microorganisms, such as the *OM27* clade, and a significant fraction of "hidden" microbial diversity to unravel raises intriguing possibilities and challenges for future explorations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16052183/s1, Figure S1: Rarefaction curves of the samples. The alpha diversity plots were obtained by using the Shannon index.

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References

1. Fuller, R.; Landrigan, P.J.; Balakrishnan, K.; Bathan, G.; Bose-O'Reilly, S.; Brauer, M.; Caravanos, J.; Chiles, T.; Cohen, A.; Corra, L.; et al. Pollution and health: A progress update. *Lancet Planet. Health* **2022**, *6*, e535–e547. [CrossRef] [PubMed]
2. Landrigan, P.J.; Fuller, R.; Acosta, N.J.; Adeyi, O.; Arnold, R.; Basu, N.; Baldé, A.B.; Bertollini, R.; Bose-O'Reilly, S.; Boufford, J.I.; et al. The Lancet Commission on pollution and health. *Lancet* **2018**, *391*, 462–512. [CrossRef] [PubMed]
3. Yadav, D.; Rangabhashiyam, S.; Verma, P.; Singh, P.; Devi, P.; Kumar, P.; Hussain, C.M.; Gaurav, G.K.; Kumar, K.S. Environmental and health impacts of contaminants of emerging concerns: Recent treatment challenges and approaches. *Chemosphere* **2021**, *272*, 129492. [CrossRef]

4. Vasilachi, I.C.; Asiminicesei, D.M.; Fertu, D.I.; Gavrilescu, M. Occurrence and fate of emerging pollutants in water environment and options for their removal. *Water* **2021**, *13*, 181. [CrossRef]
5. Geissen, V.; Mol, H.; Klumpp, E.; Umlauf, G.; Nadal, M.; van der Ploeg, M.; van de Zee, S.E.A.T.M.; Ritsema, C.J. Emerging pollutants in the environment: A challenge for water resource management. *Int. Soil Water Conserv. Res.* **2015**, *3*, 57–65. [CrossRef]
6. Snow, D.D.; Cassada, D.A.; Larsen, M.L.; Mware, N.A.; Li, X.; D'Alessio, M.; Zhang, Y.; Sallach, J.B. Detection, occurrence and fate of emerging contaminants in agricultural environments. *Water Environ. Res.* **2017**, *89*, 897–920. [CrossRef] [PubMed]
7. Gomes, A.R.; Justino, C.; Rocha-Santos, T.; Freitas, A.C.; Duarte, A.C.; Pereira, R. Review of the ecotoxicological effects of emerging contaminants to soil biota. *J. Environ. Sci. Health Part A* **2017**, *52*, 992–1007. [CrossRef]
8. Risk Assessment Guidance for Superfund Volume 1. Human Health Evaluation Manual (Part E, Supplemental Guidance for Dermal Risk Assessment), EPA/540/R/99/005 Office of Superfund Remediation and Technology Innovation. U.S. Environmental Protection Agency: Washington, DC, USA. Available online: https://www.epa.gov/sites/production/files/2015-09/documents/part_e_final_revision_10-03-07.pdf (accessed on 12 October 2020).
9. United States Environmental Protection Agency (USEPA). Contaminants of Emerging Concern Including Pharmaceuticals and Personal Care Products. Washington, DC, USA. Available online: https://www.epa.gov/wqc/contaminants-emerging-concern-including-Pharmaceuticals-and-personal-care-products (accessed on 12 October 2020).
10. Patel, M.; Kumar, R.; Kishor, K.; Mlsna, T.; Pittman, C.U., Jr.; Mohan, D. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. *Chem. Rev.* **2019**, *119*, 3510–3673. [CrossRef]
11. Baken, K.A.; Sjerps, R.M.; Schriks, M.; van Wezel, A.P. Toxicological risk assessment and prioritization of drinking water relevant contaminants of emerging concern. *Environ. Int.* **2018**, *118*, 293–303. [CrossRef]
12. Khatib, J.M.; Baydoun, S.; ElKordi, A.A. Water pollution and urbanisation trends in Lebanon: Litani River basin case study. In *Urban Pollution: Science and Management*; John Wiley & Sons Ltd.: Hoboken, NJ, USA, 2018; pp. 397–415. [CrossRef]
13. Petrie, B.; Barden, R.; Kasprzyk-Hordern, B. A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* **2015**, *72*, 3–27. [CrossRef]
14. Park, J.; Yamashita, N.; Park, C.; Shimono, T.; Takeuchi, D.M.; Tanaka, H. Removal characteristics of Pharmaceuticals and Personal Care Products: Comparison between membrane bioreactor and various biological treatment processes. *Chemosphere* **2017**, *179*, 347–358. [CrossRef]
15. Akgündüz, M.Ç.; Çavuşoğlu, K.; Yalçın, E. The potential risk assessment of Phenoxyethanol with a versatile model system. *Sci. Rep.* **2020**, *10*, 1209. [CrossRef]
16. Wang, J.; Liu, Y.; Kam, W.R.; Li, Y.; Sullivan, D.A. Toxicity of the cosmetic preservatives parabens, phenoxyethanol and chlorphenesin on human meibomian gland epithelial cells. *Exp. Eye Res.* **2020**, *196*, 108057. [CrossRef]
17. Panico, A.; Serio, F.; Bagordo, F.; Grassi, T.; Idolo, A.; De Giorgi, M.; Guido, M.; Congedo, M.; De Donno, A. Skin safety and health prevention: An overview of chemicals in cosmetic products. *J. Prev. Med. Hyg.* **2019**, *60*, E50.
18. Gilbert, P.; Beveridge, E.G.; Crone, P.B. The action of Phenoxyethanol upon respiration and dehydrogenase enzyme systems in Escherichia coli. *J. Pharm. Pharmacol. Suppl.* **1976**, *28*, 51.
19. Lowe, I.; Southern, J. The antimicrobial activity of Phenoxyethanol in vaccines. *Lett. Appl. Microbiol.* **1994**, *18*, 115–116. [CrossRef] [PubMed]
20. Dréno, B.; Zuberbier, T.; Gelmetti, C.; Gontijo, G.; Marinovich, M. Safety review of Phenoxyethanol when used as a preservative in cosmetics. *J. Eur. Acad. Dermatol. Venereol.* **2019**, *33*, 15–24. [CrossRef]
21. Ducci, L.; Rizzo, P.; Pinardi, R.; Solfrini, A.; Maggiali, A.; Pizzati, M.; Balsamo, F.; Celico, F. What Is the Impact of Leaky Sewers on Groundwater Contamination in Urban Semi-Confined Aquifers? A Test Study Related to Fecal Matter and Personal Care Products (PCPs). *Hydrology* **2022**, *10*, 3. [CrossRef]
22. Di Dio, G.; Lasagna, S.; Preti, D.; Sagne, M. Carta geologica dei depositi quaternari della provincia di Parma. *Il Quat.* **1997**, *10*, 443–450.
23. Di Dio, G. *Applicazione di Concetti e Metodi della Stratigrafia Fisica alla Ricerca di Risorse Idriche nel Sottosuolo della Pianura Emiliano-Romagnola*; Serie 3a; Giornale di Geologia: Bologna, Italy, 1998; Volume 60, pp. 35–39.
24. Regione Emilia-Romagna; ENI-AGIP. *Riserve Idriche Sotterranee Della Regione Emilia-Romagna*; Cura di, A., Di Dio, G.M., Eds.; Regione Emilia-Romagna, ENI Agip Divisione Esplorazione e Produzione; S.EL.CA: Firenze, Italy, 1998; p. 120.
25. Pinardi, R.; Feo, A.; Ruffini, A.; Celico, F. Purpose-Designed Hydrogeological Maps for Wide Interconnected Surface–Groundwater Systems: The Test Example of Parma Alluvial Aquifer and Taro River Basin (Northern Italy). *Hydrology* **2023**, *10*, 127. [CrossRef]
26. Zanini, A.; Ghirardi, M.; Emiliani, R. A multidisciplinary approach to evaluate the effectiveness of natural attenuation at a contaminated site. *Hydrology* **2021**, *8*, 101. [CrossRef]
27. Di Dio, G.; Martini, A.; Lasagna, S.; Zanzucchi, G. *Illustrative Notes of the Geological Map of Italy at 1:50,000 Scale Sheet 199 Parma Sud. Geological, Seismic, and Soil Service of the Emilia-Romagna Region, APAT-Geological Service of Italy*; S.EL.CA: Florence, Italy, 2005.
28. *UNI EN ISO 9001:2015*; Quality Management Systems Requirements. International Organization for Standardization: Geneva, Switzerland, 2015.
29. *UNI EN ISO 13485:2016*; Medical Devices—Quality Management Systems—Requirements for Regulatory Purposes. International Organization for Standardization: Geneva, Switzerland, 2016.
30. Van Boeckel, T.P.; Gandra, S.; Ashok, A.; Caudron, Q.; Grenfell, B.T.; Levin, S.A.; Laxminarayan, R. Global antibiotic consumption 2000 to 2010: An analysis of national Pharmaceutical sales data. *Lancet Infect. Dis.* **2014**, *14*, 742–750. [CrossRef]

31. Adeleye, A.S.; Xue, J.; Zhao, Y.; Taylor, A.A.; Zenobio, J.E.; Sun, Y.; Han, Z.; Salawu, O.A.; Zhu, Y. Abundance, fate, and effects of Pharmaceuticals and Personal Care Products in aquatic environments. *J. Hazard. Mater.* **2022**, *424*, 127284. [CrossRef]
32. Khan, S.; Naushad, M.; Govarthanan, M.; Iqbal, J.; Alfadul, S.M. Emerging contaminants of high concern for the environment: Current trends and future research. *Environ. Res.* **2022**, *207*, 112609. [CrossRef]
33. Lozano, I.; Pérez-Guzmán, C.J.; Mora, A.; Mahlknecht, J.; Aguilar, C.L.; Cervantes-Avilés, P. Pharmaceuticals and Personal Care Products in water streams: Occurrence, detection, and removal by electrochemical advanced oxidation processes. *Sci. Total Environ.* **2022**, *827*, 154348. [CrossRef]
34. Jarque, S.; Quirós, L.; Grimalt, J.O.; Gallego, E.; Catalan, J.; Lackner, R.; Piña, B. Background fish feminization effects in European remote sites. *Sci. Rep.* **2015**, *5*, 11292. [CrossRef]
35. Kalia, V.C. Pharmaceutical and personal care product contamination: A global scenario. In *Pharmaceuticals and Personal Care Products: Waste Management and Treatment Technology*; Butterworth-Heinemann: Oxford, UK, 2019; pp. 27–61. [CrossRef]
36. Zhang, X.; Yan, S.; Chen, J.; Tyagi, R.D.; Li, J. Physical, chemical, and biological impact (hazard) of hospital wastewater on environment: Presence of Pharmaceuticals, pathogens, and antibiotic-resistance genes. In *Current Developments in Biotechnology and Bioengineering*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 79–102. [CrossRef]
37. Segadelli, S.; Vescovi, P.; Ogata, K.; Chelli, A.; Zanini, A.; Boschetti, T.; Petrella, E.; Toscani, L.; Gargini, A.; Celico, F. A conceptual hydrogeological model of ophiolitic aquifers (serpentinised peridotite): The test example of Mt. Prinzera (North. Italy). *Hydrol. Process.* **2017**, *31*, 1058–1073. [CrossRef]
38. Allocca, V.; Celico, F.; Petrella, E.; Marzullo, G.; Naclerio, G. The role of land use and environmental factors on microbial pollution of mountainous limestone aquifers. *Environ. Geol.* **2008**, *55*, 277–283. [CrossRef]
39. CIR (Cosmetic Ingredient Review). Final report on the safety assessment of Phenoxyethanol. *J. Am. Coll. Toxicol.* **1990**, *9*, 259–278. [CrossRef]
40. Orsi, W.D.; Smith, J.M.; Liu, S.; Liu, Z.; Sakamoto, C.M.; Wilken, S.; Poirier, C.; Richards, T.A.; Keeling, P.J.; Worden, A.Z.; et al. Diverse, uncultivated bacteria and archaea underlying the cycling of dissolved protein in the ocean. *ISME J.* **2016**, *10*, 2158–2173. [CrossRef] [PubMed]
41. Gorokhova, E.; Motiei, A.; El-Shehawy, R. Understanding biofilm formation in ecotoxicological assays with natural and anthropogenic particulates. *Front. Microbiol.* **2021**, *12*, 632947. [CrossRef]
42. Yilmaz, P.; Yarza, P.; Rapp, J.Z.; Glöckner, F.O. Expanding the world of marine bacterial and archaeal clades. *Front. Microbiol.* **2016**, *6*, 1524. [CrossRef]
43. Caldwell, D.J.; Mastrocco, F.; Margiotta-Casaluci, L.; Brooks, B.W. An integrated approach for prioritizing Pharmaceuticals found in the environment for risk assessment, monitoring and advanced research. *Chemosphere* **2014**, *115*, 4–12. [CrossRef]
44. Fatta-Kassinos, D.; Meric, S.; Nikolaou, A. Pharmaceutical residues in environmental waters and wastewater: Current state of knowledge and future research. *Anal. Bioanal. Chem.* **2011**, *399*, 251–275. [CrossRef] [PubMed]
45. Khetan, S.K.; Collins, T.J. Human Pharmaceuticals in the aquatic environment: A challenge to green chemistry. *Chem. Rev.* **2007**, *107*, 2319–2364. [CrossRef] [PubMed]
46. Tran, N.; Drogui, P.; Brar, S.K. Sonochemical techniques to degrade Pharmaceutical organic pollutants. *Environ. Chem. Lett.* **2015**, *13*, 251–268. [CrossRef]
47. Li, W.C. Occurrence, sources, and fate of Pharmaceuticals in aquatic environment and soil. *Environ. Pollut.* **2014**, *187*, 193–201. [CrossRef] [PubMed]
48. Michael, I.; Rizzo, L.; McArdell, C.S.; Manaia, C.M.; Merlin, C.; Schwartz, T.; Dagot, C.; Fatta-Kassinos, D. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Res.* **2013**, *47*, 957–995. [CrossRef] [PubMed]
49. Sharma, V.K.; Anquandah, G.A.; Nesnas, N. Kinetics of the oxidation of endocrine disruptor nonylphenol by ferrate (VI). *Environ. Chem. Lett.* **2009**, *7*, 115–119. [CrossRef]
50. Taylor, D.; Senac, T. Human Pharmaceutical products in the environment—The "problem" in perspective. *Chemosphere* **2014**, *115*, 95–99. [CrossRef] [PubMed]
51. Anquandah, G.A.; Sharma, V.K.; Panditi, V.R.; Gardinali, P.R.; Kim, H.; Oturan, M.A. Ferrate (VI) oxidation of propranolol: Kinetics and products. *Chemosphere* **2013**, *91*, 105–109. [CrossRef] [PubMed]
52. Kuzmanović, M.; Ginebreda, A.; Petrović, M.; Barceló, D. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. *Sci. Total Environ.* **2015**, *503*, 289–299. [CrossRef] [PubMed]
53. Petrović, M.; Škrbić, B.; Živančev, J.; Ferrando-Climent, L.; Barcelo, D. Determination of 81 Pharmaceutical drugs by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole–linear ion trap in different types of water in Serbia. *Sci. Total Environ.* **2014**, *468*, 415–428. [CrossRef] [PubMed]
54. Qin, Q.; Chen, X.; Zhuang, J. The fate and impact of Pharmaceuticals and Personal Care Products in agricultural soils irrigated with reclaimed water. *Crit. Rev. Environ. Sci. Technol.* **2015**, *45*, 1379–1408. [CrossRef]
55. Sharma, V.K.; Anquandah, G.A.; Yngard, R.A.; Kim, H.; Fekete, J.; Bouzek, K.; Ray, A.K.; Golovko, D. Nonylphenol, octylphenol, and bisphenol-A in the aquatic environment: A review on occurrence, fate, and treatment. *J. Environ. Sci. Health Part A* **2009**, *44*, 423–442. [CrossRef]
56. Sharma, V.K.; Liu, F.; Tolan, S.; Sohn, M.; Kim, H.; Oturan, M.A. Oxidation of β-lactam antibiotics by ferrate (VI). *Chem. Eng. J.* **2013**, *221*, 446–451. [CrossRef]

57. Tölgyesi, Á.; Verebey, Z.; Sharma, V.K.; Kovacsics, L.; Fekete, J. Simultaneous determination of corticosteroids, androgens, and progesterone in river water by liquid chromatography–tandem mass spectrometry. *Chemosphere* **2010**, *78*, 972–979. [CrossRef]
58. Verlicchi, P.; Galletti, A.; Petrovic, M.; Barceló, D. Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options. *J. Hydrol.* **2010**, *389*, 416–428. [CrossRef]