

Article

Distribution of Endocrine Disruptor Chemicals and Bacteria in Saline Pétrola Lake (Albacete, SE Spain) Protected Area is Strongly Linked to Land Use

Alfonso Menchén ^{1,*}, Yolanda Espín ¹, Nicolás Valiente ^{1,2}, Beatriz Toledo ¹, Manuel Álvarez-Ortí ¹ and Juan José Gómez-Alday ¹

¹ Group of Hydrogeology, Biotechnology and Natural Resources Laboratory, Institute for Regional Development, University of Castilla-La Mancha, 02071 Albacete, Spain; Yolanda.Espin@uclm.es (Y.E.); n.v.parra@ibv.uio.no (N.V.); Beatriz.Toledo@uclm.es (B.T.); manuel.alvarez@uclm.es (M.Á.-O.); JuanJose.Gomez@uclm.es (J.J.G.-A.)

² Centre for Biogeochemistry in the Anthropocene, Department of Biosciences, Section for Aquatic Biology and Toxicology, University of Oslo, P.O. Box 1066 Blindern, 0316 Oslo, Norway

* Correspondence: Alfonso.Menchen@uclm.es

Received: 25 May 2020; Accepted: 7 June 2020; Published: 10 June 2020



Abstract: Saline lakes are subject to numerous environmental impacts related to human activities, changing the chemical and biological natural conditions of the ecosystem. Sustainable development depends on the conservation of such delicate saline ecosystems, which may hold distinctive biodiversity. Pollution is one of the major threats to surface water bodies, for example by increasing nutrient contents and organic pollutants, including endocrine disrupting chemicals. Microbially mediated redox processes exert a fundamental control on nutrient turnover and contaminant removal. This study examines the influence of land use on the distribution of endocrine disrupting chemicals as well as on the microbial community composition in lacustrine sediments from Pétrola saline Lake (SE Spain). The lake is impacted by anthropogenic activities (agriculture, farming, mining and urban wastewater spills). Applying chemical and molecular tools (sequencing of 16S rRNA gene) showed a clear influence of land use on the chemistry and bacterial abundance of the lake sediments. The sampling points closer to wastewater outflows and mining ponds (2635, 2643 and 2650) showed fewer numbers and types of endocrine disrupting chemicals as well as a smaller number of families in the microbial community. These findings improve our understanding of how land use affects both water chemistry and the abundance of organisms responsible for biogeochemical cycles.

Keywords: Hypersaline lake; endocrine disruptors; GC-MSD; 16 rRNA gene; Sediment microbiota; Bacterial bioremediation

1. Introduction

The Sustainable Development Goals established by the United Nations (UN) encourage the international community to safeguard ecosystems by 2030 due to their close relation with the conservation of biodiversity and water resources, and human health [1]. In agreement with these goals, the European Water Framework Directive (WFD) and the European Green Deal communication require European Union member states to reach good environmental status of their aquatic ecosystems. One of the major risks for water bodies and ecosystem sustainability involve emerging pollutants [2,3]. Facing this challenge calls for evaluating the distribution and fate of pollutants as well as the bacterial community structure (composition). In the case of wetlands, the attenuation capacity of pollutants is linked to the bacterial communities at the sediment–water interface [4–7]. Therefore, the relative abundance and distribution of bacteria can be an indicator for the attenuation potential of pollutants

linked to biogeochemical turnover. Moreover, bacterial distribution can be also affected by land use in the catchment areas of water bodies. In this study, we evaluated the distribution of bacterial communities and endocrine disruptors by analysing lacustrine sediments and water samples.

Pétrola wetland (SE Spain, Figure 1) is a hypersaline playa lake type encompassing a saline complex formed by 12 shallow lakes covering an area of approximately 25 km². This is one of the most important saline wetlands in the region of Castilla-La Mancha and is an important hotspot of biodiversity. It has therefore been given protection status in the form of several regulations: “Sites of Community Importance” EU 1997, “Natural Reserve” Junta de Castilla-La Mancha Regional Government (DOCM 26/2003), and Special Protection Area (Code: ES0000153 Steppe area of eastern Albacete). In addition to the botanical, faunal and hydrological characteristics of the area, the lake serves as a refuge for migratory birds; it sustains an important population of flamingos and hosts remarkable endangered species such as the white-headed duck (*Oxyura leucocephala*). The lake exhibits a marked water level fluctuation due to the semiarid climate of the region, which leads to an imbalance between water inputs and outputs. The hydroperiod is characterized by a temporarily flooded period during the wet season. Irrigation return flows and direct spills of urban wastewaters to the lake have changed the hydroperiod, and during relatively wet years, the lake no longer becomes completely dry. The area has been subjected to previous geochemical studies on the potential nitrate attenuation capacity [8,9]. The anthropogenic impact involves organic pollutants such as endocrine disrupting chemicals (EDCs). EDCs—defined as exogenous substances or mixtures that possess properties that might lead to endocrine disruption in living organisms or their progeny or (sub) populations [10]—can affect the ecological status of natural water bodies. As no previous studies have been conducted on this issue in the study area, this represents a first characterization of the endocrine disruptors. EDC were identified based on an available EDC database. The availability of organic carbon, coupled to the supply of electron acceptor species (such as NO₃⁻, SO₄²⁻), maintain the potential of sediment to support degradation reactions of chemicals. The biodegradation potential is high at the sediment–water interface, where microorganisms can use available organic and inorganic substances as a source of energy for metabolic functions or biomass production, oxide-reductases or hydrolases enzymes [11–13]. The fate of many contaminants in water bodies is governed by biogeochemical cycles. These are driven by microbial processes and thus depend on bacterial community composition.

Little is known about the distribution of endocrine disruptors and its relationship with bacterial community composition. The first objective of this study is therefore to examine the spatial distribution and sources of EDC. For this purpose, we analysed water samples from lake water and groundwater discharge points (streams and springs). In addition, the study attempts to link the bacterial composition to the distribution of EDC, providing a better understanding of the potential influence of land use on their distribution.

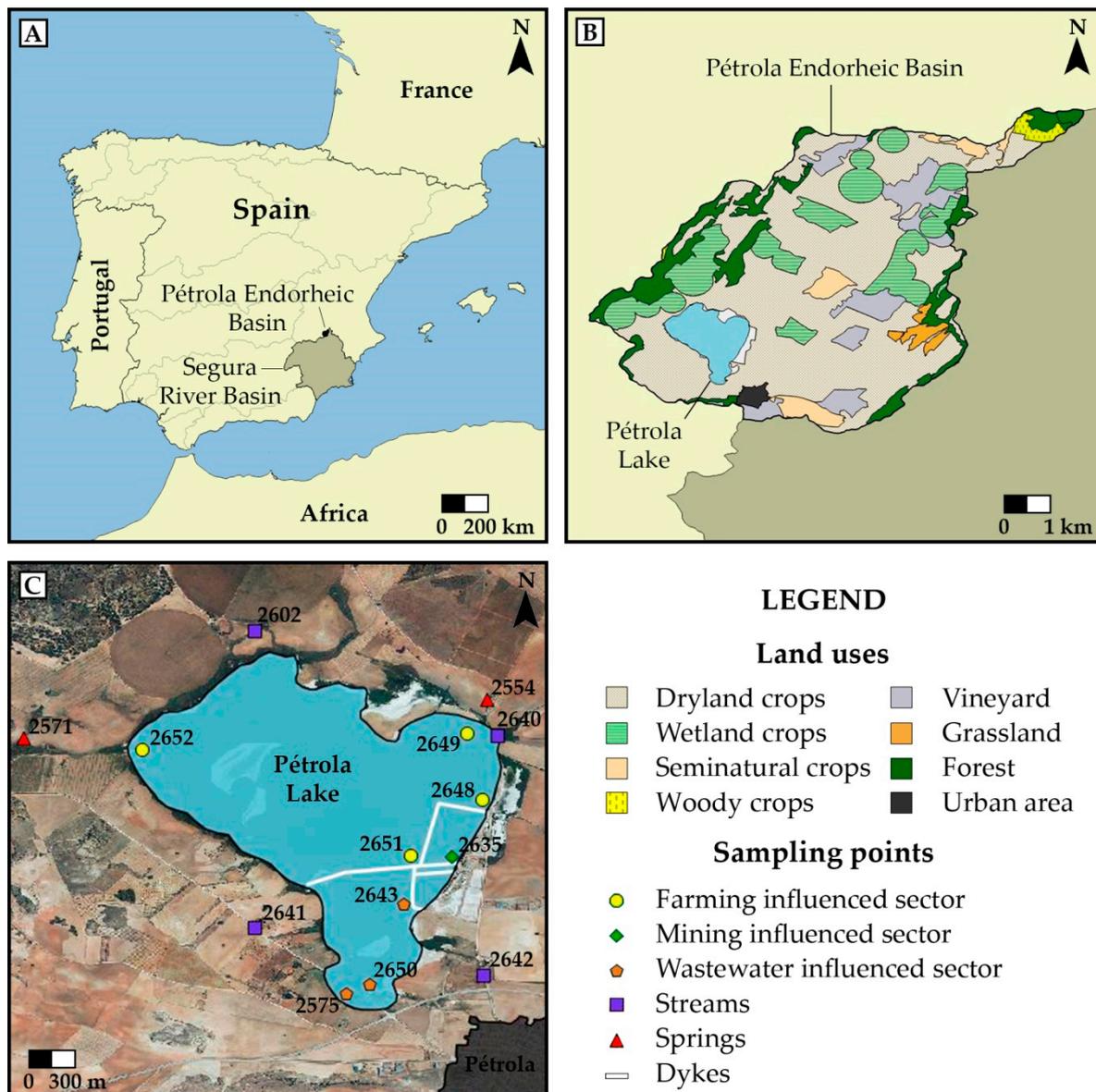


Figure 1. (A) Location of Pétrola lake at Segura River Basin in Spain. (B) Delimitation of Pétrola endorheic basin (data from Ministry for Ecological Transition and Demographic Challenge) and main land uses present in the study area (data from CORINE Land Cover, 2018). (C) Location of the water sampling points in the Pétrola Lake area.

2. Materials and Methods

2.1. Study Area

Pétrola Lake is one of the most representative saline wetlands in the Castilla-La Mancha region and is affected by noticeable anthropogenic pressures (irrigation, farming, urban wastewaters outflows). The lake–aquifer system extends over 43 km² and occupies the terminal discharge zone (the lake is connected to the aquifer) of an endorheic basin, into which various small streams discharge in a radial pattern (Figure 1). The maximum volume of stored water is reached in early May (0.78 hm³), corresponding to an estimated flooded area of 1.32 km² [14]. The maximum water depth reaches 2 m. Salinity can reach a value up to 109,400 μS/cm (November 2010) [15]. The geological context of the study area was described in Gómez-Alday et al. [8]. Two types of farming are present: dryland farming and irrigation farming. Farming encompasses approximately 75% of the total area, whereby

dryland farming contributes about 58% and irrigated cropland 17%. Irrigation return flows reach the lake through streams and springs. The impact of human activities is clearly evident in the elevated nitrate concentrations in groundwater, peaking at 149 mg/L (March 2009) [8]. The remaining area is occupied by semi-natural land cover types including Mediterranean forest and scrubland (19%) [16]. Urban wastewaters from a population of 692 inhabitants (year 2019) are discharged directly into the lake, without proper treatment. Ammonium in wastewaters can reach values of about 43 mg/L [9]. Moreover, mining activities had an important influence up until the 1990s, disrupting original lake geomorphology. Finally, brines were exploited in the 20th century by isolating part of the lake with dikes, forming evaporation ponds (Figure 1). Recently, organic-rich lake bottom sediments, mixed with a detrital fraction, have been deposited, enhanced by the eutrophic conditions.

2.2. Sample Collection

Sampling sites comprised springs and streams surrounding the lake (2554, 2571, 2602, 2640, 2641, 2642) as well as the surficial water of the lake (Figure 1). In the lake, sampling points were distributed into 5 groups according to their proximity to the main anthropogenic pressures around the lake perimeter and the compartmentation of the lake by artificial dikes. The artificial dikes were designed to minimize the effects of human pressures on lake hydroecology by isolating parts of the water body. Communication between isolated parts occurs during the high flooding level. Sites 2649, 2651 and 2652 are representative of agricultural discharge areas (irrigation return flows). Sampling point 2651 is close to the depocenter of the lake and away from the main pollutant inputs. Site 2648 is located close to the input of an artificial channel that discharges effluents from livestock directly into the lake. Site 2635 represents an isolated evaporating pond for salt extraction, with a limited communication with the lake. Sites 2575, 2643 and 2650 are separated from the rest of the lake area by an artificial dike, and all of them are influenced by urban outflows, with 2575 being the closest, about 0.25 km from the raw wastewater. Site 2643 is set 0.78 km away from the urban wastewater raw discharge point. The stream 2642 reaches this zone. Synthetic and organic fertilizers from agriculture reach the lake through small streams.

2.2.1. Water Sampling

A total of 112 water samples, collected monthly from March to December 2018, were analysed to identify endocrine disrupting chemicals at 14 sampling points (Figure 1). Surface water samples were collected at the lake sites 2648, 2649, 2650, 2651 and 2652, the evaporating pond (2635), and the wastewater-influenced area pond (2575, 2643). Springs and streams surrounding the lake (2554, 2571, 2602, 2640, 2641, 2642) were also sampled during the same period. Physico-chemical parameters were measured in situ using a HQ40d portable Multi-Parameter Meter (Hach Company, Loveland, CO, USA). The accuracy of physico-chemical determinations was ± 0.3 °C for T, ± 0.002 for pH, $\pm 0.5\%$ for EC and TDS (± 200 mS/cm), ± 0.1 mV for Eh, and ± 0.01 mg/L for dissolved oxygen. One litre of water was collected with an amber glass flask, previously rinsed several times with the water of the sample itself. Afterwards, the flask was sealed with a screw cap, avoiding headspace, refrigerated and transported to the laboratory. At the laboratory, water samples were filtered with a 0.45 μ m nylon filter and stored in a cold room (4–6 °C) until further analysis.

2.2.2. Sediment Sampling

A total of 6 sediment samples from Pétrola Lake were collected for metagenomic study at the lake sampling points 2635, 2643, 2648, 2649, 2651 and 2652, during March 2018. Sampling was performed by collecting the first 5 cm of the lake bottom sediments. Samples, collected aseptically in sterile 50 mL polypropylene tubes, were transported to the laboratory under refrigeration and darkness, for a time period not exceeding 5 h.

2.3. Endocrine Disrupting Chemicals Analysis

EDCs were analysed by gas chromatography coupled to a mass detector prior to solid phase extraction. For solid phase extraction, a Vac Elut manifold was used. First, the Bond Elut Plexa PAX type extraction cartridges were placed and conditioned by adding 2 mL of analytical grade methanol and 2 mL of MiliQ water; then, 500 mL of the sample passed through the extraction column supported by a vacuum pump, at a rate of about 40 drops per minute. The compounds retained in the solid phase were eluted twice with 5 mL of dichloromethane. Afterwards, a eluted volume of dichloromethane was evaporated with a gentle flux of nitrogen (N₂); the tube in which the eluent had been evaporated was rinsed several times with 250 µL of toluene, and then the accumulated volume of toluene was poured into a 1 mL vial insert; finally, the poured extract in the vial was analysed in Agilent 7890B Series Gas Chromatograph (GC) linked to an Agilent 5977B Mass Selective Detector (MSD) equipped with a DB-5ms capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness, J&W Scientific, Folsom, CA, USA). High purity helium (99.9999%) was used as carrier gas. One complete run lasted 34.17 min. A programmed-temperature vaporiser (PTV) injector worked in solvent vent mode. The inlet temperature was held at 100 °C for 0.5 min, raised to 280 °C at 900 °C/min, and finally decreased to 100 °C at 900 °C/min until the analysis was finished. The oven temperature was held at 100 °C for 1.5 min, raised to 180 °C at 30 °C/min, then raised to 310 °C at 5 °C/min for 4 min. MS transfer line and the ion source temperature were both set at 280 °C. The MSD was operated in full scan mode from 50 to 500 m/z. Data acquisition and processing were based on deconvolution technologies, which were accessed in ChemStation Software (version E.0200.493; Agilent Technologies, Santa Clara, CA, USA). The database used for screening analysis included 926 pesticides and endocrine disruptors as in Wylie [17]. Deconvolution was run to identify endocrine disruptor compounds according to the following criteria: the automated mass spectral deconvolution and identification system match values were higher than 60%. Chemical analyses were performed at the facilities of the Biotechnology and Natural Resources Laboratory at the Institute for Regional Development (IDR), University of Castilla-La Mancha (UCLM).

2.4. DNA Extraction and MiSeq Illumina Sequencing

Bacterial DNA from the sediment was extracted immediately after sampling to minimize significant DNA degradation. Prior to DNA extraction, all sediment samples were aseptically homogenised, and 0.5 g were used for total DNA extraction. Extraction was performed using the NucleoSpin[®] Soil DNA extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The yield and quality of the extracted DNA samples were evaluated on 1% agarose gel. DNA concentration was checked by spectrophotometric methods, using a multimode lector Cytation 5 (BioTek, Vermont, VT, USA). All extracted DNA samples were stored at −20 °C until further processing.

After quality and quantity controls, amplicon sequencing (V3–V4 region) of 16S rRNA gene [18,19] was performed on DNA extracts on a MiSeq using v3 chemistry by Stabvida (Caparica, Portugal). Final taxonomic assignment was obtained after amplicon data processing and sequence alignment using QIIME 2[™] v2018.6.0 [20] and the version 132 of SILVA database [21].

2.5. Statistical Analysis

Endocrine disrupting chemical data were analysed by the non-parametric procedure of the Kruskal–Wallis test. Analysis of variance (ANOVA) was used to determine statistical significance in microbiological characterization. Data analysis was carried out with Statgraphics Centurion and SPSS.

3. Results and Discussion

3.1. Endocrine Disrupting Chemicals Distribution

The Endocrine Database Exchanged (TEDX) and Database of Endocrine-Disrupting Chemicals and Their Toxicity Profile (DEDuCT) were employed for EDC identification. They comprise a list of chemicals that have shown evidence of endocrine disruption in scientific research. From the list of EDCs detected in the water samples, plastics, or those that could be part of plastics, were discarded in order to avoid an incorrect interpretation of results. As shown in Table 1, a total of 34 compounds classified as EDC were identified in surface waters of the lake (2643, 2648, 2649, 2650, 2651, 2652), evaporating pond (2635), wastewater (2575) ponds, and in springs and streams (2554, 2571, 2602, 2640, 2641, 2642). The most frequently detected EDC was cyprodinil (69/112; 61.6%), followed by tonalide (44/112; 39.3%), *O*-phenylphenol (43/112; 38.4%), caffeine (29/112; 25.9%) and fluorene (27/112; 24.1%).

Table 1. Endocrine disrupting chemicals identified in water samples collected from small streams and springs surrounding the lake and lake surface water. Concentrations are given in ng/L. The main land use is indicated at each point: IRL: irrigated land; DRL: dry land; WWP: wastewater pond; MP: mining pond; LS: livestock. -: not-detected.

| Land Use | Spring and Stream | | | | | | WWP | | | Petrola Lake | | | | | Total |
|--------------------------|-------------------|------|------|------|------|------|------|------|------|--------------|------|------|------|------|-------|
| | IRL | IRL | IRL | DRL | DRL | DRL | WWP | WWP | WWP | IRL | IRL | IRL | MP | LS | |
| Control Point | 2554 | 2571 | 2602 | 2640 | 2641 | 2642 | 2575 | 2643 | 2650 | 2649 | 2652 | 2651 | 2635 | 2648 | |
| Atrazine | - | 1 | 1 | 1 | - | 1 | - | - | 1 | 1 | 1 | - | - | - | 7 |
| Carbofuran | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 1 |
| Chlorfenvinphos, cis- | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 1 |
| Chlorfenvinphos, trans- | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 1 |
| Diazinon | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 1 |
| Dinocap | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | 1 |
| Methoprene | 1 | - | 1 | - | - | - | - | - | - | - | - | - | - | 1 | 3 |
| Mevinphos | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 1 |
| Propoxur | - | - | - | - | - | - | - | - | - | 1 | 1 | 1 | 1 | 3 | 7 |
| Tetramethrin II | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | 1 |
| Boscalid (Nicobifen) | - | 4 | - | - | - | - | - | - | - | 2 | 1 | - | - | 1 | 8 |
| Cyprodinil | 6 | 6 | 6 | 8 | 7 | 4 | 7 | 4 | 2 | 5 | 3 | 4 | 3 | 4 | 69 |
| Flutolanil | 1 | - | 1 | 1 | 1 | 1 | - | - | - | - | - | - | - | - | 5 |
| Methomyl | - | - | 1 | 1 | - | - | - | 1 | - | 1 | 1 | 2 | - | - | 7 |
| <i>o</i> -Phenylphenol | 6 | 3 | 3 | 5 | 4 | 5 | 7 | 1 | 2 | 1 | - | 3 | 1 | 2 | 43 |
| Oxadiazon | - | - | 1 | 1 | - | - | - | 1 | - | 1 | - | - | 1 | - | 5 |
| Propiconazole-II | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 1 |
| Pyrimethanil | - | - | - | - | - | 1 | - | 1 | - | - | - | 1 | - | - | 3 |
| Tebuconazole | - | 2 | - | - | - | - | - | 1 | - | 1 | 1 | 2 | - | 1 | 8 |
| Terbuthylazine | - | - | - | - | - | - | 2 | - | - | - | - | - | - | - | 2 |
| 2,4,6-Tribromophenol | 3 | 3 | 2 | 2 | 3 | 3 | 2 | - | - | - | - | - | - | - | 18 |
| Acenaphthylene | 1 | - | 1 | - | 1 | - | - | 1 | - | - | 1 | - | - | 1 | 6 |
| Atrazine-desethyl | - | 5 | - | 1 | - | - | - | - | - | - | - | - | - | - | 6 |
| Butylated hydroxyanisole | - | 1 | 1 | 1 | 1 | - | 2 | - | - | 1 | - | - | 1 | - | 8 |
| Caffeine | 4 | 1 | 3 | 4 | 5 | 1 | 6 | - | 2 | - | 1 | - | 1 | 1 | 29 |
| Dibenz[a,h]anthracene | - | 2 | 1 | 1 | - | 1 | - | - | - | - | - | - | 1 | 1 | 7 |
| Fluoranthene | 3 | 2 | 3 | 3 | 2 | 1 | 1 | - | - | 1 | - | 1 | - | - | 17 |
| Fluorene | 1 | 2 | 2 | 5 | 5 | 2 | 3 | 1 | 1 | - | 1 | 1 | 1 | 2 | 27 |
| Musk Ketone | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | 1 |
| Phenanthrene | 1 | - | - | - | - | - | 2 | - | - | - | - | - | - | - | 3 |
| Piperonyl butoxide | - | - | - | - | 2 | 1 | 8 | - | - | 1 | - | 1 | - | - | 13 |
| Terbuthylazine-desethyl | - | 1 | - | - | 1 | 3 | - | - | - | - | - | - | - | - | 5 |
| Tonalide | 7 | 4 | 4 | 6 | 6 | 5 | 8 | 1 | 2 | 1 | - | - | - | - | 44 |
| Triclosan | - | - | - | - | - | - | 2 | - | - | - | - | - | - | - | 2 |
| Count ¹ | 11 | 15 | 15 | 14 | 13 | 13 | 18 | 9 | 6 | 12 | 9 | 10 | 8 | 10 | |
| Sum ² | 34 | 38 | 31 | 40 | 39 | 29 | 56 | 12 | 10 | 17 | 11 | 17 | 10 | 17 | |

¹ Number of different endocrine disruptors per sampling point. ² Total number of endocrine disruptors detected per sampling point.

Nineteen of the detected EDC can be classified as pesticides or biocides: atrazine, boscalid, carbofuran, cis-chlorfenvinphos, trans-chlorfenvinphos, cyprodinil, diazinon dinocap, flutolanil,

methomyl, methoprene, mevinphos, *O*-phenylphenol, oxadiazinon, propiconazole, propoxur, pyrimethanil, tebuconazole, terbutylazine and tetramethrin; two metabolites from triazine herbicides (desethyl-atrazine and desethyl-terbutylazine); five of the identified EDC are polycyclic aromatic hydrocarbons (PAHs): acenaphthylene, dibenz[*a,h*]anthracene, fluoranthene, fluorene and phenanthrene; four EDC compounds can be grouped as body care products, such as tonalide, butylated hydroxyanisole, musk ketone and triclosan. Finally, caffeine, a legal drug, appears widespread in the different sampling points of the all water sample types.

Regarding the pesticides detected, half of them (atrazine, carbofuran, chlorfenvinphos isomers, diazinon, dinocap, methoprene, mevinphos, propoxur and tetramethrin) are banned in Spain and Europe. Surprisingly, clorfenvinphos, metoprene, mevinphos, tetramethrin and atrazine, which were banned between 2003–2004, appear in water samples several years after banning [22,23]. Those pesticides whose use is allowed appear more frequently than those prohibited. Cyprodinil and *O*-phenylphenol were the most often detected pesticides; both are broad-spectrum activity substances that can be used as pesticides in many crops and livestock, preservatives in fruits or flowers, and even as additives in the food industry [24–26].

Concerning the rest of the EDCs, there are no regulations regarding their use. However, fluoranthene (17/112; 15.2%) is a priority substance because of its negative effects on aquatic ecosystems [27]. Noticeably, tonalide (44/112; 39.3%) and caffeine (29/112; 25.9%) were the most detected EDCs; the first, tonalide, is widely used as a low-cost fragrance ingredient in cosmetics, detergents, fabric softeners, household cleaning products and air fresheners. Owing to its lipophilicity, persistence and extensive use, tonalide has been detected in water bodies, sediments and biota, including humans [28]. Tonalide concentrations are relatively high in the environment and biota, and are especially high in wastewater treatment plants [28]. Caffeine is an alkaloid that occurs in more than 60 plant species (e.g., in the seeds of the coffee, cacao and cola tree and in the leaves of the tea tree). It is a constituent in a variety of beverages (coffee, tea, caffeinated soft drinks) and in numerous food products (chocolate, pastries, dairy desserts). The global average consumption is about 70 mg per person per day but varies in the different countries [29]. This makes it a pollutant that can be found in wastewater effluents, in surface waters [30] and even in groundwater [31]. Caffeine has been proposed as an indicator of wastewater pollution [32,33].

The domestic insecticides, chlorfenvinfos isomers (2/112; 1.8%) and diazinon (1/112; 0.9%), the fragrance compound musk ketone (1/112; 0.9%) and triclosan (2/112; 1.8%), a disinfectant used in body care products, were detected only in the area affected by urban wastewaters (site 2575); at this site, the more frequent EDCs were tonalide (8/112; 7.1%) and piperonyl butoxide (13/112; 11.6%), an ingredient in insecticides to control pests in households, in food-handling establishments and for human and veterinary applications against ectoparasites. This suggests that the source is also linked to urban wastewaters. The banned pesticides dinocap (1/112; 0.9%) and tetramethrin (1/112; 0.9%), the authorized fungicide flutolanil, and the degradation products of triazine herbicides desethyl-atrazine (6/112; 5.4%) and desethyl-terbutylazine (5/112; 4.5%) were identified only in springs and streams draining agricultural areas. Finally, the banned biocides mevinphos (1/112; 0.9%) and propoxur (7/112; 6.2%) were detected only in lake water samples. In the areas of the lake receiving return flows from agriculture, such as points 2649 and 2652, we identified atrazine (7/112; 6.2%), propoxur, boscalid (8/112; 7.1%), cyprodinil, methomyl (7/112; 6.2%) and tebuconazole (8/112; 7.1%). Cyprodinil, *O*-phenylphenol, butylated hydroxyanisole (8/112; 7.1%), fluoranthene, fluorene (27/112; 24.1%), piperonyl butoxide and tonalide were identified in all sampling points.

3.2. Microbial Community Structure

Among sampling sites, bacterial taxa (as relative abundance in %) in sediment samples varied greatly (Figure 2). At the phylum level, Proteobacteria, Bacteroidetes and Firmicutes were the dominant taxa. Proteobacteria cover a range from 33.1% (site 2643) to 56.3% (site 2651), Bacteroidetes a range from 10.5% (site 2635) to 17.2% (site 2648) and Firmicutes a range from 2% (site 2643) to 11.7% (site

2635). At the class level, Gammaproteobacteria, Deltaproteobacteria (identified taxa related to sulfate reduction [34]) and Alphaproteobacteria (identified taxa related to nitrate reduction [35]) were identified in all sampling points, showing a range from 31% to 54% at sites 2643 and 2651, respectively. Besides the Proteobacteria members, the association of sulphur reducers belonging to Bacteroidia and Clostridia comprised the main class consortium, covering ranges from 6% up to >16% (sites 2651 and 2652, respectively). At the family level, the abundance of taxa increased up to 631 families. The Proteobacteria members *Desulfobacteraceae* (up to 13.5% at 2648) and *Rhodobacteraceae* (up to 8.4% at 2651) were the most represented families across the analysed sediments (Figure 2). In association with *Spirochaetaceae*, they carry out the main recycling and transformation of S-compounds [36–39]. *Ectothiorhodospiraceae* (up to 6.8% at 2649) represented the major community, which can utilize nitrogen and sulphur-compounds for anoxic metabolic pathways [40]. *Anaerolineaceae*, *Trueperaceae* and *Marinilabiaceae* are linked to the carbon cycle and the degradation of organic matter [41–43]. These families are able to recycle S-N-C under high salinity conditions [40,44,45], which may confer a turnover potential in sediments from Pétrola Lake.

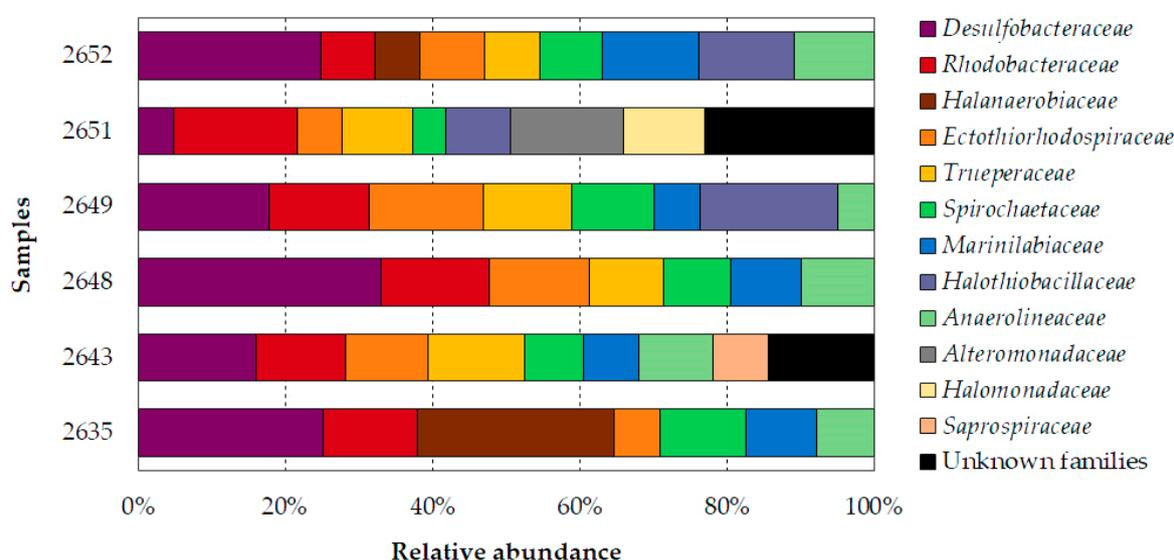


Figure 2. Relative abundance (%) of major families identified in sediment samples from Pétrola Lake. Only families with a relative abundance >2% of total bacteria population were included.

In contrast, a minor group of families was present that, together, did not represent more than 14%; their presence may depend on EDC and land use distribution. Families such as *Alteromonadaceae* [46], with large genomes containing degradative genes, and halophilic *Halomonadaceae* and *Halothiobacteraceae*, linked to N and S cycles [47,48], contributed significantly in sectors of the lake with a direct influence of agriculture return flows (sites 2649 and 2652). Noticeably, these families were not found in the mining pond (2635). Due to the poor communication with the rest of the water mass, flooding occurs only during high stand level conditions. At 2635, an increase of between 2% and 10% in the abundance of the halophilic family *Halanaerobiaceae* was found to be closely linked with the recycling of several types of C-compound [49]. Importantly, sectors of the lake far from direct inputs of contaminants, such as 2643 and 2651, exhibited a high relative abundance of unknown families.

3.3. Land Use Influence on EDC Distribution and Bacteria Community Structure

The number of EDCs differed significantly (Chi squared: 9.761; $p < 0.01$) between springs and streams, and surface water samples. The wastewater point 2575 showed the largest number of EDCs, with a total of 18 compounds detected 56 times (Table 1). In addition, pesticides or their metabolites were detected up to 98 times in springs and streams surrounding the lake. The largest number (23) was recorded at the spring sample point 2571 (Table 1), close to a major irrigation area

(Figure 1). An important part of the EDC load reaches the lake through the small surrounding streams. These streams drain through surface and groundwater runoff and dry and irrigation agricultural areas. The presence of EDCs in springs and streams reveals that one of the most relevant contamination sources to the saline lake is diffuse. Urban outflows, a point pollution source, constitute another key input of pesticides such as cyprodinil, *O*-phenylphenol, boscalid, methomyl and atrazine. Some EDCs are common in both agricultural and urban outflows: cyprodinil, *O*-phenylphenol and caffeine. Atmospheric deposition as a source of some EDCs cannot be ruled out. This is the case in some polycyclic aromatic hydrocarbons (PAHs) such as fluorene, fluoranthene or phenanthrene, which can be transported long distances and deposited with rainfall [50,51]. Other pesticides that are not volatilized attach to soil particles and are transported by wind and then deposited [52,53]. The anthropogenic pressures, agriculture and wastewater outflows, the intensity of EDC use and their persistence in the environment can explain the distribution of most of the EDC. For example, the widespread distribution of cyprodinil and *O*-phenylphenol can be explained because they are permitted pesticides in common use; in contrast, the spatial distribution of atrazine is more limited because it was banned 13 years ago, but due to its persistence, it is still detected.

Regarding the persistence in the environment of EDC, the physico-chemical properties (solubility, soil adsorption coefficient- K_{oc} , Groundwater Ubiquity Score-GUS, Octanol-water partition coefficient- K_{ow}) of a pollutant and the environmental redox conditions, which can be governed by the activity of microorganisms, can control their distribution and fate. Note that the number of compounds in the lake is lower than in streams and springs, which points to attenuation processes. It is reasonable to assume that the overall number of EDCs in the aquifer is more important than that in the surface water body, because the volume of groundwater is greater. The absence of some compounds in the lake can be explained by the analytical resolution of the employed GC-MS equipment (they are below the detection limit) or by attenuation processes linked to both physical (i.e., adsorption capacity of sediments, photodegradation) and biogeochemical attenuating processes.

From a microbial perspective, members of Alpha-, Gamma- and Deltaproteobacteria, Bacteroidia and Clostridia classes—which use carbon, sulphur and nitrogen compounds for energetic and metabolic purposes—were identified in Pétrola Lake sediments. They have also been reported in soda and salt ecosystems in Lonar Lake, Lake Grevelingen or Lake Bosten [44,54,55]. The co-existence of nitrogen or sulphur-oxidizing, and nitrate or sulphate-reducing, bacteria in Pétrola Lake suggests the presence of functional coupled cycles (i.e., redox processes related to S-N-C cycles) at the sediment–water interface. The microbial activity at the sediment–water interface may control redox conditions by utilizing different electron donors (i.e., organic matter) to sustain metabolic activity. This interpretation can be inferred indirectly by estimating the amount of organic carbon (OC) available and exploring the microbial community structure in the water body. The amount of OC in the sediment surface layer is variable, ranging from 0.68 to 6.10% [9]. Therefore, enough OM is available to enable heterotrophic processes driven by bacteria, which can help reduce contaminant loads. Both high NO_3^- attenuation ($1.25 \text{ mmol d}^{-1}\text{kg}^{-1}$, Carrey et al. [56]) and H_2S production rates ($0.36 \text{ nmol/cm}^{-3}\text{s}^{-1}$, Menchén et al. [57]) have been found in experimental assays, which suggest a high metabolic activity linked to high rates of nutrient turnover and mineralization in Pétrola Lake sediments. The identification of bacteria communities linked to C-N-S cycles open the possibility of EDC attenuation by biodegradation processes [58]. The intensity of these processes may be linked to the lake characteristics (i.e., relative isolation from pollution sources) as suggested by the variability in the spatial distribution of bacteria families per land-use zone. Bacterial communities can change rapidly in response to the available source of energy and can develop resistance mechanisms linked to the input of some inorganic (i.e., nitrate) or organic (EDC) pollutants [59–61]. Accordingly, lake zones partially isolated from the rest of the water body but affected by human pressures (i.e., 2635) showed lower relative abundances of families than those affected by farming or wastewaters. Thus, bacterial composition is modified by anthropogenic pressures, directly affecting the natural development of the saline system. Furthermore, such lake zones contained fewer endocrine disruptors, reinforcing the idea that this specific site is

isolated from the influence of other sources of contamination. This provides support for the conclusion that the functional traits of bacterial communities change in response to anthropogenic pressures [62].

4. Conclusions

Based on the Endocrine Database Exchanged (TEDX) and Database of Endocrine-Disrupting Chemicals and Their Toxicity Profile (DEDuCT), a total of 34 endocrine-disrupting chemicals were detected in the aquifer–saline system of Pétrola Lake. They can be grouped into biocides, PAHs, body care products and abuse drugs. The spatial distribution of these EDC is not homogeneous and depends on the dominant land use, e.g., pesticides were more abundant at those sites related to agricultural (irrigation) land uses. Urban wastewater outflows represent the most important source of endocrine disrupting chemicals and are characterised by body care products as well as biocides related to veterinary drugs and household products. Notably, caffeine was present in all sampling sites, regardless of land use. The presence of pesticides forbidden since 2003, such as metoprene or mevinphos, indicates the elevated persistence of certain EDCs in the environment. The number of EDCs is no doubt larger in the aquifer than in the lake, pointing to attenuation processes reducing the amount of these pollutants in the aquifer–saline lake hydrogeological system.

We outline the overall structure of the main bacterial communities in the sediments. Amplicon sequencing of the 16S rRNA gene revealed a gradient in the microbial composition affected by land use proximity, as well as a relationship between the relative isolation of certain sites from the rest of the water body and EDC appearance. The relationship between microbial composition and the pollutant source location may affect both the biodegradation capacity and the functional traits of the microbial community. The community structure changed in response to the anthropogenic pressures. Alpha-, Gamma- and Deltaproteobacteria, Bacteroidia and Clostridia were the dominant classes in all samples; all can biogeochemically transform EDC linked to S-N-C cycles. Importantly, some banned pesticides currently still appear in groundwater and surficial waters of the lake. These insights provide a better understanding of the distribution of some EDCs in the protected area, an important step in establishing and implementing future management procedures focused on their control.

Regarding management measurements, the partial isolation of lake areas affected by farming and wastewater sources does not resolve the presence of organic pollutants in the surface waters. Since this study demonstrates an excess of biocides in the environment, management measurements should be focused on the reduction in the use of biocides by, for example, promoting good agricultural practices. The wastewater treatment plant needs to be operational and should provide a treatment which not only includes the removal of nutrients but also organics. Regarding the presence of forbidden EDCs, the promotion of studies by environmental authorities focused on the transport of pollutants in groundwater would contribute to forecasting the persistence of some pollutants in the salt lake–aquifer system.

Author Contributions: Conceptualization, J.J.G.-A.; methodology, A.M., B.T. and Y.E.; writing—original draft preparation, A.M., J.J.G.-A., M.Á.-O. and Y.E.; writing—review and editing, A.M., B.T., J.J.G.-A., M.Á.-O., N.V. and Y.E.; funding acquisition, J.J.G.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by projects from the Spanish government FEDER-Ministerio de Ciencia e Innovación y Universidades-Agencia Estatal de Investigación, grant number CICYT-CGL2017-87216-C4-2-R, and from Castilla-La Mancha Regional Government, grant number SBPLY/17/180501/000296L.

Acknowledgments: This work has been funded by the research projects CGL2017-87216-C4-2-R from the National Research Program I+D+i (FEDER/Ministerio de Ciencia, Investigación y Universidades) and SBPLY/17/180501/000296L from the National Research Program I+D+i of the Junta de Comunidades de Castilla-La Mancha. We would also like to thank Michael Stachowitsch for improving the English text.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Messerli, P.; Murniningtyas, E.; Eloundou-Enyegue, P.; Foli, E.G.; Furman, E.; Glassman, A.; Hernández-Licona, G.; Kim, E.M.; Lutz, W.; Moatti, J.-P.; et al. The Future is Now—Science for Achieving Sustainable Development. In *Global Sustainable Development Report*; United Nations: New York, NY, USA, 2019.
2. Colborn, T.; Vom Saal, F.S.; Soto, A.M. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environ. Health Perspect.* **1993**, *101*, 378–384. [[CrossRef](#)] [[PubMed](#)]
3. Harrison, R.M.; Hester, R.E. *Endocrine Disrupting Chemicals*; The Royal Society of Chemistry: London, UK, 1999; Volume 12, pp. 1–26. ISBN 978-0-85404-255-5. [[CrossRef](#)]
4. Katagi, T. Behavior of pesticides in water-sediment systems. In *Reviews of Environmental Contamination and Toxicology*; Ware, G.W., Ed.; Springer: New York, NY, USA, 2006; Volume 187, pp. 133–251. ISBN 978-0-387-30237-9. [[CrossRef](#)]
5. Shibata, A.; Kodaka, R.; Fujisawa, T.; Katagi, T. Degradation of flumioxazin in illuminated water-sediment systems. *J. Agric. Food Chem.* **2011**, *59*, 11186–11195. [[CrossRef](#)] [[PubMed](#)]
6. Zhang, C.; Zhao, H.; Ping, L.; Cai, X.; Wu, M.; He, H.; Zhang, C.; Zhu, Y.; Li, Z. Degradation of rizazole in water-sediment systems. *J. Environ. Sci. Health B* **2013**, *48*, 319–323. [[CrossRef](#)] [[PubMed](#)]
7. Huang, X.; Wu, C.; Xiong, X.; Zhang, K.; Liu, J. Partitioning and degradation of triclosan and formation of methyl-triclosan in water-sediment systems. *Water Air Soil Pollut.* **2014**, *225*, 2099. [[CrossRef](#)]
8. Gómez-Alday, J.J.; Carrey, R.; Valiente, N.; Otero, N.; Soler, A.; Ayora, C.; Sanz, D.; Muñoz-Martín, A.; Castaño, S.; Recio, C.; et al. Denitrification in a hypersaline lake-aquifer system (Pétrola Basin, Central Spain): The role of recent organic matter and Cretaceous organic rich sediments. *Sci. Total Environ.* **2014**, *497–498*, 594–606. [[CrossRef](#)] [[PubMed](#)]
9. Valiente, N.; Carrey, R.; Otero, N.; Soler, A.; Sanz, D.; Muñoz-Martín, A.; Jirsa, F.; Wanek, W.; Gómez-Alday, J.J. A multi-isotopic approach to investigate the influence of land use on nitrate removal in a highly saline lake-aquifer system. *Sci. Total Environ.* **2018**, *631–632*, 649–659. [[CrossRef](#)] [[PubMed](#)]
10. Bergman, Å.; Heindel, J.J.; Kasten, T.; Kidd, K.A.; Jobling, S.; Neira, M.; Zoeller, R.T.; Becher, G.; Bjerregaard, P.; Bornman, R.; et al. The impact of endocrine disruption: A consensus statement on the state of the science. *Environ. Health Perspect.* **2013**, *121*. [[CrossRef](#)] [[PubMed](#)]
11. Tang, C.Y.; Fu, Q.S.; Criddle, C.S.; Leckie, J.O. Effect of flux (transmembrane pressure) and membrane properties on fouling and rejection of reverse osmosis and nanofiltration membranes treating perfluorooctane sulfonate containing wastewater. *Environ. Sci. Technol.* **2007**, *41*, 2008–2014. [[CrossRef](#)] [[PubMed](#)]
12. Karigar, C.S.; Rao, S.S. Role of microbial enzymes in the bioremediation of pollutants: A review. *Enzyme Res.* **2011**. [[CrossRef](#)] [[PubMed](#)]
13. Abatenh, E.; Gizaw, B.; Tsegaye, Z.; Wassie, M. The role of microorganisms in bioremediation—A review. *Open J. Environ. Biol.* **2017**, *2*, 38–46. [[CrossRef](#)]
14. Dountcheva, I.; Doña, C.; Sanz, D.; Sánchez, J.M.; Gómez-Alday, J.J. Quantitative approach to water balance in groundwater discharge hypersaline wetlands. Pétrola lake, SE Spain. Proceedings of IAH2019, the 46 Annual Congress of the International Association of Hydrogeologists, Málaga, Spain, 22–27 September 2019; Gómez-Hernández, J.J., Andreo-Navarro, B., Eds.; Asociación Internacional de Hidrogeólogos—Grupo Español: Barcelona, Spain, 2019.
15. Valiente, N.; Carrey, R.; Otero, N.; Gutierrez-Villanueva, M.A.; Soler, A.; Sanz, D.; Castaño, S.; Gómez-Alday, J.J. Tracing sulfate recycling in the hypersaline Pétrola Lake (SE Spain): A combined isotopic and microbiological approach. *Chem. Geol.* **2017**, *473*, 74–89. [[CrossRef](#)]
16. Valiente, N. A Multidisciplinary Approach for Assessing Natural Attenuation of Pollutants in a Highly Saline Lake-Aquifer System: The Case of Pétrola Lake, Spain. Ph.D. Thesis, University of Castilla-La Mancha, Albacete, Spain, August 2018.
17. Wylie, P.L. *Screening for 926 Pesticides and Endocrine Disruptors by GC/MS with Deconvolution Reporting Software and a New Pesticide Library*; Application Note; Agilent Technologies: Wilmington, DE, USA, 2006.
18. Turnbaugh, P.J.; Ley, R.E.; Hamady, M.; Fraser-Liggett, C.M.; Knight, R.; Gordon, J.I. The human microbiome project. *Nature* **2007**, *449*, 804–810. [[CrossRef](#)] [[PubMed](#)]
19. Petrosino, J.F.; Highlander, S.; Luna, R.A.; Gibbs, R.A.; Versalovic, J. Metagenomic Pyrosequencing and Microbial Identification. *Clin. Chem.* **2009**, *55*, 856–866. [[CrossRef](#)] [[PubMed](#)]

20. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [[CrossRef](#)] [[PubMed](#)]
21. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [[CrossRef](#)] [[PubMed](#)]
22. Vonberg, D.; Vanderborght, J.; Cremer, N.; Pütz, T.; Herbst, M.; Vereecken, H. 20 years of long-term atrazine monitoring in a shallow aquifer in western Germany. *Water Res.* **2014**, *50*, 294–306. [[CrossRef](#)] [[PubMed](#)]
23. Gros, M.; Blum, K.M.; Jernstedt, H.; Renman, G.; Rodríguez-Mozaz, S.; Haglund, P.; Andersson, P.L.; Wiberg, K.; Ahrens, L. Screening and prioritization of micropollutants in wastewaters from on-site sewage treatment facilities. *J. Hazard. Mater.* **2017**, *328*, 37–45. [[CrossRef](#)] [[PubMed](#)]
24. Coelhan, M.; Bromig, K.H.; Glas, K.; Roberts, A.L. Determination and levels of the biocide ortho-phenylphenol in canned beers from different countries. *J. Agric. Food Chem.* **2006**, *54*, 5731–5735. [[CrossRef](#)] [[PubMed](#)]
25. Waechter, F.; Weber, E.; Hertner, T.; May-Hertl, U. Cyprodinil: A fungicide of the anilinopyrimidine class. In *Hayes' Handbook of Pesticide Toxicology*, 3rd ed.; Syngenta Crop Protection AG: Basle, Switzerland, 2010; pp. 1903–1913. [[CrossRef](#)]
26. Mishra, S.; Saini, M.K.; Singh, M.K.; Alam, S.; Raza, S.K.; Thakur, L.K.; Mukharjee, S. Method Development and validation for identification of 2-phenyl phenol in siapton 10L by gas chromatography mass spectrometry. *World J. Pharm. Pharm. Sci.* **2015**, *4*, 783–792.
27. European Union. Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. *Off. J. Eur. Union* **2013**, *226*, 1–17.
28. Li, W.; Wang, S.; Li, J.; Wang, X.; Fan, B.; Gao, X.; Liu, Z. Development of aquatic life criteria for tonalide (AHTN) and the ecological risk assessment. *Ecotoxicol. Environ. Saf.* **2020**, *189*, 109960. [[CrossRef](#)] [[PubMed](#)]
29. Buerge, I.J.; Poiger, T.; Müller, M.D.; Buser, H.R. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. *Environ. Sci. Technol.* **2003**, *37*, 691–700. [[CrossRef](#)] [[PubMed](#)]
30. Knee, K.L.; Gossett, R.; Boehm, A.B.; Paytan, A. Caffeine and agricultural pesticide concentrations in surface water and groundwater on the north shore of Kauai (Hawaii, USA). *Mar. Pollut. Bull.* **2010**, *60*, 1376–1382. [[CrossRef](#)] [[PubMed](#)]
31. Sui, Q.; Cao, X.; Lu, S.; Zhao, W.; Qiu, Z.; Yu, G. Occurrence, sources and fate of pharmaceuticals and personal care products in the groundwater: A review. *Emerg. Contam.* **2015**, *1*, 14–24. [[CrossRef](#)]
32. Gonçalves, E.S.; Rodrigues, S.V.; Silva-Filho, E.V.D. The use of caffeine as a chemical marker of domestic wastewater contamination in surface waters: Seasonal and spatial variations in Teresópolis, Brazil. *Rev. Ambiente Água* **2017**, *12*, 192–202. [[CrossRef](#)]
33. Dvory, N.Z.; Livshitz, Y.; Kuznetsov, M.; Adar, E.; Gasser, G.; Pankratov, I.; Lev, O.; Yakirevich, A. Caffeine vs. carbamazepine as indicators of wastewater pollution in a karst aquifer. *Hydrol. Earth Syst. Sci.* **2018**, *22*, 6371–6381. [[CrossRef](#)]
34. Campbell, B.J.; Engel, A.S.; Porter, M.L.; Takai, K. The versatile ϵ -proteobacteria: Key players in sulphidic habitats. *Nat. Rev. Microbiol.* **2006**, *4*, 458–468. [[CrossRef](#)] [[PubMed](#)]
35. Labrenz, M.; Hirsch, P. Roseovarius. In *Bergey's Manual of Systematics of Archaea and Bacteria*; Bergey's Manual Trust and John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2015. [[CrossRef](#)]
36. Andreote, F.D.; Jiménez, D.J.; Chaves, D.; Dias, A.C.; Luvizotto, D.M.; Dini-Andreote, F.; Fasanella, C.C.; Lopez, M.V.; Baena, S.; Taketani, R.G.; et al. The microbiome of Brazilian mangrove sediments as revealed by metagenomics. *PLoS ONE* **2012**, *7*, e38600. [[CrossRef](#)] [[PubMed](#)]
37. Kuever, J. The Family Desulfobacteraceae. In *The Prokaryotes*; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin, Germany, 2014; pp. 45–73. [[CrossRef](#)]
38. Pujalte, M.J.; Lucena, T.; Ruvira, M.A.; Arahal, D.R.; Macián, M.C. The Family Rhodobacteraceae. In *The Prokaryotes*; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin, Germany, 2014; pp. 439–512. [[CrossRef](#)]
39. Reyes, C.; Schneider, D.; Thürmer, A.; Kulkarni, A.; Lipka, M.; Szejtrensus, S.Y.; Böttcher, M.E.; Daniel, R.; Friedrich, M.W. Potentially Active Iron, Sulfur, and Sulfate Reducing Bacteria in Skagerrak and Bothnian Bay Sediments. *Geomicrobiol. J.* **2017**, *34*, 840–850. [[CrossRef](#)]

40. Imhoff, J.F. The Family Ectothiorhodospiraceae. In *The Prokaryotes*; Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H., Eds.; Springer: New York, NY, USA, 1992; pp. 3222–3229. [[CrossRef](#)]
41. Sinkko, H.; Lukkari, K.; Sihvonen, L.M.; Sivonen, K.; Leivuori, M.; Rantanen, M.; Paulin, L.; Lyra, C. Bacteria Contribute to Sediment Nutrient Release and Reflect Progressed Eutrophication-Driven Hypoxia in an Organic-Rich Continental Sea. *PLoS ONE* **2013**, *8*. [[CrossRef](#)] [[PubMed](#)]
42. Rosenberg, E. The Family Marinilabiaceae. In *The Prokaryotes*; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin, Germany, 2014; pp. 731–732. [[CrossRef](#)]
43. Albuquerque, L.; Rainey, F.A.; da Costa, M.S. Trueperaceae. In *Bergey's Manual of Systematics of Archaea and Bacteria*; Whitman, W.B., Rainey, F., Kämpfer, P., Trujillo, M., Chun, J., DeVos, P., Hedlund, B., Dedysh, S., Eds.; Bergey's Manual Trust and John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2015. [[CrossRef](#)]
44. Lipsewiers, Y.A.; Hopmans, E.C.; Meysman, F.J.; Sinninghe Damsté, J.S.; Villanueva, L. Abundance and diversity of denitrifying and anammox bacteria in seasonally hypoxic and sulfidic sediments of the saline lake Grevelingen. *Front. Microbiol.* **2016**, *7*, 1661. [[CrossRef](#)] [[PubMed](#)]
45. González-Blanco, G.; Pérez-Pérez, V.; Orozco-Villafuerte, J.; Aguirre-Garrido, J.; Beristain-Cardoso, R.; Buendía-González, L. Kinetics and microbial structure of nitrogen cycle bacteria contained in the rhizosphere of natural wetland polluted with chromium. *Rev. Mex. Ing. Quim.* **2019**, *19*, 543–553. [[CrossRef](#)]
46. López-Pérez, M.; Rodríguez-Valera, F. The Family Alteromonadaceae. In *The Prokaryotes*; Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F., Eds.; Springer: Berlin/Heidelberg, Germany, 2014; pp. 69–92.
47. Oren, A.; Arahal, D.R.; Ventosa, A. Emended descriptions of genera of the family Halobacteriaceae. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 637–642. [[CrossRef](#)] [[PubMed](#)]
48. Tahrioui, A.; Schwab, M.; Quesada, E.; Llamas, I. Quorum sensing in some representative species of halomonadaceae. *Life* **2013**, *3*, 260–275. [[CrossRef](#)] [[PubMed](#)]
49. Oren, A. Halanaerobiaceae. In *Bergey's Manual of Systematics of Archaea and Bacteria*; Whitman, W.B., Rainey, F., Kämpfer, P., Trujillo, M., Chun, J., DeVos, P., Hedlund, B., Dedysh, S., Eds.; Bergey's Manual Trust and John Wiley & Sons, Inc.: Hoboken, NJ, USA, 2015. [[CrossRef](#)]
50. Motelay-Massei, A.; Garban, B.; Tiphagne-Larcher, K.; Chevreuil, M.; Ollivon, D. Mass balance for polycyclic aromatic hydrocarbons in the urban watershed of Le Havre (France): Transport and fate of PAHs from the atmosphere to the outlet. *Water Res.* **2006**, *40*, 1995–2006. [[CrossRef](#)] [[PubMed](#)]
51. Liu, F.; Xu, Y.; Liu, J.; Liu, D.; Li, J.; Zhang, G.; Li, X.; Zou, S.; Lai, S. Atmospheric deposition of polycyclic aromatic hydrocarbons (PAHs) to a coastal site of Hong Kong, South China. *Atmos. Environ.* **2013**, *69*, 265–272. [[CrossRef](#)]
52. Gavrilescu, M. Fate of Pesticides in the Environment and its Bioremediation. *Eng. Life Sci.* **2005**, *5*, 497–526. [[CrossRef](#)]
53. Traisup, K. Fate of two currently-used pesticides in water-sediment systems. Ph.D. Thesis, University of York, Heslington, UK, November 2012.
54. Tang, X.; Xie, G.; Shao, K.; Dai, J.; Chen, Y.; Xu, Q.; Gao, G. Bacterial Community Composition in Oligosaline Lake Bosten: Low Overlap of Betaproteobacteria and Bacteroidetes with Freshwater Ecosystems. *Microbes Environ.* **2015**, *30*, 180–188. [[CrossRef](#)] [[PubMed](#)]
55. Paul, D.; Kumbhare, S.V.; Mhatre, S.S.; Chowdhury, S.P.; Shetty, S.A.; Marathe, N.P.; Bhute, S.; Shouche, Y.S. Exploration of microbial diversity and community structure of Lonar Lake: The only hypersaline meteorite crater lake within basalt rock. *Front. Microbiol.* **2016**, *6*, 1553. [[CrossRef](#)] [[PubMed](#)]
56. Carrey, R.; Rodríguez-Escales, P.; Otero, N.; Ayora, C.; Soler, A.; Gómez-Alday, J.J. Nitrate attenuation potential of hypersaline lake sediments in central Spain: Flow-through and batch experiments. *J. Contam. Hydrol.* **2014**, *164*, 323–337. [[CrossRef](#)] [[PubMed](#)]
57. Menchén, A.; Valiente, N.; Toledo, B.; Gómez-Alday, J.J. Microscale effects of oxygen and light on bacterial sulfate reduction in organic-rich lacustrine sediments. *E3S Web Conf.* **2019**, *98*, 11004. [[CrossRef](#)]
58. Vilela, C.L.S.; Bassin, J.P.; Peixoto, R.S. Water contamination by endocrine disruptors: Impacts, microbiological aspects and trends for environmental protection. *Environ. Pollut.* **2018**, *235*, 546–559. [[CrossRef](#)] [[PubMed](#)]
59. Hussain, S.; Siddique, T.; Saleem, M.; Arshad, M.; Khalid, A. Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. *Adv. Agron.* **2009**, *102*, 159–200. [[CrossRef](#)]

60. Fierer, N.; Lauber, C.L.; Ramirez, K.S.; Zaneveld, J.; Bradford, M.A.; Knight, R. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *ISME J.* **2012**, *6*, 1007–1017. [[CrossRef](#)] [[PubMed](#)]
61. Ramirez, K.S.; Craine, J.M.; Fierer, N. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob. Chang. Biol.* **2012**, *18*, 1918–1927. [[CrossRef](#)]
62. Blunt, S.M.; Sackett, J.D.; Rosen, M.R.; Benotti, M.J.; Trenholm, R.A.; Vanderford, B.J.; Hedlund, B.P.; Moser, D.P. Association between degradation of pharmaceuticals and endocrine-disrupting compounds and microbial communities along a treated wastewater effluent gradient in Lake Mead. *Sci. Total Environ.* **2018**, *622*, 1640–1648. [[CrossRef](#)] [[PubMed](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).