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Article

How Efficient Are Semi-Natural Ponds in Assimilating Wastewater Effluents? Application to Fuente de Piedra Ramsar, Mediterranean Salt Lake (South of Spain)

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Abstract: This work concerns the case study of a Mediterranean Ramsar salt lake (Fuente de Piedra, southern Spain) that receives the treated wastewater of the local village treatment plant. The wastewater goes through a system of canals, water dams, and three semi-natural ponds that were built in 2005. This work aims to investigate the capacity of the system to assimilate the impact of wastewater effluents on Lake Fuente de Piedra. For this, four points were sampled on 27–29 April 2016, at the inlet and the outlet points of the first and the third semi-natural ponds, with three replicates each. Temperature, pH, and conductivity at the inlet were 19.62 °C, 7.99, and 3262.67 µS/cm, respectively, and increased through the pond system by 7.59%, 8.04%, and 37.34%, respectively. Phytoplankton concentration indicators decreased from the inlet point to the outlet point (chlorophyll *a* from >500 to <20mg/L), as did the biovolume (from $>5 \times 10^{10}$ to 4.3×10^9 µm^3/mL). Zooplankton biovolume, in contrast, increased three orders of magnitude from the inlet (3.5×10^7 µm^3/mL) to the outlet point (1.6×10^9 µm^3/mL). Heterotrophic bacteria (1.29×10^5 cfu/mL) and faecal enterococci (1033 ± 351 cfu/100 mL) were high at the inlet point, but decreased at the outlet point by almost three orders of magnitude. Total phosphorous and total nitrogen decreased 40.3% and 23.1% through the pond system. The results showed an improvement in water quality in its passage through the built system. Additionally, as permanent wetlands with acceptable water quality, the water system attracts wild fauna during the dry summer, leading to the conclusion that these semi-natural or artificial wetlands should be extrapolated to other aquatic ecosystems (Mediterranean wetlands) that receive contributions of residual waters. Better functioning of the treatment plant is desirable to improve the conservation of the Ramsar and adjacent wetlands systems.

Keywords: wastewater effluent; semi-natural ponds; phytoplankton; zooplankton; faecal bacteria; Ramsar

1. Introduction

Today, the importance of wetlands worldwide has clearly been proved [1]. Most attention has been traditionally focused on large temperate lakes, while Mediterranean wetlands have received much less consideration [2]. These Mediterranean wetlands represent unique repositories of biodiversity, which hold exclusive communities of aquatic organisms playing an important role in the regional biodiversity conservation [3–6]. However, despite being recognized as important hot spots for the support of aquatic biodiversity, they experience a strong detrimental human disturbance [7–9].

Amongst these human disturbances, cultural eutrophication of freshwater ecosystems has been recognized worldwide as a serious environmental issue for more than half a century [10–13] and remains a major water quality issue. In the European Union, general concerns about water quality led to the Water Framework Directive [14], which demands a good ecological status for all ground and surface water.

One of the most obvious problems of cultural eutrophication is the release of sewage in natural water bodies [6,15]. Apart from the widely known adverse effects of nutrient enrichment in the ecosystem, pollutants and waterborne pathogens also represent a serious threat to public health [16–18]. In this sense, it is interesting to note that water treatment in Mediterranean areas is a challenging task, since villages can duplicate resident population during the tourist season, exceeding water treatment capacity. In order to resolve these problems, constructed wetlands technology has been developed since 1980s according to the effective role of wetlands as natural purifiers of water [19]. Indeed, transit throughout a wetland system increases hydraulic retention time, subsequently reducing nutrients because of biogeochemical interactions and exchange of pollutants between water, plant, microbial, and sediment compartments [20]. Moreover, an extensive and efficient reduction in pollutants from municipal, agricultural, mining, or industrial effluents and urban runoff have also been reported [21]. Pollutant removal is accomplished by the interdependent action of several physical, chemical, and biological processes. Reduction in both nutrients and contaminants is an ideal solution to be coupled with conventional wastewater treatment processes that rely on chemical disinfection and other processes in order to comply with the stringent safety required for water reuse [22]. Accordingly, a significant, though variable, amount of pathogenic and indicator microorganisms can be removed, depending on the microorganisms' different behaviour and survival in every wetland [23].

The use of natural and constructed wetlands for wastewater treatment has been widely displayed in the literature [19,24]. Natural wetlands have special characteristics that make them suitable for wastewater effluent purification [24]. In our case, the relevant differentiation between constructed wetlands mentioned in the bibliography and the systems under examination in this study was that a coupling of natural and semi-natural wetlands was used to purified wastewater effluent before discharges in a Ramsar wetland. This set of wetlands was previously modified in order to expand their dimension and volume to increase the purifying capacity.

All in all, our hypothesis is that the transit of water from the wastewater treatment plant through a semi-natural pond system results in an increase in the quality of the water before being discharged to the Ramsar wetland.

2. Materials and Methods

2.1. Study Area

Fuente de Piedra is an endorheic playa-lake ecosystem [25] located in Andalusia (southern Spain). It was recognized as a Ramsar site in 1983 and declared as regional Nature Reserve in 1984 [26]. One of the major problems of this ecosystem is the discharge of treated wastewater from the nearby Fuente de Piedra town, even though it fulfils the standards for the treatment of urban wastewater (RD 509—see Table 1).

Table 1. Values of different parameters of water quality from the treatment plant EDAR Fuente de Piedra II.

Water Quality Parameters	Decrease (%)	Inlet Mean (mg/L)	Outlet Mean (mg/L)	Flow Mean (m^3/h)	Hydraulic Retention Time (d)
Biochemical oxygen demand (BOD)	84 ± 15	181 ± 95	25 ± 17		
Chemical oxygen demand (COD)	74 ± 12	451 ± 220	104 ± 41		
Total Suspended Solids (TSS)	52 ± 30	197 ± 63	164 ± 31		
Winter, Spring and Autumn				13.54	80
Summer				16.67	65

In 1996, the Government of Andalusia purchased Laguneto estate, which has 6.1 hectares (ha). In 1999, a 0.88 ha plot including the northern end of Laguneto was added to the Nature Reserve. Since then, treated wastewater discharged from the treatment plant can either go directly to the Fuente de Piedra wetland or go through several small semi-natural ponds: Laguneto (27,801 m^2, and a maximum depth of 1.42 m), Laguna de los Juncares (9222 m^2 and a maximum depth of 0.39 m), and Los Juncares (19,828 m^2 and a maximum depth of 0.20 m—Figure 1). Considering that the mean depth of each pond is about 50% of the maximum depth, and a mean flow in spring is 13.54 $m^3 \cdot h^{-1}$, a retention time of 61, 13, and 6 days was calculated for the Laguneto, Laguna de los Juncares, and Los Juncares ponds, respectively. According to a higher flow rate, the retention time in summer is 81% of the retention time in spring. The decision between one or another pathway is taken by the managing organisms of the natural reserve. The actions carried out were financed by Project LIFE03NAT/E/000055 and consisted of (i) desiccation of Laguneto wetland and removal of organic matter and debris [27], (ii) dredging 866 m^3 of mud from canals and building new canals including floodgates to allow regulation of water flow, (iii) the construction of some islands in the centre of the Laguneto wetland to increase the nesting habitat for birds, and (iv) revegetation of the perimeter of Laguneto and the channels previously mentioned. The vegetation surrounding Laguneto is composed of *Tamarix canariensis*, *Tamarix africana*, *Phragmites australis*, *Scirpoides holoschoenus*, *Juncus acutus*, and *Bolboschoenus maritimus*. Moreover, 2000 m of perimeter channels of Lake Fuente de Piedra were dredged, and five new floodgates were constructed in the five points where human influents enter the lake [28].

Figure 1. Location map of the study area, sampling points, and water flow through the semi-natural ponds system (**blue arrows**) direct to the Ramsar wetland. Before restoration in 1996, water flows followed the **red arrows**, but this is no longer possible.

2.2. Physico-Chemical and Biological Variables

In order to monitor the assimilation capacity of the ponds system, four points (A to D) were sampled on 27–29 April 2016, covering the area from the treated wastewater inlet to each wetland of these semi-natural ponds to the system's outlet (Figure 1). Three replicates were obtained in each sampling point. Physico-chemical parameters (temperature, conductivity, pH) were measured with a Hanna Multiparameter sensor HI 9829 (Hanna Instruments, Woonsocket, RI, USA). Maximum depth was measured in each wetland as a hydrological variable. Water samples for total nutrient analyses were taken in sterile polyethylene vials and immediately frozen (−20 °C). Total nitrogen and total phosphorus were further analysed in the laboratory with Nanocolor 985 083 and Nanocolor 985 076 analysis kit (Macherey-Nagel GmbH & Co.KG, Düren, Germany), respectively.

Bacteriological analyses were carried out in only three sampling points (A, B, and D) after filtration with nitrocellulose membranes of 0.45 μm pore size. Membranes were incubated on Oxoid nutrient agar at 22 and 37 °C for 48 h for the determination of heterotrophic bacteria, Difco m FC agar medium at 44.5 °C over 24 h for the determination of faecal coliforms, and on Difco m *Enterococcus* agar medium at 37 °C over 48 h for the determination of faecal enterococci. Faecal coliforms and faecal enterococci were analysed according to the APHA 9222-D and APHA 9230-C standard methods respectively.

Total chlorophyll *a* concentration and phytoplankton composition were measured with a submersible FluoroProbe (bbe Moldaenke GmbH, Schwentinental, Germany). The fluorospectrometer discriminated between main phytoplanktonic groups (i.e., diatoms and dinoflagellates, dinoflagellates, blue-green algae, green algae, and cryptophytes) based on the relative fluorescence intensity of chlorophyll *a* (Chl*a*) at 680 nm, following sequential light excitation by 5 Light-Emitting Diodes at 450, 525, 570, 590, and 610 nm wavelength [29,30].

The abundance and size estimation of phytoplankton cells between 15 and 100 μm Equivalent Spherical Diameter (ESD) and zooplankton (250 and 1000 μm ESD) were analysed with a FlowCAM (Fluid Imaging Technologies, Inc. Scarborough, ME, USA) using the autoimage mode. This analysis provides individual pictures of each particle in the vision field. After data acquisition, phytoplankton and zooplankton abundance were estimated by manual selection of phytoplankton and zooplankton, respectively [31]. For phytoplankton, 30 mL of sample preserved with formol (4% f.c.) was passed through a 100 μm flow cell and analysed with a 100-fold magnification (10× objective). For zooplankton, 50 mL of a concentrated sample preserved with formol (4% f.c.) was passed through a 1000 μm flow cell and analysed with a 20-fold magnification (2× objective). Due to low concentration at sampling points A and B, the zooplankton was concentrated previously by a factor of 40.

3. Results

3.1. Abiotic Conditions

Table 2 shows the longitudinal profile of the environmental variables through the semi-natural wetland system. Temperature ranged between 19.62 °C and 21.95 °C, with a net increment of 7.95% between point A inlet in Laguneto to point D outlet in the Fuente de Piedra salt lake. pH value increased 8.04% from point A inlet to point B outlet of Laguneto wetland. Then, it decreased again as it flowed toward the Fuente de Piedra salt lake and decreased only 0.65% between point A and D. Conductivity ranged between 2561 and 4481 μS/cm, increasing 37.34% between point A inlet and point D outlet to the Ramsar site. During the sampling period, the three semi-natural ponds registered their maximum depth.

Total phosphorus (TP) and total nitrogen (TN) experienced a similar pattern. Both nutrients decreased throughout Laguneto (54.00% and 50.34%, respectively) but increased again before reaching Lake Fuente de Piedra (30.43% and 54.79%, respectively). In both cases, there is a net decrease between the inlet into Laguneto (point A) and the outlet into Lake Fuente de Piedra (40% and 23.13%, respectively).

Table 2. Longitudinal profile of physicochemical variables through the semi-natural ponds system.

Abiotic Parameters	Point A	Point B	Point C	Point D
Temperature (°C)	19.62 ± 0.10	20.83 ± 0.04	21.95 ± 0.05	21.17 ± 0.09
pH	7.99 ± 0.03	8.63 ± 0.01	7.45 ± 0.02	7.94 ± 0.02
Electric conductivity (µS/cm)	3262.67 ± 4.37	2561.00 ± 1.87	2688.75 ± 25.86	4481.00 ± 4.24
Total phosphorus (mg/L)	5.00 ± 3.46	2.30 ± 0.58	2.30 ± 0.58	3.00 ± 0.00
Total nitrogen (mg/L)	14.70 ± 0.58	7.30 ± 0.58	11.30 ± 2.89	11.30 ± 2.08

3.2. Biological Response

Table 3 shows the concentration of total heterothophic bacteria and faecal indicators (faecal coliforms and faecal enterococci) in wastewater flowing through the ponds system. Total heterotrophic bacteria grown at both 22 and 37 °C decreased by three orders of magnitude from point A to point D. Abundance of faecal coliforms was lower in wastewater effluent inlet (point A) than in the outlet of Laguneto wetland (point B), increasing one order of magnitude and decreasing, in the same order, in the outlet to the Ramsar wetland (point D). Faecal enterococci, in contrast, showed the highest abundance in wastewater effluent inlet (point A), decreasing three times in the outlet of Laguneto wetland (point B). Finally, faecal enterococci concentration released to the Ramsar wetland (point D) was very low, with only 1 cfu/100 mL.

Table 3. Concentration of total heterothophic bacteria and faecal indicators (faecal coliforms and faecal enterococci) in wastewater effluent flowing through the ponds system, grown in agar mediums. A, B, and D are the sampling points.

Bacteria	A	B	D
Heterotrophic bacteria at 22 °C (cfu/mL)	$(1.29 \pm 0.60) \times 10^5$	$(2.10 \pm 1.39) \times 10^4$	388 ± 151
Heterotrophic bacteria at 37 °C (cfu/mL)	$(2.39 \pm 2.23) \times 10^5$	$(3.18 \pm 1.17) \times 10^4$	247 ± 135
Faecal coliforms (cfu/100 mL)	65 ± 40	655 ± 18	17 ± 21
Faecal enterococci (cfu/100 mL)	1033 ± 351	388 ± 68	1 ± 1

Figure 2 shows the relative contribution of identifiable phytoplankton groups by fluorescence fingerprints to chlorophyll-a concentration. Results indicate that phytoplankton was dominated by green algae in all sampling points except point D. A significant drop (>75%) was observed between points B and C, and between this last one and point D, before the inlet to the Fuente de Piedra lake. At this last sampling point, the phytoplankton community was dominated by bluegreen algae (Figure 2).

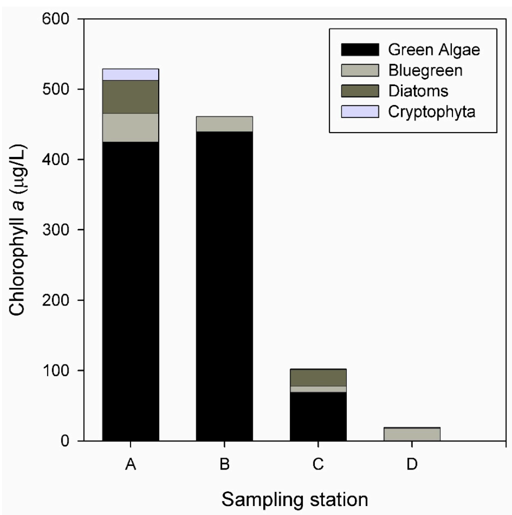

Figure 2. Longitudinal profile of total chlorophyll *a* and relative contribution of phytoplankton identifiable groups by fluorescence fingerprints.

Phytoplankton and zooplankton biovolume present an opposite pattern through the semi-natural pond system (Figure 3). While phytoplankton showed a market decrease (12.7 fold) from point A to D, zooplankton suffered an increment with a maximum value in Los Juncares pond (increasing from point A to C by factor 75.2). This increment in zooplankton biovolume is due to a proliferation of cladoceran species, which dominated the zooplankton community.

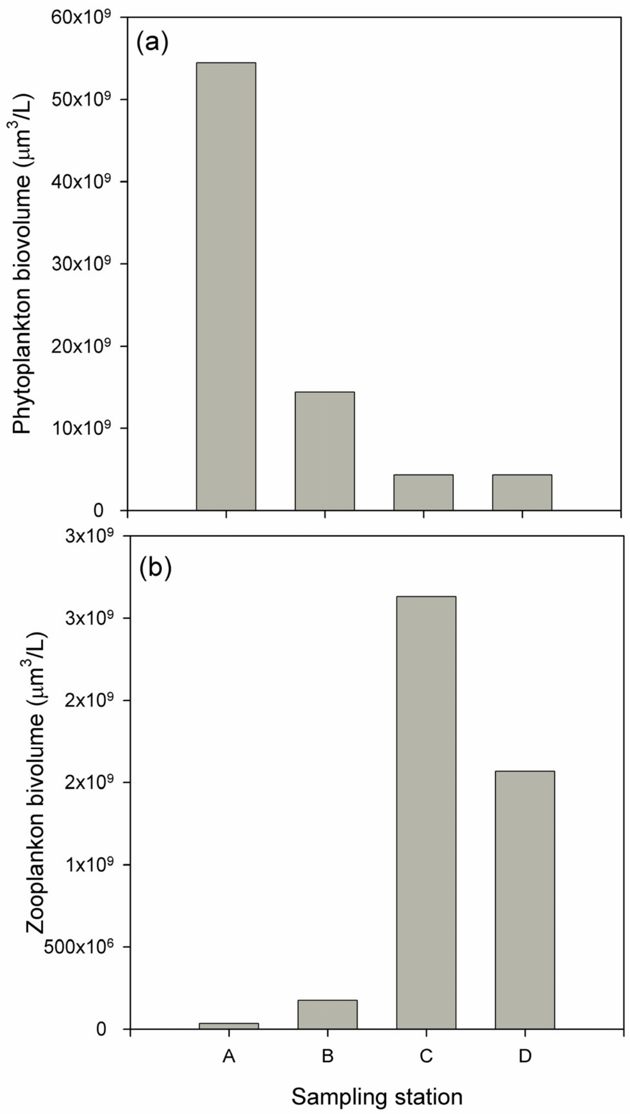

Figure 3. Longitudinal profile of: (**a**) Phytoplankton biovolume 5–100 μm ESD; (**b**) Zooplankton biovolume 250–1000 μm ESD.

4. Discussion

Natural wetlands are widely used as wastewater effluent discharge sites [32]. The high depuration capacity of wetlands has stimulated the development of emerging technology worldwide to construct artificial wetlands systems for treatment of wastewater [15,32]. In the last few years, the volume of wastewater reuse has been increasing with a number of different purposes: agricultural activities, irrigation of green areas, waterfalls, road cleaning, car washing, or firefighting, amongst others [33]. In the Mediterranean region, wastewater recycling and reuse has been practiced since antiquity [34], but regulations on wastewater reuse today is an essential topic [35]. This is more obvious in dry periods, in which the lack of water increases wastewater effluents reuse [35].

Results of physicochemical analysis show that these ponds act as sites in which large environmental changes take place, the most important change being the differences in temperature. Many wetlands processes, such as several biogeochemical processes that regulate the removal of nutrients, are influenced by temperature [36]. These processes have a direct influence on overall treatment efficiency, with high implications in water quality. Therefore, the typical alternation of turbid and clear water phases that occurs with a temporal pattern in natural lakes [37] are here associated to a spatial scale, from the inlet of treated wastewater effluent of Laguneto wetland to the outlet flowing into the Fuente de Piedra salt lake. The turbid water phase occurred in the Laguneto wetland, which receives a wastewater effluent inlet. High concentration of nutrients, together with high temperature values, allow the phytoplankton community to show high values of chlorophyll *a* concentration and biovolume, dominated by green-algae phytoplankton groups. By contrast, the clear water phase was linked to a top-down control by cladoceran species over an edible phytoplankton community, which results in a grazing-driven collapse of phytoplankton populations. With these high feeding rates, the dense cladoceran population transfers phytoplankton biomass to higher trophic levels (invertebrate and waterbird communities) and increments the water transparency before it is released into Lake Fuente de Piedra. In this sense, the existence of a marked increment in the abundance of waterbirds has been associated, in another Mediterranean Ramsar site (Albufera de Adra, south of Spain), with clear water phases [38].

As the discharge comes from a wastewater treatment plant, high heterotrophic and faecal bacteria (faecal coliforms and faecal enterococci) were observed at the inlet of the semi-natural treatment system. High numbers of faecal bacteria in point A could indicate an incomplete depuration of wastewater in the treatment plant. However, as has been recognized elsewhere, wastewater wetlands generally perform well for bacterial pollution [24]. In our system, faecal coliforms decreased about 73.8 $\pm$ 3.2% between inlet and outlet, which suggests that algae could help to inhibit coliforms growth [39,40]. These results indicate that our wetland system is an excellent method to reduce faecal enterococci almost to zero. While faecal streptococii decreased continuously from the inlet to the outlet, faecal coliforms increased by a factor 10 from point A to B. Part of this pollution could be produced by bird faeces [41,42]. This wetland has an elevated density of waterbirds, e.g., *Phoenicopterus roseus*, *Anas clypeata*, *Fulica atra*, *Oxyura leucocephala*, and some gull species (*Chroicocephalus ridibundus*, *Larus fuscus* and *Larus michahellis*). The last census conducted in the spring of 2016 by the managers of Lake Fuente de Piedra showed values of 625 gulls, 117 common coots, 99 northern shovelers, and 68 flamingos in Laguneto. However, faecal bacteria decreased below 200 cfu/100 mL at sampling station D, which is an excellent value for bath water [43]. If wastewater effluent was directly introduced into the Ramsar wetland (red gross arrow in Figure 1), faecal bacteria could be higher. This action also generates water quality deterioration because, in Mediterranean areas, a large amount of organic matter and nutrients has been observed in the channels that contain wastewater effluent [9].

Our wetland system shows similar efficiency in bacteria and nutrient reduction to other wastewater treatment systems [44,45]. However, natural wetland-systems are usually cheaper to build and maintain [46,47]. In our case, the cost of the wetland system was about 131,050 euros, and provides additional purification of wastewater effluent released by a wastewater treatment plant before entering a protected Ramsar wetland. Considering the low cost of construction and maintenance and that wetland ecosystems are among the most endangered ecosystems worldwide [1], our results suggest the importance of adding natural wetland systems to traditional wastewater treatment plants in order to improve released water quality. Furthermore, reducing water consumption as a measure of conservation of available water resources for the future in response to measures imposed by climate change leads to a proportional reduction in the volume of wastewater effluent, but not in the contaminant loading [48]. Thus, more efficient wastewater treatment plants are necessary, which might be achieved by coupling natural wetland systems to traditional treatment plants, avoiding economic and ecological problems at the same time. The use of artificial wetlands for the treatment of wastewater with lower costs of installation, operation, and maintenance make them an appropriate alternative to traditional treatment plants [32], and wetlands could be used to minimize bad functioning or temporally overload of treatment plant capacity.

Furthermore, in an arid area such as southern Spain, a constructed wetland provides an additional support for waterbirds and other wetland-related species. In this sense, a constructed wetland serves as a refuge for waterbirds, amphibians, and aquatic invertebrates groups. This is relevant in Fuente de Piedra because, as was observed during the sampling period, the semi-natural wetlands maintain water (1.42, 0.39 and 0.20 m depth for Laguneto, Laguna de Los Juncares, and Los Juncares, respectively) when the salt lake was completely dry. Moreover, regarding wildlife that feed in natural wetlands, it is important to monitor water quality bioassays and biomarkers, allowing the detection of toxic chemicals and possible cumulative effects. The monitoring of biota tissues would allow the detection of bioaccumulation of toxins in the organisms and compare them with Environmental Quality Standards [49]. In our case, health problems are low because the wetland system is not accessible to visitors, as it is included in a protected area acting only as an observation site for waterbirds. The discharged wastewater effluents guarantee the existence of small wetlands during dry summers, which are an attraction point for birds. In fact, the small wetland system adjacent to the Ramsar wetland of Fuente de Piedra includes bird-watching points with guided observation running in summer. The presence of wetlands and diverse waterbirds throughout the year is a key factor for tourism. Hence, the wastewater treatment by artificial wetlands guarantees tourist activity and economic development in the area.

In order to extrapolate the results obtained in this study to other Mediterranean wetlands, it is important to note that the short sampling period in this study might not be representative for the functioning of the semi-natural ponds system throughout the year or even during a longer period of time. However, our objective was to study the biogeochemical assimilation capacity of the semi-natural system. For this purpose, a sampling period after a very dry period was chosen (the rainfall during September 2015–April 2016 was 279.9 $L \cdot m^{-2}$, with the mean value being 379.02 $L \cdot m^{-2}$). During this period of time, Lake Fuente de Piedra and all the surrounding wetlands were dry. This guarantees that our results only show the biogeochemical assimilation capacity without the influence of dilution processes. Thus, although it was a short sampling period, the environmental conditions when the data were taken provide an interesting insight into the functioning of this system. In fact, we really sampled pure wastewater effluent through the pond system. However, it is also important to point out that a longer sampling period would be necessary in future in order to describe the functioning of the pond system throughout the year and the effects of dilution processes. Additionally, the undiluted assimilation capacity could feed models of treatment wetlands [50].

Finally, it is important to mention that one of the major implications of Life programs is their social repercussion. In our case, constructed wetlands were developed as a sustainable solution for Fuente de Piedra village and for the reuse of water in a representative wetland. As mentioned previously, these actions have a great impact on both tourist activity and environmental education.

5. Conclusions

The wetland system fulfils three main functions: (i) it improves the quality of discharged water from the treatment plant, (ii) it increases wetland hydrodiversity (it induces the existence of wetlands, at a local scale, with different hydroperiod), and (iii) it provides more water during dry years, guaranteeing the presence of birdlife, which is important for local tourism. The results obtained allow us to recommend that semi-natural or artificial wetlands should also be considered in other aquatic ecosystems (Mediterranean wetlands) that receive wastewater effluent discharges.

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