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Article

# Evaluation of Microbiological and Physicochemical Contamination of Groundwater in the Coastal Wetland of Pantanos de Villa (Lima-Peru)

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## Abstract

(1) Background: Groundwater quality in tropical wetlands remains poorly studied. Los Pantanos de Villa, a key wetland located on Peru's desert coast, is recognized as a Protected Natural Area and a Ramsar site that faces ongoing threats. This study aimed to assess groundwater contamination in the wetland using microbiological (*Escherichia coli*, total and fecal coliforms) and physicochemical parameters, as well as water quality indices (WQIs); (2) Methods: In 2023 annual sampling was conducted at 12 locations (4 test pits and 8 piezometers), during the high recharge season (March) and low recharge season (September). Parameters were compared to national (Peru) and international (U.S.) standards. Two WQIs—the Canadian Council of the Ministry of the Environment (CCME) and the National Sanitation Foundation (NSF)—were applied; (3) Results: Microbiological contamination levels were dangerously high at all sampling sites. Physicochemical parameters exceeded permissible limits in at least one season. The CCME-WQI classified 100% of samples as having poor quality, and the NSF-WQI classified 75% as poor and 25% as medium quality; (4) Conclusions: Groundwater in the Pantanos de Villa wetland exceeds national and international contamination thresholds. Microbiological pollution is particularly concerning. WQIs confirm a degraded state.

**Keywords:** protected natural area; groundwater; water quality; wetland

## 1. Introduction

Wetlands are the most productive ecosystems in the world and host an important diversity of species [1]. They provide important ecosystem services and carbon sinks, which are relevant for mitigating the effects of climate change, improving water quality, and conserving biodiversity [2,3]. Despite this, wetlands are often subject to anthropogenic impacts, such as urbanization, agriculture and drainage [2,4], which directly affect the quality of water resources and cause their degradation.

At the Latin American level, studies on water resources tend to focus on surface water sources, such as rivers and lakes, and groundwater studies are not given the same priority [5]. Likewise, due to geographical diversity, these studies are complicated to carry out in certain areas [6]. In Peru, coastal wetlands represent a reservoir of freshwater and a constant recharge of aquifers [5,7], and these environments form a biological corridor and host very important biodiversity [8].

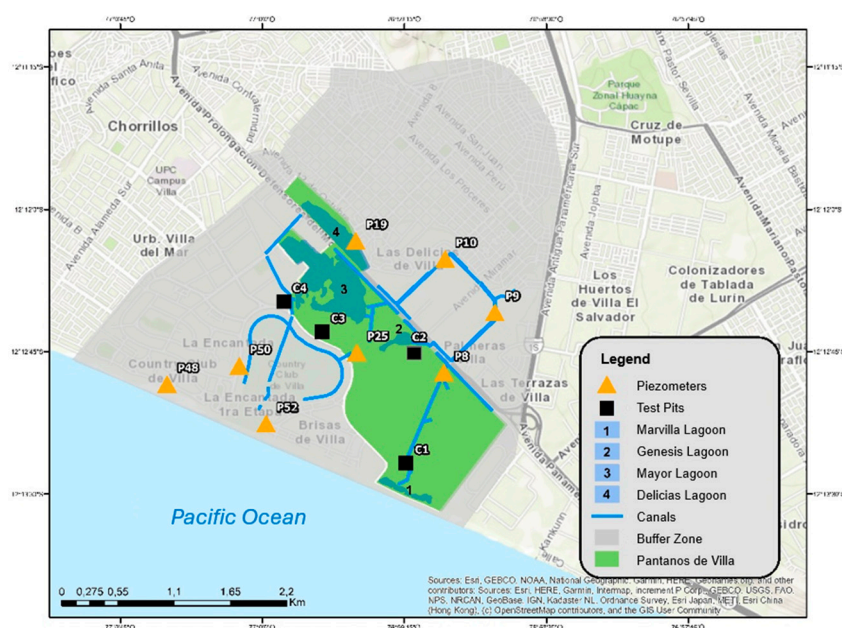
A notable wetland within the study area is the Pantanos de Villa Natural Protected Area, which was incorporated into the National System of Protected Areas by the Peruvian state in 2006 as a wildlife refuge [9]. In a similar manner, the urban ecosystem in question is overseen by the Municipal Authority of Los Pantanos de Villa (PROHVILLA). The international importance of this wetland has been underscored by its designation as a RAMSAR site in 1997 [10]. However, this wetland is located within the metropolitan area of Lima and is surrounded by urban areas. Consequently, it is subject to persistent anthropogenic pressure, which can have detrimental effects on water resources and the

ecosystem. The present study evaluated the groundwater quality of the Pantanos de Villa NPA, considering both microbiological and physicochemical indicators.

## 2. Materials and Methods

### 2.1. Study Area

This research was conducted in the coastal wetland of Los Pantanos de Villa ( $12^{\circ}12' S$ ,  $076^{\circ}59' W$ ) and its buffer zone (Figure 1) located in Lima, Peru. This wetland was classified as a Natural Protected Area (NPA) by Resolution No. 358-2001-INRENA. The buffer zone is characterized by being adjacent to the NPA and being under special protection (RP N°169-2016-SERNANP). The wetland has an area of 263.27 ha [11].



**Figure 1.** Map of groundwater quality sampling points inside and outside the Pantanos de Villa wetland, Lima, Peru.

The wetland is characterized as a hydromorphic ecosystem and its importance is due to its biological diversity and water resources, as well as its high scientific and cultural value [12]. In terms of geomorphology, the wetland has a minimum altitude of 1 meters above sea level (m.a.s.l.) and a maximum altitude of 5 m.a.s.l., since it is in the lower part of the hydrogeographic basin of the Rimac River valley. The climate is typical of the Peruvian coastal desert, with average annual temperatures between 17.2 and 23.0 °C, relative humidity between 84.4 and 99%, and low rainfall. The area surrounding the wetland is used for urban, industrial and service activities [10,13].

### 2.2. Sample Method

The sample set included 12 sampling points, 6 of which were situated within the wetland environment and 6 in the surrounding area. The sampling points comprised 4 pits and 8 piezometers, as illustrated in Figure 1. The coding was based on the hydrographic network formed by the piezometers owned by PROHVILLA. Sampling was conducted in two stations in the year 2022, considering that a higher water recharge occurs in the month of March (summer) and there is lower water recharge in September (winter) [14]. The U.S. Geological Survey methodology was employed in the National Field Manual for the purpose of groundwater quality sampling. The National Field Manual of the U.S. Geological Survey methodology was employed for groundwater quality sampling [15].

The collection of groundwater samples was executed through the implementation of two distinct methodologies. The initial approach entailed the utilization of the PROHVILLA operational

piezometers. The samples were extracted using a bailer (Figure 2). This method involves extracting the sample three times to minimize disturbance of the water column [16]. The second phase of the experiment involved the construction of 4 pits, with dimensions of approximately 1 x 1 x 1 meters. Two of these pits were positioned within the confines of the wetland, while the remaining two were situated in the external environment. Subsequent to the creation of the test pits, with the prescribed depth, a bailer was utilized to extract the groundwater sample. After extraction of the sample, the extracted soil was deposited at the place of origin [15].



**Figure 2.** Surface water of the Pantanos de Villa wetland (A) and groundwater (B).

To determine groundwater quality, an *in situ* evaluation of physicochemical parameters, such as pH, total suspended solids (TSS), electrical conductivity (EC) and temperature, was first performed using a Hanna Model HI 98311 Multiparameter. An *ex situ* evaluation was then performed for microbiological (*Escherichia coli*, total coliforms and fecal coliforms) and physicochemical parameters (total phosphorus, nitrates NO<sub>3</sub><sup>-</sup>, chemical oxygen demand [COD], biochemical oxygen demand [BOD] oils and fats). These samples were sent to the laboratory R-LAB S.A.C., certified by INACAL (Lima-Peru).

The parameters were analyzed by comparison with the Environmental Water Quality Standards of the Peruvian Regulations, hereinafter referred to as EQS - Water, of category 4: Conservation of the Aquatic Environment, subcategory E1: Lagoons and lakes, which includes natural surface water bodies that are part of fragile ecosystems, NPAs and/or buffer zones, and that have characteristics that require an additional level of protection [17]. This national regulation was used as a reference, as Peru does not have a specific regulation for groundwater. Likewise, it was decided to use a complementary international standard from the United States, which is the Groundwater Quality Standards 15A NCAC 2L .0202. [18].

### 2.3. Water Quality Index

In order to comprehensively assess the groundwater quality, the calculation of the Water Quality Index (WQI) of Peru was carried out by the National Water Authority [19]. This index follows the methodology of the WQI of the Canadian Council of the Ministry of the Environment (CCME). The purpose of establishing the methodology for calculating the WQI is to express the condition or state of water quality based on established ranges.

The index establishes a rating by comparing the monitoring results that do not meet the Environmental Quality Standards (EQS), the number of parameters that exceed EQS-Water and the magnitude of the exceedance [20]. The established rating ranges from poor to excellent (Table 1).

**Table 1.** Interpretation of the water quality index of Peru rating.

CCME_WQI	Rating	Interpretation
95-100	Excellent	Water quality is protected with no threats or damage. Conditions are very close to natural or desired levels
80-94	Good	Water quality deviates somewhat from natural water quality. However, desirable conditions may be with some minor threats or damage.
65-79	Favorable	Natural water quality is occasionally threatened or impaired. Water quality often deviates from desirable values. Many uses need treatment.
45-64	Regular	Water quality does not meet quality objectives; desirable conditions are often threatened. Many of the uses need treatment.
0-44	Poor	Water quality does not meet quality objectives, almost always threatened or impaired. All uses need prior treatment.

CCME-WQI: Canadian Council of the Ministry of the Environment water quality index.

The other WQI used for the evaluation was that established by the National Sanitation Foundation (NSF) of the United States. This index was calculated using the ICATEST software, in which the parameter data are entered, and the index is calculated based on the weighting factor generated by the software. It also has its own interpretation with established colors and values (Table 2).

**Table 2.** Interpretation of the National Sanitation Foundation water quality index of the United States.

NSF-WQI	Classification
91-100	Excellent
71-90	Good
51-70	Average
26-50	Poor
0-25	Very poor

NSF-WQI: National Sanitation Foundation- water quality index.

#### 2.4. Data Analysis

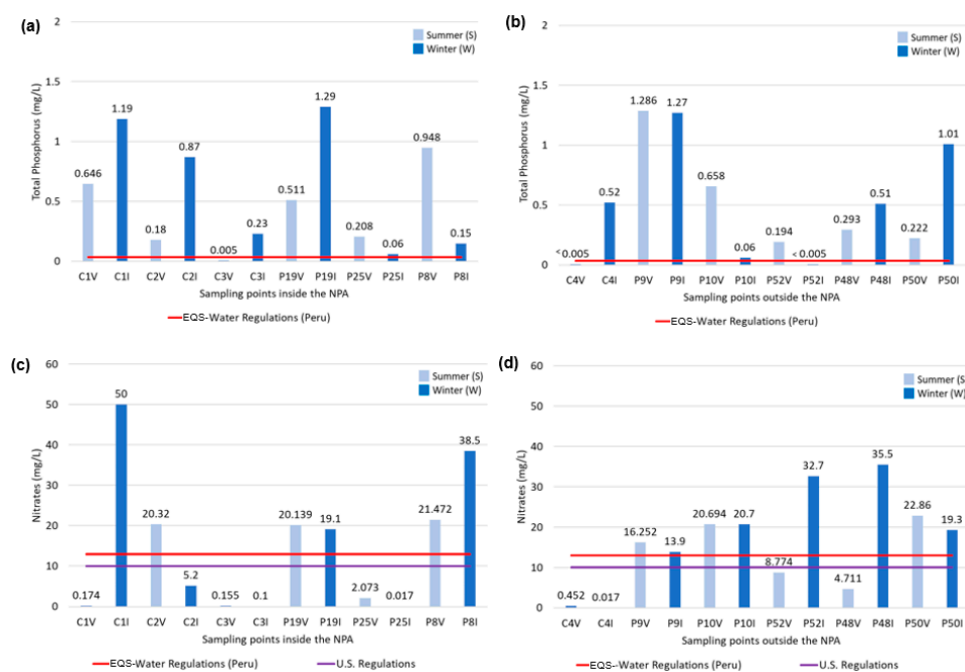
Statistical analysis was performed considering two groups: the buffer zone (outside the wetland) versus the interior of the Pantanos de Villa wetland, as well as the winter versus the summer season. The variable analyzed was the quality of the groundwater, considering microbiological and physicochemical parameters. To verify the normal distribution of the data, the Shapiro-Wilk test was performed, which was not fulfilled for the physicochemical and microbiological data, and thus, the non-parametric Mann-Whitney U test was applied. IBM SPSS Statistics v.26 software was used for all the data analyses.

### 3. Results

#### 3.1. Physicochemical Parameters

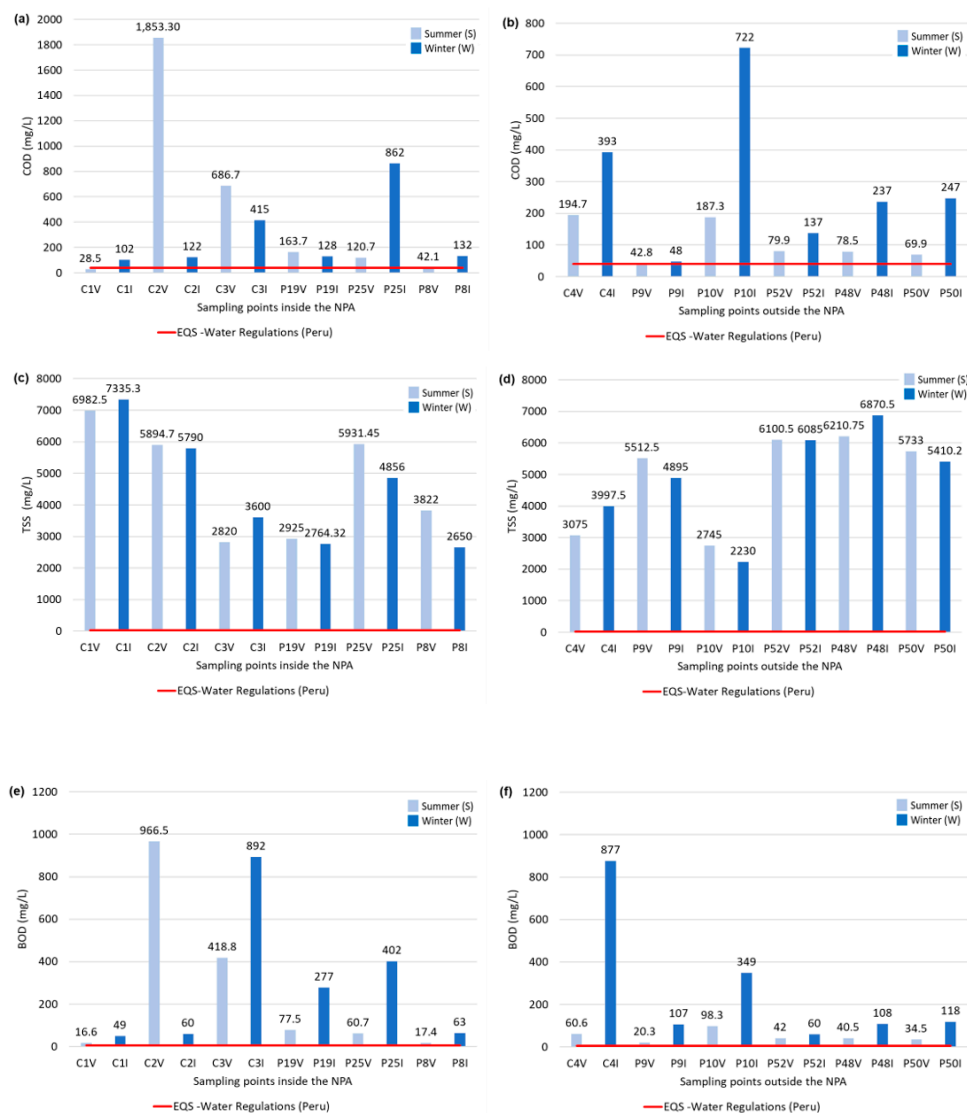
The pH value obtained was an average of 7.29 (summer - winter), with values within the ranges of the EQS - Water and the international standards for groundwater in the United States [18]. As for temperature, the values were also obtained according to Peruvian standards, with an average of 21.49°C (ranging from 19.80 - 24.20°C). Regarding oils and fats, an average (summer - winter) of 16.88 mg/L was obtained (ranging from <1.6 to 30.6 mg/L). Three sampling points outside the wetland (P52, P48 and C4) and two points inside the wetland (C2 and P25) exceeded the limits of the national regulations.

Another parameter evaluated was total phosphorus, with which 75% of the points evaluated inside and outside the wetland exceed the EQS - Water. Of the 25% that did not exceed the regulations, one point was located inside the NPA (C3V) and two were outside (C4V and P52I) (Figure 3 a and b). For the nitrate parameter, 50% of the sampling points inside the NPA exceeded the national standard of 13 mg/L (Peru) and the international standard of 10 mg/L (USA), while outside the wetland, 66.6% of the total exceeded the standard (Figure 3 c and d). Regarding EC, 100% of the points exceeded the national regulations, both inside and outside the NPA. This is due to the characteristics of the location of the wetland, which is a coastal ecosystem adjacent to the sea.



**Figure 3.** Comparison of physicochemical parameters inside and outside the wetland (NPA) (a) total phosphorus (mg/L) inside the NPA and (b) outside the NPA. (c) nitrates (mg/L) inside the NPA and (d) outside the NPA.

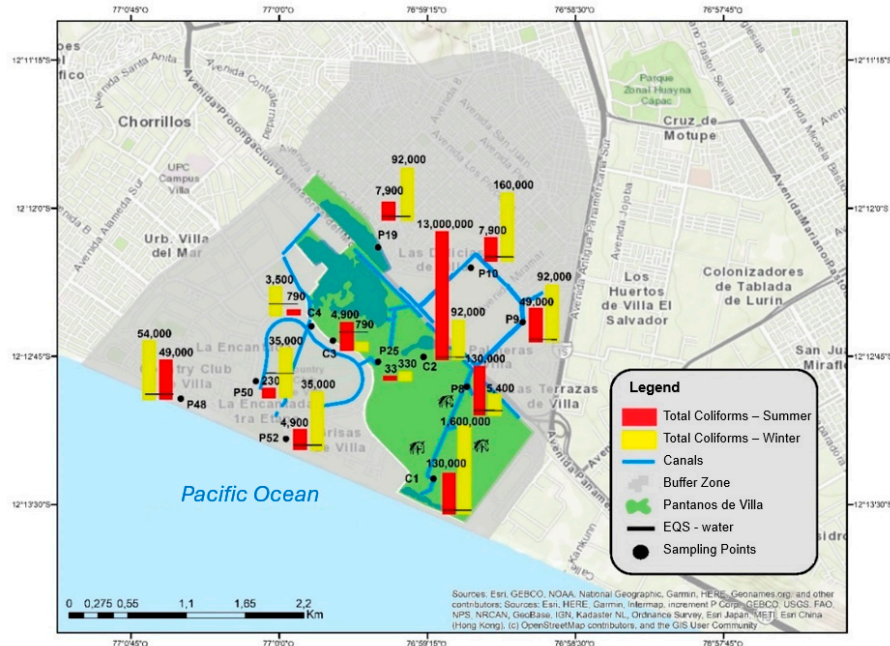
When analyzing the COD parameter, all the values exceeded the EQS - water. The sampling point with the highest value (C2) was 1853.3 mg/L located inside the wetland and near the Genesis Lagoon (Figure 4 a and b). For TSS, all the points exceeded national and international (U.S.) regulations. The sampling point with the highest concentration was C1, located within the wetland adjacent to the Marvilla Lagoon (Figure 4 c and d). The BOD results showed that all sampling points, inside and outside the NPA in the summer and winter seasons, exceeded the EQS water value, and the highest value was found inside the NPA (C2V) in the summer season (966 mg/L). This point is located adjacent to the Genesis Lagoon (Figure 4 e and f).



**Figure 4.** Comparison of physicochemical parameters inside and outside the wetland (a) COD (mg/L) inside the NPA and (b) COD outside the NPA. (c) TSS (mg/L) inside the NPA and (d) TSS outside the NPA. (e) BOD (mg/L) inside the NPA and (f) BOD5 outside the NPA.

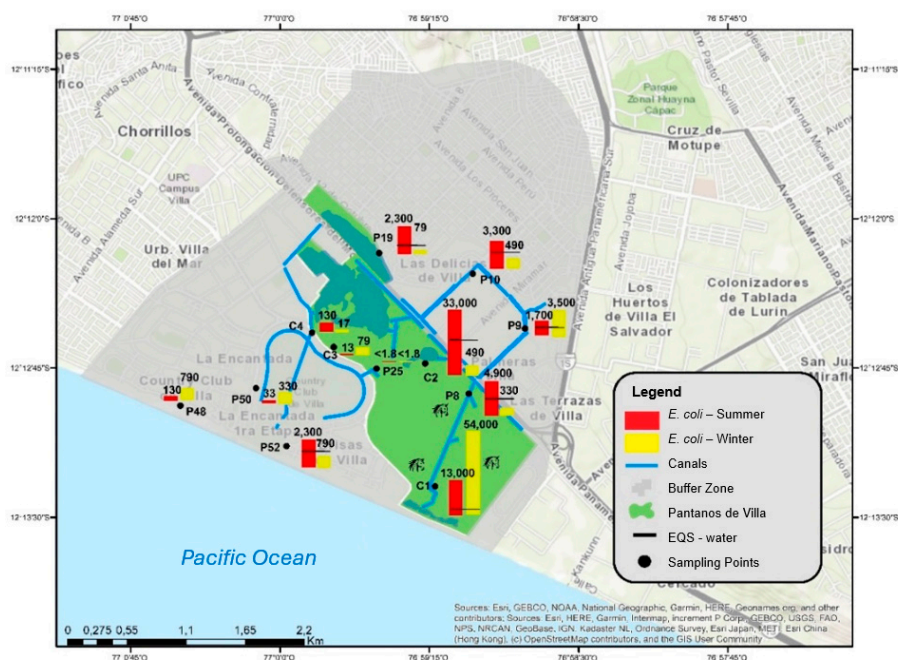
### 3.2. Microbiological Parameters

For total coliforms, a value of 50 most probable number per 100 milliliters (MPN/100 mL) is established for EQS-water (Peru) and 100 MPN/100 mL for U.S. standards. The analysis showed that all the groundwater points evaluated in the wetland, except for one (P25V), exceeded the national and international environmental quality standards. A particular case was the summer sampling point (C2V), located within the NPA, where the highest value of 13,000,000 MPN/100 mL was found (Figure 5). Similarly, the behavior of the two outlier points with a higher concentration was observed.



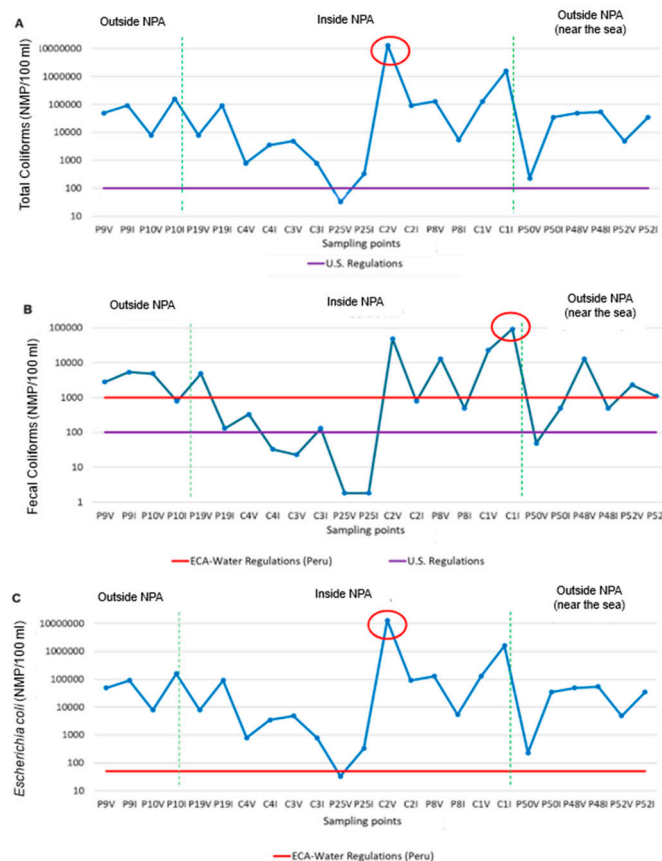
**Figure 5.** Total coliforms evaluated in groundwater during the summer and winter of 2023, inside and outside the Pantanos de Villa Wetland.

According to the results obtained for fecal coliforms, within the NPA, 42% of the points evaluated exceeded the EQS - Water and 75% exceeded the international standards (U.S.). Outside the NPA, 50% of the points evaluated exceeded the national standard and 83% exceeded the international standard (U.S.). In the case of *E. coli*, 67% of the total points evaluated within the NPA exceeded the EQS - Water. One of the points (P25) that did not exceed the standards was found in both seasons with values of 1.8 MPN/100 mL (Figure 6). Outside the NPA, all of the points evaluated exceed the national standard.



**Figure 6.** *Escherichia coli* found in groundwater inside and outside Los Pantanos de Villa during the summer and winter of 2023.

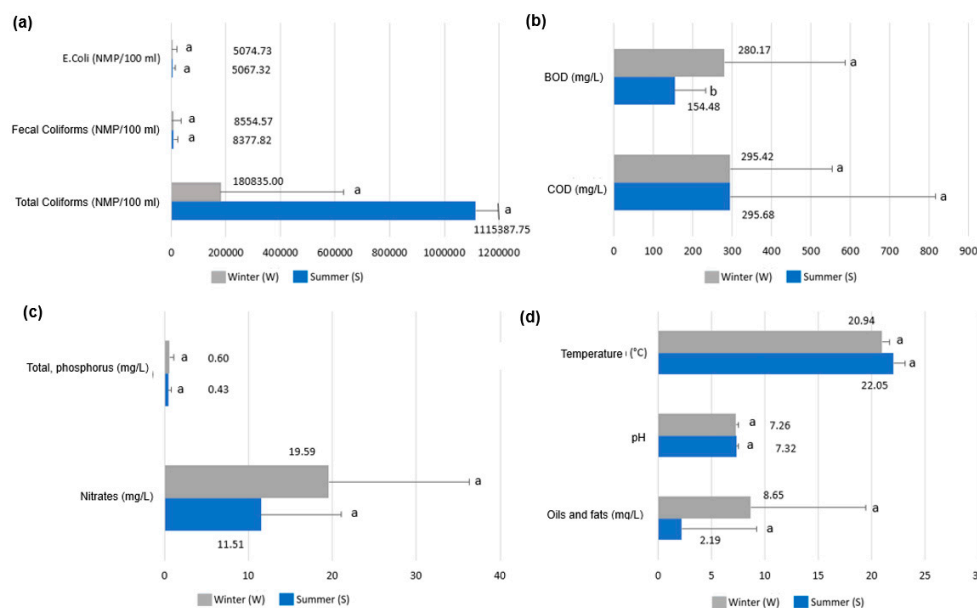
Likewise, analysis according to the direction of the water flow was carried out, from the starting zone, outside the wetland, where the water enters the wetland, and where the watercourse reaches the Pacific Ocean. The highest total coliform values were found inside the NPA (Figure 7), with these concentrations decreasing at points P25 and P50. Similarly, the highest concentrations of fecal coliforms were observed within the NPA at points C1 and C2. Finally, the highest and lowest concentrations of *E. coli* were recorded within the NPA. This shows the absence of a defined pattern in the microbiological contamination of the watercourse, although sampling points with critical values were identified at C1 and C2, within the NPA, where the microbiological parameters analyzed exceeded both national and international water quality standards.



**Figure 7.** Comparison of microbiological parameters according to the water flow of the Pantanos de Villa wetland and national and international regulations. (a) Total coliforms (MPN/100mL) (b) Fecal coliforms (MPN/100mL) (c) *Escherichia coli* (MPN/100mL).

Statistical analysis of the data, performed using the non-parametric Mann-Whitney U test, showed that none of the physicochemical and microbiological parameters presented significant differences ( $p < 0.05$ ) between the areas inside and outside the wetland. Both total and fecal coliforms and *E. coli* concentrations were elevated in both sites. Similarly, COD and BOD parameters showed high mean values in both areas. EC and TSS, which are expected to be elevated due to the nature of the wetland and its direct relationship with the ocean, also showed elevated levels inside and outside. The lack of significant differences suggests that the contamination is similar inside and outside the NPA.

The same test was carried out to evaluate the physicochemical and microbiological parameters by season (summer-winter). As a result, only the BOD showed a significant difference ( $p < 0.05$ ) according to the sampling season. The average value was lower in the summer than in the winter. It should be noted that the other parameters, such as microbiological parameters, had different high, albeit non-significant, averages in both sampling seasons, (Figure 8).



**Figure 8.** Comparison of the physicochemical and microbiological parameters in the Pantanos de Villa wetland in the winter and summer. **(a)** Microbiological parameters (MPN/100mL) **(b)** COD and BOD **(c)** Nitrates and total phosphorus. **(d)** Temperature, pH, and oils and fats. Each graph shows the average values and standard error. Similar letters (a) indicate no significant difference between groups according to the Mann-Whitney U test.

### 3.3. Water Quality Index (Peru)

Based on the CCME, the WQI - Peru indicated "poor quality" groundwater at all 12 sampling points inside and outside the wetland, and in both the summer and winter seasons (Table 3). The water quality would not meet the conservation objectives of Peruvian regulations and is threatened.

### 3.4. National Sanitation Foundation Water Quality Index (U.S.)

The result of the NSF-WQI was similar to that of the WQI-Peru, with nine sampling points presenting "poor quality", while three sampling points (C3, C4 and P25) had "medium quality". These latter three sampling points were very close to each other, have similar physical characteristics and show a better quality according to NSF-WQI compared to the other points evaluated (Table 3).

**Table 3.** Water quality indices of the groundwater in the Pantanos de Villa wetland.

Code	Location	Season	CCME-WQI (Peru)	NSF-WQI (U.S.)
C1	Inside NPA	Summer	26.26	Poor
		Winter	15.85	Poor
C2	Inside NPA	Summer	10.80	Poor
		Winter	21.30	Poor
C3	Inside NPA	Summer	35.53	Poor
		Winter	34.66	Poor
C4	Outside NPA	Summer	40.15	Poor
		Winter	26.55	Poor
P8	Inside NPA	Summer	16.08	Poor
		Winter	25.33	Poor
P9	Outside NPA	Summer	15.85	Poor

		Winter	15.85	Poor	33.25	Poor
P10	Outside NPA	Summer	16.11	Poor	34.67	Poor
		Winter	25.43	Poor	43.01	Poor
P19	Inside NPA	Summer	16.11	Poor	35.61	Poor
		Winter	25.17	Poor	38.13	Poor
P25	Inside NPA	Summer	30.59	Poor	63.2	Average
		Winter	31.07	Poor	64.46	Average
P52	Outside NPA	Summer	16.33	Poor	43.56	Poor
		Winter	20.83	Poor	41.7	Poor
P48	Outside NPA	Summer	25.92	Poor	47.47	Poor
		Winter	15.99	Poor	35.34	Poor
P50	Outside NPA	Summer	29.57	Poor	47.25	Poor
		Winter	25.17	Poor	36.21	Poor

NPA: national protected area; CCME-WQI: Canadian Council of the Ministry of the Environment-Water Quality Index; NSF-WQI: National Sanitation Foundation-Water Quality Index.

## 4. Discussion

### 4.1. Evaluation of Physicochemical Parameters

Regarding the physicochemical parameters evaluated, pH and temperature levels were found to be in accordance with both national and international standards. These findings were consistent with the results reported in the study by Puma *et al.* in 2022 [21]. However, oils and fats were found to exceed the national regulations (EQS-water) in three sampling points located outside the NPA. As low temperatures prevail during the winter months, water solubility is diminished. This phenomenon leads to the accumulation of substances at the surface of the water, forming layers that can exceed regulatory limits. Excessive accumulation of these substances poses a threat to aquatic ecosystems, potentially causing harm to aquatic life [22].

The total phosphorus levels measured at all sampling points exceeded the limits established by Peruvian regulations during at least one of the periods evaluated. A particularly notable increase in total phosphorus was observed during the winter months, which may be attributed to the contribution of water recharge, a factor that has been shown to favor increased concentrations of this element. Conversely, the application of detergents as a source of phosphorus outside the wetland has been demonstrated to elevate the trophic status of the water resource. This occurs because these compounds are ultimately deposited in the lagoons of the ecosystem [23]. Similarly, nitrates in groundwater typically show low concentrations; however, these levels may be increased by anthropogenic activities, such as agriculture, domestic and industrial effluents, and the use of detergents in washing activities in the channels external to the wetland [23,24].

The EC of the water samples collected from the Pantanos de Villa site exceeded the national standard at all sampling points. This is attributable to the geographical location of the Pantanos de Villa, which is a coastal wetland adjacent to the sea. The higher salinity content of the soil and water at this location is reflected in the results [25]. This has been documented in a comparable manner in the coastal wetland of Ventanilla (Lima), where values that surpass the EQS - Water benchmark have been observed [26].

With regard to the BOD, all the sampling points exceeded the Peruvian standard at both stations. The available organic matter is a factor that generates an increase in BOD concentrations. The reason for this is that high concentrations require more oxygen for its decomposition, thereby increasing BOD [2]. With respect to COD, point C2, situated in proximity to the Genesis lagoon, exhibited the

most elevated value (1,853.3 mg/L). These values exhibited a substantial increase in comparison with the study conducted in the wetland by Puma *et al.* in 2022 [21].

The TSS concentrations at all the sampling points exceeded the established standards, exhibiting a direct correlation with EC. The relationship between EC and TSS concentrations is such that an increase in EC results in an increase in TSS concentration. This can be attributed to the presence of salts and minerals in the groundwater, which is a common occurrence in arid regions [27]. In the Poza la Arenilla Wetland (Lima), the maximum surface water value recorded was 171.2 mg/L [23], whereas within the NPA, Pantanos de Villa exhibited a maximum value of 7335.3 mg/L. This increase could be attributed to the filtration of waste from homes with silos and horse feces, which can raise the levels of solids in the water [28].

#### 4.2. Evaluation of Microbiological Parameters

The elevated levels of total coliforms observed suggest a nutrient-rich source that sustains the bacterial community. This may be associated with the proximity of residences to wetland channels and the absence of septic system utilization, which has a detrimental effect on groundwater quality [29]. Regarding fecal coliforms, 75% of the sampling points did not comply with the established regulations, with the highest recorded values found in proximity to the Marvilla Lagoon, where avian and mammalian feces may contribute to their proliferation [30].

Concurrently, 58% of the points presented levels exceeding the established limits for *E. coli*, a bacterium that is characteristic of the intestinal microflora and can be transported from the soil surface to the groundwater [21]. The elevated levels of coliform bacteria observed in the vicinity of lagoons (C1 and C2) can be attributed to the presence of wetland gulls. It has been reported that these birds excrete substantial quantities of coliform bacteria in their feces, thereby serving as a significant source of contamination for surface and groundwater bodies [31]. Furthermore, in certain locations, such as C1, the presence of equines has been observed to potentially impact water quality. This is attributed to the fact that equine feces have been found to contribute to the presence of total and fecal coliforms in the soil and water table [32]. As posited by Solarte *et al.* in 2006, these three microbiological parameters are interrelated in nature, as they are derived from human and animal feces, as well as from wastewater and water bodies exposed to fecal contamination [33].

#### 4.3. Water Quality Indexes (WQI)

The findings of this study indicate the presence of a critical situation in the Pantanos de Villa wetlands, with the Peruvian WQI rating groundwater quality as "Poor" at all the points evaluated. In contrast, the United States WQI (NSF) classified 75% of the points evaluated as "Poor" and the remaining points as "Medium. The findings, both within and outside the NPA, underscore the deterioration of groundwater quality and its implications for the ecosystem. Conversely, surface water quality in Laguna Marvilla was classified as "Regular," exhibiting a lesser degree of impact from horse excreta and the biological activity of the ecosystem [34]. At the regional level, studies in the Santay wetland (Guayaquil, Ecuador) within a protected area indicated between "good" and "acceptable" water quality [35], suggesting that concentrations of total coliforms, fecal coliforms, and *E. coli* have a significant impact on WQIs. Consequently, the variability in water quality can be influenced by both anthropogenic factors and natural processes, underscoring the necessity of monitoring and management [36].

**Author Contributions:** Conceptualization, V.M., T.P and D.R.; methodology, V.M and T.P.; software, V.M. and T.P.; validation, V.M., T.P. and D.R.; formal analysis, V.M. and T.P.; investigation, V.M. and T.P.; resources, V.M. and T.P.; data curation, V.M.; writing—original draft preparation, V.M.; writing—review and editing, V.M.; visualization, V.M.; supervision, D.R.; project administration, V.M., T.P. and D.R.; funding acquisition, V.M. and T.P. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** All relevant data are contained within this paper.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Abbreviations

The following abbreviations are used in this manuscript:

NPA	Natural Protected Area
TSS	Total Suspended Solids
EC	Electrical Conductivity
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
EQS	Environmental Quality Standards
m.a.s.l.	meters above sea level
PROHVILLA	Municipal Authority of Los Pantanos de Villa
WQI	Water Quality Index
NSF	National Sanitation Foundation
CCME	Canadian Council of the Ministry of the Environment

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