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Fecal indicator bacteria in Alalay Lagoon, Cochabamba, Bolivia: sanitary assessment and implications for opportunistic pathogen monitoring

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Abstract

Alalay Lagoon, located in Cochabamba, Bolivia, is affected by chronic fecal and chemical contamination, raising environmental and public health concerns. This study assessed the use of fecal indicator bacteria (FIB): total coliforms (TC), fecal coliforms (FC), and *Escherichia coli*, as sanitary indicators and supporting signals for microbiological risk in an urban eutrophic lagoon. Water samples from three lagoon sites were collected monthly from April 2019 to January 2020, with additional samples collected from inflow channels in January 2020, and were analyzed by culture-based methods. FIB levels exceeded Bolivian regulatory limits (fecal coliforms > 1,000 CFU/100 mL), supporting the influence of wastewater inputs. Spearman correlation analysis showed strong associations among classical fecal indicators, particularly TC and FC ($\rho = 0.74$, $p < 0.001$), whereas correlations between FIB and opportunistic bacterial groups were weak, negative, or even non-significant. *Streptococcus faecalis* showed a significant negative correlation with FC and a positive association with *Clostridium* spp., suggesting selective persistence patterns under lagoon conditions. The results indicate that FIB are useful for sanitary assessment of fecal contamination, but they should not be interpreted as definitive predictors of opportunistic pathogens. Complementary molecular-based and physicochemical monitoring is recommended to improve the risk assessment of impacted urban water bodies such as the Alalay Lagoon.

Keywords: fecal contamination; water quality; microbial indicators; environmental microbiology; public health.

Introduction

Lagoons are lentic freshwater systems that are sensitive to climatic and geomorphological variability and naturally undergo chemical fluctuations (Prado-Fernández, 2020). However, their equilibrium is increasingly compromised by climate change (Rita *et al.*, 2009) and by anthropogenic activities that modify physicochemical parameters, negatively affecting biodiversity, ecosystem services, and ecological structure (Søndergaard and Jeppsen, 2007; Pomati *et al.*, 2016). These disturbances trigger water quality degradation, the proliferation of toxic algae producing cyanotoxins, hypoxia, biotic homogenization, and disruption of trophic networks (National Research Council, 2000).

In urban ecosystems, these effects are amplified by direct and indirect human activity and the proximity of urban settlements that promote nutrient enrichment in water bodies (Bashir *et al.*, 2020). This is the case of Alalay lagoon, an Andean water body located southeast of the city of Cochabamba, Bolivia, which is undergoing accelerated eutrophication due to anthropogenic pressure (Rejas and Fernández, 2022). This situation is evidenced by the continuous inflow of untreated effluents from the Rocha River, which exhibit high levels of organic pollution (Toledo and Amurrio, 2006), as well as by unregulated wastewater discharges from nearby sources (Ayala *et al.*, 2007; Ampuero Alcoba, 2009; Pérez-Fernández *et al.*, 2020). The combined contribution of these sources introduces organic matter and nutrients such as nitrogen and phosphorus, thereby altering the physicochemical parameters of the aquatic ecosystem. Consequently, recurrent hypoxia, toxic algal blooms, progressive loss of aquatic biodiversity, fish kills, and persistent alkalization (pH 8–9) have been reported (Morales *et al.*, 2017; UMSS, 2016).

Water quality in Alalay lagoon has become a case of particular interest due to its high organic load, the presence of heavy metals reported by Pérez (2005, as cited in Morales and Rivera, 2012), the accumulation of toxic sediments (Castellón, 2001), and severe eutrophication—conditions that favor the proliferation of pathogenic microorganisms and fecal indicator bacteria (FIB) (Korajkic *et al.*, 2018; Daga-Quisbert *et al.*, 2024). These bacteria, widely used as bioindicators of contamination by human excreta, are associated with waterborne diseases that remain a leading cause of morbidity and mortality worldwide (Harwood *et al.*, 2005; WHO, 2001).

Moreover, aquatic environments with high organic matter loads may facilitate the persistence and dissemination of opportunistic pathogens, as well as antibiotic-resistant bacteria and virulence genes, thereby increasing public health risks, particularly among vulnerable populations (Daga-Quisbert, 2023). Several studies have documented these threats in comparable settings, highlighting the need for more efficient and preventive surveillance systems (Payment and Locas, 2011; Quillaguamán *et al.*, 2021).

Within this framework, the present study evaluated the usefulness of fecal indicator bacteria (FIB) as sanitary indicators of contamination by human excreta through standardized bacteriological assays. In addition, we propose their integration into preventive monitoring programs aimed at the early detection of microbiological risks in aquatic ecosystems affected by anthropogenic activities.

Materials and Methods

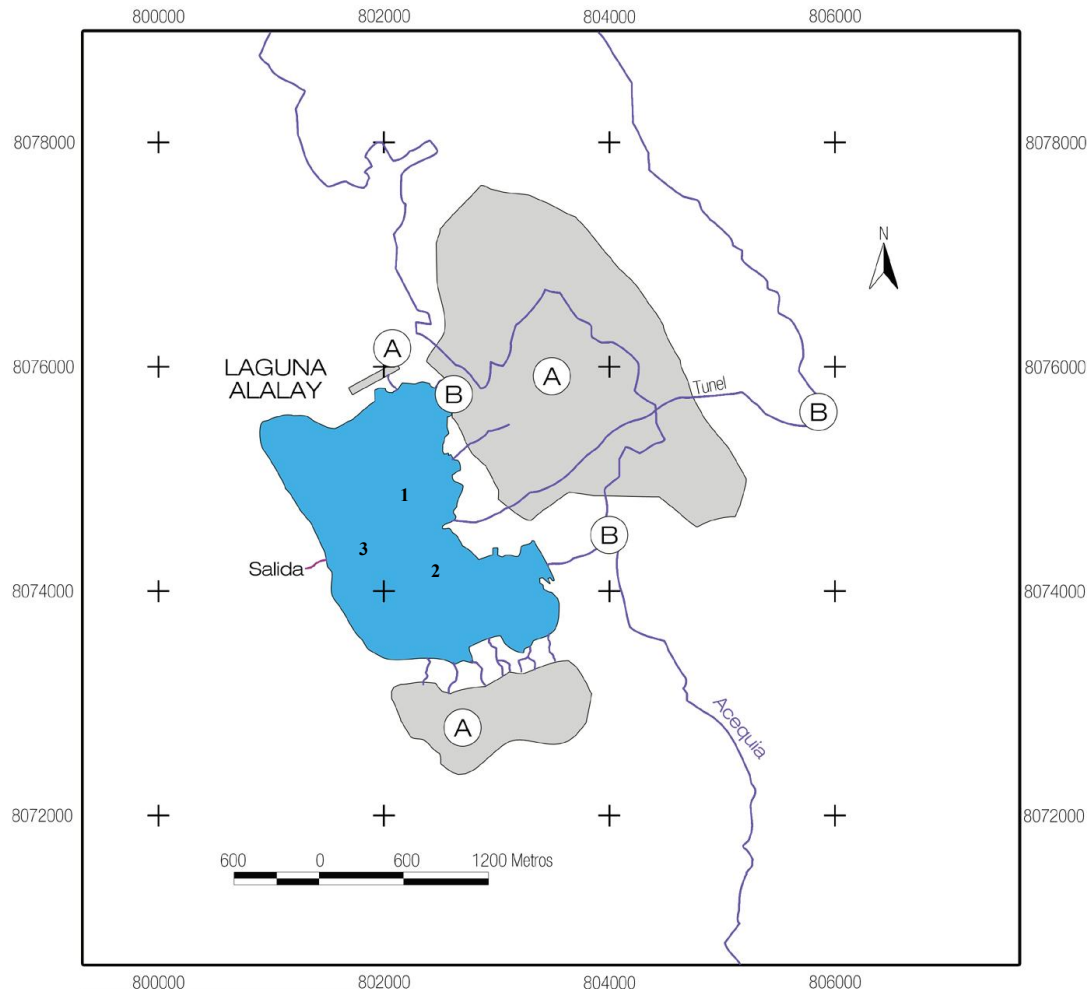
Study area characterization

Alalay lagoon is located southeast of the city of Cochabamba in Bolivia (17°23'43" S, 66°09'35" W; Fig. 1) at 2,570 m a.s.l. The city has low annual precipitation, approximately 450 mm and sparse vegetation cover within the lagoon catchment (Acosta, 2018). It is a shallow urban lagoon with a maximum recorded depth of 5 m and a quadrangular shape delimited by the "Circuito Bolivia" avenue and adjacent urban infrastructure, including the Country Club sport field. The lagoon covers an area of approximately 219.5 ha and has a perimeter of 8,121 m; its maximum water storage volume is approximately 3 000 000 m³ (Acosta, 2018; UMSS, 2016).

The lagoon was originally engineered to regulate discharges from the Rocha River during flood events. In recent years, local authorities have redirected management efforts toward ecosystem restoration, recreational use, and environmental conservation (Rejas and Fernández, 2022).

The Rocha River is the main inflow, providing approximately 60% of the lagoon's water volume, and the lagoon occasionally receives additional inputs from the Angostura reservoir. Substantial volumes also enter through runoff during the rainy season and via multiple stormwater channels connected to the lagoon, where contaminated water from diffuse sources in the southern sector may intermittently circulate (Ayala *et al.*, 2007; Acosta, 2018).

Alalay lagoon is characterized by mineralized waters, high electrical conductivity, and a slightly to strongly alkaline pH. Dissolved oxygen concentrations can range from anoxia to supersaturation, and nutrient levels (phosphorus and nitrogen) are high, resulting in trophic conditions that vary from eutrophic to hypertrophic (Acosta, 2018; UMSS, 2016).



- 88 REFERENCES
- 89 Inflow channels
- 90 Water body
- 91 Diffuse water input
- 92 Point-source water input
- 93 Internal sampling points

92 Figure 1. Map of Alalay lagoon illustrating its tributary network, as well as the spatial distribution of both diffuse
 93 and point-source water inputs. Spanish labels in the original map correspond to: salida (Exit), túnel (Tunnel),
 94 metros (meters), acequia (irrigation ditch) (Adapted from CREMPLA, 2012)

95 **Sampling and microbiological analysis**

96 Samples were collected monthly from April to December 2019, except in November. Sampling sites
 97 in Alalay lagoon were located along the southeastern margin and were differentiated as follows: Site 1
 98 (southeast), Site 2 (central lagoon), and Site 3 (northwest; outlet/discharge point), as indicated in
 99 Fig. 1. In addition, in January 2020, samples were collected from five adjacent inflow points where
 100 reclaimed/reuse water enters Alalay Lagoon (Fig. 1).

101 Samples were collected in sterile 1-L wide-mouth polypropylene bottles, preserved with EDTA and
 102 10% sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$), transported at 4 °C under a cold chain in containers with ice
 103 packs, and analyzed on the day of arrival at the laboratory.

All samples were analyzed in duplicate at the Microbiology Laboratory of the Center for Water and Environmental Sanitation (CASA), Universidad Mayor de San Simón (UMSS). Fecal contamination indicators were assessed, including total coliforms (TC), thermotolerant coliforms (TTC), also known as Fecal Coliforms (FC), *Escherichia coli* (*E. coli*), and heterotrophic bacteria (heterotrophic plate count/total bacterial count). In addition, non-fecal bacterial groups and opportunistic pathogens, including *Pseudomonas* spp., *Clostridium* spp., and *Streptococcus faecalis*, were evaluated and also considered alternative indicators of fecal contamination. Bacteriological parameters were analyzed using the membrane filtration method. Plates for TC, heterotrophic bacteria, *Pseudomonas* spp., and *Clostridium* spp. were incubated at 37 °C for 24-48 h, with *Clostridium* spp. incubated under anaerobic conditions. Plates for FC, *E. coli*, and *S. faecalis* were incubated at 44 °C for 24-48 h using selective and differential media. Procedures followed the CASA laboratory protocol and Standard Methods for the Examination of Water and Wastewater (APHA, 2023). Results were expressed as CFU/100 mL for all groups, except heterotrophic bacteria, which were reported as CFU/mL due to their high abundance, which could exceed the countable range on culture plates.

A targeted isolation protocol was also applied in duplicate to Alalay Lagoon samples for the detection of enteric pathogens such as *Salmonella* spp. and *Shigella* spp., and waterborne environmental pathogens such as *Vibrio* spp. These microorganisms were handled with particular care due to their sensitivity and limited survival in aquatic environments. For this purpose, a previously homogenized aliquot of each sample was incubated in liquid pre-enrichment media: tetrathionate broth at 41 °C for 4 h for *Salmonella* spp. and *Shigella* spp. (APHA, 2017), and alkaline peptone water supplemented with 1% NaCl at 37 °C for 24 h for *Vibrio* spp. (Sandoval, 1991). After pre-enrichment, samples were again inoculated in duplicate onto selective and differential media: SS agar for *Salmonella* and *Shigella* and TCBS agar for *Vibrio* spp., followed by incubation at 37 °C for 24 h. Colonies with typical morphology were then selected and subjected to confirmatory biochemical tests, with identification performed only for isolated enteric pathogens.

The data were compiled and systematized for four analyses. First, monitoring temporal and spatial patterns of bacterial communities, second, the TC/FC index (Goyal, Gerba, and Melnick, 1977) was used as an auxiliary index to infer whether bacterial contamination was predominantly fecal or environmental. Third, Spearman rank correlations were conducted using SPSS software version 27 to assess associations among fecal indicators and opportunistic bacterial groups. Fourth, correspondence analysis (CA) was used to explore associations among bacterial groups, sampling sites, and sampling months.

Results

Microbial counts were log₁₀-transformed for correlation analyses and for graphical comparison, since the original counts expressed as CFU showed high dispersion and differences of several orders of magnitude among sampling points. This transformation reduced the effect of extreme values, improved the approximation to normality, and facilitated comparison among the different microbial groups. The original units were retained for descriptive interpretation, heterotrophic bacteria were reported as CFU/mL according to the laboratory enumeration procedure and the remaining bacterial groups as CFU/100 mL. *Salmonella* spp., *Shigella* spp., and *Vibrio* spp. were analyzed qualitatively as presence/absence in the samples.

Heterotrophic bacteria exhibited seasonal variation, with concentrations ranging from 3 to 5 log₁₀ units (Fig. 2). The lowest concentration was recorded in August at three sampling points coinciding with the dry season, whereas concentrations increased in December, associated with the rainy season, which replenishes Alalay Lagoon mainly with water from the Rocha River, particularly the sampling point 1 which has more proximity to irrigation ditches.

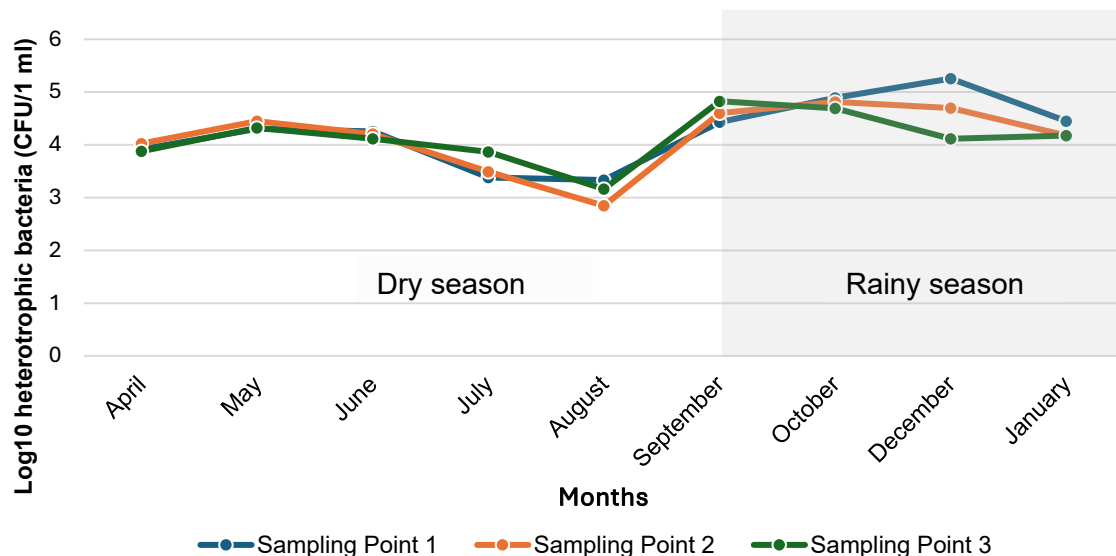


Figure 2. Monthly dynamics of log₁₀ heterotrophic bacteria at Sampling Points 1, 2, and 3 in Laguna Alalay during the dry and rainy seasons. Bacterial concentrations are expressed as log₁₀ CFU/1 mL.

Regarding indicator bacteria, total and fecal coliform concentrations tended to increase during both the dry season (May–June) and slightly in the rainy season (December–January), likely due to the inflow of wastewater into the lagoon, which would increase the fecal bacterial load. In contrast, in September and October, the load decreased relatively and remained more stable (Fig. 3). In addition, the mean fecal coliform count in the inflow channels to Alalay Lagoon was 1.23×10^6 colony-forming units (CFU) per 100 mL, a value that far exceeds the limit of 1000 CFU/100 mL established by Bolivia's Environmental Law No. 1333 and its current complementary regulations.

Using the coliform data, the TC/FC index was calculated and yielded a value of 7.63, which is classified as low according to Geldreich and Kenner (1969). This value indicates a predominant contribution of fecal-origin bacteria throughout the entire sampling period. Accordingly, a greater predominance of thermotolerant coliforms was observed, corroborating the persistent fecal contamination that has been documented for decades in Alalay Lagoon and highlighting the main pollution sources entering this ecosystem.

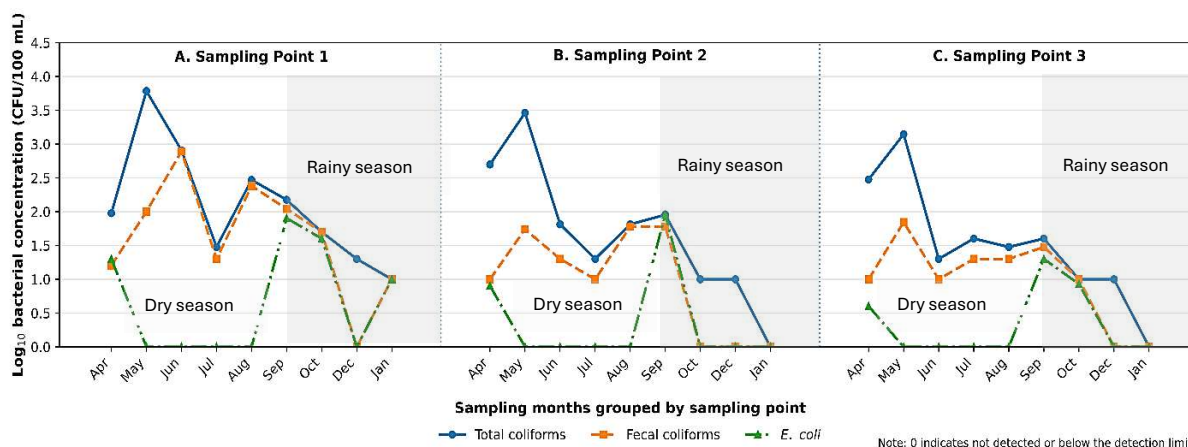


Figure 3. Monthly variation of log₁₀ bacterial concentrations of total coliforms, fecal coliforms, and *E. coli* at three sampling points in Laguna Alalay. Values of 0 indicate non-detection or levels below the detection limit.

Among the opportunistic pathogen groups and alternative indicators, *Pseudomonas* was the most abundant and persistent bacterial group across the three sampling points. Its concentration was detected in all months and reached the highest values, especially at sampling Point 1 in August with approximately 5.2 Log₁₀ CFU/100 mL, and at Sampling Points 2 and 3 in January, with around 5.0–5.1 Log₁₀ CFU/100 mL. This pattern contrasted with the classical indicators during those months (0, 1, and 2 log), highlighting the high persistence of *Pseudomonas* under different environmental conditions, possibly through competition and inhibition of other bacteria even under adverse conditions.

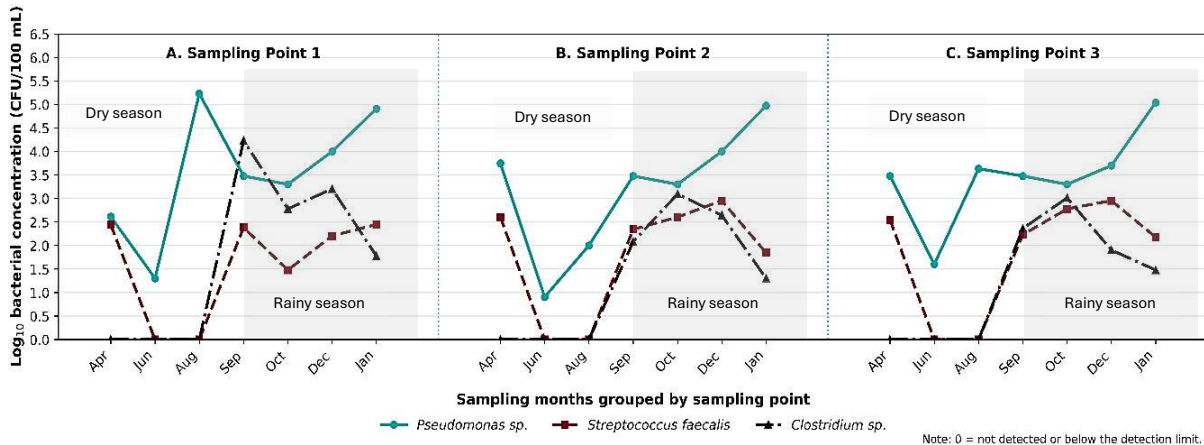


Figure 4. Monthly variation of log₁₀ bacterial concentrations of *Pseudomonas* spp, *Streptococcus faecalis*, and *Clostridium* spp at three sampling points in Laguna Alalay. Values of 0 indicate non-detection or levels below the detection limit.

In contrast, *Streptococcus faecalis* showed a more irregular pattern, with moderate concentrations generally between 1.5 and 3.0 Log₁₀ CFU/100 mL. It was not detected in June and August at the three sampling points, suggesting intermittent contamination. Nevertheless, its counts were, in some cases, higher than those of *E. coli*, particularly during the rainy season, supporting the interpretation that Alalay Lagoon receives fecally contaminated inputs from nearby sources, such as clandestine household sewer connections and inflow channels originating from the Rocha River (Fig. 4).

Regarding anaerobic bacteria, *Clostridium* spp. were detected mainly from September onward. Its highest value occurred at Sampling Point 1 in September, reaching approximately 4.2 Log₁₀ CFU/100 mL, while at Sampling Points 2 and 3 its values remained lower, mostly between 1.3 and 2.8 Log₁₀ CFU/100 mL. This pattern suggests uneven contamination across the lagoon, likely influenced by rainy-season inflow from the Rocha River carrying fecally contaminated material into Alalay Lagoon.

Within the category of enteric pathogens of fecal origin, no characteristic isolates of *Salmonella* spp. or *Shigella* spp. were observed in the analyzed samples. Likewise, although microbiological protocols for the isolation of *Vibrio* spp. were applied, its presence could not be confirmed in the studied samples. The sample destined for this analysis were collected in December 2019, a period during which the urban reservoir annually accumulates wastewater from the Rocha River.

Data distribution was assessed using the Shapiro-Wilk test on log₁₀-transformed data to evaluate normality. Spearman's rank correlation revealed clear positive associations among the coliform indicators. Total coliforms were strongly positively correlated with fecal coliforms ($\rho \approx 0.74$, $p < 0.001$) while fecal coliforms were positively associated with *E. coli* ($\rho = 0.38$, $p < 0.10$) even though it did not reach the standard significance threshold but is reported as marginal trend. These associations confirm that the indicator groups respond coherently to fecal contamination gradients in the lagoon.

Correlations between classical fecal indicators and opportunistic bacterial groups were weaker and, in most cases, non-significant (Fig. 5). *Streptococcus faecalis* showed a significant negative association with fecal coliforms ($\rho = -0.53$, $p < 0.05$) and a positive association with *Clostridium* spp. ($\rho = 0.48$, $p < 0.05$) suggesting some co-occurrence among persistent fecal indicators. Total coliforms showed a marginal negative trend with *Clostridium* spp. ($\rho = -0.43$, $p < 0.10$), and fecal coliforms showed a marginal negative trend with *Pseudomonas* spp. ($\rho = -0.40$, $p < 0.10$). Moreover, *Pseudomonas* spp., showed weak or negative associations with coliforms and even with opportunistic pathogens, highlighting its distinct ecological behavior in this polluted ecosystem. All these correlation results are supported by Kendall's tau-b test that evaluated the strength and direction of the associations between the bacterial groups involved. Further details are provided in Supplementary material SM1

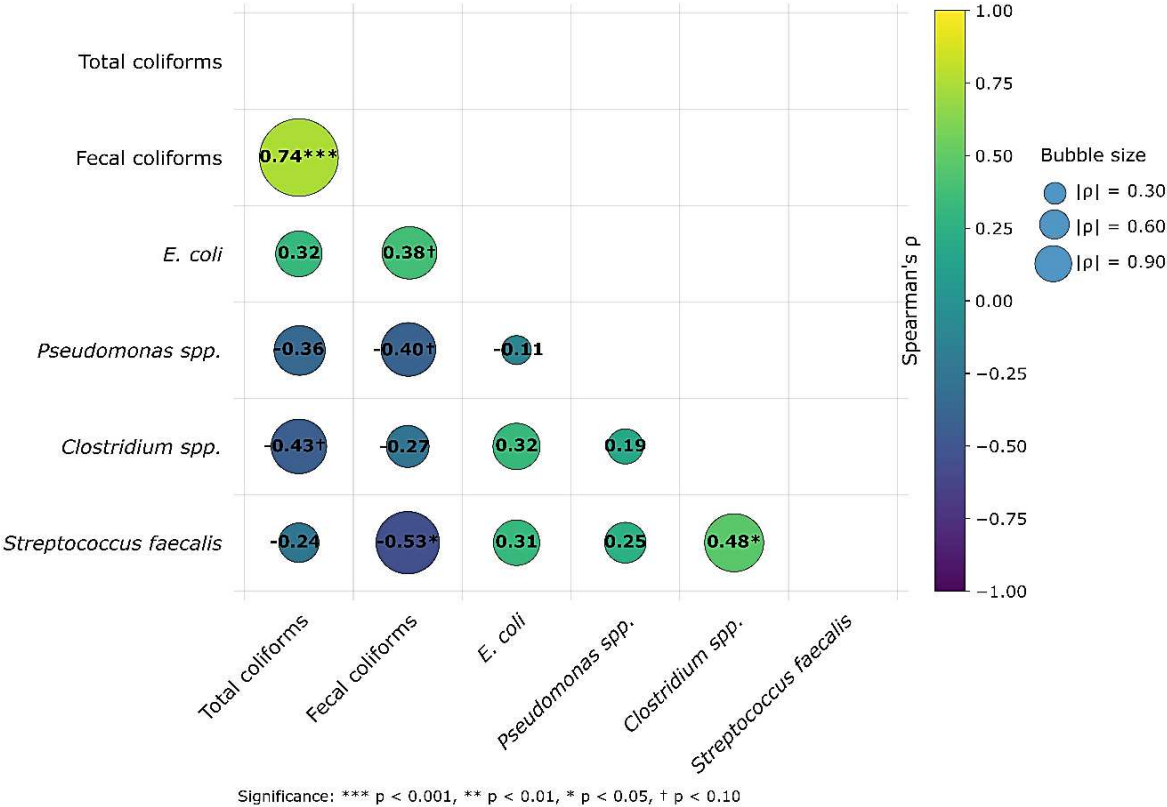


Figure 5. Spearman correlation bubble plot among log10-transformed microbial indicators. Values inside bubbles represent Spearman's rho coefficients. Bubble size is proportional to the absolute value of ρ . The color gradient indicates the direction and magnitude of the correlation. Asterisks denote statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. † $p < 0.10$ (marginal trends).

Overall, these patterns suggest that some opportunistic pathogens may persist independently from classical coliforms, and also that coliform indicators are reliable predictors of fecal contamination, whereas opportunistic bacteria like *Streptococcus faecalis*, *Clostridium* spp., and *Pseudomonas* spp. display selective correlation patterns, reflecting variable responses to environmental factors. The inclusion of marginal trends allows these observations to be exploratory for modeling purposes, providing insight into potential interactions among microbial groups.

We also performed a correspondence analysis (CA) to explore the association between bacterial groups, sampling sites and sampling months. This analysis identified three bacterial assemblages, structured mainly according to two criteria: (i) the sampling site (P1, P2, P3) and (ii) the month in which fieldwork was conducted (Fig. 6). The analysis separated the samples into three main

assemblages: a) classical fecal indicators, b) *Pseudomonas* spp., and c) *Clostridium* spp. with heterotrophic bacteria.

The first bacterial assemblage comprising fecal indicator bacteria, was associated with sampling site P1 in September, a month generally characterized by alkaline waters, high electrical conductivity, and elevated orthophosphate levels (Pérez-Fernández *et al.*, 2020; Acosta, 2018). These conditions favor the development of fecal streptococci. The low rainfall during this period likely increases salt concentrations, which may explain the elevated bacterial counts.

In contrast, the distribution of coliforms and *E. coli* at P1 in September is more complex, as their highest concentrations occur during both the dry (May–June) and rainy (December–January) seasons. Although September shows lower counts, the increase in total nitrogen reported for this month (Acosta, 2018; Quillaguamán *et al.*, 2021) may stimulate their growth, given their dependence on nitrogen sources associated with ammonia oxidation processes in fecal matter (Rodier *et al.*, 2009).

The second group, *Pseudomonas*, showed a seasonal association with the rainy season (January), without a clear spatial preference within the lagoon. Its proliferation has been linked to alkaline pH (> 8) and high salinity, as reported in the cited studies (Pérez-Fernández *et al.*, 2020; Nuñez *et al.*, 2020).

Since the physicochemical information used for interpretation was obtained from previous years, it was used only as contextual support and not as contemporaneous environmental data from the present sampling period. Nevertheless, available reports suggest that these parameters have not varied substantially from year to year, particularly after the 2016 algal bloom period (Morales *et al.*, 2017). Moreover, the results reported by different authors (Acosta, 2018; Pérez-Fernández *et al.*, 2020; Quillaguamán *et al.*, 2021) are generally consistent and broadly comparable. Further details are provided in Supplementary Material SM2.

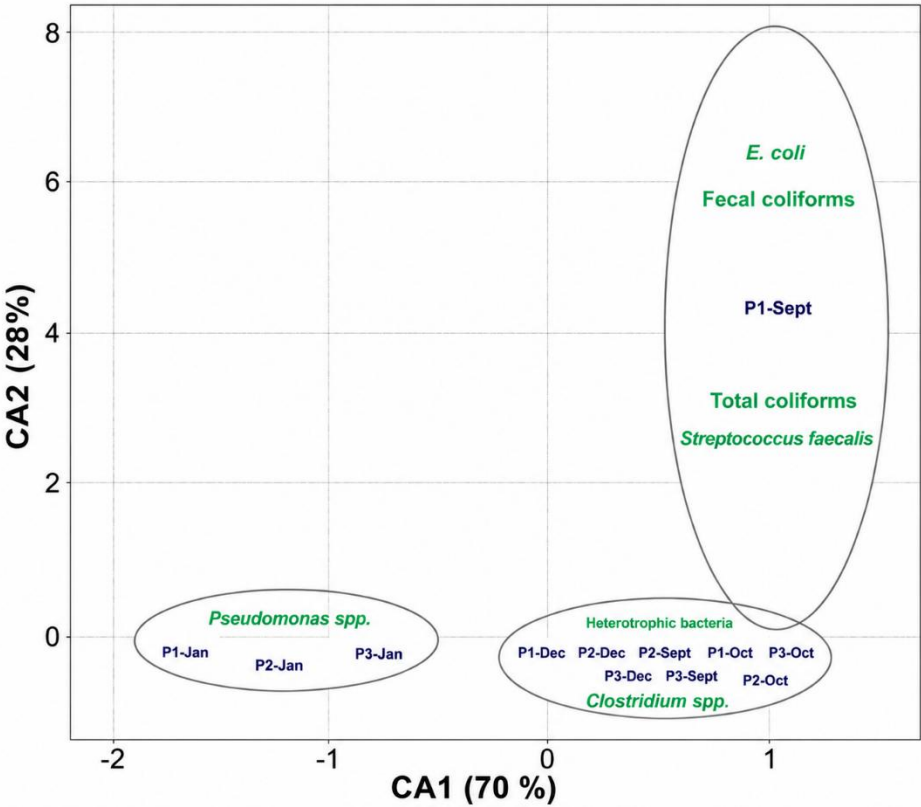


Figure 6. Correspondence analysis of the bacterial groups. The identified bacteria exhibit a tendency to cluster according to sampling site and month, revealing three distinct groups (circles): the first composed of fecal bacteria, the second of *Pseudomonas* spp., and the third of *Clostridium* spp. and heterotrophic bacteria.

Discussion

The results of this study indicate that fecal coliform (FC) and total coliform (TC) concentrations in the inflow channels to Alalay Lagoon (during December and January) show a high mean count of 1.23×10^6 CFU/100 mL, exceeding the limits established by Bolivian environmental legislation (RMCH, 1992), which allows up to 1,000 CFU/100 mL for wastewater discharges into water bodies. These findings support the interpretation that wastewater inputs continue to exert a strong influence on the sanitary condition of Alalay Lagoon.

In contrast, FC and TC counts within the lagoon were lower, likely due to dilution effects. This suggests that the microbial load decreases markedly with increasing distance from the entry point to the sampling site, a pattern also observed in a study of the Rio Grande along the Mexico–United States border, where high microbial loads associated with wastewater discharges progressively declined downstream (Flores-Márquez, 2011). Therefore, the spatial distribution of microbial contamination in Alalay Lagoon appears to be influenced by both wastewater inputs and internal hydrological dynamics.

The TC/FC ratio is used as an auxiliary index to infer whether the detected bacteria originate primarily from fecal or environmental sources (e.g., insects, plants, or soils) (Goyal *et al.*, 1997; EPA, 1980; Rabeh and Azab, 2006). In a previous study, Iriarte and Romero (2021) calculated this index for Alalay Lagoon and reported an average value of 7.68, indicating a predominance of fecal bacteria throughout the year. The present study yielded a very similar value (7.63), together with continuous detection of fecal bacterial groups across the sampling period. This reinforces the evidence of persistent fecal contamination in Alalay lagoon, documented for years (Pérez-Fernández *et al.*, 2020), and will help to identify the main pollution sources entering this ecosystem.

FC and TC are commonly used as bacterial indicators because they provide indirect evidence of environmental conditions that may favor pathogen occurrence in aquatic systems. To evaluate this relationship, Mirón-Canelo and Alonso-Sardón (2008) described the attributable risk approach, hereafter RA, which was subsequently adapted to microbiological water-quality assessment by Barrera-Escorcia *et al.* (2013). In this context, RA estimates the excess probability of detecting a pathogen associated with the presence of a given microbial indicator. Using this approach in Alalay lagoon, Iriarte, Orellana-Salazar, and Romero (2021) reported high attributable risk values for TC, suggesting that this indicator was more strongly associated with *Pseudomonas* spp., *Clostridium* spp., and *Escherichia coli*. By contrast, FC was more closely associated with *E. coli*, while *Clostridium* spp. and *Pseudomonas* spp. occurred at lower percentages. In the present study, this background supports the need to interpret classical fecal indicators as useful sanitary signals, but not as definitive predictors of opportunistic bacterial groups. Future studies could complement correlation analyses with attributable risk, sensitivity-based indices and Quantitative Microbial Risk Assessment (QMRA) to better evaluate the predictive value of fecal indicators in this type of urban aquatic ecosystem.

Despite the criticisms surrounding correlation analyses between indicator bacteria and pathogens in aquatic environments—due to uncertainties in relating concentrations of both groups and predicting occurrence at the population level (Payment and Locas, 2011; Dufour *et al.*, 2003)—authors such as Harwood *et al.* (2017) and Von Sperling, Mihelcic, and Verbyla (2019) support their use because they can assist in interpreting ecological and sanitary events occurring in the ecosystem. An example of this is the massive fish kill accompanied by a cyanobacterial algal bloom reported in Alalay Lagoon in 2016 (Carrillo, 2016; Morales *et al.*, 2017).

Regarding statistical correlation, microbial indicators in the sampled water body showed a strong positive correlation between total coliforms and fecal coliforms ($p = 0.744$, $p < 0.01$), which is

consistent with the expectation that general coliforms tend to co-occur with fecal-specific bacteria. This outcome is supported by findings from Edberg *et al.* (2000), who observed similar strong associations in freshwater systems impacted by human waste, suggesting that total coliform levels can serve as an initial indicator of fecal contamination related to hygienic deficiencies despite their inherent limitations as non-specific microbial indicators.

Moreover, Spearman correlation analysis between fecal indicators and opportunistic pathogens, showed negative coefficients and non-significant values ($0.05 < p < 0.10$), which should not be necessarily interpreted as absence of health risk. Instead, it indicates that opportunistic bacteria may persist independently from classical fecal indicators where a similar reuse-water study faced the same limitation, when indicator microorganisms (FC, TC, coliphages) did not show significant correlations with environmental pathogens such as *Cryptosporidium*, *Clostridium perfringens*, and enteroviruses (Harwood *et al.*, 2005). Unexpectedly, *Streptococcus faecalis* and fecal coliforms showed a negative correlation, with *S. faecalis* sometimes increasing while fecal coliforms declined. This likely reflects differences in environmental tolerance, as *S. faecalis* is more resilient to stressors such as temperature, nutrient limitation, and chemical pollutants, whereas fecal coliforms like *E. coli* are more sensitive (Ashbolt 2004; Payment and Locas 2011). In addition, the physicochemical conditions of Alalay Lagoon—including variations in nutrient levels, organic matter, and hydrological inputs from urban runoff and the Rocha River—may favor the persistence of *S. faecalis* while temporarily reducing fecal coliform abundance (Quillaguamán *et al.*, 2021; Acosta, 2008; Pérez-Fernández *et al.*, 2020). These factors together help explain the observed inverse relationship and underscore the need to consider both microbial and physicochemical dynamics when assessing water quality.

The high concentrations of fecal indicator bacteria (FIB) found in Alalay Lagoon reflect chronic fecal contamination, consistent with previous studies (Iriarte, Orellana-Salazar, and Romero, 2021). The lack of correlation between FIB and pathogens agrees with findings from eutrophic environments, where factors such as pH and nutrient availability can influence microbial survival and dynamics (Harwood *et al.*, 2005). Nevertheless, the persistence of *S. faecalis* and *Clostridium* spp. further supports the need to include more alternative indicators in monitoring program such as *Methanobrevibacter smithii*, proposed as a non-conventional fecal indicator in wastewater (Romanazzi *et al.*, 2016).

The absence of confirmed isolates of waterborne environmental bacteria with pathogenic potential, such as *Vibrio* spp and enteric pathogenic bacteria such as *Salmonella* spp. and *Shigella* spp. does not eliminate the sanitary relevance of the findings. These waterborne pathogens are not classical fecal indicators, and their detection depends on several factors, including sample timing, bacterial viability, environmental stress, and methodological sensitivity. Accordingly, continued testing of bacterial pathogen groups in parallel with classical fecal indicators is recommended, especially in polluted urban water bodies where direct or indirect human exposure may occur.

Culture-based assays remain useful in resource-limited monitoring contexts, but they should be complemented with molecular tools when the goal is detecting pathogens including dormant forms such as viable but non-culturable (VBNC) cells, identify source-specific contamination, such as microbial source tracking (MST) and assess the presence of antibiotic resistance genes or virulence markers. In this sense, previous metagenomic studies in Alalay Lagoon have reported more sensitive indicators such as *Bacteriodes*, *Bifidobacterium*, *Enterococcus* and *Blautia*, genetic markers associated with virulence and antimicrobial resistance (Quillaguamán *et al.*, 2021; Daga-Quisbert *et al.*, 2024). These findings do not replace culture-based monitoring, but they highlight the value of integrating complementary molecular tools in future surveillance strategies.

Conclusions

The results of this study demonstrate that Alalay Lagoon exhibits persistent fecal contamination, with the presence of fecal indicators, and opportunistic bacterial groups, representing an ecological and

public health concern for Cochabamba city. Although enteric pathogenic bacteria such as *Salmonella* spp and *Shigella* spp were not confirmed in the analyzed samples, their detection should be tested in parallel with classical fecal indicators to improve sanitary interpretation and strengthen microbiological monitoring programs.

Microbial loads, measured by heterotrophic bacteria, showed seasonal fluctuations, increasing during the rainy season due to wastewater and organic matter inputs from the Rocha River, and declining in the dry season, likely influenced by reduced nutrients and microbial interactions. These results highlight that sampling time affects observed microbial concentrations, underlining the need to account for seasonal variability in water quality monitoring.

Total coliform (TC) and fecal coliform (FC) counts in inflow channels of Alalay Lagoon consistently exceeded the limit established by Bolivia's Environmental Law No. 1333, confirming wastewater inputs as the primary contamination source. Correlation analysis revealed strong positive associations between TC and FC as well as a moderate association between *S. faecalis* with *Clostridium* spp. In contrast, *S. faecalis* displayed a significant negative correlation with FC, suggesting that this indicator did not follow the same occurrence pattern across the analyzed samples. The positive association between *S. faecalis* and *Clostridium* spp. may reflect shared fecal origin, persistence in sediments or organic matter, or an older fecal contamination signal.

These results highlight the usefulness of fecal indicators for identifying fecal contamination. However, low or undetected levels of classical indicators do not necessarily imply the absence of microbiological concern, as opportunistic and anaerobic bacterial groups may persist independently. The inclusion of marginal trends, such as TC with *Clostridium* spp. and FC with *Pseudomonas* spp., further suggests that microbial interactions may be influenced by seasonal and environmental conditions. Therefore, incorporating molecular or viability-based methods could strengthen monitoring and microbial source-tracking programs in impacted urban water bodies such as Alalay Lagoon.

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AI-assisted language editing

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with English language editing, including grammar correction, wording, readability, and clarity, as English is not the authors' first language. The authors reviewed and verified all AI-assisted edits and remain fully responsible for the accuracy, originality, scientific content, interpretations, and conclusions presented in the manuscript.

Author contribution

Javier Orellana: responsible for the original concept and participate in the environmental sampling and bacterial assessment in laboratory. Mercedes Iriarte: assisted with sample processing and reviewing the sampling protocol. All authors wrote and approved the final version of the manuscript.

Data availability

Supplementary data to this article can be found online.

Declaration

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Competing interests

The authors declare no competing interests.

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