

Article

Seasonal Dynamics of Antibiotic Resistance Genes in Irrigation Canals and a Protected Wetland Receiving Treated Wastewater (Central Chile)

Oscar López-Sandoval ¹, Daniela López-Leyton ^{2,3,*}  and Claudia Vélez ³

¹ Escuela de Medicina Veterinaria, Facultad de Recursos Naturales y Medicina Veterinaria, Universidad Santo Tomás, Santiago 8370003, Chile; olopez@santotomas.cl

² Centro Bahía Lomas, Facultad de Ciencias, Universidad Santo Tomás, Santiago 8370003, Chile

³ Facultad de Ciencias, Universidad Santo Tomás, Santiago 8370003, Chile; cvelez@santotomas.cl

* Correspondence: dlopezleyton@santotomas.cl

Abstract

The presence of antibiotic resistance genes (ARGs) in wastewater and their dissemination into aquatic ecosystems represents a significant threat to ecological integrity and public health. This study focuses on the Batico Lagoon Natural Sanctuary (receiving effluents from a WWTP) and on irrigation canals used for agriculture (without WWTP influence). The aim was to analyze seasonal ARG dynamics and relate their occurrence to water physicochemical characteristics. Five sampling campaigns were conducted, during which physicochemical analyses and molecular detection were performed to identify the *bla*TEM, *sul1*, *tetW*, and *ermB* genes. Dissolved oxygen ranged from 2.8 to 5.0 mg/L, COD in untreated wastewater reached 287.84 mg/L, and the lagoon exhibited hypereutrophic conditions with total nitrogen averaging 4.56 mg/L (BL1) and 4.39 mg/L (BL2). *bla*TEM was the most prevalent gene, detected in 71% of samples, followed by *sul1* (63%) and *tetW* (50%). *ermB* was the least prevalent, present in only 25% of samples. Significant seasonal variations were observed: 33% of samples were ARG-positive in winter, 38% in spring, 50% in fall, and 88% in summer, with all four genes detected during the latter. Seasonal factors, especially summer temperatures and human activity, may increase ARG dissemination, highlighting the need for better WWTP effluent management.

Keywords: antibiotic resistance genes; nature sanctuary; batico lagoon; irrigation canals; wastewater treatment plant; ecosystem conservation; wetland



Academic Editors: Limin Ma,
Chong Chen and Jing Hu

Received: 17 April 2026

Revised: 10 June 2026

Accepted: 16 June 2026

Published: 14 July 2026

Copyright: © 2026 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 \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Antibiotics are essential pharmaceuticals used worldwide in human medicine, veterinary applications, and agriculture. However, their excessive and often unregulated use has led to their widespread presence in natural ecosystems, raising serious environmental and public health concerns. A significant proportion of administered antibiotics—ranging from 30% to 90%—is excreted unchanged through urine and feces, subsequently reaching water bodies through wastewater discharges, agricultural runoff, and improper disposal [1]. Consequently, antibiotic residues have been detected in various aquatic environments, including lakes, rivers, oceans, drinking water supplies, and wastewater treatment plants (WWTPs) [2]. These contaminants not only affect water quality but also alter microbial community structures, favoring the proliferation of antibiotic-resistant bacteria and antibiotic resistance genes (ARGs) [3]. In 2019, bacterial antimicrobial resistance (AMR) was

associated with approximately 4.95 million deaths globally, with 1.27 million deaths directly attributable to AMR—surpassing the burden of HIV/AIDS and malaria [4].

ARGs in aquatic ecosystems are mainly influenced by their emission sources and by meteorological and hydrological factors. Furthermore, the environmental impact of these pollutants is closely related to the degree of anthropization; for example, fecal pollution is a determining factor in the presence and concentration of ARGs in environments affected by human activities [5,6].

The global extent of antibiotic contamination varies significantly depending on local consumption patterns, wastewater management practices, and regulatory frameworks. Studies have reported high antibiotic concentrations in manure and slurry (e.g., 20,000 µg/kg of sulfonamides in Switzerland), posing contamination risks when used as fertilizers [7]. In China, the world's largest producer and consumer of antibiotics, environmental contamination is particularly concerning due to direct wastewater discharges and the use of animal waste as fertilizer [8]. Additionally, antibiotic concentrations in water bodies vary considerably depending on the type of antibiotic and study focus. For example, Wang et al. [9] reported sulfonamide concentrations of up to 16,216 ng/L in Taizhou Bay, China, while Boger et al. [10] reported 4.11 µg/L of beta-lactams in the Barigui River, Brazil. This variation underscores the need for region-specific studies to understand local contamination dynamics and assess the potential risks posed by antibiotic residues. Therefore, the presence of antibiotics such as oxytetracycline, sulfonamides, erythromycin, and clarithromycin in natural environments could serve as an indicator of contamination and as a reference for assessing water quality.

In Latin America, agricultural systems have emerged as relevant reservoirs of ARGs, with studies reporting their presence in soils, irrigation water, livestock waste, and river systems under intensive farming practices. For example, research in Brazil identified genes such as *sul1*, *sul2*, *blaTEM*, and *int11* in agricultural matrices [11], while investigations in Argentina reported multidrug-resistant bacteria in food-production animals [12]. These findings highlight regional risks for water quality and food safety and reinforce the need to evaluate ARG dynamics in agroecosystems.

In Chile, the reuse of treated wastewater and biosolids for agricultural purposes is becoming increasingly common, particularly in areas experiencing water shortages. According to a report by the Superintendency of Sanitary Services [13], the total volume of treated wastewater reached 1.252 billion cubic meters. Of this, 74% was discharged into continental surface water bodies, 21% into the sea and just 5% was reused directly, primarily for agricultural purposes (60%). However, this situation is critical given that wastewater treatment plants are not designed to remove emerging contaminants such as antibiotic residues and ARGs. Moreover, national regulations—including DS No. 90/2000 [14] (discharges into water bodies), DS No. 4/2009 [15] (sludge management), Law 21.075 on greywater reuse, and technical standards [16], NCh 1333 [17] (irrigation water) and NCh 409/1 [18] (drinking water)—do not establish monitoring requirements or limits for these contaminants [19]. Furthermore, there are no specific environmental regulations for livestock waste, a source of antibiotics, hormones, heavy metals, pathogens and ARGs that is frequently applied to agricultural soils or discharged into water bodies [20].

These regulatory omissions create scenarios of high environmental exposure, whereby treated water, biosolids and livestock waste are applied to aquatic and agricultural ecosystems without specific regulation, contributing to the selection, persistence and horizontal transfer of ARGs [19,20].

Although ARGs are not yet included in Chilean environmental regulations, strategic initiatives are emerging. The National Action Plan on AMR (2021–2025) [21] incorporates the environmental dimension within a One Health framework and highlights the need for

scientific evidence on ARGs in environmental matrices. Internationally, the World Health Organization (WHO), the Food and Agriculture Organization of the United Nations (FAO), and the United Nations Environment Programme (UNEP) have emphasized the environmental drivers of AMR and called for systematic surveillance in aquatic systems. In this context, our study provides critical data that can support future regulatory development and inform environmental and water quality policies.

One of the primary concerns associated with antibiotic contamination is its role in promoting the spread of ARGs in aquatic environments. Even at low concentrations, antibiotics exert selective pressure on bacterial communities, favoring the persistence of resistant strains [22]. ARGs can be transferred horizontally among bacteria, facilitating the dissemination of resistance mechanisms across different ecosystems, including drinking water supplies, agricultural fields, and the human microbiome [23]. Previous studies have demonstrated the presence of ARGs in vegetables irrigated with contaminated water and in soils fertilized with manure containing antibiotic residues, underscoring the potential risks to food safety and human health [24]. Moreover, studies have detected antibiotics such as azithromycin, sulfamethoxazole, and trimethoprim at concentrations of 152, 590, and 1172 ng/L before treatment and at 96, 390, and 290 ng/L in WWTP effluents, highlighting the inefficiency of conventional treatment processes in fully eliminating these contaminants [25].

The occurrence and distribution of ARGs in aquatic environments are influenced not only by emission sources but also by key physicochemical parameters. Temperature, dissolved oxygen, pH, and electrical conductivity have been shown to modulate horizontal gene transfer and bacterial resistance dynamics in water bodies [26–29]. Additionally, dissolved organic matter and nitrogen compounds ($\text{NH}_4^+\text{-N}$) have been positively associated with ARG abundance in aquatic systems [26,30]. These parameters are routinely monitored in wastewater treatment plants and aquatic ecosystems, making them relevant indicators for assessing ARG dissemination risk.

In this context, this study focuses on the Maipo River Basin, a highly vulnerable system that supplies water to the Metropolitan Region—home to 40% of Chile’s population—while facing severe water stress and multiple sources of pollution, including WWTP discharges and agricultural runoff [31]. Specifically, the aims were to: (i) characterize the physicochemical properties of water in river systems connected to the Batuco Lagoon Natural Sanctuary, which receives effluents from the “Cadellada” WWTP, and compare them with irrigation canals in urban (San Bernardo) and rural (Curacaví) settings not directly influenced by the WWTP; and (ii) analyze the seasonal variations in the prevalence of ARGs associated with the most widely used antibiotic families in Latin America— β -lactams (*blaTEM*), macrolides (*ermB*), sulfonamides (*sul1*), and tetracyclines (*tetW*). This study was designed as a surveillance program to identify the presence or absence of ARGs and the spatial and temporal patterns of their distribution, thus laying the groundwork for subsequent quantitative studies. It provides key information on antibiotic contamination and the spread of ARGs in the Maipo River basin, contributing to a better understanding of their environmental impact and serving as a basis for future water quality management strategies.

2. Materials and Methods

2.1. Study Area

This study was conducted in three different zones within the Metropolitan Region of Santiago, Chile ($33^\circ 26' 16''$ S, $70^\circ 39' 1''$ W), selected to evaluate water quality parameters with distinct environmental and anthropogenic conditions: raw wastewater, treated effluents from WWTP, natural wetlands, and irrigation canals. Six sampling points were distributed among:

Zone 1: Cadellada WWTP and Batuco Lagoon Natural Sanctuary, Lampa.

Zone 2: Las Mercedes, Rural Irrigation Canal, Curacaví.

Zone 3: Calera Canal, Urban Irrigation Canal, Catemito, San Bernardo.

The geographic distribution and characteristics of the sampling points are detailed in Figure 1 and Table 1.

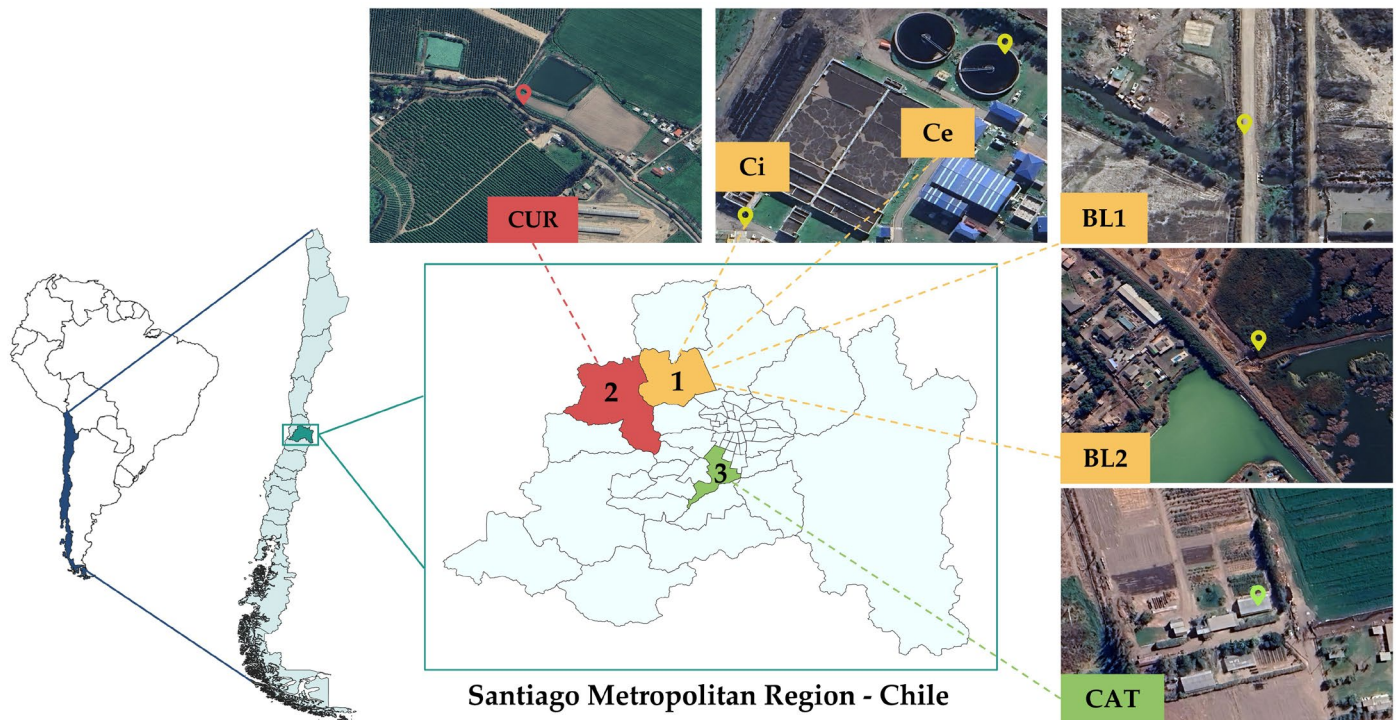


Figure 1. Sampling points map. Zone 1: raw wastewater with pretreatment WWTP (Ci); WWTP outlet decanter (Ce); Batuco Lagoon inlet tributary (BL1); Batuco Lagoon outlet (BL2). Zone 2: rural irrigation canal (CUR). Zone 3: urban irrigation canal (CAT).

Table 1. Main characteristics of zones and sampling points.

Zone	Sampling Points		Georeference	System Type		Characteristics of Sampling Points
	Name	Acronym		Water Type	Characteristics	
1	Cadellada influent	Ci	33°12'41"S 70°47'14" W	WWTP *	Raw wastewater	Pretreatment (desander and degreaser)
	Cadellada effluent	Ce	33°12'37" S 70°47'08" W	WWTP *	Secondary effluent	Post-secondary sedimentation, pre-chlorination
	Batuco Lagoon 1	BL1	33°11'55" S 70°48'58" W	Natural wetland	Water column	Main tributary influenced by treated wastewater
	Batuco Lagoon 2	BL2	33°12'58" S 70°50'04" W	Natural wetland	Water column	Outflow point of Batuco Lagoon
2	Curacaví	CUR	33°25'18" S 71°05'00" W	Irrigation canal	Rural irrigation	Agricultural usage, Urban–rural influence
3	Catemito	CAT	33°36'34" S 70°44'13" W	Irrigation canal	Rural irrigation	Agricultural usage, Urban influence

Note: * WWTP: wastewater treatment plant.

The sampling sites were selected based on their relevance to wastewater treatment efficiency, wetland water quality, and irrigation practices in both rural and urban contexts.

The main characteristics of each site are summarized in Table 1, and additional details are provided in the Supplementary Material (Text S1) [32,33].

2.2. Sampling Strategy

Five sampling campaigns were conducted between August 2022 and April 2023, covering all seasons of the Southern Hemisphere: August (Winter, W), September (Spring, S), December (Summer, Sm), January, and April (Fall, F). The results are presented as mean values for the fall–winter (F/W) and spring–summer (S/Sm) seasons, and the seasonal physicochemical characterization was carried out according to the methodology described by López et al. [34].

At each site, duplicate water column samples (500 mL) were collected, stored at 4 °C in the dark to prevent microbial activity and photochemical reactions, and subsequently analyzed in the laboratory [35]. In situ measurements were also conducted at each sampling point to assess the following parameters: (a) temperature, (b) pH, (c) oxidation-reduction potential (ORP), (d) dissolved oxygen (DO), (e) electrical conductivity (EC), and (f) turbidity. These measurements were obtained using Hanna HI98191 and OAKTON PCD650 multiparameter instruments and a Hanna HI98193 oximeter for DO.

Additionally, laboratory analyses were performed in duplicate to determine total and soluble chemical oxygen demand (COD), nitrite (NO_2^- -N), nitrate (NO_3^- -N), ammonium (NH_4^+), total nitrogen (TN), phosphate (PO_4^{3-} -P), and total phosphorus (TP) at the Centro Bahía Lomas laboratory in Concepción.

2.3. Analytical Methods

2.3.1. Physicochemical Parameters

Water samples collected at each sampling point were filtered through a 0.45 µm pore-sized Whatman membrane filter. The physicochemical parameters were analyzed following the protocols outlined in the Standard Methods [35]. Chemical Oxygen Demand (COD) was determined using the colorimetric method (5210-B). Nitrate (NO_3^- -N) was quantified via UV spectrophotometry, whereas nitrite (NO_2^- -N) and ammonium (NH_4^+ -N) concentrations were assessed using the colorimetric method. Total nitrogen (TN) was analyzed using the Spectroquant-Nova 60 system with Merck reagent kits. For in situ measurements, an OAKTON PC650-480485 portable multiparameter probe was used, while dissolved oxygen (DO) was measured using a HANNA OXI 330i/set HI 9146-04 portable oximeter.

2.3.2. DNA Extraction

Biomass samples were centrifuged at $5000 \times g$ for 5 min. DNA was extracted in duplicate from 50 mL of water samples using the FastDNA[®] SPIN Kit for Soil and the FastPrep[®] Instrument (MP Biomedicals, Santa Ana, CA, USA), following the manufacturer's protocol (Base Catalog Number 6560200). The quality of the extracted DNA was assessed by agarose gel electrophoresis and ultraviolet absorbance using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). DNA quantification was performed with the QuantiFluor dsDNA kit (Promega, Madison, WI, USA) in a 96-well microplate reader (SpectraMax M5, Molecular Devices, San Jose, CA, USA). Finally, DNA was diluted to $50 \text{ ng } \mu\text{L}^{-1}$ using sterile water and stored at -20°C until further analysis.

2.3.3. PCR Amplification

Conventional endpoint PCR was employed for the qualitative screening (presence/absence) of antibiotic resistance genes (ARGs). Because the objective was to identify the presence of these genes rather than to quantify their abundance, quantitative metrics such as cycle threshold (Ct) values, limits of detection (LoD), limits of quantification (LoQ),

and standard curves were not determined. PCR amplification of ARGs was performed using specific primers (*bla*TEM, *erm*B, *sul*1, *tet*W) targeting β -lactam, macrolide, sulfonamide, and tetracycline resistance genes [36–39]. To ensure comparability among samples, a consistent amount of template DNA (50 ng μ L^{−1}) was used in all reactions. Each reaction was carried out in a final volume of 50 μ L, consisting of a reagent mix (buffer, dNTPs, MgCl₂, primers, and Taq polymerase) and 5 μ L of template DNA. To ensure the accuracy, specificity, and absence of contamination, each PCR run included both negative controls (Milli-Q water) and positive controls (DNA from an isolate known to carry the target resistance genes) [40]. Detailed thermal cycling conditions, primer sequences, and specific reagent concentrations are provided in the Supplementary Material (Text S2, Tables S1–S3).

2.3.4. Visualization of Gel Electrophoresis Results

PCR products were analyzed by agarose gel electrophoresis using 3% agarose gel (Conda, Madrid, Spain, ref. 8066) prepared in 1X TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA), supplemented with 5 μ L of RedSafe™ (Intron Biotechnology, Seongnam-si, Republic of Korea, ref. 21141) per 100 mL of gel. An amount of 10 μ L of PCR product was loaded into each well, mixed with 6X loading buffer (Thermo Fisher, Wilmington, DE, USA, ref. R0611). Two GeneRuler 100 bp DNA Ladder molecular weight markers (0.5 μ g/ μ L, Thermo Fisher, Wilmington, DE, USA, ref. 15628050) were included in each gel to flank the PCR products. The amplified DNA fragments were visualized under UV light using a transilluminator.

2.4. Statistical Analyses

The physicochemical parameters and the presence/absence of ARGs in water samples from six sampling points, categorized by seasonal periods—fall/winter (F/W) and spring/summer (S/Sm)—were statistically analyzed. The Shapiro–Wilk test was used to assess the normality of data distribution. For normally distributed variables, parametric analyses were conducted, including multiple linear regression and one-way ANOVA, followed by Tukey’s multiple comparison test. When data did not meet the normality assumption, the non-parametric Kruskal–Wallis test was applied to determine significant differences among sampling points and seasons. In addition, univariable logistic regression analyses were performed to assess the potential association between physicochemical water variables (temperature, pH, ORP, EC, DO, turbidity, and nutrient concentrations) and the presence of individual ARGs (*bla*TEM, *erm*B, *sul*1, and *tet*W). The results, expressed as odds ratios (OR) with 95% confidence intervals (CI) and *p*-values, are presented in the Supplementary Material (Table S4).

Based on the nature of the data and the objectives of the analysis, a principal component analysis (PCA) was performed on the original measurements of each parameter, without applying prior normalization or standardization, following the recommendations of Pavez-Muñoz et al. [41]. PCA was performed to identify underlying patterns and potential clustering of sampling sites and seasonal periods based on physicochemical parameters and the presence of ARGs. All multivariate analyses were performed using R software (version 4.3.2, R Core Team, 2023) with the packages vegan (version 2.6-4) for principal component analysis and ggplot2 (version 3.4.2) for data visualization (<https://www.R-project.org>, accessed on 1 July 2025). Complementary statistical analyses were performed using GraphPad Prism version 9.5.1 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com, accessed on 1 July 2025), with a significance level set at 0.05.

3. Results and Discussion

3.1. In Situ Parameters

The average values of the in situ parameters are presented in Figure 2 and Supplementary Material (Table S5).

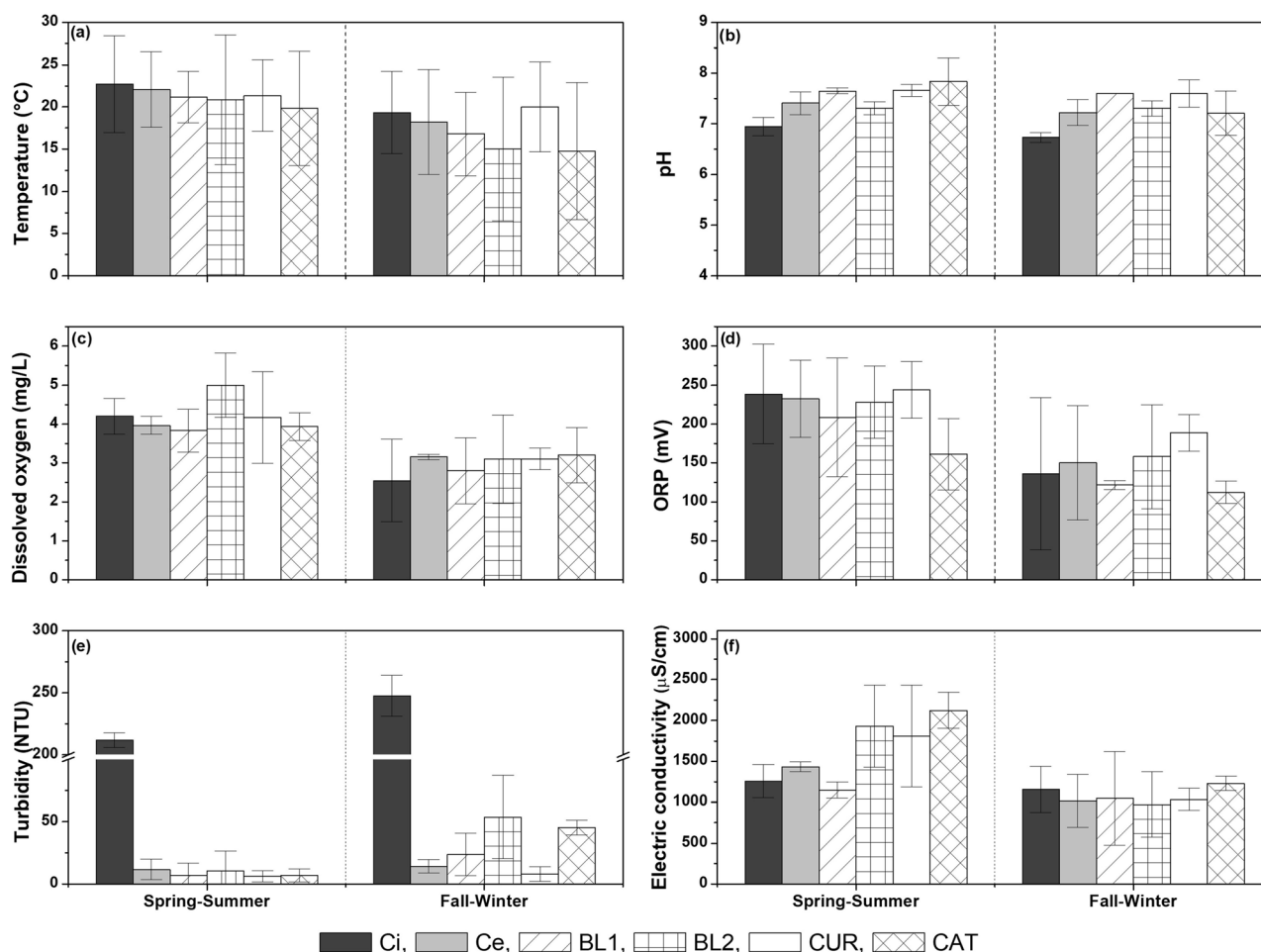


Figure 2. Averages and standard deviations of in situ parameters: (a) temperature; (b) pH; (c) oxidation-reduction potential (ORP); (d) dissolved oxygen (DO); (e) electrical conductivity (EC); and (f) turbidity, across different sampling sites.

The mean water temperature was 19.34 °C, with higher values in spring–summer (21.3 °C) than fall–winter (16.8 °C), consistent with the Mediterranean climate of the Santiago Metropolitan Region and exceeding historical regional averages by up to 7.05 °C, possibly reflecting warming trends associated with El Niño and La Niña events [42]. Mean pH was 7.37 ± 0.32 across all sites, with slightly lower values at Ci (6.84 ± 0.15) attributable to the higher acid content of raw wastewater [43]. DO ranged from 1.80 to 5.70 mg/L, with higher values in spring–summer (4.18 mg/L), reflecting algal and macrophyte dynamics in Batuco Lagoon and temperature-driven microbial activity in irrigation canals. ORP confirmed predominantly aerobic conditions across all sites (218 mV spring–summer; 145 mV fall–winter) [44]. Turbidity was significantly higher at Ci (230 NTU) than at other sites (15 NTU), with a seasonal pattern of higher values in fall–winter associated with increased runoff. Mean EC was 1347 ± 393 μS/cm, with higher values in spring–summer; irrigation canal values (1034–2124 μS/cm) approached limits potentially harmful to sensitive crops according to NCh 1333 [17].

These physicochemical parameters are relevant to ARG dissemination through multiple mechanisms. Elevated temperatures stimulate bacterial metabolic activity, horizontal gene transfer, and the expression of resistance mechanisms such as efflux pumps [26,27,45]. Near-neutral pH across most sites suggests that microbial equilibrium was maintained, as acidic conditions are known to favor ARG propagation through plasmids and extracellular DNA [28]. DO levels in Batuco during fall–winter approached microaerobic thresholds; Jong et al. [29] demonstrated that conjugative ARG transfer frequencies decrease significantly under microaerobic conditions, suggesting partial limitation of horizontal gene transfer during colder months. Both elevated turbidity and dissolved organic matter fractions may favor ARG mobility and transfer [46], while the high ionic content observed may promote ARG dissemination by inducing osmotic stress and stabilizing extracellular DNA [27].

3.2. Characterization Physicochemical

COD concentrations at Ci were consistent with untreated wastewater ranges (70–1200 mg/L) [13,47,48], with higher total organic load in fall–winter (536.8 mg/L), while Ce concentrations (12.86–51.25 mg/L) were within typical ranges for secondary treated effluent in accordance with DS No. 90/2000 [14,47–49]. Batuco Lagoon and irrigation canals showed the same seasonal trend, with COD values ranging from 20–30 mg/L in irrigation canals to 26–37 mg/L in Batuco Lagoon, remaining within regulatory limits for irrigation water. The dissolved organic matter fraction is particularly relevant in the context of ARG dissemination: Li et al. [26] demonstrated that low-molecular-weight fractions (≤ 10 kDa) can increase plasmid-mediated conjugative ARG transfer by up to 7.1 times [50], and Sorn et al. [51] showed that both high and low organic carbon conditions can facilitate antibiotic resistance through distinct mechanisms. These findings highlight Ci as the critical point with the most favorable conditions for horizontal gene transfer, while suggesting that even the lower COD levels recorded in Batuco Lagoon and irrigation canals may represent a latent risk for ARG dissemination.

The seasonal nutrient characterization is presented in Table 2. At Ci, nitrogen concentrations were within typical ranges for untreated domestic wastewater (35–100 mg/L) [47], with the Cadellada WWTP achieving NH_4^+ -N removal efficiencies of 91.97%, consistent with reported secondary treatment performance [52]; the increase in NO_3^- -N at Ce reflects aerobic nitrification. In Batuco Lagoon, NH_4^+ -N and NO_3^- -N concentrations at BL1 and BL2 exceeded the hypereutrophic threshold established by the DGA (≥ 1.2 mg/L) [53], representing a critical risk for ecosystem biodiversity and favoring resistant and opportunistic species [54]. In irrigation canals, CUR showed higher NH_4^+ -N (10.19 mg/L) and NO_3^- -N (3.90 mg/L) than CAT (5.32 and 2.01 mg/L, respectively).

The nitrogen profile observed across sampling points may be modulating ARG abundance. Zhang et al. [30] reported a positive correlation between NH_4^+ -N and ARG abundance and a negative correlation with NO_3^- -N. The elevated NH_4^+ -N concentrations at Ci (54.72 mg/L), CUR (10.19 mg/L), and CAT (5.36 mg/L) suggest favorable conditions for resistant bacteria in anoxic microenvironments, consistent with Sun et al. [55], who demonstrated that NH_4^+ -N and NO_3^- -N applications significantly increase ARG relative abundance through enrichment of the Proteobacteria phylum.

Phosphorus concentrations at Ci and Ce were within typical ranges for raw and secondary treated effluent, respectively [47]. In Batuco Lagoon, average phosphorus concentrations confirmed hypereutrophic conditions (>0.1 mg/L) [53], which, combined with elevated nitrogen levels, may favor excessive phytoplankton growth and alter ecosystem structure and functioning. Phosphorus in irrigation canals remained within acceptable ranges for irrigation water use.

Table 2. Seasonal physicochemical characterization of samples throughout the monitoring period.

Sample	Season	Parameter (Average $\pm$ SD)			
		COD _T (mg/L)	NO ₃ ⁻ -N (mg/L)	NH ₄ ⁺ -N (mg/L)	TP (mg/L)
Ci	S-Sm	457.50 $\pm$ 35.46	3.02 $\pm$ 0.65	50.46 $\pm$ 4.94	6.13 $\pm$ 1.44
	F-W	536.8 $\pm$ 8.25	1.94 $\pm$ 0.01	58.08 $\pm$ 9.18	6.10 $\pm$ 0.36
Ce	S-Sm	51.25 $\pm$ 26.10	23.69 $\pm$ 6.30	5.11 $\pm$ 1.43	0.910 $\pm$ 0.77
	F-W	25.94 $\pm$ 9.14	19.48 $\pm$ 6.91	3.45 $\pm$ 2.34	0.088 $\pm$ 0.04
BL1	S-Sm	9.18 $\pm$ 3.12	3.96 $\pm$ 0.61	1.91 $\pm$ 1.32	1.83 $\pm$ 0.38
	F-W	10.63 $\pm$ 1.66	1.22 $\pm$ 1.49	1.34 $\pm$ 0.08	1.10 $\pm$ 0.28
BL2	S-Sm	5.71 $\pm$ 2.21	1.62 $\pm$ 1.10	1.43 $\pm$ 0.99	1.70 $\pm$ 1.31
	F-W	10.50 $\pm$ 2.40	2.31 $\pm$ 2.91	1.44 $\pm$ 0.48	0.90 $\pm$ 0.28
CUR	S-Sm	6.74 $\pm$ 1.69	5.91 $\pm$ 1.29	13.42 $\pm$ 6.59	3.00 $\pm$ 0.82
	F-W	15.26 $\pm$ 1.47	1.89 $\pm$ 0.03	6.96 $\pm$ 0.34	3.20 $\pm$ 0.02
CAT	S-Sm	7.10 $\pm$ 3.72	2.31 $\pm$ 2.82	4.40 $\pm$ 4.18	0.60 $\pm$ 0.14
	F-W	17.43 $\pm$ 12.26	1.70 $\pm$ 2.16	6.23 $\pm$ 3.58	1.65 $\pm$ 0.21

Note: COD_T: total chemical oxygen demand; NO₃⁻-N: nitrate nitrogen; NH₄⁺-N: ammonium nitrogen; TP: total phosphorus; S-Sm: spring-summer; F-W: fall-winter.

3.3. Presence of Antibiotic Resistance Genes

The ARGs present in the sampling points in each season of the year can be seen in Table 3.

Table 3. Presence of ARGs at sampling points by season of the year.

Season	Ci	Ce	BL1	BL2	CUR	CAT
Winter	<i>blaTEM</i> <i>sul1</i>	<i>blaTEM</i>	<i>blaTEM</i> <i>sul1</i>	<i>sul1</i>	**	<i>blaTEM</i> <i>sul1</i>
Spring	<i>blaTEM</i> <i>tetW</i>	<i>blaTEM</i> <i>tetW</i>	<i>blaTEM</i>	<i>tetW</i>	<i>tetW</i>	<i>blaTEM</i> <i>tetW</i>
Summer	<i>ermB</i> <i>sul1</i> <i>tetW</i>	<i>blaTEM</i> <i>ermB</i> <i>sul1</i>	<i>blaTEM</i> <i>ermB</i> <i>sul1</i> <i>tetW</i>	<i>blaTEM</i> <i>ermB</i> <i>sul1</i>	<i>blaTEM</i> <i>ermB</i> <i>sul1</i> <i>tetW</i>	<i>blaTEM</i> <i>ermB</i> <i>sul1</i> <i>tetW</i>
Fall	<i>blaTEM</i> <i>sul1</i>	<i>sul1</i> <i>tetW</i>	<i>blaTEM</i> <i>tetW</i>	<i>blaTEM</i> <i>sul1</i>	<i>blaTEM</i> <i>sul1</i> <i>tetW</i>	<i>sul1</i>

Note: ** Not detected.

Figure 3 illustrates the frequency of ARG-positive samples at the six sampling points. All sites (100%) tested positive for the presence of any ARGs, including the Batuco Lagoon Nature Sanctuary sites. The sites with the highest frequency of positive samples were Ci, BL1 and CAT, with an average of 56% of positive samples. In contrast, ARGs were found in 50% of the samples from Ce and CUR, and those with the lowest positivity for the ARGs under study were found in BL2 (44%), although no statistically significant differences were observed between the different aquatic environments studied ($p \geq 0.05$). These findings are consistent with previous research, such as that of Langbehn et al. [56], which documented the widespread presence and distribution of antibiotics and ARGs in aquatic ecosystems, soils and agricultural, industrial and human runoff waters.

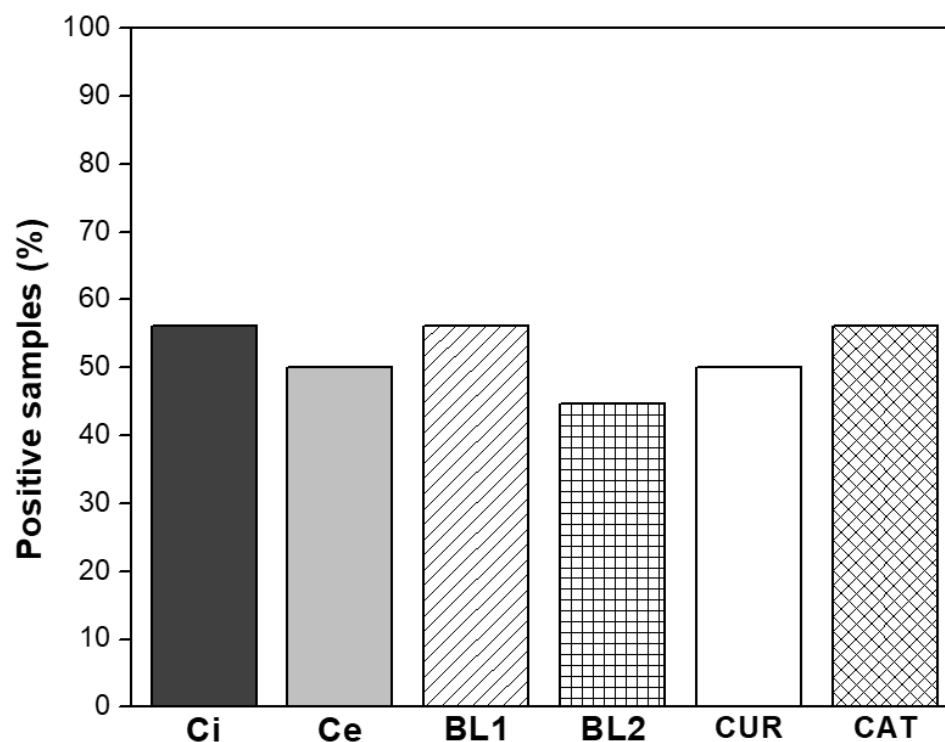


Figure 3. Frequency of ARG-positive samples by sampling site.

In line with these patterns, growing evidence from Latin America indicates that agricultural systems also play a key role in ARG dissemination. The presence of ARGs in environments linked to agricultural production represents an emerging risk to food security in Latin America. In Brazil, Ferreira et al. [11] reviewed 36 studies on antimicrobial resistance in agricultural environments, including soils, irrigation water, and animal waste, identifying genes such as *sul1*, *sul2*, *blaTEM*, and *intI1*, which are associated with intensive agricultural practices and fertilization systems.

Evidence from Argentina further reinforces this trend. Multidrug-resistant *Salmonella* strains isolated from farm pigs showed high resistance to β -lactams and quinolones, with more than 50% of isolates classified as multidrug-resistant, highlighting risks associated with the animal–human food chain [12]. Taken together, these findings from Brazil and Argentina, along with studies in Chile, demonstrate that agricultural environments and rural water resources can act as reservoirs and transmission routes for ARGs, posing a potential threat to regional food safety.

In the case of samples collected at the WWTP “Cadellada” (Ci and Ce), it was observed that the positivity of ARGs showed variations of less than 7%, which are not significant ($p > 0.05$), between influent (Ci) and effluent (Ce). These results are consistent with previous studies that identify wastewater treatment plants as one of the main sources of ARG release into the environment [57]. This is also consistent with the findings of Sun et al. [36], who reported that although wastewater treatment reduced the concentrations of ARGs by 1–2 orders of magnitude, more than 69.95% of the resistance genes remained enriched in the effluent. At a regional scale, Bueno et al. [37] documented a significant increase of 17 ARGs in a watershed in southern Chile, Región de los Ríos, affected by three WWTPs.

Urban Wastewater Treatment Plants (UWWTP) bring together Antibiotic Resistant Bacteria (ARB), and antibiotic residues as chemical micropollutants favor selection for these microorganisms. At the same time, this offers a rich nutrient supply and bacterial cell interaction, which facilitates antibiotic resistance horizontal gene transfer [58]. Although only some of the ARB released into the environment through UWWTP effluent

are capable of causing disease in humans or animals, the risk of enriching environmental resistance through selection or horizontal gene transfer and consequently contributing to the emergence of resistance in pathogenic bacteria cannot be overlooked [59].

Optimizing the performance of UWWTPs is essential to reducing the release of ARB and ARGs. Leiva et al. [60] determined that systems employing chlorination can achieve reductions of >3.0 log of ARGs in the final effluent, outperforming UV disinfection. It has also been established that chlorination operated at ~0.5 mg/L of free residual chlorine with a 30 min contact time can reduce ARB by 3.8–5.6 log and decrease genes such as *tetM* and *blaTEM* by 0.40–1.45 and 1.04–2.45 log, respectively [61]. These findings suggest that ensuring adequate residual chlorine concentrations and minimum contact times represents an immediately implementable and low-cost operational improvement for many UWWTPs.

Additionally, advanced tertiary technologies offer even higher removal efficiencies. Ozonation at doses between 5 and 15 mg/L has been reported to reduce 90–99% of ARGs and more than 3 log of ARB [61]. UV disinfection, under optimized fluence, removes 1–2 log of ARB and ARGs and achieves > 90% elimination, while membrane systems (Ultrafiltration/Nanofiltration/Reverse Osmosis) can reach removals exceeding 3–6 log [62]. These technologies represent suitable options in urban settings where technical and infrastructural capacities support their implementation.

Nature-based solutions can also be strategically integrated in rural areas, where treated effluents are commonly discharged into irrigation canals. Leiva et al. [63] reported that constructed wetlands can reduce ARGs by 0.1–2.1 log, while vertical-flow and hybrid systems can remove >90% of *Enterococci* and *Enterobacteria* and reduce multiple ARGs by 90–95% [61]. Their incorporation as downstream polishing stages provides an additional low-energy barrier to mitigate the spread of antimicrobial resistance in agricultural environments.

Samples obtained from the Batocho Lagoon (BL1 and BL2) and irrigation canals (CUR and CAT) were positive for ARGs (≥51%) with variations of less than 6% between them. These results are congruent with those observed by Bartholin et al. [64], who found that 100% of Gram-negative bacilli isolated from 11 aquatic ecosystems in Chile (including lakes, rivers, a waterfall and a river basin) showed resistance to at least one antibiotic. In addition, extended-spectrum β -lactamase-encoding genes, specifically *blaTEM* and *BlaCTX-M*, were detected in four of these samples.

The detection of ARGs in irrigation canals (CAT and CUR) and in the Batocho Lagoon (BL1 and BL2) is supported by the findings of Stocker et al. [65] who reported a positive correlation between bacterial resistance and rainfall. This correlation could be explained by increased surface runoff, which transports suspended particles, dissolved elements and microorganisms into open aquatic systems. Additionally, the sampling points are located in areas of agricultural activity, where the practice of fertigation seems to contribute to the persistent presence of ARGs. In Chile, bio-fertigation with treated household and agricultural waste is common and has been identified as one of the main pathways for transferring ARGs into soil–plant and water systems [63].

In agricultural contexts, treated wastewater and biosolids are increasingly used for irrigation and soil fertilization, especially in areas facing water scarcity. However, national standards such as NCh 1333/1978 [17] (irrigation water) and DS N° 4/2009 [15] (sludge management/biosolid application) do not include parameters for emerging contaminants such as antibiotics or ARGs [19,63]. This regulatory omission, coupled with insufficient environmental monitoring, may facilitate the dissemination of ARGs in agricultural soils and irrigation systems. In Chile, approximately 30% of the 170,000–200,000 tons of biosolids produced annually by wastewater treatment plants are applied to agricultural soils [66], and the reuse of treated wastewater for irrigation exceeds 60 million cubic meters per year, primarily in water-scarce central regions such as Valparaíso and Metropolitana [67].

Although the use of antibiotics in livestock production is regulated in Chile (including prescription requirements and restrictions on growth promoters), current policies do not mandate the monitoring or treatment of antibiotic residues and ARGs in livestock waste. Resolutions such as SAG's Exempt Resolution No. 1129/2022 [68] and No. 6801/2020 [69] establish requirements for the prudent use, registration, and commercialization of veterinary antimicrobials, but they do not extend to the environmental control of their residues or associated resistance genes [63,68,69].

This omission is critical, as manure and slurry are commonly applied to agricultural soils or discharged into water bodies, potentially contributing to the environmental dissemination of ARGs. Taken together, these practices represent potential vectors for ARG proliferation in soil and irrigation networks, especially in the absence of environmental monitoring or regulatory thresholds for emerging contaminants. This context is consistent with our findings of ARG persistence in irrigation canals and wetlands, even in systems not directly influenced by WWTPs [20,63]. Therefore, to improve environmental and water quality monitoring and management strategies, ARGs should be routinely monitored. In this context, it is suggested to use *sul1* as an indicator of anthropogenic impact and the potential for horizontal gene transfer, *blaTEM* and *tetW* due to their widespread presence and relevance to β -lactam and tetracycline resistance, and *ermB* in sites with known macrolide use, such as agricultural runoff [6].

3.4. Spatial and Seasonal Determination of Antibiotic Resistance Genes in Aquatic Systems

Figure 4 shows the seasonal occurrence of the ARGs studied in this work. The results reveal that the most commonly found gene throughout the period and at all sampling points was *blaTEM*, with a frequency of 71%, followed by *sul1* with 63% and *tetW* with 50%. On the other hand, the *ermB* gene had the lowest frequency (25%). These findings are consistent with those reported by Jia et al. [70], who documented a high relative frequency of *sul1*, *tetA*, *tetC*, *tetZ*, *gyrA*, *ermF*, *cmlA* and *blaTEM* ARGs in the Ba River, China. In their study, the sum of these genes accounted for 92.3% to 99.8% of the total ARGs in water samples and 63.2% to 99.5% in sediment samples. The predominant presence of these ARGs is attributed to the influence of residual antibiotics from urban areas, hospitals and nearby sewage treatment plants [71,72].

	Winter						Spring						Summer						Fall					
	Ci	Ce	BL1	BL2	CUR	CAT	Ci	Ce	BL1	BL2	CUR	CAT	Ci	Ce	BL1	BL2	CUR	CAT	Ci	Ce	BL1	BL2	CUR	CAT
<i>blaTEM</i>	+	+	+	−	−	+	+	+	+	−	−	+	−	+	+	+	+	+	+	−	+	+	+	−
<i>ermB</i>	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−
<i>sul1</i>	+	−	+	+	−	+	−	−	−	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+
<i>tetW</i>	−	−	−	−	−	−	+	+	−	+	+	+	+	−	+	−	+	+	−	+	+	−	+	−

Figure 4. Seasonal presence/absence of ARGs at sampling points. (+): ARG detected. (−): ARG not detected. Percentages are shown for descriptive purposes only; statistical analyses were conducted using binary presence/absence data.

The abundance of the *blaTEM* gene in watercourses is a significant indicator of anthropogenic contamination related to antibiotic resistance [68,69]. In this study, the results reflect this trend, with 50% of *blaTEM*-positive samples in the CUR and BL2 sampling points, located in areas with lower anthropogenic incidence, such as rural areas and a nature sanctuary. In contrast, the sampling point located at the entrance of the Batuco Lagoon (BL1) and coming directly from the wastewater treatment plant showed 100%

positive samples for *blaTEM*. This could be related to the fact that the tributary is superficial and open, passing through urban and rural areas, so it could be contaminated with fecal waste through stormwater runoff, domestic animals, human waste and wildlife [73]. While studies in Chile indicate that there is no association between resistance profiles or genes and geographical areas [74], research in Ecuador shows that the risk of ARGs in surface watercourses increases with greywater contamination from household sewage, revealing a Gram-positive bacteria resistance of 19% to the cephalothin and 8% to ampicillin, while in Gram-negative bacteria, the highest prevalence of resistance was observed in coliforms against ampicillin (34%) and cephalothin (17%), so that greywater can be considered as a reservoir of antibiotic-resistant bacteria, thus increasing their health risk, a situation similar to that observed in this study. Urban areas, such as CAT, presented 75% positivity, as did the samples obtained from Ci and Ce, areas that are directly influenced by the wastewater treatment plant analyzed.

These findings are in line with Sodhi et al. [75], who pointed out that water treatment systems do not have effective processes to degrade beta-lactam antibiotic residues, thus increasing the likelihood of developing bacterial resistance. However, studies carried out in Spain suggest that *blaTEM* and *sul1* genes are the most prevalent in agricultural fields and crops, with positivity percentages above 85% [76]. When considering the set of ARGs present in the plant microbiome, they are also found in the plant microbiome of the endosphere, phyllosphere and rhizosphere [77]. The presence of ARGs has been detected both on the surface of fruits and vegetables and in root and leaf endophytic bacteria and phyllosphere microorganisms in vegetables grown in manure-fertilized soils [24,26,28,43]. Similarly, studies indicate that *blaTEM* is the predominant beta-lactam ARG among beta-lactam resistance genes in other food chains, such as milk, describing between 94.55% and 88.59% prevalence in milk [78].

Although *sul1* is the second most commonly isolated ARG in the different sampling sites, followed by *tetW* and *ermB* in third and fourth place, respectively, and although the presence of these genes is often associated with urbanization, sulfonamides, in combination with trimethoprim, are widely used in both veterinary practice and human medicine. Several studies have documented the frequency of sulfonamide (*sul1* and *sul2*) and tetracycline (*tetG*, *tetA*, *tetO*, *tetC*, *tetX*, *tetM* and *tetQ*) genes in the Beijiing River and Pearl River in China, particularly in areas with a high degree of urbanization or in areas heavily influenced by human activities [79]. *Sul1* has also been detected in hospital wastewater, irrigation canals and wastewater in Nigeria [80], as well as in surface water near wastewater treatment plants [81]. In Spain, studies have shown significant differences in the concentrations of antibiotics and ARGs, such as the macrolide gene (*ermB*), the fluoroquinolone gene (*qnrS*) and the tetracycline gene (*tetW*), in the Ter River, both upstream and downstream of a wastewater treatment plant effluent discharge [81]. The findings of this study are also consistent with those reported by Bueno et al. [37], who identified that the most abundant genes in wastewater treatment plants in Chile were *sul1*, *qnrS*, *sul2*, and *strB*.

The low frequency observed for *ermB* compared to the other genes is probably due to a combination of factors. However, from a methodological point of view, primers for *ermB* have been used and validated in environmental and clinical studies [82,83]. Regarding the actual prevalence of this gene in the sampled environments, from an ecological perspective, *ermB* encodes resistance to macrolides, antibiotics that are prevalent in animal production systems. Our sampling sites (although anthropically influenced) did not include intensive farms, which probably explains its low prevalence. This is consistent with studies in different regions where *ermB* appears less frequently than genes associated with sulfonamides or β -lactams [84]. Likewise, environmental factors such as substrate pH, organic load,

bacterial composition, and water flow dynamics can influence the relative persistence of *ermB*, making it less detectable than integron or highly mobile genes [85,86].

Regarding the relative presence of ARGs in the sampling points, divergent results are observed, as the distribution of positive samples for the four ARGs under study shows seasonal variations. In winter, neither the *ermB* nor *tetW* genes were detected, and considering all samples collected in this season, ARGs were found in 33% of them. In spring, neither *ermB* nor *sul1* were identified, and the percentage of positive samples increased to 38%. During fall, the *ermB* gene was absent, but positive samples increased to 50%. In contrast, summer showed the presence of all ARGs and the highest percentage of positivity, reaching 88% in the samples analyzed.

Studies have suggested that seasonal effects on the presence of ARGs may be due to temperature variations, which affect the behavior of microbial communities. For example, Garcia et al. [87] evaluated the seasonal variations of the ARGs *sul1*, *sul2*, and *ermB* and observed a positive correlation (0.99) between *sul1* reduction and temperature decrease. However, the results for the other ARGs were inconclusive. In contrast, Li et al. [88] reported a 16% reduction in ARGs during summer compared to winter, while Fang et al. [89] reported an 18% reduction in ARGs during winter compared to summer. Furthermore, Sabri et al. [90] observed negative correlations between temperature and the abundance of ARGs, and Abou-Kandil et al. [91] attributed these results to an intensification of metabolic activity and biomass responsible for ARG biodegradation during summer. These findings show that there is still no consensus on the seasonal effect on the presence of ARGs in aquatic environments, although in this study, summer and fall were the seasons showing the highest presence and diversity.

3.5. Principal Component Analysis of the Interaction Between Antibiotic Resistance Genes and Physicochemical Parameters

When evaluating the PCA biplot, in the context of the presence (1) or absence (0) of the ARGs found (*blaTEM*, *ermB*, *sul1*, and *tetW*), no distinct clusters are observed between the presence or absence of the resistance genes studied (Figure 5). PC1 and PC2 together explained 63.97% of the total variance. Although PC3 adds 10.95% (cumulative 74.92%), the biplot visualization was limited to PC1 and PC2, as these captured the primary patterns of interest in relation to ARG presence/absence. The complete PCA output is provided in the Supplementary Material (Table S6).

Previous studies have indicated that water quality parameters correlate significantly with concentrations of bacteria and antibiotic resistance genes [92]. Among these, nitrate and dissolved organic matter stand out as key factors, showing positive associations with resistance to antibiotics such as tetracycline, affecting between 6.3% and 12.7% of the total *E. coli* population [93]. On the other hand, Brissaud et al. [94] and He et al. [95] found that the stratification of pond water quality parameters, in particular temperature, is a determining factor in generating differences in fecal microbial concentrations at different sampling depths, which influences the likelihood of developing antibiotic resistance.

Similarly, dissolved nutrients, both organic and inorganic, have been found to be positively associated with fecal bacteria concentrations in ponds and lakes [96]. The main explanations for this phenomenon include the availability of nutrients that favor bacterial growth and persistence and the coincidence of nutrient inputs from precipitation events, which can transport both nutrients and fecal bacteria into water bodies [93]. In addition, the role of dissolved organic matter has been highlighted as a critical factor in the availability and transport of antibiotics in the environment, mediated by electrochemical interactions [97].

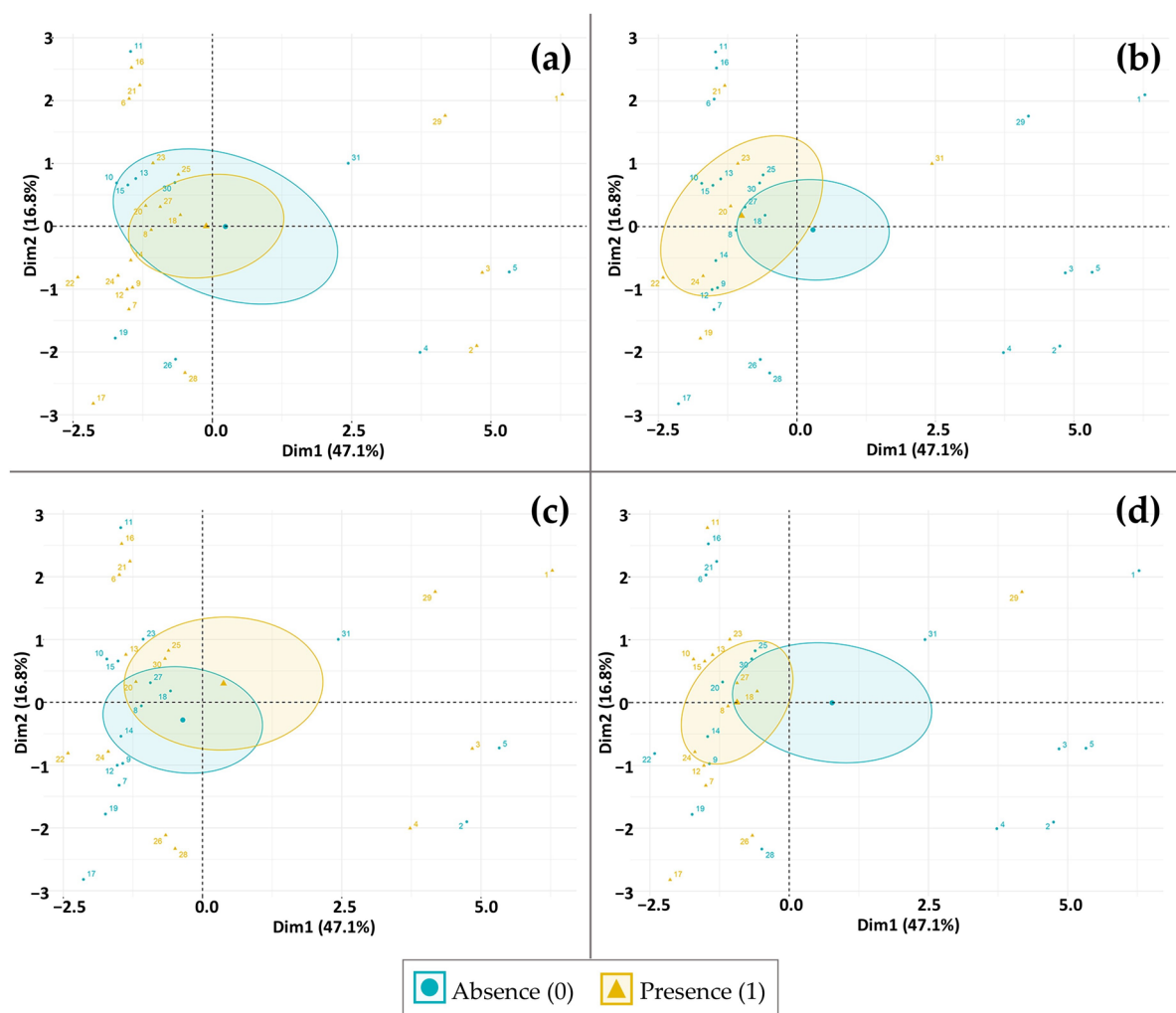


Figure 5. Biplot of the principal component analysis showing the distribution of samples and key physicochemical variables in relation to the presence (1) or absence (0) of each resistance gene: (a) *blaTEM*, (b) *ermB*, (c) *sul1* and (d) *tetW*.

Another factor that must be considered in future studies is the level of chlorophyll-a, which is commonly used to determine the trophic state of wetlands or lakes, as well as the phytoplankton biomass—values that are higher in the summer months in some Chilean lakes [98], thereby promoting the reproduction of invasive algal bloom species. On the other hand, the propagation of phycosphere ARGs is promoted through horizontal transfer under nutrient limitations (N or P), increasing the complexity among algae, bacteria and ARGs in wetlands [99].

It is important to note that, during the sampling period in this study, no extreme climatic events were observed, contrary to what has been widely studied in China, Europe and the United States, as they have been shown to influence the occurrence of antibiotic resistance in different ecosystems such as mountains and snow, rivers where water temperature has increased [100] and regions of these countries where an increase in temperature above normal averages has been described as a result of climate change [101]. Although extreme weather events (such as heavy rainfall, flooding or runoff) have also been identified as factors affecting antibiotic resistance [102], no clear patterns or distinct clusters were evident in this study that would allow a direct link between physicochemical factors and the presence or absence of antibiotic resistance genes in the aquatic environments studied.

Our results provide relevant empirical evidence on the seasonal variability and distribution of ARGs in freshwater systems subject to different anthropogenic pressures. These

findings align with national and international strategic frameworks that recognize the environmental dimension of AMR. In particular, Chile's National Action Plan on AMR (2021–2025) [21] identifies as a priority the generation of scientific information to support the creation of regulatory instruments. Likewise, global initiatives such as the WHO Global Action Plan and the joint UNEP–FAO [103] report recommend incorporating ARG surveillance into aquatic ecosystems and wastewater monitoring. This study directly contributes to these objectives by identifying regulatory gaps and providing data that can support the design of environmental standards for water reuse scenarios.

4. Conclusions

This research has demonstrated a significant dispersion of ARGs in canals and urban and rural irrigation stations that depend on the Maipo River basin, Metropolitan Region of Santiago de Chile. ARGs were detected even at sites with no direct WWTP influence, suggesting that indirect pathways such as agricultural runoff and surface drainage contribute to their dissemination across the basin. The identification of ARGs in the Batocho Lagoon Nature Sanctuary reinforces the evidence that WWTPs are reservoirs of ARGs and potential sources of genetic contamination. This represents a latent risk to the Sanctuary's biodiversity, as the continued accumulation of these genes may alter ecological processes and increase the likelihood of resistant bacteria transmission, with consequences for human and animal health.

Although no clear patterns were observed directly linking physicochemical water parameters to ARG presence or absence, the results suggest that ecological dynamics, microbiological interactions, or unidentified anthropogenic pressures may influence their distribution. These findings provide a valuable scientific basis for strengthening regulatory frameworks in Chile and Latin America and for promoting ARG monitoring in environmental and water-quality management. Routine surveillance of *sul1* as an indicator of anthropogenic impact, *blaTEM* and *tetW* due to their widespread presence and clinical relevance, and *ermB* in sites with known macrolide use is strongly recommended. Since WWTP effluent is the only tributary feeding Batocho Lagoon, maintaining this flow while adopting advanced tertiary treatments (ozonation, UV disinfection, or membrane filtration) and nature-based solutions as a complementary polishing stage is essential to reduce ARG loads before discharge.

Future Areas of Research

The findings highlight the urgent need for further research to assess the abundance, persistence, and mobility of ARGs across aquatic and terrestrial environments, including an investigation of whether associated fauna may act as reservoirs or vectors of ARGs and resistant bacteria—an emerging threat to biodiversity and public health. A complementary study on ARG detection in *Xenopus laevis* as a potential disseminator of antibiotic resistance in Chilean freshwater ecosystems is currently under development. Future studies should incorporate quantitative approaches such as qPCR alongside antibiotic concentration measurements (sulfonamides, tetracyclines) to establish correlations with detected resistance genes. Expanding the analysis to a broader range of ARGs and mobile genetic elements, such as integrons (*intI1*), and extending sampling to sediments, biofilms, and local fauna would provide a more comprehensive understanding of ARG persistence and dissemination pathways. More detailed longitudinal sampling within the WWTPs would help identify treatment stages with lower ARG removal efficiency, supporting targeted intervention design.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w18141702/s1>: Text S1. Detailed description of the study area and sampling sites; Text S2. PCR amplification protocol and thermocycler conditions; Table S1. Reagents and concentrations used for PCR; Table S2. Primers and amplicons of the ARGs under study; Table S3. Thermocycler temperature programming; Table S4. Univariable logistic regression results showing the association between physicochemical water variables and the presence of ARGs; Table S5. In-situ parameters at sampling sites by seasonal period; Table S6. Comprehensive summary table of the Principal Component Analysis (PCA), indicating the importance of each variable measured in water, standard deviation (SD), and percentage of explanation of variability linked to each principal component.

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

Funding: This research was funded by the National Agency for Research and Development (ANID), Grant for Installation in Academia, Call 2021, grant number PAI77200034.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to ongoing related studies.

Acknowledgments: The authors thank Raul Alejandro Alegría Morán for his support in statistical analyses and manuscript revision, and Miguel Castro Retamal from the Applied Microbiology Laboratory, Faculty of Sciences, Universidad Santo Tomás, Santiago, for providing laboratory facilities and technical support in PCR analyses. The authors used AI-assisted tools (Claude Sonnet 4.6, Anthropic, San Francisco, CA, USA) during the preparation of the response-to-reviewers letter to improve clarity and language. The manuscript content was reviewed and validated by all authors, who take full responsibility for the integrity of the work.

Conflicts of Interest: The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial resistance
ANID	National Agency for Research and Development
ARGs	Antibiotic resistance genes
BL1	Batuco Lagoon inlet tributary
BL2	Batuco Lagoon outlet
<i>bla</i> TEM	Beta-lactam resistance gene
CAT	Urban irrigation canal, Catemito
Ce	Cadellada effluent wastewater
Ci	Cadellada influent wastewater
COD	Chemical oxygen demand
CUR	Rural irrigation canal, Curacaví
DGA	Dirección General de Aguas
DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
DS	Decreto Supremo

EC	Electrical conductivity
<i>ermB</i>	Macrolide resistance gene
F/W	Fall–winter
FAO	Food and Agriculture Organization
NCh	Norma Chilena
NTU	Nephelometric Turbidity Unit
ORP	Oxidation-Reduction Potential
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
ROS	Reactive Oxygen Species
S/Sm	Spring–summer
<i>sul1</i>	Sulfonamide resistance gene
<i>tetW</i>	Tetracycline resistance gene
TN	Total nitrogen
TP	Total phosphorus
UNEP	United Nations Environment Programme
UWWTP	Urban Wastewater Treatment Plant
WHO	World Health Organization
WWTP	Wastewater treatment plant

References

1. Ebele, A.J.; Abdallah, M.A.E.; Harrad, S. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg. Contam.* **2017**, *3*, 1–16. [\[CrossRef\]](#)
2. Maghsodian, Z.; Sanati, A.M.; Mashifana, T.; Sillanpää, M.; Feng, S.; Nhat, T.; Ramavandi, B. Occurrence and distribution of antibiotics in the water, sediment, and biota of freshwater and marine environments: A review. *Antibiotics* **2022**, *11*, 1461. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Zhi, S.; Shen, S.; Zhou, J.; Ding, G.; Zhang, K. Systematic analysis of occurrence, density and ecological risks of 45 veterinary antibiotics: Focused on family livestock farms in Erhai Lake basin, Yunnan, China. *Environ. Pollut.* **2020**, *267*, 115539. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Murray, C.J.L.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Aguilar, G.R.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet* **2022**, *399*, 629–655. [\[CrossRef\]](#)
5. Zhou, C.S.; Wu, J.W.; Dong, L.L.; Liu, B.F.; Xing, D.F.; Yang, S.S.; Wu, X.K.; Wang, Q.; Fan, J.N.; Feng, L.P.; et al. Removal of antibiotic resistant bacteria and antibiotic resistance genes in wastewater effluent by UV-activated persulfate. *J. Hazard. Mater.* **2020**, *388*, 122070. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Berendonk, T.U.; Manaia, C.M.; Merlin, C.; Fatta-Kassinos, D.; Cytryn, E.; Walsh, F.; Martinez, J.L. Tackling antibiotic resistance: The environmental framework. *Nat. Rev. Microbiol.* **2015**, *13*, 310–317. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Haller, M.Y.; Müller, S.R.; McArdell, C.S.; Alder, A.C.; Suter, M.J.F. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography–mass spectrometry. *J. Chromatogr. A* **2002**, *952*, 111–120. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Zhu, Y.G.; Johnson, T.A.; Su, J.Q.; Qiao, M.; Guo, G.X.; Stedtfeld, R.D.; Hashsham, S.A.; Tiedje, J.M. Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 3435–3440. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Wang, N.; Shen, W.; Zhang, S.; Cheng, J.; Qi, D.; Hua, J.; Qiu, H. Occurrence and distribution of antibiotics in coastal water of the Taizhou Bay, China: Impacts of industrial activities and marine aquaculture. *Environ. Sci. Pollut. Res.* **2022**, *29*, 81670–81684. [\[CrossRef\]](#)
10. Boger, B.; Surek, M.; Vilhena, R.D.O.; Fachi, M.M.; Junkert, A.M.; Santos, J.M.; Domingos, E.-L.; Cobre, A.F.; Momade, D.R.; Pontarolo, R. Occurrence of antibiotics and antibiotic resistant bacteria in subtropical urban rivers in Brazil. *J. Hazard. Mater.* **2021**, *402*, 123448. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Ferreira, P.F.A.; Bertholoto, D.M.; Nunes, J.F.; Sant’Anna, G.S.L.; Patricio, T.C.D.C.; Xavier, J.F.; de Souza, H.D.F.; Coelho, S.M.O.; de Souza, M.M.S.; Coelho, I.D.S. Antimicrobial resistance in agricultural environments in Brazil—A concern from a One Health perspective. *Rev. Bras. Ciênc. Solo* **2025**, *49*, e0240152. [\[CrossRef\]](#)
12. Parada, J.; Galas, M.; Faccone, D.; Tamiozzo, P.; Carranza, A. Antibiotic resistance and associated resistance determinants in different *Salmonella enterica* serovars isolated from pigs in Argentina. *Vet. World* **2022**, *15*, 1215. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Superintendencia de Servicios Sanitarios (SISS). *Informe Sobre Reutilización de Aguas Servidas Tratadas*; Ministerio de Obras Públicas: Santiago, Chile, 2024.

14. *Decreto Supremo N° 90/2000*; Norma de Emisión para la Regulación de Contaminantes Asociados a las Descargas de Residuos Líquidos a Aguas Marinas y Continentales Superficiales. Ministerio Secretaría General de la Presidencia: Santiago, Chile, 2000.
15. *Decreto Supremo N° 4/2009*; Reglamento para el Manejo de Lodos Generados en Plantas de Tratamiento de Aguas Servidas. Ministerio Secretaría General de la Presidencia: Santiago, Chile, 2009.
16. *Ley N° 21.075*; Ley Sobre Reuso de Aguas Grises. Congreso Nacional de Chile: Valparaíso, Chile, 2018.
17. *Norma Chilena NCh 1333/1978*; Requisitos de Calidad del Agua para Diferentes Usos. Instituto Nacional de Normalización (INN): Santiago, Chile, 1978.
18. *Norma Chilena NCh 409/1*; Norma Chilena de Calidad del Agua Potable. Instituto Nacional de Normalización (INN): Santiago, Chile, 2005.
19. Mansilla, S.; Portugal, J.; Bayona, J.M.; Matamoros, V.; Leiva, A.M.; Vidal, G.; Piña, B. Compounds of emerging concern as new plant stressors linked to water reuse and biosolid application in agriculture. *J. Environ. Chem. Eng.* **2021**, *9*, 105198. [\[CrossRef\]](#)
20. Villamar, C.A.; Vera-Puerto, I.; Rivera, D.; de la Hoz, F. Reuse and recycling of livestock and municipal wastewater in Chilean agriculture: A preliminary assessment. *Water* **2018**, *10*, 817. [\[CrossRef\]](#)
21. Ministerio de Salud de Chile. *Plan Nacional Contra la Resistencia a los Antimicrobianos: Chile 2021–2025*; Ministerio de Salud: Santiago, Chile, 2021.
22. Bengtsson-Palme, J.; Larsson, D.J. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. *Environ. Int.* **2016**, *86*, 140–149. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Manaia, C.M.; Macedo, G.; Fatta-Kassinos, D.; Nunes, O.C. Antibiotic resistance in urban aquatic environments: Can it be controlled? *Appl. Microbiol. Biotechnol.* **2016**, *100*, 1543–1557. [\[PubMed\]](#)
24. Guo, Y.; Qiu, T.; Gao, M.; Sun, Y.; Cheng, S.; Gao, H.; Wang, X. Diversity and abundance of antibiotic resistance genes in rhizosphere soil and endophytes of leafy vegetables: Focusing on the effect of the vegetable species. *J. Hazard. Mater.* **2021**, *415*, 125595. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Petrović, M.; Hernando, D.; Díaz-Cruz, S.; Barceló, D. Liquid chromatography–tandem mass spectrometry for the analysis of pharmaceutical residues in environmental samples: A review. *J. Chromatogr. A* **2005**, *1067*, 1–14. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Li, H.; Jiang, E.; Wang, Y.; Zhong, R.; Zhou, J.; Wang, T.; Liu, M. Natural organic matters promoted conjugative transfer of antibiotic resistance genes: Underlying mechanisms and model prediction. *Environ. Int.* **2022**, *170*, 107653. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Wang, S.; Li, W.; Xi, B.; Cao, L.; Huang, C. Mechanisms and influencing factors of horizontal gene transfer in composting system: A review. *Sci. Total Environ.* **2024**, *955*, 177017. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Huang, H.; Chen, Y.; Zheng, X.; Su, Y.; Wan, R.; Yang, S. Distribution of tetracycline resistance genes in anaerobic treatment of waste sludge: The role of pH in regulating tetracycline resistant bacteria and horizontal gene transfer. *Bioresour. Technol.* **2016**, *218*, 1284–1289. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Jong, M.-C.; Harwood, C.R.; Blackburn, A.; Snape, J.R.; Graham, D.W. Impact of redox conditions on antibiotic resistance conjugative gene transfer frequency and plasmid fate in wastewater ecosystems. *Environ. Sci. Technol.* **2020**, *54*, 14752–14762. [\[CrossRef\]](#)
30. Zhang, J.; Chen, J.; Wang, C.; Wang, P.; Gao, H.; Feng, B.; Fu, J. Vertical variation of antibiotic resistance genes and their interaction with environmental nutrients in sediments of Taihu lake. *J. Environ. Manag.* **2024**, *370*, 122661. [\[CrossRef\]](#)
31. Cuencas Regenerativas, de la crisis a la Seguridad Hídrica. Hoja de Ruta Maipo y Maule. In *Escenarios Hídricos 2030-EH2030*; Fundación Chile: Santiago, Chile, 2022.
32. Instituto Nacional de Estadística-Chile (INE). Censos de Población y Vivienda. 2024. Available online: <https://censo2024.ine.gob.cl/> (accessed on 16 April 2026).
33. Ministerio del Medio Ambiente (MMA). *Declara Santuario de la Naturaleza Laguna de Bato*; Ministerio del Medio Ambiente (MMA): Santiago, Chile, 2024.
34. López, D.; Fuenzalida, D.; Vera, I.; Rojas, K.; Vidal, G. Relationship between the removal of organic matter and the production of methane in subsurface flow constructed wetlands designed for wastewater treatment. *Ecol. Eng.* **2015**, *83*, 296–304. [\[CrossRef\]](#)
35. APHA/AWWA/WEF. *Standard Methods for the Examination of Water and Wastewater*, 23rd ed.; American Public Health Association: Washington, DC, USA, 2017.
36. Sun, S.; Wang, Q.; Wang, N.; Yang, S.; Qi, H. High-risk antibiotics positively correlated with antibiotic resistance genes in five typical urban wastewater. *J. Environ. Manag.* **2023**, *342*, 118296. [\[CrossRef\]](#)
37. Bueno, I.; Verdugo, C.; Jimenez-Lopez, O.; Alvarez, P.P.; Gonzalez-Rocha, G.; Lima, C.A.; Travis, D.A.; Wass, B.; Zhang, Q.; Ishii, S.; et al. Role of wastewater treatment plants on environmental abundance of Antimicrobial Resistance Genes in Chilean rivers. *Int. J. Hyg. Environ. Health* **2020**, *223*, 56–64. [\[CrossRef\]](#)
38. Beretsou, V.G.; Michael-Kordatou, I.; Michael, C.; Santoro, D.; El-Halwagy, M.; Jäger, T.; Besselink, H.; Schwartz, T.; Fatta-Kassinos, D. A chemical, microbiological and (eco)toxicological scheme to understand the efficiency of UV-C/H₂O₂ oxidation on antibiotic-related microcontaminants in treated urban wastewater. *Sci. Total Environ.* **2020**, *744*, 140835. [\[CrossRef\]](#) [\[PubMed\]](#)

39. Moura, A.; Henriques, I.; Smalla, K.; Correia, A. Wastewater bacterial communities bring together broad-host range plasmids, integrons and a wide diversity of uncharacterized gene cassettes. *Res. Microbiol.* **2011**, *162*, 244–250.
40. Bartlett, J.M.; Stirling, D. *PCR Protocols*; Humana Press: Totowa, NJ, USA, 2003; Volume 226, pp. 3–525.
41. Pavez-Muñoz, E.; González, C.; Fernández-Sanhueza, B.; Sánchez, F.; Escobar, B.; Ramos, R.; Fuenzalida, V.; Galarce, N.; Arriagada, G.; Neira, V.; et al. Antimicrobial usage factors and resistance profiles of Shiga toxin-producing *Escherichia coli* in backyard production systems from Central Chile. *Front. Vet. Sci.* **2021**, *7*, 595149. [[CrossRef](#)] [[PubMed](#)]
42. Cordero, R.R.; Feron, S.; Damiani, A.; Carrasco, J.; Karas, C.; Wang, C.; Kraamwinkel, C.T.; Beaulieu, A. Extreme fire weather in Chile driven by climate change and El Niño–Southern Oscillation (ENSO). *Sci. Rep.* **2024**, *14*, 1974. [[CrossRef](#)] [[PubMed](#)]
43. Rocco, M.; Rubio, M.A. Chemical behavior of chromium, iron, lead, molybdenum, manganese and zinc in the surface water of two urban Lagoons in Santiago, Chile. *Fresenius Environ. Bull.* **2010**, *19*, 438–446.
44. Liu, C.; Du, Y.; Zhong, J.; Zhang, L.; Huang, W.; Han, C.; Gu, X. From macrophyte to algae: Differentiated dominant processes for internal phosphorus release induced by suspended particulate matter deposition. *Water Res.* **2022**, *224*, 119067. [[CrossRef](#)] [[PubMed](#)]
45. Liao, H.; Lu, X.; Rensing, C.; Friman, V.P.; Geisen, S.; Chen, Z.; Zhu, Y. Hyperthermophilic composting accelerates the removal of antibiotic resistance genes and mobile genetic elements in sewage sludge. *Environ. Sci. Technol.* **2018**, *52*, 266–276. [[PubMed](#)]
46. Li, W.; Zhang, G. Detection and various environmental factors of antibiotic resistance gene horizontal transfer. *Environ. Res.* **2022**, *212*, 113267. [[CrossRef](#)] [[PubMed](#)]
47. Henze, M.; Harremoës, P.; la Cour Jansen, J.; Arvin, E. *Wastewater Treatment: Biological and Chemical Processes*, 3rd ed.; Springer: Berlin, Germany, 2001; p. 422.
48. Xie, Y.; Chen, Y.; Lian, Q.; Yin, H.; Peng, J.; Sheng, M.; Wang, Y. Enhancing real-time prediction of effluent water quality of wastewater treatment plant based on improved feedforward neural network coupled with optimization algorithm. *Water* **2022**, *14*, 1053. [[CrossRef](#)]
49. Tchobanoglous, G.; Stensel, H.D.; Tsuchihashi, R.; Burton, F.L.; Abu-Orf, M.; Bowden, G. *Wastewater Engineering: Treatment and Resource Recovery*, 5th ed.; McGraw-Hill: New York, NY, USA, 2014.
50. Ravndal, K.T.; Opsahl, E.; Bagi, A.; Kommedal, R. Wastewater characterisation by combining size fractionation, chemical composition and biodegradability. *Water Res.* **2018**, *131*, 151–160. [[CrossRef](#)] [[PubMed](#)]
51. Sorn, S.; Sulfikar, M.; Lin, M.-Y.; Shuto, M.; Noguchi, M.; Honda, R.; Yamamoto-Ikemoto, R.; Watanabe, T. Potential impact factors on the enhancement of antibiotic resistance in a lake environment. *J. Water Health* **2022**, *20*, 1017–1026. [[CrossRef](#)] [[PubMed](#)]
52. Leiva, A.M.; Monsalves, N.; Gómez, G.; Vidal, G. Fate of antibiotic resistance genes in organic wastes from sewage treatment plants in the framework of circular economy. In *Occurrence and Behavior of Emerging Contaminants in Organic Wastes and Their Control Strategies*, 1st ed.; Huang, K., Bhat, S.A., Eds.; Elsevier: Amsterdam, The Netherlands, 2024; p. 282.
53. Dirección General de Aguas (DGA). *Normas y Criterios para la Calidad de Aguas: Red de Control de Calidad de Aguas en Lagos y Lagunas*; Ministerio de Obras Públicas, Gobierno de Chile: Santiago, Chile, 2015.
54. Habit, E.; Parra, O. *Biodiversidad de Ecosistemas de Agua Dulce*; Ministerio del Medio Ambiente: Santiago, Chile, 2016.
55. Sun, S.; Lu, C.; Zhang, Y.; Wang, Y.; Wang, H.; Wang, Z. Antibiotic resistance gene abundance and bacterial community structure in soils altered by ammonium and nitrate concentrations. *Soil Biol. Biochem.* **2020**, *149*, 107965. [[CrossRef](#)]
56. Langbehn, R.K.; Michels, C.; Soares, H.M. Antibiotics in wastewater: From its occurrence to the biological removal by environmentally conscious technologies. *Environ. Pollut.* **2021**, *275*, 116603. [[CrossRef](#)] [[PubMed](#)]
57. Rizzo, L.; Manaia, C.; Merlin, C.; Schwartz, T.; Dagot, C.; Ploy, M.C.; Michael, I.; Fatta-Kassinos, D. Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: A review. *Sci. Total Environ.* **2013**, *447*, 345–360. [[CrossRef](#)] [[PubMed](#)]
58. Harnisz, M. Total resistance of native bacteria as an indicator of changes in the water environment. *Environ. Pollut.* **2013**, *174*, 85–92. [[CrossRef](#)] [[PubMed](#)]
59. Manaia, C.M.; Rocha, J.; Scaccia, N.; Marano, R.; Radu, E.; Biancullo, F.; Cerqueira, F.; Fortunato, G.; Iakovides, I.C.; Zammit, I.; et al. Antibiotic resistance in wastewater treatment plants: Tackling the black box. *Environ. Int.* **2018**, *115*, 312–324. [[CrossRef](#)] [[PubMed](#)]
60. Leiva, A.M.; Gómez, G.; González-Rocha, G.; Piña, B.; Vidal, G. Performance of full-scale rural wastewater treatment plants in the reduction of antibiotic-resistant bacteria and antibiotic resistance genes from small-city effluents. *J. Environ. Chem. Eng.* **2024**, *12*, 112322. [[CrossRef](#)]
61. Hazra, M.; Watts, J.E.; Williams, J.B.; Joshi, H. An evaluation of conventional and nature-based technologies for controlling antibiotic-resistant bacteria and antibiotic-resistant genes in wastewater treatment plants. *Sci. Total Environ.* **2024**, *917*, 170433. [[CrossRef](#)] [[PubMed](#)]
62. Hiller, C.X.; Hübner, U.; Fajnorova, S.; Schwartz, T.; Drewes, J.E. Antibiotic microbial resistance (AMR) removal efficiencies by conventional and advanced wastewater treatment processes: A review. *Sci. Total Environ.* **2019**, *685*, 596–608. [[CrossRef](#)] [[PubMed](#)]

63. Leiva, A.M.; Piña, B.; Vidal, G. Antibiotic resistance dissemination in wastewater treatment plants: A challenge for the reuse of treated wastewater in agriculture. *Rev. Environ. Sci. Biotechnol.* **2021**, *20*, 1043–1072. [\[CrossRef\]](#)
64. Bartholin, M.J.; Barrera-Vega, B.; Berrocal-Silva, L. Antibiotic-resistant bacteria in environmental water sources from southern Chile: A potential threat to human health. *Microbiol. Res.* **2023**, *14*, 1764–1773. [\[CrossRef\]](#)
65. Stocker, M.; Smith, J.; Pachepsky, Y. Spatial variation of tetracycline-resistant *E. coli* and relationships with water quality variables in irrigation water: A pilot study. *Appl. Microbiol.* **2023**, *3*, 504–518. [\[CrossRef\]](#)
66. Fernández, G.; Sandoval, M.; Vidal, G. *Serie N°6: Reciclaje en la Agricultura: Disposición de Bisólidos al Suelo*; Serie Comunicacional CRHIAM; Universidad de Concepción: Concepción, Chile, 2019.
67. Sepúlveda, R.; Leiva, A.M.; Cornejo, P.; Vidal, G. *Serie N°7: Salinización de Suelos Agrícolas por el Reúso de Aguas Servidas Tratadas*; Serie Comunicacional CRHIAM; Universidad de Concepción: Concepción, Chile, 2018.
68. Servicio Agrícola y Ganadero (SAG). *Resolución Exenta N° 1129/2022: Requisitos Para el Registro y Uso de Antimicrobianos Veterinarios*; Ministerio de Agricultura: Santiago, Chile, 2022.
69. Servicio Agrícola y Ganadero (SAG). *Resolución Exenta N° 6801/2020: Regulaciones Sobre Uso y Comercialización de Antimicrobianos Veterinarios*; Ministerio de Agricultura: Santiago, Chile, 2020.
70. Jia, J.; Guan, Y.; Cheng, M.; Chen, H.; He, J.; Wang, S.; Wang, Z. Occurrence and distribution of antibiotics and antibiotic resistance genes in Ba River, China. *Sci. Total Environ.* **2018**, *642*, 1136–1144. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Shi, W.; Zhang, H.; Li, J.; Liu, Y.; Shi, R.; Du, H.; Chen, J. Occurrence and spatial variation of antibiotic resistance genes (ARGs) in the Hetao Irrigation District, China. *Environ. Pollut.* **2019**, *251*, 792–801. [\[CrossRef\]](#) [\[PubMed\]](#)
72. Lachmayr, K.L.; Kerkhof, L.J.; Di Rienzo, A.G.; Cavanaugh, C.M.; Ford, T.E. Quantifying nonspecific TEM β -lactamase (*bla*TEM) genes in a wastewater stream. *Appl. Environ. Microbiol.* **2009**, *75*, 203–211. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Thomas, J.L.; Slawson, R.M.; Taylor, W.D. *Salmonella* serotype diversity and seasonality in urban and rural streams. *J. Appl. Microbiol.* **2013**, *114*, 907–922. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Valenzuela-Flores, J.H. *Resistencia a los Antibióticos en Cepas de Salmonella spp. Aisladas Desde Cauces de Agua de la Región Metropolitana y su Asociación con el Área Geográfica*; Universidad de Chile: Santiago, Chile, 2016.
75. Sodhi, K.K.; Kumar, M.; Singh, D.K. Insight into the amoxicillin resistance, ecotoxicity, and remediation strategies. *J. Water Process Eng.* **2021**, *39*, 101858. [\[CrossRef\]](#)
76. Cerqueira, F.; Matamoros, V.; Bayona, J.; Piña, B. Antibiotic resistance genes distribution in microbiomes from the soil-plant-fruit continuum in commercial *Lycopersicon esculentum* fields under different agricultural practices. *Sci. Total Environ.* **2019**, *652*, 660–670. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Chen, Q.L.; Hu, H.W.; Zhu, D.; Ding, J.; Yan, Z.Z.; He, J.Z.; Zhu, Y.G. Host identity determines plant associated resistomes. *Environ. Pollut.* **2020**, *258*, 113709. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Qi, Z.; Jin, S.; Guo, X.; Tong, H.; Ren, N.; You, S. Distribution and transmission of β -lactamase resistance genes in meal-to-milk chain on dairy farm. *Environ. Pollut.* **2023**, *331*, 121831. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Ling, Z.; Yang, Y.; Huang, Y.; Zou, S.; Luan, T. A preliminary investigation on the occurrence and distribution of antibiotic resistance genes in the Beijiang River, South China. *J. Environ. Sci.* **2013**, *25*, 1656–1661. [\[CrossRef\]](#)
80. Adekanmbi, A.O.; Adejoba, A.T.; Banjo, O.A.; Saki, M. Detection of *sul1* and *sul2* genes in sulfonamide-resistant bacteria (SRB) from sewage, aquaculture sources, animal wastes and hospital wastewater in South-West Nigeria. *Gene Rep.* **2020**, *20*, 100742. [\[CrossRef\]](#)
81. Lekunberri, I.; Villagrasa, M.; Balcázar, J.L.; Borrego, C.M. Contribution of bacteriophage and plasmid DNA to the mobilization of antibiotic resistance genes in a river receiving treated wastewater discharges. *Sci. Total Environ.* **2017**, *601*, 206–209. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Pei, R.; Kim, S.C.; Carlson, K.H.; Pruden, A. Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Res.* **2006**, *40*, 2427–2435. [\[CrossRef\]](#) [\[PubMed\]](#)
83. von Wintersdorff, C.J.; Penders, J.; Stobberingh, E.E.; Oude Lashof, A.M.; Hoebe, C.J.; Savelkoul, P.H.; Wolffs, P.F. High rates of antimicrobial drug resistance gene acquisition after international travel, The Netherlands. *Emerg. Infect. Dis.* **2014**, *20*, 649. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Bento, J.T.; Gomes-Gonçalves, S.; Cruz, R.; Esteves, F.; Baptista, A.L.; Aires Pereira, M.; Caseiro, P.; Carreira, P.; Figueira, L.; Mesquita, J.R.; et al. The prevalence of antimicrobial resistance genes in the environments of small ruminant farms from Central Portugal. *Antibiotics* **2025**, *14*, 576. [\[CrossRef\]](#) [\[PubMed\]](#)
85. Proia, L.; Von Schiller, D.; Sánchez-Melsió, A.; Sabater, S.; Borrego, C.M.; Rodríguez-Mozaz, S.; Balcázar, J.L. Occurrence and persistence of antibiotic resistance genes in river biofilms after wastewater inputs in small rivers. *Environ. Pollut.* **2016**, *210*, 121–128. [\[CrossRef\]](#) [\[PubMed\]](#)
86. Reichert, G.; Hilgert, S.; Alexander, J.; de Azevedo, J.C.R.; Morck, T.; Fuchs, S.; Schwartz, T. Determination of antibiotic resistance genes in a WWTP-impacted river in surface water, sediment, and biofilm: Influence of seasonality and water quality. *Sci. Total Environ.* **2021**, *768*, 144526. [\[CrossRef\]](#) [\[PubMed\]](#)

87. García, J.; García Galán, M.J.; Day, J.W.; Boopathy, R.; White, J.R.; Wallace, S.; Hunter, R.G. A review of emerging organic contaminants (EOCs), antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) in the environment: Increasing removal with wetlands and reducing environmental impacts. *Bioresour. Technol.* **2020**, *307*, 123228. [\[CrossRef\]](#) [\[PubMed\]](#)
88. Li, S.; Zhang, R.; Hu, J.; Shi, W.; Kuang, Y.; Guo, X.; Sun, W. Occurrence and removal of antibiotics and antibiotic resistance genes in natural and constructed riverine wetlands in Beijing, China. *Sci. Total Environ.* **2019**, *664*, 546–553. [\[CrossRef\]](#) [\[PubMed\]](#)
89. Fang, H.; Zhang, Q.; Nie, X.; Chen, B.; Xiao, Y.; Zhou, Q.; Liang, X. Occurrence and elimination of antibiotic resistance genes in a long term operation integrated surface flow constructed wetland. *Chemosphere* **2017**, *173*, 99–106. [\[CrossRef\]](#) [\[PubMed\]](#)
90. Sabri, N.; Schmitt, H.; van der Zaan, B.; Gerritsen, H.; Rijnaarts, H.; Langenhoff, A. Performance of full scale constructed wetlands in removing antibiotics and antibiotic resistance genes. *Sci. Total Environ.* **2021**, *786*, 147–368. [\[CrossRef\]](#)
91. Abou-Kandil, A.; Shibli, A.; Azaizeh, H.; Wolff, D.; Wick, A.; Jadoun, J. Fate and removal of bacteria and antibiotic resistance genes in horizontal subsurface constructed wetlands: Effect of mixed vegetation and substrate type. *Sci. Total Environ.* **2021**, *759*, 144193. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Amato, M.; Dasí, D.; González, A.; Ferrús, M.A.; Castillo, M.A. Occurrence of antibiotic resistant bacteria and resistance genes in agricultural irrigation waters from Valencia city (Spain). *Agric. Water Manag.* **2021**, *256*, 107097. [\[CrossRef\]](#)
93. Korajkic, A.; Wanjugi, P.; Brooks, L.; Cao, Y.; Harwood, V.J. Persistence and decay of fecal microbiota in aquatic habitats. *Microbiol. Mol. Biol. Rev.* **2019**, *83*, e00005–19. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Brissaud, F.; Tournoud, M.G.; Drakides, C.; Lazarova, V. Mixing and its impact on faecal coliform removal in a stabilisation pond. *Water Sci. Technol.* **2003**, *48*, 75–80. [\[CrossRef\]](#) [\[PubMed\]](#)
95. He, J.; Valeo, C.; Chu, A. Variation in water quality of a stormwater pond from diurnal thermal stratification. *J. Water Resour. Hydraul. Eng.* **2015**, *4*, 181–190. [\[CrossRef\]](#)
96. Skarbøvik, E.; Perović, A.; Shumka, S.; Nagothu, U.S. Nutrient inputs, trophic status and water management challenges in the transboundary Lake Skadar/Shkodra, Western Balkans. *Arch. Biol. Sci.* **2014**, *66*, 667–681. [\[CrossRef\]](#)
97. Christou, A.; Agüera, A.; Bayona, J.M.; Cytryn, E.; Fotopoulou, V.; Lambropoulou, D.; Manaia, C.M.; Michael, C.; Revitt, M.; Schröder, P.; et al. The potential implications of reclaimed wastewater reuse for irrigation on the agricultural environment. *Water Res.* **2017**, *123*, 448–467. [\[CrossRef\]](#) [\[PubMed\]](#)
98. Rodríguez-López, L.; González-Rodríguez, L.; Duran-Llacer, I.; Cardenas, R.; Urrutia, R. Spatio-temporal analysis of chlorophyll in six Araucanian lakes of Central-South Chile from Landsat imagery. *Ecol. Inform.* **2021**, *65*, 101431. [\[CrossRef\]](#)
99. Liu, Q.; Jia, J.; Hu, H.; Li, X.; Zhao, Y.; Wu, C. Nitrogen and phosphorus limitations promoted bacterial nitrate metabolism and propagation of antibiotic resistome in the phycosphere of *Auxenoclorella pyrenoidosa*. *J. Hazard. Mater.* **2024**, *468*, 133786. [\[CrossRef\]](#) [\[PubMed\]](#)
100. Yu, Q.; Han, Q.; Shi, S.; Sun, X.; Wang, X.; Wang, S.; Yang, J.; Su, W.; Nan, Z.; Li, H. Metagenomics reveals the response of antibiotic resistance genes to elevated temperature in the Yellow River. *Sci. Total Environ.* **2023**, *859*, 160324. [\[CrossRef\]](#) [\[PubMed\]](#)
101. McGough, S.F.; MacFadden, D.R.; Hattab, M.W.; Mølbak, K.; Santillana, M. Rates of increase of antibiotic resistance and ambient temperature in Europe: A cross-national analysis of 28 countries between 2000 and 2016. *Euro Surveill.* **2020**, *25*, 1900414. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Burnham, J.P. Climate change and antibiotic resistance: A deadly combination. *Ther. Adv. Infect. Dis.* **2021**, *8*, 2049936121991374. [\[CrossRef\]](#) [\[PubMed\]](#)
103. United Nations Environment Programme (UNEP). *Bracing for Superbugs: Strengthening Environmental Action in the One Health Response to Antimicrobial Resistance*; UNEP: Nairobi, Kenya, 2023.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.