

# Water quality of artificial canals used for agricultural purposes affected by urban and agricultural activities through a chemical and microbial perspective

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## ABSTRACT

Water from artificial canals in reclaimed floodplains is primarily used for crop irrigation; however, its quality is often compromised by chemical and microbial hazards, which may pose a threat to crop safety and quality. The main objectives of the present work were a) to analyse the chemical and microbial properties of water in a network of artificial canals; b) to identify the relationships among such parameters; and c) to detect spatial shifts in water quality (upstream and downstream) along some key canals. The canals were grouped into four sectors based on water origin: Sector A (urban), Sector B (wastewater treatment plants), Sector C (rural), and the Canale Emiliano Romagnolo (CER, Po River). The three years data showed a concentration decrease of most of the chemical targets, with the following order, sector B > sector A > sector C > CER. For microbial parameters, Sectors A and B exhibited higher biological pollution than Sector C and CER. Results were generally under the Italian legislation limits for water reuse. The multiple linear regression models revealed a generally positive correlation between microbial populations and sectors influenced by urban activities (Sectors A and B), while the relationships between microbial populations and chemical properties were less clear. Sodium adsorption ratio was the main parameter distinguishing canals in Sector B, whereas canals in sector A were characterized by overall higher P-PO<sub>4</sub> and N-NO<sub>3</sub> concentrations compared to sector C and CER. Upstream-downstream comparison generally indicated either stable or improved water quality, with the exception of a canal affected by the intrusion of poor-quality water. Overall, this study demonstrates that wastewater likely plays a dominant role in shaping water quality within artificial floodplain canals, highlighting the pronounced vulnerability of these canals to point-source pollution.

## 1. Introduction

Water is the cornerstone of economic gain and supports the wildlife. Humans get different benefits from freshwater, which includes water for human consumption (drinking and domestic) and water for agricultural, urban and industrial activities (Jackson et al., 2001). Globally, almost 70 % of water is used for agricultural purposes (Brown and Matlock, 2011), in particular for irrigation. The increasing scarcity of freshwater, drastically affected by overconsumption and shifting precipitation patterns, impose the implementation of the use of non-traditional water sources in agriculture, such as wastewater for treatment plants and artificial canals.

In the last century most of the riverine floodplains at global scale

were reclaimed through the construction of artificial canals, levees and dams (Knox et al., 2022). The artificial canals are important providers of hydraulic safety, flood risk mitigation and water storage for seasonal irrigation (Poesio et al., 2023; Shastry et al., 2020). Generally, these canals suffer from pollution and microbial hazards deriving from human, industrial and cropping activities (Das Sarkar et al., 2020; Lucas et al., 2022). The main chemical pollutant parameters are nitrates, sulphates, phosphates, copper and iron, which are often associated with agricultural-based activities (fertilizers, pesticides and herbicides). In waterbodies, they are responsible for eutrophication, excessive algal blooms and low oxygen concentrations (Das Sarkar et al., 2020; Lucas et al., 2022). But, also chemical parameters such as dissolved oxygen (DO), chemical and biological oxygen demand (COD and BOD) and

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electrical conductivity (EC) (Elkiran et al., 2019; Sharma et al., 2020; Shil et al., 2019) represent key parameters to be evaluated. In addition, pathogenic microorganisms (i.e.: *Escherichia coli*, Salmonella and fecal streptococci) derived from wastewater plants and livestock/human activities must be monitored to avoid a massive presence of water-borne pathogens within the irrigation waters (Henriot et al., 2022; Hubbart et al., 2022). Therefore, the safety and quality are becoming a major concern, as there is a potential risk of transferring such contaminants to the soil and crops (Perulli et al., 2024). From a regulatory point of view, the World Health Organization recommends a flexible guideline for a use of treated wastewater (TW) in agriculture at global level, but most countries decided to use more restricted internal regulations. In Italy, for example, TW from a wastewater treatment plant can be used in agriculture for irrigation purposes according to the Decree of Ministry for Environment (D.M., 2003) (direct use) and to the Decree of Ministry for Environment (D.M., 2006) (indirect use), but no discrimination in the pollutant thresholds is foreseen for the different cultivated crops (Deloitte, 2015). In the meantime, at European level, the parliament developed the Regulation 2020/741 (in force since June 2023) on minimum requirements for the direct TW use in agriculture (European Commission, 2020). The new EU Regulation allows the adoption of the most appropriate and sustainable treatment from a technical and economic standpoint, based on the crops present in the area to be served (Cosenza et al., 2024). The regulation, that has been implemented in the Italian D.lgs No. 39/2023 (D.lgs, 2023), classifies the reclaimed water quality into four classes (A to D), and each class establishes the limits to be achieved for Biochemical Oxygen Demand (BOD) total suspended solids (TSS), turbidity, and *E. coli*. The limits set by the European regulation on water reuse are stricter than the discharge limits currently established by Italian legislation (D.M., 2006), which is the legislative reference adopted by the reclamation authorities for indirect use of TW. For this purpose, reclamation consortia authorities, within the scope of their competences, have the right to create and manage canal networks and pumping systems, allowing the use of TW for crop irrigation. Hence, multi-year spatial monitoring of water quality is pivotal for an efficient management of water resource (Gikas, 2014; Neale et al., 2003). The evaluation of water quality and the study of the driving forces may be crucial to provide useful information for improving irrigation practices and safeguarding soil and crop quality (Schnoor, 2014; Singh et al., 2005), especially in reclaimed floodplains. In this context, the use of models capable of identifying the key factors influencing aquatic conditions is essential for monitoring programs and for supporting water resource management at the national level. In this regard, the application of various multivariate statistical techniques such as cluster analysis, principal component analysis (Kumar et al., 2019; Ustaoglu and Tepe, 2019; Vittori Antisari et al., 2010), artificial neural network (Bui et al., 2020; Khoi et al., 2022; Noori et al., 2020), tree-based models (Bui et al., 2020; Khoi et al., 2022; Tiyasha et al., 2021) and multiple liner regression models (Ahmed et al., 2019; Elkiran et al., 2019; Kouadri et al., 2021) help to better understand the main driving factors affecting water quality.

The main objective of the present work was to compare the water quality of 27 artificial canals network intended for irrigation, supplied by 4 hydraulic districts crossing the metropolitan area of Bologna (Italy). The study focused on: 1) three-years monitoring (2020–2022) of chemical and microbiological parameters; 2) the relationships among such parameters; 3) water properties shifts (up- and downstream analysis) along some pivotal canals.

The present work wants to investigate in more detail the local situation around Bologna (Italy) and give a first response to the quality water assessment in the light of the Italian legislation for the water reuse in agriculture and the new EU regulation.

## 2. Materials and methods

### 2.1. Study area

The study area is managed by the Renana Reclamation Consortium Authority and it is located along the southern boundary of the Po River sedimentary basin (Bianchini et al., 2012; Carminati and Martinelli, 2002). The area is drained by the Reno River which ensures proper rainwater runoff and supplies surface water for crop irrigation. The climate is temperate with a mean annual air temperature of 14.5 °C and a mean cumulative annual precipitation of 575 mm, with fall season as the wettest one.

The floodplain macro-district covers 41 % of the total district extension in the metropolitan area of Bologna; the artificial drainage network is divided into 32 main basins, 20 located to the right of the Reno River and 12 to the left. It is an important network of artificial remediation canals counting around 2000 km of length and crossing an area of around 1400 km<sup>2</sup> (Fig. 1). In recent decades, thanks to 48 pumping stations equipped with 109 pumps, it has distributed an average of 60 million m<sup>3</sup> of water per year for irrigation purposes, enabling the irrigation of approximately 18,000 ha of land. The study area included 4 hydraulic sectors (Sector A, B, C and CER) according to the water origin source. The Sector A includes two artificial canals (Navile and Savena Abbandonato) which draw water from the Reno River and the Savena River, respectively. They cross, as underground canals, the city of Bologna; then, as open-air canals, they flow towards north/north-east and expand over the floodplains. The Sector B is the area of artificial canals collecting water from 8 wastewater treatment plants within a radius of 60 km. Sector C is a large area located at north, north-east of Bologna, built up of artificial canals fed by CER and used for runoff water collection and irrigation. The CER (Canale Emiliano-Romagnolo) is one of the most important hydraulic infrastructures in northern Italy. It is a large cemented canal, 135 km long, covering an area of approximately 3000 km<sup>2</sup>. The CER was built for irrigation purposes along the Emilia Romagna region, distributing water from the Po River to the provinces of Ferrara, Bologna, Ravenna, Forlì-Cesena and Rimini.

### 2.2. Sampling

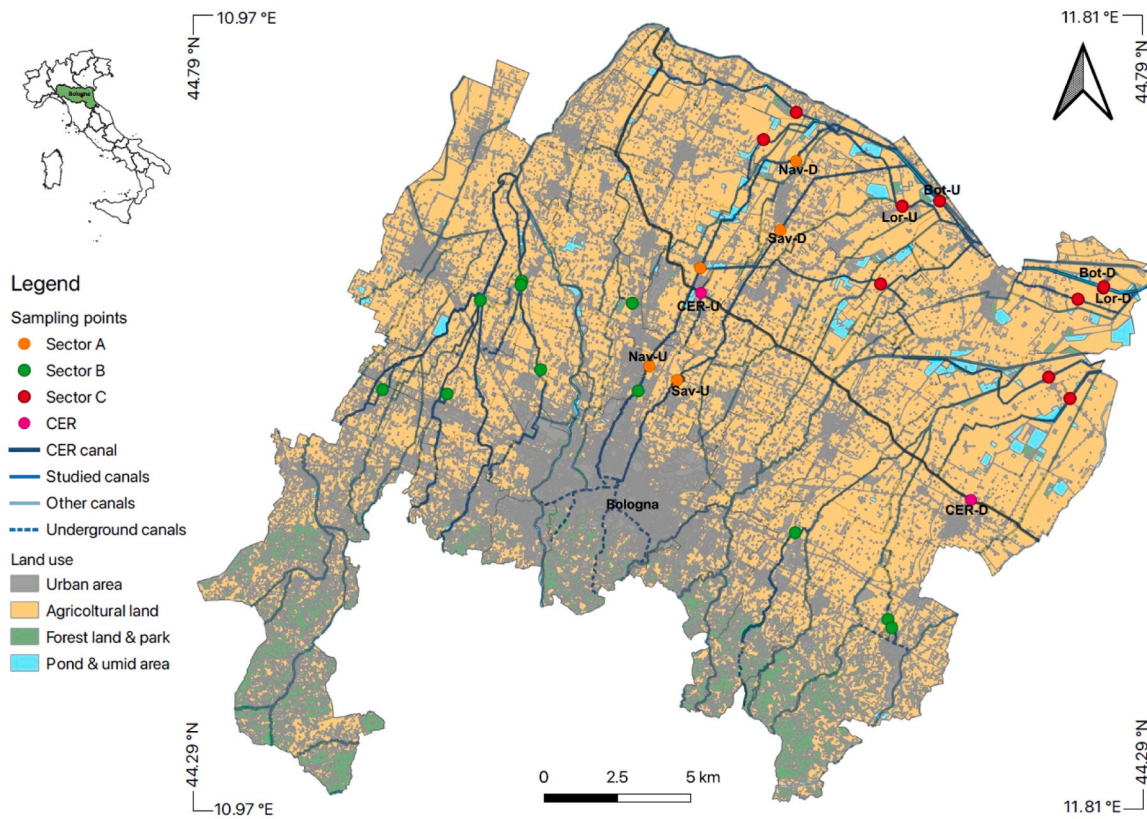
Monthly water sampling was carried out at 27 sites within the study area (Fig. 1) between April and September in 2020, 2021, and 2022, spanning the entire irrigation season: 5 sampling sites in Sector A, 11 sampling sites in Sector B, 10 sampling sites within Sector C and 2 sampling sites (one upstream and one downstream) 23 km apart along the CER, which can be considered as benchmark. Sector A and Sector C included upstream and downstream sampling points along some of the canals: specifically, “Navile” and “Savena Abbandonato” for sector A, and “Lorgana” and “La Botte” for sector C. The distance between upstream and downstream sampling points was about 18, 16, 15 and 12 km for Navile, Savena Abbandonato, Lorgana and La Botte, respectively. Concerning Sector B, no canals included upstream and downstream sampling; the water sampling was performed roughly 10 m downstream the treated wastewater discharges.

In Fig. 2 the temperature of air and water canals during the 3-years monitoring period are reported.

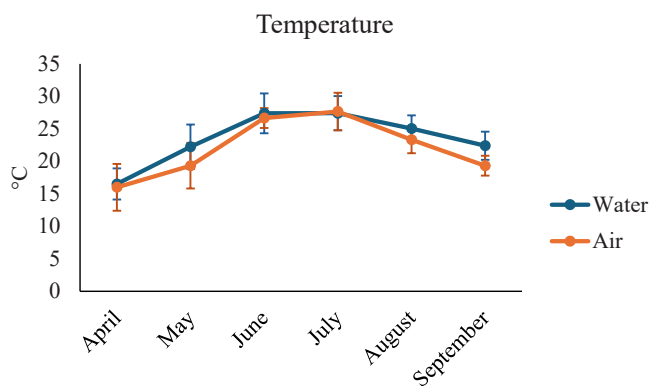
The water samples were stored in sterile Pyrex glasses and placed in cooler bags. The pH, water dissolved oxygen (DO), the ammonium (NH<sub>4</sub>-N), nitrate (NO<sub>3</sub>-N) nitrogen concentrations were measured in the field with a multiparameter probe Aquaread AP2000 (Aquaread Ltd., UK).

### 2.3. Chemical laboratory analyses

In the laboratory, water samples were filtered with Whatman 42 filter paper in order to determine the dissolved nitrogen (TN)



**Fig. 1.** Map of the study area. CER: Canale Emiliano Romagnolo; Nav: Navile canal; Sav: Savena Abbandonato canal; Lor: Lorgana canal; Bot: La Botte canal; U: upstream; D: downstream.



**Fig. 2.** Monthly mean air and water canal temperatures of the study area.

concentrations using TOC-V CPN analyser (Shimadzu, Japan) and the concentrations of total Cl, B, Ca, Mg, Na and Si using inductive coupled plasma optical emission spectrometry (ICP-OES, Arcos II, Kleve, Germany, Ametek Spectro) after acidification with high-purity HNO<sub>3</sub> (Suprapur, Merck, Germany). The concentrations of phosphates (PO<sub>4</sub><sup>3-</sup>) and sulphates (SO<sub>4</sub><sup>2-</sup>) were determined by Integrated Capillary High-Pressure Ionic Chromatography (Dionex, ICS 4000 Thermo Scientific). The carbonate (CaCO<sub>3</sub>) content was measured by titration with 0.02 M HCl (Ferronato et al., 2013). The chemical oxygen demand (COD) and the biological oxygen demand (BOD) were measured by a Hach Lange appropriate kit (Vittori Antisari et al., 2020). The chemical analyses are summarized in Table 1.

The sodium adsorption ratio (SAR), defined as the ratio of sodium concentration (Na) to the combined concentration of calcium and magnesium (Ca + Mg) in a given water sample was calculated.

$$SAR = \frac{Na}{\sqrt{\frac{Ca+Mg}{2}}}$$

For assessing the organic pollution due to anthropogenic activities within each sector, the organic pollution index (OPI) was calculated in accordance with Biedunkova et al. (2025) and Chen et al. (2023)

$$OPI = \frac{BODi}{BODr} + \frac{CODi}{CODr} + \frac{NO3 - Ni}{NO3 - Nr} + \frac{PO4i}{PO4r}$$

where BODi, CODi, NO<sub>3</sub>-Ni and PO<sub>4</sub>i are the concentrations of the monitored parameters, while BODr (40 mg L<sup>-1</sup>), CODr (160 mg L<sup>-1</sup>), NO<sub>3</sub>-Nr (20 mg L<sup>-1</sup>) and PO<sub>4</sub>r (30.7 mg L<sup>-1</sup>) are the maximum permissible concentration of such parameters in water according to the Decree of Ministry for Environment (D.M., 2006).

#### 2.4. Water microbiological analyses

Water samples were analysed for the enumeration of total coliforms, fecal coliforms, *Escherichia coli* and fecal streptococci (Tcol, Fcol, Ecol and Strep, respectively), according to ISO 9308-1:2014 and ISO 7899-2:2000, respectively. 100 mL of samples and their aliquots (1:10, 1:100 and 1:1000) were filtered through nitrocellulose membranes (0.45 µm pore size, 47 mm diameter, Sartorius). Membranes were placed onto Chromogenic Coliform Agar ad KF streptococcus agar (Oxoid, Thermofisher, Milan, Italy), and incubated at 37 °C for 24 h (Tcol and Ecol), 44 °C for Fcol ad 37 °C for 48 h (Strep). *E. coli* identity (5–10 blue/purple colonies from the countable dilution) was then confirmed by checking indole production and cytochrome oxidase activity.

Membranes with putative fecal streptococci (red-maroon or pink colonies) were then transferred to plates with Bile Aesculine Azide Agar (100072, Merck), and incubated for 2 h at 44 °C. Colonies that turned

**Table 1**

Summary of the analytical procedures for determining pH, dissolved oxygen (DO), ammonium nitrogen (NH<sub>4</sub>-N), nitrate nitrogen (NO<sub>3</sub>-N), total nitrogen (TN), Cl, B, Ca, Mg, Na, Si, PO<sub>4</sub><sup>3-</sup>, SO<sub>4</sub><sup>2-</sup>, CaCO<sub>3</sub>, chemical oxygen demand (COD) and biological oxygen demand in water samples.

Parameter	Instrument/ Methodology	Limit of detection	Accuracy	Reference
pH	Aquaread AP2000 (Aquaread Ltd., UK)	0.01	10 %	Astel et al., (2016); Obolewski et al., (2018)
DO	Aquaread AP2000 (Aquaread Ltd., UK)	0.01 mg L <sup>-1</sup>	1 %	Astel et al., (2016); Obolewski et al., (2018)
NH <sub>4</sub> -N	Aquaread AP2000 (Aquaread Ltd., UK)	0.1 mg L <sup>-1</sup>	10 %	Astel et al., (2016); Obolewski et al., (2018)
NO <sub>3</sub> -N	Aquaread AP2000 (Aquaread Ltd., UK)	0.05 mg L <sup>-1</sup>	10 %	Astel et al., (2016); Obolewski et al., (2018)
TN	TOC-V CPN analyser (Shimadzu, Japan)	0.005 mg L <sup>-1</sup>	3 %	Vittori Antisari et al., (2020)
Cl	Inductive coupled plasma optical emission spectrometry (Arcos II, Kleve, Germany, Ametec Spectro)	1 mg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)
B	Inductive coupled plasma optical emission spectrometry (Arcos II, Kleve, Germany, Ametec Spectro)	5 µg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)
Ca	Inductive coupled plasma optical emission spectrometry (Arcos II, Kleve, Germany, Ametec Spectro)	0.05 mg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)
Mg	Inductive coupled plasma optical emission spectrometry (Arcos II, Kleve, Germany, Ametec Spectro)	0.001 mg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)
Na	Inductive coupled plasma optical emission spectrometry (Arcos II, Kleve, Germany, Ametec Spectro)	0.05 mg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)
Si	Inductive coupled plasma optical emission spectrometry (Arcos II, Kleve, Germany, Ametec Spectro)	1 µg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)
PO <sub>4</sub> <sup>3-</sup>	Integrated Capillary High-Pressure Ionic Chromatography (Dionex, ICS 4000 Thermo Scientific)	50 µg L <sup>-1</sup>	5 %	Ferronato et al., (2013)
SO <sub>4</sub> <sup>2-</sup>	Integrated Capillary High-Pressure Ionic Chromatography (Dionex, ICS 4000 Thermo Scientific)	10 µg L <sup>-1</sup>	5 %	Ferronato et al., (2013)
CaCO <sub>3</sub>	Titration	5 mg L <sup>-1</sup>	10 %	Ferronato et al., (2013)
COD	Hach Lange	5 mg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)

**Table 1 (continued)**

Parameter	Instrument/ Methodology	Limit of detection	Accuracy	Reference
BOD	Hach Lange	4 mg L <sup>-1</sup>	5 %	Vittori Antisari et al., (2020)

dark brown to black with a typical dark halo were considered to be fecal streptococci colonies.

*Salmonella* spp. was detected according to the UNI EN ISO 19250:2013 procedure. Results were recorded as Log<sub>10</sub> of colony forming units (CFU) 100 mL<sup>-1</sup> for Tcol, Fcol, Ecol and Strep, and absence/presence for *Salmonella* spp.

## 2.5. Statistical analyses

The effect of sector types on water quality parameters was tested through the linear mixed-effects model using the “nlme” package with sampling months and sampling points as random factors. To evaluate the differences among sectors, the paired comparison was performed using “emmeans” package.

The water chemical parameters were analysed by principal component (PC) analysis to identify key chemical variables featuring water of the sectors. Afterwards, the scores of the PCs were evaluated for their implementation within the multiple regression models. In particular, the obtained scores were used within the multiple linear regression models built to fit relationships between water microbial properties (*i.e.*, Tcol, Fcol, Ecol and Strep) and the water chemical parameters and sector type.

The classification tree was used as predictive model to select the most significant predictors among chemical and microbial parameters for sector type. Prior performing the model, the dataset was splitted into training subset that included the data of water samples collected in 2020 and 2021, and test subset that included those of 2022. The classification tree model was developed on the training data subset and was implemented by caret package. The accuracy of the model was assessed through the confusion matrix function.

The Kruskal-Wallis *H* test was used to analyse the difference of water properties between upstream and downstream. All the statistical analysis was done using the R 4.3.0 software.

## 3. Results

### 3.1. The linear mixed-effects model

#### 3.1.1. Chemical and microbiological results

From an overall picture, most of the chemical analysed parameters showed decreasing values in the following order: sector B > sector A > sector C > CER (Table 2). The microbial parameters showed higher values within sectors A and B than sector C and CER. Waters showed slightly alkaline characteristics with pH values between 7.96 and 7.52. Although CER showed the highest pH, it had the lowest amount of carbonate (77 mg L<sup>-1</sup>), while the highest carbonate content was found within the sector B (174 mg L<sup>-1</sup>). The EC showed the highest mean value within the canals receiving the water from the wastewater plants (sector B); values significantly decreased in Sector A (784 µS cm<sup>-1</sup>), then in Sector C and in CER the value was the lowest one (470 µS cm<sup>-1</sup>).

The DO content had the highest values within CER (8.56 mg L<sup>-1</sup>) and the lowest one within Sector A (7.67 mg L<sup>-1</sup>). The sector B generally showed the highest concentrations of nutrients (*i.e.*, N forms, PO<sub>4</sub>, SO<sub>4</sub>, Cl, Ca, Mg, Si, B and Na) and the lowest ones within the CER (Table 2). Consequently, SAR values showed a decreasing trend in order Sector B > Sector A > Sector C > CER. Taking into account the organic pollution, both BOD and COD showed the highest values within Sector A and B (21.7 and 255 mg L<sup>-1</sup>; 19.1 and 199 mg L<sup>-1</sup>); COD decreased significantly within Sector C and CER. BOD remain constant in sector C

**Table 2**

Mean  $\pm$  standard deviation of the investigated chemical and microbial parameters. Different letters within the same row indicate significant differences according to linear mixed-effects model ( $P < 0.05$ ).

	Sector A	Sector B	Sector C	CER
pH	7.52 $\pm$ 0.28 c	7.56 $\pm$ 0.22 c	7.77 $\pm$ 0.14 b	7.96 $\pm$ 0.18 a
EC ( $\mu\text{S cm}^{-1}$ )	784 $\pm$ 197 b	1139 $\pm$ 281 a	623 $\pm$ 161c	470 $\pm$ 127 d
DO ( $\text{mg L}^{-1}$ )	7.67 $\pm$ 0.82 b	7.97 $\pm$ 0.64 b	8.10 $\pm$ 0.86 ab	8.56 $\pm$ 0.83 a
TN ( $\text{mg L}^{-1}$ )	7.55 $\pm$ 4.62 b	15.65 $\pm$ 7.67 a	3.49 $\pm$ 2.29 c	3.70 $\pm$ 2.79 c
NO <sub>3</sub> -N ( $\text{mg L}^{-1}$ )	2.53 $\pm$ 2.03 b	6.76 $\pm$ 4.17 a	1.15 $\pm$ 0.58 c	0.56 $\pm$ 0.53 d
NH <sub>4</sub> -N ( $\text{mg L}^{-1}$ )	1.87 $\pm$ 1.43 b	3.31 $\pm$ 2.57 a	0.82 $\pm$ 0.84 c	1.41 $\pm$ 1.15 b
BOD ( $\text{mg L}^{-1}$ )	21.7 $\pm$ 5.4 a	19.1 $\pm$ 7.9 ab	19.9 $\pm$ 5.8 ab	12.7 $\pm$ 6.3 c
COD ( $\text{mg L}^{-1}$ )	255 $\pm$ 125 a	199 $\pm$ 136 ab	157 $\pm$ 105 c	154 $\pm$ 177 c
CaCO <sub>3</sub> ( $\text{mg L}^{-1}$ )	146 $\pm$ 30 b	174 $\pm$ 39 a	118 $\pm$ 20 c	77 $\pm$ 15 d
PO <sub>4</sub> ( $\text{mg L}^{-1}$ )	1.60 $\pm$ 0.69 a	2.32 $\pm$ 2.67 a	0.37 $\pm$ 0.36 b	0.12 $\pm$ 0.04 c
SO <sub>4</sub> ( $\text{mg L}^{-1}$ )	64.9 $\pm$ 16.0 b	83.5 $\pm$ 23.9 a	50.4 $\pm$ 13.2 c	38.8 $\pm$ 11.3 d
Cl ( $\text{mg L}^{-1}$ )	98 $\pm$ 33 b	150 $\pm$ 38 a	67 $\pm$ 28 c	23 $\pm$ 9 d
B ( $\mu\text{g L}^{-1}$ )	293 $\pm$ 229 b	450 $\pm$ 211 a	191 $\pm$ 108 c	79 $\pm$ 54 d
Ca ( $\text{mg L}^{-1}$ )	82.7 $\pm$ 14.8 a	89.6 $\pm$ 25.4 a	64.4 $\pm$ 10.3 b	59.4 $\pm$ 11.8 b
Mg ( $\text{mg L}^{-1}$ )	14.7 $\pm$ 2.7 b	21.2 $\pm$ 4.2 a	15.7 $\pm$ 2.2 b	13.7 $\pm$ 2.3 b
Na ( $\text{mg L}^{-1}$ )	67.9 $\pm$ 22.2 b	133.5 $\pm$ 46.2 a	43.5 $\pm$ 20.5 c	17.3 $\pm$ 7.3 d
SAR	6.71 $\pm$ 1.79 b	12.78 $\pm$ 4.24 a	4.76 $\pm$ 1.95 c	1.98 $\pm$ 0.60 d
Si ( $\mu\text{g L}^{-1}$ )	840 $\pm$ 299 b	1129 $\pm$ 443 a	631 $\pm$ 144 c	599 $\pm$ 230 c
Tcol ( $\log_{10}$ UFC/100 mL)	5.01 $\pm$ 0.52 a	5.06 $\pm$ 0.65 a	4.30 $\pm$ 0.55 b	4.35 $\pm$ 0.58 b
Fcol ( $\log_{10}$ UFC/100 mL)	4.73 $\pm$ 0.53 a	4.78 $\pm$ 0.66 a	3.86 $\pm$ 0.66 b	4.14 $\pm$ 0.56 b
Ecol ( $\log_{10}$ UFC/100 mL)	3.29 $\pm$ 0.71 a	3.34 $\pm$ 0.61 a	2.51 $\pm$ 0.50 b	2.10 $\pm$ 0.35 b
Strep ( $\log_{10}$ UFC/100 mL)	3.67 $\pm$ 0.76 a	3.87 $\pm$ 0.72 a	2.74 $\pm$ 0.53 b	2.23 $\pm$ 0.43 c

EC: electrical conductivity; DO: dissolved oxygen; TN: total nitrogen; BOD: biological oxygen demand; COD: chemical oxygen demand; SAR: sodium adsorption ratio; Tcol: total coliforms; Fcol: faecal coliforms; Ecoli: *E. coli*; Strep: Faecal streptococcus; CER: Canale Emiliano Romagnolo.

(19.1  $\text{mg L}^{-1}$ ) and had the lowest value within the CER (12.7  $\text{mg L}^{-1}$ ). The OPI showed the highest values in Sectors A and B (2.23 and 2.14, respectively), while the lowest one was observed in CER (1.17).

Microbiological analysis evidenced higher values of Tcol in sectors A and B ( $\log_{10}$  UFC/100 mL 5.01 and 5.06  $\log_{10}$  UFC/100 mL, respectively) compared to sectors C and CER (4.74 and 4.75  $\log_{10}$  UFC/100 mL, respectively). Similarly, Fcol showed the highest values in Sector A and B (4.73 and 4.78  $\log_{10}$  UFC/100 mL, respectively). Overall, Fcol enumeration evidenced very close values to Tcol. Ecol showed higher values in sectors A and B (5.01 and 5.06  $\log_{10}$  UFC/100 mL, respectively) than in sector C and CER (2.51 and 2.10  $\log_{10}$  UFC/100 mL, respectively). Finally, Strep load ranked from 3.87 up to 2.23  $\log_{10}$  UFC/100 mL showing a decreasing trend with the following order: sector A = sector B > sector C > CER.

### 3.2. Upstream-downstream comparison

With regard to chemical parameters, CER did not show any difference between upstream and downstream sampling points (Table 3). Regarding to sector C, the canals Lorgana and La Botte showed a general decrease in the values of EC, SAR, COD and content of nutrients from upstream to downstream. In sector A, Savena canal showed an increase of values for the most of chemical parameters, whereas Navile canal showed a somehow similar behaviour found for the canals of sector C (Table 3).

Microbial parameters did not show a significant decrease between up- and downstream, with some exception; Savena (sector A) and La Botte (sector C) showed a significant decrease in both Ecol and Strep with a 1 log decrease in Savena (Table 3). Savena also had a significant decrease of Tcol population (4.72 and 4.38  $\log_{10}$  UFC/100 mL, upstream and downstream, respectively).

### 3.3. Water microbial property predictors

The PC analysis (Fig. 3a) showed that most of the total data set variance was related to PC1 (41.25 %), PC2 (12.28 %) and PC3 (8.14 %).

The first PC has been mainly and positively driven by EC, NO<sub>3</sub>-N, NH<sub>4</sub>-N, TN, CaCO<sub>3</sub>, PO<sub>4</sub>, SO<sub>4</sub>, Cl, Ca, Na, Si and SAR (Fig. 3b). The PC2 was mainly driven by NO<sub>3</sub>-N content (-0.55), CaCO<sub>3</sub> content (-0.50)

and SAR (+0.55). Finally, the PC3 was strongly correlated with DO content (+0.69), BOD (-0.55) and COD (-0.59).

The multiple linear regression models used to determine the most important factors (i.e., the PC1, PC2 and PC3 scores of the water chemical properties and sector type) responsible for the variation in water microbial properties (i.e., Tcol, Fcol, Ecol and Strep) showed  $p$  values  $<$  0.05, indicating that such model fits well with the obtained data (Tables 4–7). The models pointed out a positive correlation between the microbial population and those sectors affected by urban activities with exception of Ecol which showed a negative correlation with water of sector B.

Concerning the correlation between the chemical properties, gathered from the PC1–3, and the investigated microbial populations, unclear trends were observed. For example, the multiple linear regression model showed a negative correlation between Tcol and EC, NO<sub>3</sub>-N, NH<sub>4</sub>-N, TN, CaCO<sub>3</sub>, PO<sub>4</sub>, SO<sub>4</sub>, Cl, Ca, Na, Si and SAR, while such correlation was positive for Ecol, and any correlation was missing for Fcol and Strep. Similarly, the correlation with PC3 was negative for Tcol, Fcol and Strep, but positive for Ecol. However, it is important to note that PC3 explained only 8 % of the total variance.

### 3.4. Driving water features for sector type distinguishment

The classification tree had four splits and five terminal nodes, with an overall accuracy of about 81 and 64 % for the training and the test subsets, respectively (Fig. 4).

The most important variables of the classification tree were SAR and the contents of PO<sub>4</sub>, NO<sub>3</sub>-N and Cl. The first split was determined by SAR threshold of 7.2. Through this split, 45 % of our observations had a SAR value of 7.2 or greater which mostly included (81 %) water samples collected from the sector B. The 55 % of the observations that were characterized by a SAR value less than 7.2 mostly included water samples of sector C (65 %). The threshold of PO<sub>4</sub> content of 0.85  $\text{mg L}^{-1}$  drove the split of the group containing the 55 % of observations. Specifically, the observations characterized by a PO<sub>4</sub> content less than 0.85  $\text{mg L}^{-1}$  included mainly water samples of sector C and to lesser extent those of CER. These two sectors were further distinguished by the Cl content whose values were less than 20  $\text{mg L}^{-1}$  within the water samples collected from the CER. The observations characterized by a PO<sub>4</sub> content of 0.85  $\text{mg L}^{-1}$  or more, instead, included the water

Table 3

Mean  $\pm$  standard deviation of the investigated chemical and microbial parameters upstream (Up) and downstream (Down) the canals La Botte, Lorgana, Navile, Savena and Canale Emiliano Romagnolo (CER). For each canal, different letters within the same row indicate significant differences according to Kruskal-Wallis H test ( $P < 0.05$ ).

	La Botte		Lorgana		Navile		Savena		CER	
	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
pH	7.67 $\pm$ 0.28	7.80 $\pm$ 0.26	7.64 $\pm$ 0.26 b	7.85 $\pm$ 0.23 a	7.19 $\pm$ 0.35 b	7.67 $\pm$ 0.38 a	7.82 $\pm$ 0.44	7.62 $\pm$ 0.39	7.96 $\pm$ 0.19	7.96 $\pm$ 0.30
EC ( $\mu\text{S cm}^{-1}$ )	684 $\pm$ 97 a	583 $\pm$ 105 b	825 $\pm$ 200 a	718 $\pm$ 192 b	1016 $\pm$ 168 a	820 $\pm$ 179 b	468 $\pm$ 195 b	791 $\pm$ 240 a	460 $\pm$ 102	471 $\pm$ 97
DO ( $\text{mg L}^{-1}$ )	7.59 $\pm$ 0.97	8.49 $\pm$ 1.05	7.39 $\pm$ 0.90 b	8.06 $\pm$ 1.22 a	7.36 $\pm$ 0.89 b	8.00 $\pm$ 0.79 a	8.14 $\pm$ 1.04	8.06 $\pm$ 1.09	8.23 $\pm$ 0.99	8.45 $\pm$ 0.87
TN ( $\text{mg L}^{-1}$ )	3.45 $\pm$ 1.61	2.57 $\pm$ 1.78	4.19 $\pm$ 2.42	4.05 $\pm$ 2.80	11.5 $\pm$ 6.1 a	7.3 $\pm$ 5.0 b	2.26 $\pm$ 1.49 b	7.92 $\pm$ 5.96 a	2.64 $\pm$ 1.10	2.45 $\pm$ 1.00
NO <sub>3</sub> -N ( $\text{mg L}^{-1}$ )	1.18 $\pm$ 0.98	0.84 $\pm$ 0.66	1.56 $\pm$ 1.34	1.33 $\pm$ 0.83	3.93 $\pm$ 3.78	2.68 $\pm$ 3.41	0.79 $\pm$ 0.78 b	2.64 $\pm$ 3.52 a	0.43 $\pm$ 0.44	0.32 $\pm$ 0.19
NH <sub>4</sub> -N ( $\text{mg L}^{-1}$ )	0.81 $\pm$ 0.60	0.63 $\pm$ 0.68	0.92 $\pm$ 0.74	0.88 $\pm$ 0.81	2.97 $\pm$ 3.47	1.57 $\pm$ 1.58	0.49 $\pm$ 0.30 b	2.05 $\pm$ 2.86 a	0.97 $\pm$ 0.49	0.97 $\pm$ 0.52
BOD ( $\text{mg L}^{-1}$ )	20.7 $\pm$ 8.7	19.9 $\pm$ 11.5	23.9 $\pm$ 14.2	21.2 $\pm$ 11.5	25.3 $\pm$ 9.7	23.1 $\pm$ 10.1	14.8 $\pm$ 11.8 b	22.3 $\pm$ 10.9 a	15.0 $\pm$ 11.3	10.8 $\pm$ 7.4
COD ( $\text{mg L}^{-1}$ )	212 $\pm$ 162 a	137 $\pm$ 142 b	260 $\pm$ 139 a	139 $\pm$ 140 b	318 $\pm$ 190	302 $\pm$ 250	81 $\pm$ 48 b	201 $\pm$ 79 a	112 $\pm$ 249	178 $\pm$ 335
CaCO <sub>3</sub> ( $\text{mg L}^{-1}$ )	127 $\pm$ 41	113 $\pm$ 44	148 $\pm$ 44	127 $\pm$ 42	180 $\pm$ 62	159 $\pm$ 53	96 $\pm$ 27 b	142 $\pm$ 50 a	73 $\pm$ 11	73 $\pm$ 10
PO <sub>4</sub> ( $\text{mg L}^{-1}$ )	0.40 $\pm$ 0.24 a	0.14 $\pm$ 0.08 b	1.17 $\pm$ 0.51 a	0.43 $\pm$ 0.21 b	2.02 $\pm$ 0.82	1.92 $\pm$ 0.76	0.38 $\pm$ 0.21 b	1.88 $\pm$ 0.70 a	0.09 $\pm$ 0.05	0.11 $\pm$ 0.06
SO <sub>4</sub> ( $\text{mg L}^{-1}$ )	54.3 $\pm$ 7.45	50.1 $\pm$ 10.9	63.8 $\pm$ 14.5 a	52.2 $\pm$ 16.8 b	83.8 $\pm$ 15.8 a	69.8 $\pm$ 14.2 b	40.3 $\pm$ 16.3 b	61.9 $\pm$ 15.8 a	34.9 $\pm$ 4.89	35.2 $\pm$ 4.96
Cl ( $\text{mg L}^{-1}$ )	79.5 $\pm$ 15.6	64.5 $\pm$ 14.0	104 $\pm$ 23 a	76 $\pm$ 26 b	132 $\pm$ 44 a	110 $\pm$ 30 b	46 $\pm$ 22 b	98 $\pm$ 30 a	21 $\pm$ 6	20 $\pm$ 7
B ( $\mu\text{g L}^{-1}$ )	210 $\pm$ 129	194 $\pm$ 144	284 $\pm$ 180	229 $\pm$ 162	314 $\pm$ 240	249 $\pm$ 178	211 $\pm$ 175	331 $\pm$ 425	113 $\pm$ 100	52 $\pm$ 64
Ca ( $\text{mg L}^{-1}$ )	69.1 $\pm$ 8.32	60.2 $\pm$ 10.8	82.8 $\pm$ 10.9 a	69.3 $\pm$ 16.1 b	98.5 $\pm$ 11.2	89.1 $\pm$ 14.7	58.6 $\pm$ 15.3 b	80.6 $\pm$ 11.1 a	55.7 $\pm$ 9.3	56.3 $\pm$ 9.5
Mg ( $\text{mg L}^{-1}$ )	16.3 $\pm$ 2.0	15.4 $\pm$ 2.2	16.4 $\pm$ 2.5	16.3 $\pm$ 4.5	17.3 $\pm$ 2.5	15.8 $\pm$ 2.9	10.8 $\pm$ 3.0 b	14.6 $\pm$ 2.3 a	13.3 $\pm$ 2.0	13.2 $\pm$ 1.9
Na ( $\text{mg L}^{-1}$ )	53.7 $\pm$ 9.4 a	43.0 $\pm$ 7.1 b	68.8 $\pm$ 11.0 a	52.3 $\pm$ 14.2 b	96.5 $\pm$ 20.7 a	76.0 $\pm$ 18.1 b	30.9 $\pm$ 18.9 b	64.3 $\pm$ 15.4 a	15.0 $\pm$ 5.6	14.9 $\pm$ 6.0
SAR ( $\text{mmol(c) L}^{-1}$ )	5.81 $\pm$ 0.95 a	4.96 $\pm$ 0.83 b	6.92 $\pm$ 1.07 a	5.59 $\pm$ 1.33 b	8.96 $\pm$ 1.80 a	7.39 $\pm$ 1.36 b	3.59 $\pm$ 1.79 b	6.56 $\pm$ 1.32 a	1.84 $\pm$ 0.73	1.81 $\pm$ 0.84
Si ( $\mu\text{g L}^{-1}$ )	692 $\pm$ 241	636 $\pm$ 262	729 $\pm$ 260	672 $\pm$ 202	1169 $\pm$ 319 a	933 $\pm$ 344 b	469 $\pm$ 216	577 $\pm$ 324	563 $\pm$ 329	555 $\pm$ 301
Tcol ( $\log_{10}$ UFC/100 mL)	4.13 $\pm$ 0.54	4.17 $\pm$ 0.76	4.52 $\pm$ 0.38	4.39 $\pm$ 0.96	4.77 $\pm$ 0.40	4.53 $\pm$ 0.23	4.72 $\pm$ 0.44 a	4.38 $\pm$ 0.40 b	4.27 $\pm$ 0.69	4.27 $\pm$ 0.56
Fcol ( $\log_{10}$ UFC/100 mL)	3.56 $\pm$ 0.75	3.71 $\pm$ 0.88	4.14 $\pm$ 0.52	3.82 $\pm$ 1.05	4.31 $\pm$ 0.72	4.11 $\pm$ 0.60	4.26 $\pm$ 0.61	4.07 $\pm$ 0.59	3.97 $\pm$ 0.64	3.90 $\pm$ 0.92
Ecol ( $\log_{10}$ UFC/100 mL)	2.42 $\pm$ 0.46 a	2.12 $\pm$ 0.46 b	2.87 $\pm$ 0.60	2.38 $\pm$ 0.75	2.92 $\pm$ 0.94	2.94 $\pm$ 0.88	3.68 $\pm$ 0.53 a	2.71 $\pm$ 0.70 b	1.92 $\pm$ 0.19	2.18 $\pm$ 0.55
Strep ( $\log_{10}$ UFC/100 mL)	2.62 $\pm$ 0.41 b	2.21 $\pm$ 0.65 a	3.00 $\pm$ 0.31	2.66 $\pm$ 0.80	3.32 $\pm$ 0.58	2.94 $\pm$ 0.62	3.24 $\pm$ 0.51 a	2.59 $\pm$ 0.59 b	2.05 $\pm$ 0.32	2.32 $\pm$ 0.60

EC: electrical conductivity; DO: dissolved oxygen; TN: total nitrogen; BOD: biological oxygen demand; COD: chemical oxygen demand; SAR: sodium adsorption ratio; Tcol: total coliforms; Fcol: faecal coliforms; Ecol: *E. coli*; Strep: Faecal streptococcus.

samples of sectors A and B which were distinguished one each other by the NO<sub>3</sub>-N content. In fact, the water samples of sector B had a NO<sub>3</sub>-N content of 6.3 mg L<sup>-1</sup> or more.

## 4. Discussion

### 4.1. Water properties overview

It is a matter of fact that water flowing within artificial canals continuously changes its properties on both short spatial and temporal scales (Vehoungstræte and Rose, 2014; Won et al., 2013). But, as observed in this study, a kind of stable and consistent trend may be associated to the water origin according to the hydraulic sector. Chemical analysed parameters were in line with the wastewater re-use Italian legislation (D.M., 2006) with the exception of two COD values that were slightly above the limit value (160 mg L<sup>-1</sup>; D.M., 2006) (Sectors A and B). While, the BOD values were considerably lower in all sectors and below the Italian limit legislation (40 mg L<sup>-1</sup>; D.M., 2006), showing a less impact of the organic pollution.

Ecol values were below the legislation limit of 5000 UFC/100 mL (3.7 UFC log<sub>10</sub>/100 mL; D.M., 2006) in all sectors. *E. coli* is often used as indicator of fecal contamination in different kind of water to evaluate the potential presence of waterborne pathogens, although the vast majority of *E. coli* strains are non-pathogenic (Blaustein et al., 2013). Basically, *E. coli* survival rates are known to be dependent on temperature and several other factors, such as salinity, pH, predation and nutrient contents (Bordalo et al., 2002; Davies and Evison, 1991; Harmel et al., 2010; Jamieson et al., 2004; McCambridge and McMeekin, 1980; Sinton et al., 2002). Moreover, it has a relatively low die-off or inactivation rate coefficient, and it is able to travel long distances underground (Foppen and Schijven, 2006).

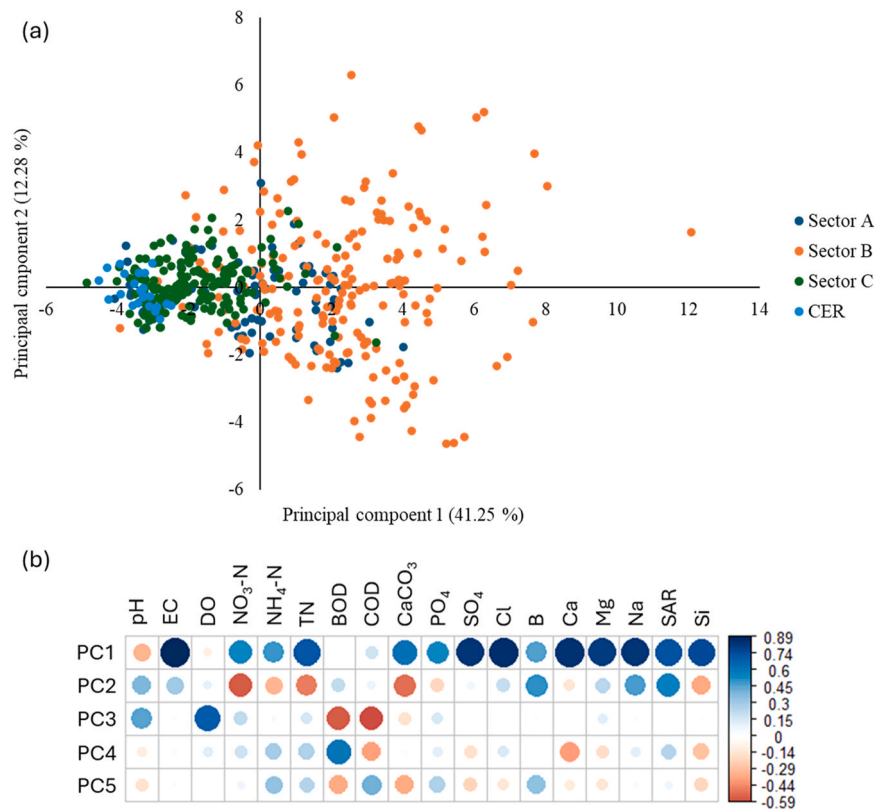
Overall, the three years data showed a concentration decrease of all chemical targets, with the following order, Sector B > Sector A > Sector C > CER, whereas for microbial parameter Sectors A and B always fell in the same group with higher biological pollution compared to Sector C and CER.

These findings would confirm that urbanization and anthropization are responsible for the pollution widespread in watercourses (Gorgoglione et al., 2020; Jayasiri et al., 2022). In fact, early studies demonstrated the negative influence of water quality due to urban runoff (Ewen et al., 2007).

Sector A and Sector B included artificial canals mainly affected by human pollution (grey and black waters), which notably influence the inorganic/organic residues in watercourses (Ferreira et al., 2021; Suthi et al., 2023). Sector B represents the worst situation, since the artificial canals are collectors of water from urban treatment plants. Sector C is a sector comprising several artificial canals located in the middle of the floodplain and receiving water from the CER. Conversely, CER is a cemented canal not receiving nutrients and organic compounds from agricultural land and urban areas (Giri, 2021). In fact, the CER showed the highest DO value, which indicates a scarcity of organic molecules and nutrients; their increase would imply a higher risk of oxygen depletion in the water (Anh et al., 2023; Freire et al., 2021).

The pH values of water samples were in line with those observed in earlier studies (Marchina et al., 2015; Marconi et al., 2011). The highest amount of carbonates observed within Sector B might be attributed to the common practice used within wastewater treatment plants to use lime or carbonate-rich materials (Hassan et al., 2023; Houshyar and Bacenetti, 2023) for nutrients immobilization. (Tustenberger and Castro-Munoz, 2022; Santos et al., 2024).

The highest EC values observed in Sector B (1139  $\mu\text{S cm}^{-1}$ ) confirmed the deterioration of water quality due to the input of wastewater into the canals, which is known to carry a high salt load (de Sousa et al., 2014; Shulll-Trauring et al., 2020). Moreover, results evidenced the highest concentrations of ions like Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup> (Pawlowicz, 2008). The high value of chloride ions may be also



**Fig. 3.** Principal component analysis results (a) of water chemical properties and their correlation with principal components (b). EC: electrical conductivity; DO: dissolved oxygen; TN: total nitrogen; BOD: biological oxygen demand; COD: chemical oxygen demand; SAR: sodium adsorption ratio; CER: Canale Emiliano Romagnolo.

**Table 4**

Multiple linear regression model coefficients between the amount of total coliform bacteria and sector type and individuals' coordinate of principal component (PC) 1, 2 and 3.

Parameter	Coefficient	Standard error	T value	Pr(> t )
Intercept	4.58998	0.07701	59.603	< 2e-16
Sector B	0.23389	0.09957	2.349	0.019241
Sector C	-0.50148	0.09573	-5.239	2.45e-07
CER	-0.39313	0.16761	-2.345	0.019418
Individuals' coordinate PC1	-0.03760	0.01643	-2.288	0.022589
Individuals' coordinate PC2	0.03399	0.02059	1.651	0.099438
Individuals' coordinate PC3	-0.10042	0.02593	-3.873	0.000123
Overall model				
Residual standard error	Degrees of freedom	Multiple R-squared	Adjusted R-squared	p-value
0.6672	471	0.1746	0.1641	<2.2e-16

associated to the sanitation systems of wastewaters by chlorine-based solutions (Shang and Blatchle, 2001; Winward et al., 2008). In addition, the Sector B showed the highest SAR values which could pose salinity and sodicity risks to soils if the water is used for irrigation, with consequent damage to soil properties and, in turn, to crops (Jahany and Rezapour, 2020; Rezapour et al., 2017). The higher concentration of salts and nutrients in Sector B, compared with the other sectors, was confirmed by the PCA. Most of the variation among sectors occurred along PC1, which was positively correlated with nutrient and salt contents. Samples from Sector B clustered mainly on the right side of the PCA scatter plot, indicating that wastewater treatment plants play a key role in deteriorating water quality by discharging these substances into

**Table 5**

Multiple linear regression model coefficients between the amount of fecal coliform bacteria and sector type and individuals' coordinate of principal component (PC) 1, 2 and 3.

Parameter	Coefficient	Standard error	T value	Pr(> t )
Intercept	4.16356	0.08998	46.273	< 2e-16
Sector B	0.28098	0.11634	2.415	0.01611
Sector C	-0.51026	0.11185	-4.562	6.47e-06
CER	-0.20500	0.19584	-1.047	0.29575
Individuals' coordinate PC1	-0.01288	0.01920	-0.671	0.50286
Individuals' coordinate PC2	0.02073	0.02406	0.862	0.38926
Individuals' coordinate PC3	-0.09443	0.03030	-3.117	0.00194
Overall model				
Residual standard error	Degrees of freedom	Multiple R-squared	Adjusted R-squared	p-value
0.7796	471	0.1697	0.1592	<2.2e-16

the artificial canals. Concerning the organic pollution, the BOD:COD ratio was less than 0.2 in all the considered sectors, suggesting the presence of organic matter characterized by low degradability within water (Anh et al., 2023). However, both Sectors A and B showed OPI values greater than 2, indicating organic contamination linked to anthropogenic activities (Biedunkova et al., 2025; Chen et al., 2023) and, consequently, higher concentration of persistent organic pollutants. These sectors are located in areas influenced by wastewater effluents and urban groundwater inputs, which are the main sources of organic contaminants (Cojoc et al., 2024; Kolpin et al., 2002).

**Table 6**  
Multiple linear regression model coefficients between the amount of Escherichia coli bacteria and sector type and individuals' coordinate of principal component (PC) 1, 2 and 3.

Parameter	Coefficient	Standard error	T value	Pr(> t )
Intercept	3.10512	0.09681	32.076	< 2e-16
Sector B	-0.43386	0.12517	-3.466	0.000576
Sector C	-0.54884	0.12034	-4.561	6.51e-06
CER	-0.82716	0.21070	-3.926	9.94e-05
Individuals' coordinate PC1	0.08941	0.02066	4.328	1.84e-05
Individuals' coordinate PC2	-0.02588	0.02589	-1.000	0.317977
Individuals' coordinate PC3	0.06643	0.03259	2.038	0.042102
Overall model				
Residual standard error	Degrees of freedom	Multiple R-squared	Adjusted R-squared	p-value
0.8387	471	0.156	0.1452	3.234e-15

**Table 7**  
Multiple linear regression model coefficients between the amount of total streptococcus bacteria and sector type and individuals' coordinate of principal component (PC) 1, 2 and 3.

Parameter	Coefficient	Standard error	T value	Pr(> t )
Intercept	2.98127	0.07855	37.956	< 2e-16
Sector B	0.52056	0.10156	5.126	4.34e-07
Sector C	-0.37091	0.09764	-3.799	0.000164
CER	-0.65537	0.17096	-3.834	0.000143
Individuals' coordinate PC1	0.01711	0.01676	1.021	0.307892
Individuals' coordinate PC2	-0.08384	0.02100	-3.992	7.60e-05
Individuals' coordinate PC3	-0.14374	0.02645	-5.435	8.79e-08
Overall model				
Residual standard error	Degrees of freedom	Multiple R-squared	Adjusted R-squared	p-value
0.6805	471	0.3644	0.3563	<2.2e-16

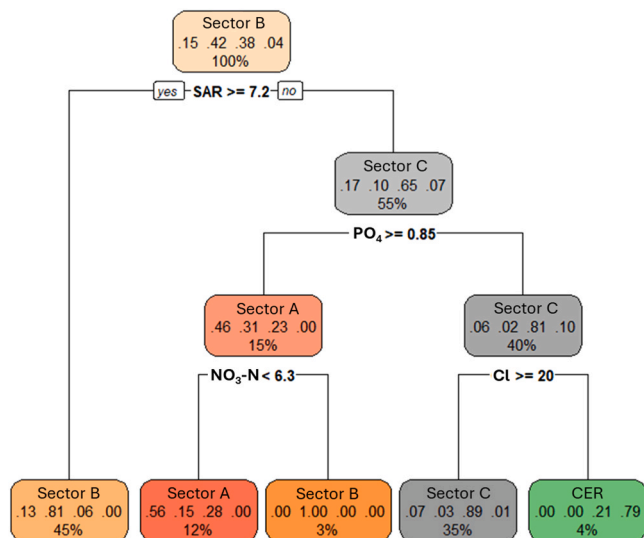
The highest Ecol average values were found in Sector A and B, thus further showing a significant influence of urban black waters and sewage, associated to the rich-nutrient load compared to Sector C and the CER. This is in agreement with some authors (Foppen and Schijven, 2006) reporting studies on *E. coli* adhesion characteristics to inorganic and organic compound due to its negative surface charge.

Noteworthy, the absence of *Salmonella* spp. in all investigated canals, although it is recognized the survival capacity in a broad range of water conditions (Liu et al., 2018); probably it can be attributed to the absence of untreated sewage effluents (Levantesi et al., 2012).

Concerning Tcol, Fcol and Strep, the Italian legislation does not establish any threshold for irrigation purposes. However, analyses have been performed to have a wider information on the total bacterial spread, in particular for fecal coliform and fecal streptococci. In general, fecal microorganisms survival is affected by complex interactions among environmental parameters (sampling area, hydrometeorological, physico-chemical, land use and cover management, sedimentation and resuspension, soil types and vegetation) (Lothrop et al., 2018). Their presence and load, as for Ecol, are rather representative of the microbial contamination since they have a certain lifespan and resistance to environmental pressure. In this context of drivers influencing microbial loads in the investigated canals, the multiple linear regression models confirmed the pivotal role of urban-related activities in increasing microbial hazards. Indeed, the multiple linear regression model highlighted a positive correlation of microbial indicators with Sectors A and B, except for Ecol in Sector B, which is positively influenced by the required disinfection treatment and it undergoes a sudden drop. Conversely, water from Sector C and the CER exhibited lower levels of microbial contamination, showing significantly lower concentrations of the target microorganisms, in particular Ecol and Strep. The linear regression model revealed a negative correlation between the sectors type (C and CER) and the microbial load. As already mentioned, both sectors largely differ from Sectors A and B; water from Sector C is less affected by urban contamination sources and consequently by fecal pollution. As already noted, the artificial canals in Sector C lie within a floodplain characterized by scattered nonpoint sources, such as fertilizer use, soil erosion, livestock activity, wildlife waste and occasional sewage inputs. Moreover, it receives water from the CER, thus improving its final water quality. Instead, the inconsistent relationships between the microbial communities and the water chemical parameters would confirm that such communities are highly sensitive to a plethora of biotic and abiotic factors, and their interactions (García-Armisen et al., 2014; Humbert et al., 2009; Savio et al., 2014).

The selected hydraulic sectors are, to some extent, representative of different chemical and microbiological pictures since water, as already mentioned, has different origin. Overall, the marked chemical distinctiveness of waters in Sector B was also highlighted by the PCA, which suggests the likely predominant role of wastewater (grey and black waters) in influencing water quality of the artificial canals (Brion et al., 2015; Dutta et al., 2020), and therefore indicates a high vulnerability of the studied canal network to the pollution from point sources.

Although the monthly sampling of canal waters over the three-year monitoring period provided a solid overview of nutrient and microbial dynamics, these processes can still undergo abrupt fluctuations on the scale of hours to days. Such short-lived events are difficult to anticipate. Intense rainfall, for example, can rapidly alter discharge, resuspend sediments, and introduce nutrient pulses that shift both microbial communities and canal chemistry (Bernal et al., 2013). Similar, short-term surges may also arise from slurry spreading or accidental releases of fertilizers (Carpenter et al., 1998; Withers et al., 2000). Sudden increases in treated wastewater discharge—whether due to hydraulic overloads, operational failures or storm-related



**Fig. 4.** Classification tree showing the main water properties shifting canals of sector A, B, C and Canale Emiliano Romagnolo (CER).

overflows—can likewise deliver pulses of nutrients, organic compounds and microorganisms, modifying water quality within hours (Bukaveckas et al., 2020; Passerat et al., 2011).

#### 4.2. Upstream-downstream comparison

The absence of parameter shifts between upstream and downstream in CER confirms the isolation of such canal from the surrounding environment due to the cemented banks.

The decrease in chemical property values from upstream to downstream in the two canals belonging to Sector C (La Botte and Lorgana) may be partly attributed to the greater uptake of nutrients and organic molecules by the riparian vegetation, as well as to their adsorption onto bed sediments (Dunea et al., 2021; May et al., 2023; Poesio et al., 2023; Tootoonchi et al., 2024). Concerning Sector A, while Navile exhibited a similar behaviour to that observed for the canals of Sector C, the Savena showed a deterioration in chemical water quality from upstream to downstream. This is likely due to the inflow into the Savena of a canal called “diversivo” originating from the Navile. It is a collector of water overflowing from the Navile to the Savena, before the downstream sampling point. While this hydraulic diversion could mitigate the destructive force of the Navile during possible flood events, it simultaneously deteriorates the chemical quality of the Savena’s water. Conversely, data for all microbial groups partially reflect the patterns observed across the different sectors. The downstream decrease in Ecol and Strep indicates an improvement in water quality both in the Savena and La Botte: in the Savena due to the greater distance from the highly anthropized upstream area, and in La Botte thanks to the inflow of better-quality water from the CER.

#### 4.3. Key drivers of sector differentiation

Our findings highlighted that the main feature characterizing the waters of sector B was SAR. To date, there is a growing global interest in using treated wastewater as a sustainable water source for agricultural purposes (e.g., Hosney et al., 2023; Pratap et al., 2023), particularly in Mediterranean countries such as Italy (Canaj et al., 2021; Colella et al., 2021; Mesa-Pérez and Berbel, 2020). However, in our study the higher SAR values observed in canals receiving wastewater compared to other canals underscore the need to address the potential sodicity risk in soils (Jahany and Rezapour, 2020; Simhayov et al., 2023) associated with wastewater use. Excessively high Na concentration can increase soil susceptibility to crusting, runoff, erosion, and reduced infiltration and hydraulic conductivity (Mandal et al., 2008; Quirk, 2001). However, these waters are not used directly for irrigation; instead, they are blended with waters from other sources by the Renana reclamation consortium, mitigating the sodicity risk. The classification tree confirmed the marked distinction of CER water attributable to its concrete embankments and the absence of feeder canals. These features prevent the intrusion of nutrients and salts from surrounding land. Notably, P-PO<sub>4</sub> content emerged as the main factor distinguishing Sector A from Sector C, with generally higher values in the former than in the latter. This pattern is consistent with the extensive use of phosphorus-based fertilizers in agricultural areas (Faridmarandi and Naja, 2014; Rodriguez et al., 2022), which can be transported to canals through leaching. Therefore, it is crucial that future research more clearly identifies both point and non-point sources of nutrient contamination in the investigated canals.

## 5. Conclusion

The present study carried out a comprehensive three-years investigation of the physical, chemical and microbiological properties of water in 27 artificial canals across 4 hydraulic sectors in the metropolitan area of Bologna (Italy). Although the analysed water is suitable for irrigation, our findings confirm that urbanization and anthropization are major

contributors to pollution in watercourses. Canals receiving water from wastewater treatment plants and crossing urban areas exhibited higher load of nutrients, organic carbon and specific microbiological indicators compared to canals flowing through agricultural lands. The classification tree showed a marked distinction of canals receiving urban treated water, primarily driven by higher SAR values, which may increase the sodicity risk of soils due to wastewater use. In contrast, canals crossing agricultural lands were characterized mainly by elevated P-PO<sub>4</sub> contents, likely related to the application of phosphorus-based fertilizers. Despite this, two canals in agricultural area showed an improvement of water quality from upstream to downstream, likely due to the combined effect of recent precision agricultural practices limiting nutrient and organic compound leaching, and nutrient uptake by riparian vegetation. CER water was distinguished by its concrete embankments and lack of feeder canals, a pattern confirmed by the absence of significant differences between upstream and downstream sampling points. To identify the main drivers affecting the microbial loads in the investigated canals, the multiple linear regression model indicated a positive correlation between microbial indicators and sectors affected by urban activities. Conversely, microbial communities showed inconsistent relationship with water chemical parameters, highlighting their sensitivity to multiple biotic and abiotic factors and their complex interactions.

This study has certain limitations, including reliance on a single monthly measurement and a limited geographical scope, which may affect the generalizability of the findings. Expanding the research to a broader geographic area and increasing the frequency of sampling would refine model parameters and enhance applicability to real-world scenarios.

Nonetheless, the selected sectors are somewhat representative of the chemical and microbiological variability typically found in reclaimed floodplains, reflecting the diverse water origins. Overall, our findings suggest that wastewater (both grey and black water) likely plays a major role in determining the water quality of artificial canals highlighting the high vulnerability of these systems to point-sources pollution.

#### CRediT authorship contribution statement

**Francesca Gaggia:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Mauro De Feudis:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Conceptualization. **William Trenti:** Investigation. **Elia Pagliarini:** Writing – original draft, Investigation. **Livia Vittori Antisari:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Diana Di Gioia:** Writing – review & editing, Resources, Funding acquisition.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Data availability

Data will be made available on request.

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