Microbiological Monitoring of Superficial Water of Lis Valley Irrigation District. Impacts of Agricultural Activities

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Abstract. Society's demand for the preservation of water resources has led to major technological advances in irrigation management, simultaneously promoting socio-economic development and natural resources conservation. This study aims to assess the impact of agricultural activities at the Lis Valley Irrigation District (LVID) on the quality of superficial water. This information will support the improvement and the minimization of risks regarding the conservation of groundwater and surface water bodies in the valley. The physicochemical and microbiological quality of water samples collected at the inlets and outlet of the LVID was determined according to the analytical reference methods. Results showed that the water at the inlets of the LVID contains microbiological contamination, due to external sources. In general, the concentrations of total coliforms (TC) in the inlet water were 1.4 times lower than in the outflow water; however, an inverse situation was found with faecal coliforms (FC), faecal enterococci (FE), and ampicillin-resistant (Amp^r) *Enterobacteriaceae*. These data reveal that the LVID's agricultural activities have not aggravated (and have even improved) those microbiological indicators of the outlet water when compared to those of the water which reaches the entrance of the district, available for agriculture. Concluding, the monitoring in a continuum of a few years will allow the assessment of risk situations of microbiological contamination to support the improvement of regional planning and agricultural management, being recommended to increase the sampling representativeness.

Keywords: Microbiological indicators · Coliforms · Enterococci · *Enterobacteriaceae* · Irrigation · Conservation of water bodies

1 Introduction

The development, sustainability, and entrepreneurial competitiveness of Portuguese agriculture depend heavily on irrigation [1], namely on the improvement of irrigation water

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management. Also, in the climate change scenarios that indicate a decrease in available water resources [2], water-saving is a strategic priority to irrigation development. The management of irrigation districts with water running systems has specific operative and water distribution characteristics; therefore, the water reuse from the drainage ditches allows mitigating the water shortage, with higher distribution equity and water productivity. This rises however some concerns regarding the health safety of farmers, food and consumer safety, as well as soil salinization. Thereafter, it is important to control the physicochemical and microbiological water quality, to supply the information system with data required for the rational management of the collective irrigation. In this context, water monitoring is a basic process for improving the management of the irrigation district, providing information about irrigation and drainage water quality, both spatially and temporally, identifying health, and environmental risks, and the required farm practices adjustments to cope with putative water scarcity and quality problems. These aspects of the irrigation systems governance should support farmers in adopting more environmentally friendly practices, thus contributing to irrigation sustainability [3].

This paper describes a study carried out on the framework of Lis Valley Water Management Operational Group, integrated on the agricultural European Innovation Partnership (EIP-AGRI) [4]. It aimed to assess the impact of agricultural activities at the Lis Valley Irrigation District (LVID) on the microbiological quality of superficial water, focusing on the results of the 2018 to 2020 seasons. This information will support the improvement and the minimization of risks regarding the conservation of groundwater and surface water bodies.

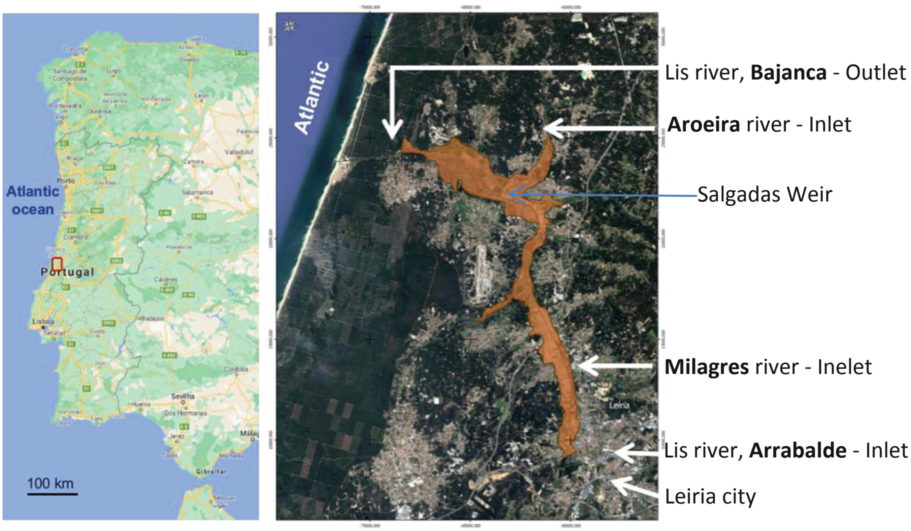
2 Materials and Methods

2.1 Study Area

This study was conducted in the LVID with about 2000 ha, mainly with modern alluvial soils of high agricultural quality, although some have poor drainage conditions. The main crops grown include forage maize, forage grass, horticultural, orchards and rice. The climate in the Lis River watershed has a Mediterranean influence, with hot and dry summers, as well as an Atlantic influence, with predominant frontal winter surfaces, moving from west to east, which are liable for most of the precipitation [5]. This is a public irrigation district managed by a Water Users' Association (WUA, located in the counties of Leiria and Marinha Grande, in the Coastal Center of Portugal (Fig. 1)). The irrigation water is supplied through several small temporary weirs from the Lis River and its tributaries, and the conveyance system comprises, for each sector, the main canal, gravity-fed by a river diversion from those weirs. During the irrigation peak period, the downstream irrigated areas are recharged by pumping from the river or drainage ditches [5].

2.2 Water Sampling

The methodology for monitoring the water quality of the irrigation and drainage networks followed published guidelines [8]. The sampling aimed to assess the representativeness



A B

Fig. 1. Location of Lis valley irrigation district in Portugal (red rectangle) [6] (A) and the sampling sites in the LVID [7] (B).

of the agricultural actives at the LVID in the water quality released downstream, into the environment. The sites studied are located at the main inlets: Lis River at Leiria, Arrabalde [39°45′8,29″; 8°48′28,49″], Milagres River [39°47′04,542″; 8°49′29,750″], Aroeira River [39°53′34,644″; 8°50′54,316″], and at the outlet of the perimeter: Lis River at Bajanca [39°53′00,295″; 8°56′05,295″]. The sampling at the downstream Lis river section (Bajanca bridge site) was carried out at low tide, to avoid the influence of brackish seawater (Fig. 1). Within the scope of this article, these sites are hereinafter referred to as, Arrabalde, Milagres, Aroeira, and Bajanca, respectively.

Sampling for microbiological quality was conducted during the irrigation periods to guarantee seasonal representativeness. It was carried out in three periods: i) at the beginning of the irrigation season when there is plenty of water in the water lines, ii) at the end of the summer, before the autumn rains, when there is a shortage of water in the water lines and iii) in the middle of those periods, during the peak of the irrigation period. Water samples were collected in sterile plastic bottles and maintained at 4 ºC until the microbiological processing, which was always carried out within four hours after sampling.

2.3 Physicochemical Analysis

The analysis of the physicochemical quality of the water samples was performed with a precalibrated *in-situ* portable multiparametric probe (SmarTROLL RDO Handheld, Fort Collins, CO 80524, USA) measuring, pH, Electrical Conductivity (EC, µS/cm), Saturation of Dissolved Oxygen (SDO, %), Temperature (T, °C), Total Dissolved Solids (TDS, ppm). Nitrates (NO_3^-, mg/L) were evaluated with ion chromatography [9]. The results obtained were compared to the maximum recommended values (MRV) of the Portuguese Irrigation Water Quality Standards [10].

2.4 Microbiological Analysis

Microbiological analysis of water samples included the enumeration of Total Coliforms (TC), Fecal Coliforms (FC), and Fecal Enterococci (FE) by the dilution method with multiple fermentation tube technique, the results being expressed as the Most Probable Number (MPN/100 mL). The mesophilic microorganism counts at 37 °C and 22 °C were carried out by the plate count incorporation method, the results being expressed as Colony Forming Units (CFU/mL), following the analytical reference methods [10].

The ampicillin-resistant bacteria in water samples were enumerated with the membrane filtration technique. Decimal dilutions of each sample were prepared in 0.9% sterile saline solution, from which 100 mL were filtered through cellulose membranes of 0.45 µm in pore size (Millipore, Bedford, MA, USA) under a vacuum system, and the filters aseptically placed on the surface of a selective medium for *Enterobacteriaceae* VRBG (Violet Red Bile Glucose) agar (Oxoid, Hampshire, England) supplemented with 20 µg/mL of sodium ampicillin (AppliChem, Darmstadt, Germany). Plates were aerobically incubated at 37 °C for 24 h to 48 h. All the *Enterobacteriaceae* isolates resistant to ampicillin (Amp^r) were counted [11].

The salinity of inlet water samples is significantly lower than those of the valley outlet samples. This is explained by the hydrogeological characteristics of saline springs on the downstream area and by the discharge of the LVID drainage ditches, as well as by the high tide of Atlantic water. This significant increase of water salinity of Lis river towards the mouth prevails, although the Aroeira contributes with much less saline water. The river water downstream Salgadas weir, located in the middle of the irrigation district (Fig. 1), is not feasible for irrigation.

3 Results and Discussion

3.1 Physicochemical Parameters of Water Quality

The average results of the physicochemical analysis obtained from 2018 to 2020 are presented in Table 1. The pH values of water samples from the irrigation and drainage are within an acceptable range [10], in general slightly above 7.0, except for the Aroeira river, a Lis tributary.

The saturation dissolved oxygen values of inlet and outlet water samples are similar and above 86% (Table 1). Although there are no references to irrigation water [12], it is recommended a value higher than 50% for drinking water, allowing to conclude that dissolved oxygen in these water bodies is good or acceptable.

The average nitrate content of inlet water samples (14.27 ± 3.7 mg/L) is lower than at the outlet (16.8 ± 6.8). Aroeira samples had fewer nitrates than other sites. Countering this trend, the content of upstream water samples evidence the intensive production systems, outside of the LVID and also the practice of soil manure amendment. Although the data is still scarce and does not include groundwater, it indicates that irrigation in the Lis Valley has no significant negative effect on the nitrate load on water resources [10].

Table 1. Physicochemical parameters of water samples, per site for Lis valley inlet and outlet water (average ± standard deviation).

Site	Role	Water quality parameters				
		pH (6.5–8.4)*	EC, μS/cm (1000)*	SDO, %	TSD, ppm (640)*	Nitrates, mg/L (50)*
Arrabalde		7.80 ± 0.37	591.50 ± 18.9	87.26 ± 11.94	370.00 ± 30.01	17.0 ± 3.0
Milagres	Inlets	7.73 ± 0.31	493.80 ± 14.8	88.09 ± 10.87	298.33 ± 32.48	16.8 ± 5.7
Aroeira		6.84 ± 1.17	286.97 ± 38.37	91.07 ± 10.87	298.33 ± 102.92	9.0 ± 2.5
Bajanca	Outlet	7.39 ± 0.29	1272.00 ± 850	86.33 ± 8.37	803.44 ± 571.90	16.8 ± 6.8

* Maximum recommended values, according to the Portuguese Irrigation Water Quality Legislation [10]. Legend: EC - Electrical Conductivity; SDO - Saturation of Dissolved Oxygen; TDS - Total Dissolved Solids

3.2 Microbiological Parameters of Water Quality

The analysis of water microbiological quality, assessed through the enumeration of indicator groups at the main inlet and outlet of the perimeter (Fig. 2A, B), shows an effect of the agricultural activities within the Valley on the water quality.

The TCs are widespread in the basin with average counts above 700 MPN/100 mL in all sites, being the counts in drainage water 1.4 times higher than those in irrigation water. Nevertheless, those enumerations, both from irrigation and drainage water samples are in between the maximum recommended value (500 MPN/100 mL) and the maximum admissible value (10,000 MPN/100 mL) regarding aquaculture and bathing purposes [10].

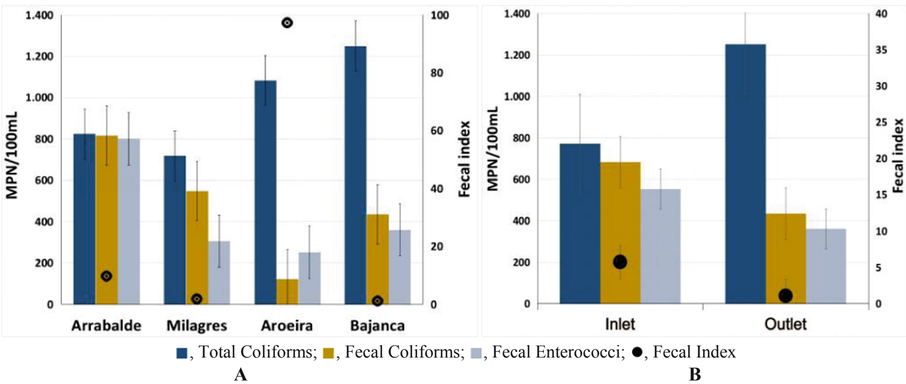


Fig. 2. Total Coliforms, Fecal Coliforms, Fecal Enterococci and Fecal Index (average counts and standard deviation) per sampling site (A) and at inlets and outlets of the LVID (B).

The FC counts in all samples are above 100MPN/100 mL, therefore not fulfilling the quality environmental objectives established for irrigation water [10]. The higher average

FC counts were registered in the inlet samples, namely in those collected at Arrabalde (816.5 MPN/100 mL) and Milagres (547.7 MPN/100 mL). These high FC values of irrigation water (Lis river water at Arrabalde weir) is explained by contamination from external sewage sources from irrigated area [13]. Nevertheless, the FC counts in the outlet water were 1.14 times lower than that in the average inlet water. This might be explained by the soil effect on the reduction of *Enterobacteriaceae* numbers [14] and also due to the type of crops grown at the outflow of the valley (pastures), which soils are not manure-amended, contrary to those with other crops in upstream areas of the valley. Considering aquaculture and bathing uses, the FC counts of LVID effluent water are above the maximum recommended value (100 MPN/100 mL), but below the maximum admissible value (2,000 MPN/100 mL) [10].

The quality standard set for irrigation water [15] refers to quality requirements for the reuse of urban wastewater treated in irrigation of agricultural, forestry, ornamental, nursery, lawn, and other green spaces. Relative to the microbiological quality requirements, that standard [15] has taken into consideration the use of irrigated crops (e.g. human or animal consumption, or industrial crops) and the respective irrigation method applied, less restrictive and more flexible than it is set in the Portuguese legislation. [10]. According to Monte and Albuquerque [16], the Portuguese legislation [10] is very demanding, since the water in most rivers does not have an FC content below 100 MPN/100 mL.

Regarding the FE (Fig. 1), the higher average counts were recorded at the valley inlet, specifically at Arrabalde weir (800.9 MPN/100 mL), with a similar explanation given to FC [13]. Although, the FE counts in the outlet water were 1.26 times lower than those at the inlets, they are above the maximum recommended value (100 MPN/100 mL), considering aquaculture and bathing uses of LVID effluent water.

The analysis of the Fecal Index proposed by Pepper and co-authors [17] (Table 2) reveals that at the initial section of Lis River (Arrabalde) and Milagres there is evidence of fecal contamination of human origin, thus entering the LVID. Differently, the water sampled at the final sections of Lis river evidence fecal contamination of animal waste in mixed pollution, possibly related to the use of animal manure as a soil fertilizer.

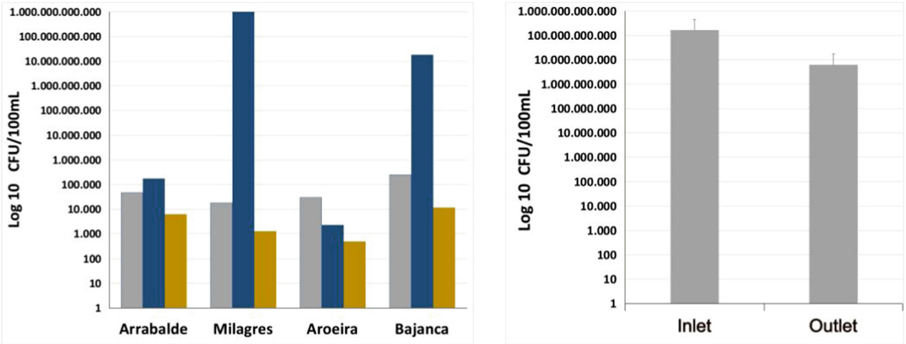
Table 2. Source of pollution according to the Fecal Index (FC/FE).

FC/FE	Source of pollution
>4.0	Human pollution
2.0–4.0	Human waste in mixed pollution
0.7–2.0	Animal waste in mixed pollution
<0.7	Wild animal pollution

FC, Fecal Coliforms; FE, Fecal Enterococci;
Source: [17].

3.3 Enumeration of Ampicillin-Resistant Bacteria

The mean counts of *Enterobacteriaceae* isolates resistant to ampicillin showed high variability throughout the irrigation season in Milagres and Bajanca sites (Fig. 3A), with particularly high enumerations in 2019 samples. The water samples with higher mean counts were collected in Milagres river (seven orders of magnitude higher than those from Aroeira and Arrabalde134). The average counts of the inputs are two orders of magnitude higher than the output (Fig. 3B).



■, 19/sept/2018; ■, 26/May/2019; ■, 1/June/2020

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Fig. 3. Average Amp^r *Enterobacteriaceae* per sampling site and sampling dates (A) and at the LVID inlet and outlet (B).

The frequent resistance to antibiotics found in the aquatic systems, namely to the β-lactams (ex. ampicillin), is an additional critical point associated with faecal contaminants, placing the water quality and public health in a critical situation. The identification of situations of microbiological contamination risk in irrigation water of LVID, whose main responsibility is external to the Lis valley, requires special precautionary measures, regarding the safety of farmers and consumers. These risks will also need to be assessed for the real influence of agricultural activity within the irrigation district, particularly at the drainage network level.

4 Final Considerations

The analysis performed to the physicochemical and microbiological parameters of water samples reveals that a significant source of the problems identified in the water is external to the irrigation district.

The agricultural activities in the LVID seems to have a positive impact in the quality of the outlet water, despite the poor quality of the water resources reaching the district and used for irrigation (the counts of faecal coliforms and ampicillin resistant bacteria were reduced 1.14 times and two orders of magnitude, respectively). The monitoring of soil and water matrixes play an important role supporting decisions in the most severely affected sectors.

The WUA and farmers should adopt the operating mode and technologies to minimize the negative impacts of the low-quality irrigation water, which monitoring within the district is crucial to allow effective adjustments [8, 19, 20]. The organic load of livestock applied to the soil, in certain areas of the district, makes the microbiological risk possibly an emerging issue, due to the presence of antibiotic resistance genes [21]. The downstream part of the district has hydrogeological characteristics of significant salinity which is a risk factor for soil due to capillary rise or drainage water reuse [22].

The poor quality of the fresh water resources available for irrigation leads to multiple risk problems, requiring mitigation measures, namely: i) Prudent use of manure as soil fertilizer, which should be appropriately composted [23], mainly when applied to horticultural crops, to protect environmental and public health problems due to microorganisms and other contaminants, like antibiotics [21]; and ii) Choice of irrigation technology, which should be adjusted according to the risk assessment for the farmer and the consumer of the product. For fresh crops, preference should be given to drip irrigation; surface furrow irrigation has no special restrictions for other crops and sprinkler irrigation is preferentially dedicated to foraging crops or crops with post-harvest processing. Farmers should be informed about special hygiene and safety precautions where microbiological contamination is most at risk [24].

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