Evaluation of the microbiological water quality in a rice-based agroecosystem in Center of Portugal

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Abstract: The demand of society for the preservation of water resources has led to great advances in technology and agricultural management to limit their negative impacts, favours socioeconomic development and promote the conservation of natural resources. This communication refers to a case study of the paddy rice agroecosystem of the Lis Valley Irrigation District (LVID), Center of Portugal, sponsored by the MEDWATERICE project (www.medwaterice.org), which considers the monitoring of the microbiological quality of irrigation water supply, surface and subsurface drainage water in the farmers' fields, representative of those rice agroecosystem. The microbial quality indicators applied to the irrigation water and rice drainage, were the counts of total coliforms (TC), faecal coliforms (FC), faecal enterococci (FE) and ampicillin-resistant Enterobacteriaceae (E-Ampr) performed according to analytical reference methods. The results show that the water resources at the entrances to the LVID contained high microbiological contamination due to sources outside the perimeter. The rice agroecosystem had a beneficial impact on the load of TC, FC and FE of the drainage water, with a decrease of two orders of magnitude between the values of these counts at the entrances and at the exits of the rice fields. However, E-Ampr counts increased in the rice paddy soil because of the specificity of this wetland ecosystem and the activity of wildlife. This study assessed the risks of microbiological contamination of irrigation water and the beneficial effect of the rice agroecosystem in their control, particularly farmers microbiological contamination, or environment problems, having provided information to support water management and mitigate those problems.

Introduction

The development, sustainability and entrepreneurial competitiveness of the Portuguese agriculture depends heavily on irrigation [1], namely on the improvement of the irrigation water management. In addition, in the climate change scenarios that indicate a decrease in available water resources [2], water saving is a strategic priority to the 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, the 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].

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 predominant climate in the Lis River watershed results from Mediterranean and Atlantic influences. The Mediterranean influence is reflected mainly in summer, because of the high temperatures and sunshine and the absence of precipitation. The Atlantic influence is characterized by the predominant winter front surfaces which, moving from west to east, are responsible 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 comprise, for each sector, a main canal, gravity-fed by a river diversion from those weirs. During the irrigation peak period, the downstream irrigated areas are re-charged by pumping from the river or drainage ditches [5].

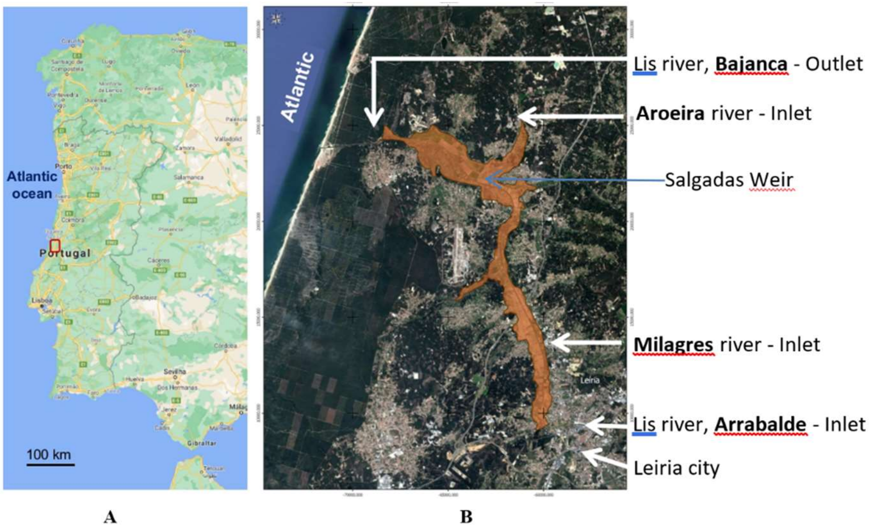


Figure 1. Location of Lis Valley Irrigation District in Portugal (red rectangle) [6] (A) and the sampling sites in the LVID [7] (B).

2.2 *Water sampling*

The methodology for monitoring water quality of the irrigation and drainage networks followed published guidelines [8]. The sampling aimed to assess the representativeness 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 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 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 4ºC until the microbiological processing, which was always carried out within four hours after sampling.

2.3 *Physicochemical analysis*

The physicochemical quality of the water samples was evaluated with a precalibrated in-situ portable multiparametric probe (SmarTROLL RDO Handheld, Fort Collins, CO 80524, USA), for the following parameters: pH, Electrical Conductivity (EC, µS/cm), Saturation of Dissolved Oxygen (SDO, %), Temperature (T, ℃), 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/100mL). 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), in accordance with 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 100mL were filtered through cellulose membranes of 0.45μm in pore size (Millipore, Bedford, MA, USA) under a vacuum system and the filters aseptically placed on the surface of selective medium for *Enterobacteriaceae* VRBG (Violet Red Bile Glucose) agar (Oxoid, Hampshire, England) supplemented with 20μg/mL of sodium ampicillin (AppliChem, Darmstadt, Germany). Plates were aerobically incubated at 37ºC for 24h to 48h. All the *Enterobacteriaceae* isolates resistant to ampicillin (Ampr) were counted [11].

The water salinity of affluent 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 a 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 obtained 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.

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	Inlets	7.80±0.37	591.5±18.9	87.26±11.94	370.0±30.0	17.0 ± 3.0
Milagres	Inlets	7.73±0.31	493.8±14.8	88.09±10.87	298.3±32.5	16.8 ± 5.7
Aroeira	Inlets	6.84±1.17	287.0±38.37	91.07±10.87	298.3±102.9	9.0 ± 2.5
Bajanca	Outlet	7.39±0.29	1272±850	86.33±8.37	803.4±571.9	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

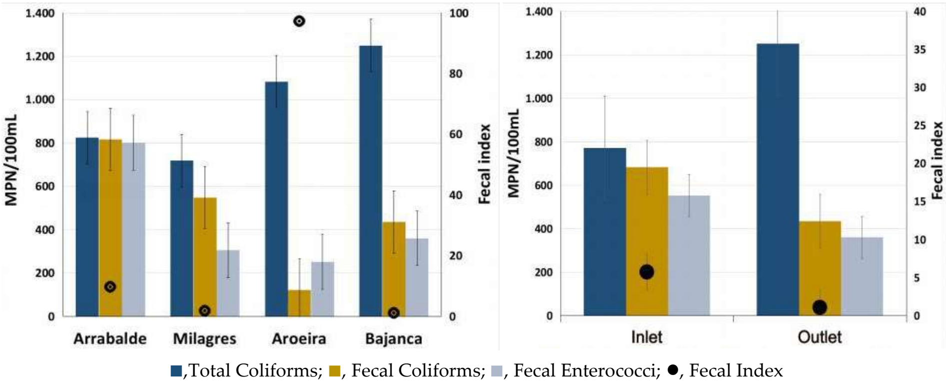
The saturation dissolved oxygen values of inlet and outlet water samples are similar and above 86% (Table 1). Although there are no references to irrigation water [12], it is recommended a value higher than 50% for drinking water, allowing to conclude that dissolved oxygen in these water bodies is good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

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

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 (Figure 2 A,B), shows an effect of the agricultural activities within the Valley on the water quality.

The TC are widespread in the basin with average counts above 700MPN/100mL 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 (500MPN/100mL) and the maximum admissible value (10,000MPN/100mL) regarding aquaculture and bathing purposes [10].



A **B**

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

The FC counts in all samples are above 100MPN/100mL, therefore not fulfilling the quality environmental objectives establish for irrigation water [10]. The higher average FC counts were registered in the inlet samples, namely in those collected at Arrabalde (816.5MPN/100mL) and Milagres (547.7MPN/100mL). 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 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 (100MPN/100mL), but below the maximum admissible value (2,000MPN/100mL) [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] have 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 faecal coliforms content below 100 MPN/100mL.

Regarding the FE (Figure 1), the higher average counts were recorded at the valley inlet, specifically at Arrabalde weir (800.9MPN/100mL), 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 (100MPN/100mL), considering aquaculture and bathing uses of LVID effluent water.

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

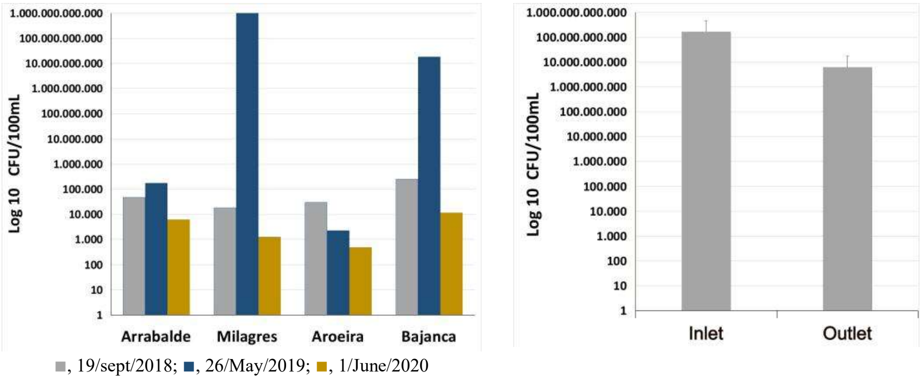
Table 2. Source of pollution according to the Fecal Index (FC/FE).

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 (Figure 3A), with particularly high enumerations in 2019 samples. The water samples with higher mean counts were collected in Milagres river (seven order of magnitude higher than those from Aroeira and Arrabalde). The average counts of the inputs are two order of magnitude higher than the output (Figure 3B).



A **B**

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

5. Conclusions

The analysis of the physicochemical and microbiological quality of water samples reveals: i) A significant source of problems is external to the irrigation district, as various measurements demonstrate. Thus, it is up to the WUA and to farmers to adapt the operating mode and technologies to minimize the negative impacts of these problems. Water quality monitoring within the district is crucial to know the local reality over time and allow adjustments to be as effective as possible [8,19,20]. ii) The organic load of livestock applied to the soil in certain areas of the district makes the emerging microbiological risk possibly, due to the presence of antibiotic resistance genes [21]. iii) 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]. Surface drainage water, groundwater, and soil monitoring play an important role in assessing the problem to determine action in the most severe cases.

Poor water quality led to multiple risk problems, requiring protection, adaptation, or mitigation measures: i) Judicious use of manure as soil fertilizer, which should be appropriately composted in farms and slaughterhouses treatment plants [23]. Cautions should be reinforced when applied to horticultural crops, or crop handling, to protect environmental and public health problems due to microorganisms and other contaminants, like antibiotics [21]; ii) Choice of irrigation technology should consider irrigation water quality and 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 forage 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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