Neotropical Biology and Conservation
7(1):16-20, january-april 2012
 - doi: 10.4013/nbc.2012.71.03

Microbiological quality of the rice-field water in the South Brazil

Qualidade microbiológica da água da cultura do arroz irrigado, no sul do Brasil

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Abstract

Total and fecal coliforms are bio-indicators of the environmental impact caused by anthropic action and are considered indicators of the microbiological quality of the water. In the cultivation of rice by irrigation much water is used and care in water management is required in order to increase the volume and quality of production while reducing the environmental impact. The objective of this research is to evaluate the quality of the irrigation water used in five rice-growing regions of the State of Rio Grande do Sul during the vegetative and reproductive phases. The results demonstrate that the indices of total and fecal coliforms in the rice field plots of the fields in all the rice producing regions of Rio Grande do Sul evaluated in this study were lower in the reproductive than in the vegetative phases. The irrigation channel presented low indices of fecal coliforms but a higher incidence of total coliforms. The data demonstrated that irrigated rice cultivation can alter the microbiological quality of the water in the plots by its potential capacity of circulating the nutrients with the result that the drainage water presents a smaller quantity of contaminating microbial agents.

Key words: water, irrigation, fecal coliforms, rice fields.

Resumo

Os coliformes totais e fecais são bioindicadores de impacto ambiental causado por ação antrópica, sendo considerados indicadores de qualidade microbiológica da água. Na cultura do arroz irrigado, onde é necessário o uso da água, devem-se ter cuidados relacionados ao manejo, visando aumento de produção e qualidade do produto, com redução do impacto ambiental. O objetivo da pesquisa foi avaliar a qualidade microbiológica da água de irrigação, em cinco regiões orizícolas do Rio Grande do Sul, nas fases vegetativa e reprodutiva. As amostras de água foram submetidas à análise da qualidade microbiológica, sendo adotado o método de indicadores de poluição que estabelece simultaneamente a concentração de coliformes fecais e totais, através da análise bioquímica pelo método Colilert® (IDEXX). Os resultados mostram que os índices de coliformes totais e fecais nas parcelas de arroz, de todas as regiões produtoras do Rio Grande do Sul, avaliadas no presente estudo, foram inferiores na fase reprodutiva da cultura quando comparados a sua fase vegetativa. O canal de irrigação apresentou baixos índices de coliformes fecais, no entanto, a presença de coliformes totais mostrou-se mais elevada. Os dados apresentados demonstram que a orizicultura pode alterar a qualidade microbiológica da água presente nas parcelas, devido ao seu potencial de ciclagem de nutrientes, fazendo com que a água de drenagem apresente menor quantidade de agentes microbianos contaminantes.

Palavras-chave: água, irrigação, coliformes, campos de arroz.

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Introduction

The total and fecal coliforms are bio-indicators of the environmental impact caused by antropical action. These bacteria are considered indicators of the microbiological quality of water and food and the Escherichia coli bacterium is used as an indicator of fecal contamination which is found principally in the intestine of human beings and warm-blooded animals. This species of bacteria is of considerable relevance in medical microbiology because of the water-carried diseases (such as diarrhea) that can be caused by inadequate basic sanitation conditions and/or poor quality of drinking water. These diseases have been responsible for many epidemics and for high rates of infantile mortality related to the drinking water used for human consumption (Greig *et al*., 2007).

The presence of pathogenic micro-organisms in water used for consumption, irrigation and recreation is considered one of the sources of risk to human health. The various domestic animals usually found in an agricultural area are frequently a major source of pathogenic micro-organisms on the ground surface or in the watershed of aquatic systems (Jamieson *et al*., 2003; Donkor *et al*., 2010).

Both surface and subterranean water resources, are suffering the consequences of various activities that are being developed on a large scale in the hydro-graphic basins and which alter both the quantity and the quality of the available water. Because of the problems in obtaining good quality water due to the increase of domestic and industrial waste, water of a lower quality is now often used for irrigation purposes (Galaviz-Vila, 2010).

Rice is a graminea classified in the C-3 plant group and is cultivated in Brazil mainly in wetlands where it has adapted to the aquatic environment. In this cultivation system controlled irrigation is widely used and the plantations occupy about one million hectares in the subtropical sub-region (the States of Rio Grande do Sul and Santa Catarina). These high-tech plantations produce excellent harvests (SOSBAI, 2005).

As it is necessary to use additional water for the development of this culture, supplies have to be taken from rivers, dams, lakes and weirs. To ensure the productivity, the quality of the product, minimal environmental impact and reduction of the production cost, the procedures for management of this water are extremely important and should be considered (Gomes and Pauletto, 1999). In this context, it should be remembered that the cultivation of irrigated rice, by submersion in the clay, requires about 2000 liters of water to produce one kilo of skinned rice, in fact, rice is one of the most demanding crops as regards water consumption. Considering the importance of this cereal in human alimentation and the quantity of water utilized in its production, it is important to study the quality of water entering the irrigation channel and that being returned to the water table via the drainage channels after use (Klostermann, 2001) with a view to the reuse of this resource in better hygienic conditions.

The objective of this study is therefore; to evaluate the microbiological quality of irrigation water used rice farmers in five regions in the in South Brazilian during the vegetative and reproductive phase of crop growth determining the importance of wetland rice fields in the alteration of the populations of the pathogenic bacteria in the irrigation water.

Material and methods

Triplicate samples of water for our study were collected between October 2005 and May of 2006 from areas corresponding to one hectare, in each one of the five irrigated rice production regions in the State of Rio Grande do Sul (Plains, Northern Coast, Southern Coast, Central Depression and Western Border, corresponding of the Dom Pedrito, Santo Antônio da Patrulha, Camaquã, Cachoeira do Sul and Uruguaiana, respectively – Figure 1). For each region three random collection points, chosen by raffle, were chosen in the irrigation channels and/or in the rice field plots of the plantations. Each sample consisted of 100 mL of water obtained by mixing ten 10 mL sub-samples, suitably flasked and conserved at 4ºC. During the culture cycle, two collections were made – one in the vegetative phase (November/December 2005) and the other in the reproductive period (March of 2006).

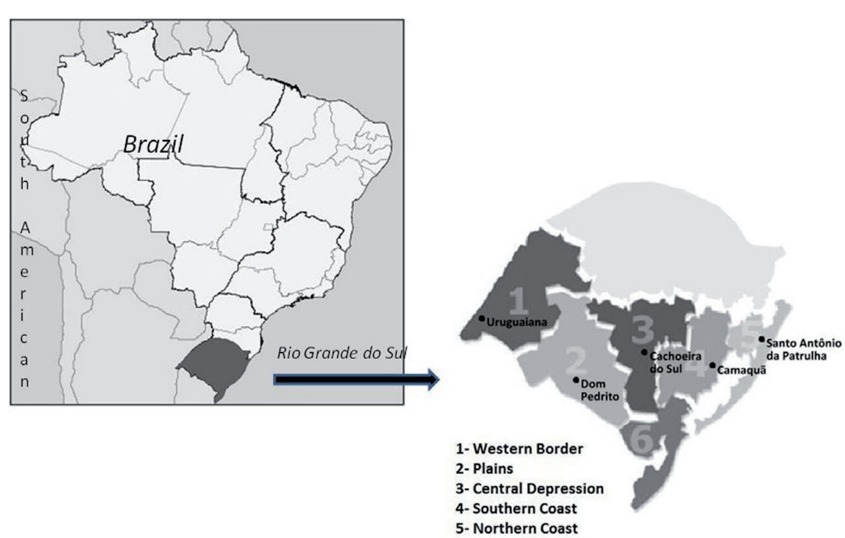

Figure 1. Map of study area and its water quality monitoring sites.

Quality analysis of the water samples was performed using the method of pollution indicators that simultaneously establishes the concentrations of total and fecal coliforms. The concentration of coliforms is expressed in the MPN/100 mL (Most Probable Number/100 mL) of the water sample. According to the WHO (1989) water samples presenting values above 1000 UFC in a 100 mL concentration of coliforms, may be used only for forage and cereal cultures. The quantity of contamination was determined by biochemical analysis using the Colilert® (IDEXX) method which makes use of defined substrate technology (Defined Substrate Technology – DST). The nutrient indicators (ONPG and MUG) are the principle source of carbon in the Colilert® system and these are metabolized by the β-D-Galactosidase and β-D-Glucoronidase enzymes to identify the coliforms and the E. coli, respectively. In biologically secure, class 1 cabinets (one for each sample) five dilutions in saline solutions (10-1, 10-2, 10-3, 10-4 e 10-5) were made and the last dilution 10-5 was applied in the Colilert® system. Afterwards the suspension was completely homogenized by applying 100 mL in 97 (48/49) wells of the Quanti-Tray/2000® equipment. This material was sealed and placed in a bacteriological oven at a 37oC, for 24h. During the development of the coliforms in the presence of ONPG and the synthesis of β-D-Glucoronidase, the growth medium alters from uncolored to yellow. Also, the growth of E. coli in the presence of MUG and synthesis of β-D-Glucoronidase fluoresces when exposed to ultraviolet light (365nm). The data is obtained by quantification of the wells - the yellow well for the total coliforms and the emission of florescence for the fecal coliforms. The results are converted into the most probable number in 100 mL of water (NMP/100 mL) using a Quanti-Tray® Most Probable Numbers Table provided by the Colilert® system (Clesceri *et al*., 1998).

The data on the microbiological quality of the water was evaluated between the five rice producing regions, the phases of the cultivations and the irrigation points. The parameters evaluated corresponded to the quantification of the coliforms total and fecal and the values were submitted to a Variance Analysis (ANOVA) and the measurements compared by Tukey at a probability of 5% utilizing the Microsoft Systat 11.

Results and discussion

The data presented in Tables 1 and 2 correspond respectively to the measurements of the indices of total and fecal coliforms present in the water in the irrigation channels and in the rice field plots of the plantations in the different rice producing regions of the State of Rio Grande do Sul. These values in the cultivated plots correspond to the bacteria present in samples collected in the vegetative phase (that is, soon after the irrigation of the plantation area) and in the productive phase (just before the area was drained) of the rice.

The indices of total and fecal coliforms were compared amongst the five regions, both in the water collected from the irrigation channels and in the rice field plots. The total coliforms found in the irrigation channels differ significantly between the five regions ($F_{4,\ 10}$= 5.647; P<0.05). The values from the region of Plains are the highest and are much different from the other regions. No significant differences are observed between the results for the fecal coliforms in the irrigation channels ($F_{4,\ 10}$= 1.500; P>0.05).

In the rice field plots, the indices of total coliforms differed significantly in the five production regions ($F_{4,\ 10}$= 15.788; P<0.05) and again, the numbers from Plains were the highest and differed from the other region in a relevant manner. However, the fecal coliform count in the rice plots

Table 1. Indices means of total coliforms present in the water samples in the irrigation channels and in the rice field plots, of the two cultivation phases, in the five rice producing regions of the State of Rio Grande do Sul (2005/2006).

Water samples	Irrigation channels		Rice field plots F1		F2	
Regions	M	SD	M	SD	M	SD
Plains	12.92	8.15	79.5	9.86	10.66	1.15
Northern Coast	0	0	3.43	3.15	0	0
Southern Coast	2.22	1.7	8.33	4.71	0	0
Central Depression	0.85	1.1	4.13	2.76	4.86	1.63
Western Border	2.42	1.68	0	0	0	0

Note: The concentration of coliforms expressed in MPN/100 mL. F_1 (Phase vegetative); F_2 (Phase reproductive); M (means); SD (Standard Deviation).

Table 2. Indices means of faecal coliforms present in the water samples in the irrigation channels and in the rice field plots, of the two cultivation phases, in the five rice producing regions of the State of Rio Grande do Sul (2005/2006).

Water samples	Irrigation channels		Rice field plots F1		F2	
Regions	M	SD	M	SD	M	SD
Plains	0.17	0.29	2.03	2.1	0	0
Northern Coast	0	0	0	0	0	0
Southern Coast	0.17	0.29	0.66	0	0	0
Central Depression	0	0	0	0	0	0
Western Border	0.5	0.5	0	0	0	0

Note: The concentration of coliforms expressed in MPN/100 mL. F_1 (Phase vegetative); F_2 (Phase reproductive); M (means); SD (Standard Deviation).

demonstrated no significant differences ($F_{4, 10}$= 15.788; P<0.05).

The indices of total coliforms did not show significant differences between the collection points, the irrigation channel and the rice field plots in the Plains region ($F_{1,4}$= 5.972; P>0.05), the Northern Coast ($F_{1,4}$= 3.556; P>0.05), the Southern Coast ($F_{1,4}$= 1.358; P>0.05) or in the Western Border ($F_{1,4}$= 6.244; P>0.05). Only in the Central Depression, did the evaluated difference demonstrate significance ($F_{1, 4}$= 22.709; P<0.05), where the indices registered in the irrigation channel are lower than those in the rice field plots.

Comparing the coliforms total in the two phases of cultivation within the rice field plots, significant differences are observed in Plains ($F_{1, 4}$= 47.068; P<0.05) and in the Southern Coast ($F_{1, 4}$= 9.408; P<0.05). In these regions the indices fall of in a relevant manner from the vegetative to the reproduction phases. However, in the regions of the Central Depression ($F_{1, 4}$= 0.157; P>0.05), and the Northern Coast ($F_{1, 4}$= 3.556; P>0.05) the values were not significantly different. In the Western Border region, in both phases of the cultivation no total coliforms were detected.

When the collection points are compared, the fecal coliforms were not significantly different in Plains, ($F_{1, 4}$= 1.911; P>0.05), Southern Coast ($F_{1, 4}$= 0.500; P>0.05) or in the Western Border ($F_{1, 4}$= 3.000; P>0.05). In the Northern Coast and Central Depression, fecal coliforms were not present. Comparing the data on the two phases (vegetative and productive) it was found that the differences were not significant in the Plains region ($F_{1, 4}$= 2.951; P>0.05) nor in the Southern Coast ($F_{1, 4}$= 4.000; P>0.05). In the remaining regions (Northern Coast, Central Depression and Western Border) fecal coliforms were not registered in the 100 mL sample of water. This data shows that the water utilized for irrigation is not being affected by the agents that cause microbial contamination – the water used for the irrigation of the rice is of good quality.

The water used for irrigation of rice cultivations in wetlands may have been obtained from various sources – such as, weirs, dams, lakes, rivers and others.

The series of results obtained in the present research show that the indices of total and fecal coliforms in the rice field plots, in all the production regions of the State of Rio Grande do Sul, were lower in the reproductive phase of the rice cultivation than in the vegetative phase of the same plantation. The irrigation channel presented low indices of fecal coliforms but the amount of total coliforms detected was higher. The data presented demonstrate that the rice cultivations could alter the micro bacterial quality of the water present in the rice field plots, due to their potential for cycling the nutrients with the result that the drainage water retains a lower quantity of pathogenic bacterial agents.

The data in the research is in agreement with the results found by Sant'ana *et al*., (2003) considering that the presence of total coliforms; (an indicator of microbiological quality) does not necessarily mean fecal contamination. However, when the E. coli, bacteria (considered a fecal coliform) is found in the sample the presence of enteropathogens is certain. In this context, the data presented by Stickler (1989) reveal that the presence of total coliforms, in the absence of *E. coli*, may indicate that the irrigation source is being contaminated by surface water and, in this case, the detection of *Enterococcus* indicates contamination with a fecal origin.

These indices registered in the Central Depression can be explained by studies on pathogenic agents that influence the microbiological quality of the waters. Considerable research (Farooq *et al*., 2008; Siqueira *et al*., 2009) describes the increase of coliforms during the summer months related to the quality of fresh water, bacterial contamination of water near to rural properties and also of water into which sanitary waste has been discharged. This may be due to innumerable factors, such as: the increase and/ or alteration of the distribution of the animals on farms; greater agricultural activity, including the application of fertilizers for production reasons (Hunter *et al*., 2002). The highest level of fecal coliforms in agricultural locations can occur due to the presence of domestic animals defecating near to the surface of the water, run-off from fertilized soil into surface water, effluent liquids draining from stables or breeding sheds or even fluids from domestic waste (Boyer, 2008). This data presented by other authors bears out the results of the present study as regards the total coliforms found in the five regions of irrigated rice plantations in Rio Grande do Sul all of which may be showing the influence of factors such as the phytosanitary treatments utilized in rice cultivations and the presence of domestic animals in the vicinity of these agricultural areas.

There is evidence that plantations of irrigated rice can act as a biological filter, removing nutrients from the eutrofized hydraulic medium for use by the plants and thus reducing the concentration of nutrients in the water draining out of the plantation as compared to what it was at the entry point (Furtado and Luca, 2003). On the other hand, competitors are present in the aquatic environment and the count of these entero-bacterium drops rapidly, presumably because they cannot successfully compete with nutrients containing other bacteria present in the above-mentioned environment (Wcislo and Chrost, 2000).

In aquatic ecosystems, the reductions in total coliforms and *E. coli* are associated to natural phenomena of the auto-purification process in which the bacteria that indicate fecal contamination are removed or eliminated and, in consequence of some entero-bacterium, as a function of physical, chemical and biological processes associated to the auto-purification processes, amongst which appear sedimentation, competition, litic action of specific bacteriophage and the synergistic effect of solar radiation, together with high values of pH and of dissolved oxygen (Deller *et al*., 2006; Garfield and Walker, 2008;Van Elsas *et al*., 2010).

According to the WHO (1989) recommendations for unrestricted irrigation, the concentration of fecal coliforms should be lower than 1000 UFC/100 mL, and therefore, water used for irrigation that presents values higher than 1000 UFC/100 mL of fecal coliform concentration, may be used only for forage and the cultivation of cereals - such as the rice culture evaluated in this study.

The authors Furtado and Luca (2003) proved that the density of total and fecal coliforms in water taken from rivers is greater than that of the irrigation water found in the areas of rice plantation, where the direct planting system was the one which presented the lowest bacterial density. These authors state that in the rice field plots, where the sheet of water is shallow, the solar radiation penetrates strongly into the water and causes an antimicrobial effect, contributing to the reduction of the populations of micro-organisms sensitive to ultraviolet radiation.

Therefore, the results of this research should be transferred to rice cultivators in the State of Rio Grande do Sul, demonstrating the way in which both the cultivation and the harvest can be improved, and also how to monitor the quality of the water utilized for irrigation.

Acknowledgments

The authors acknowledge the support received from the Technical Team at the EEA/IRGA. This research was realized with financial support from CNPq/ CTHidro, provided by a scholarship for Master to the PPG in Biology at the Unisinos (Universidade do Vale do Rio dos Sinos).

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Submitted on May 3, 2011
Accepted on November 28, 2011