

# Influence of different sources of contamination on the microbiological quality of lettuce in the Teresópolis region, RJ, Brazil

*Influência de diferentes fontes de contaminação na qualidade microbiológica de alface na região de Teresópolis, RJ*

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## ABSTRACT

Irrigation water and cultivated soil have been identified as possible sources of contamination in several crops. In certain vegetables that are eaten raw, such as lettuce, this contamination can lead to public health problems. Aiming to evaluate the influence of these sources on the quality of lettuce grown in the Córrego Sujo Basin, Teresópolis, RJ, an important agricultural pole whose production services the metropolitan region of Rio de Janeiro, water from different sources (spring, weir and river) was collected in this region, as well as samples of soil and lettuce irrigated with these waters, to carry out conventional microbiological analyzes (counts of total heterotrophic bacteria and thermotolerant coliforms) and molecular analyzes (PCR-DGGE). The count of fecal coliforms in lettuce suggests that there is an influence of irrigation water and the cultivated soil on the contamination of these vegetables. The grouping of bacterial communities in the different samples obtained by the PCR-DGGE technique shows that irrigation water has a greater influence on the contamination of these vegetables in relation to the soil where they are grown. These results corroborate the need to monitor water bodies used for irrigation and demonstrate that the PCR-DGGE technique is of great value for the study of microbial communities and, when associated with specific primers, can help in the detection of pathogens in food.

**Keywords:** water irrigation; soil cultivation; *Lactuca sativa*; fecal coliforms; denaturing gradient gel electrophoresis.

## RESUMO

A água de irrigação e o solo de cultivo têm sido apontados como possíveis fontes de contaminação em diversas culturas. Em determinadas hortaliças consumidas cruas, como a alface, essa contaminação pode causar problemas de saúde pública. Objetivando avaliar a influência dessas fontes na qualidade das alfaces cultivadas na Bacia do Córrego Sujo, Teresópolis, RJ; importante polo agrícola com produção voltada à região metropolitana do Rio de Janeiro, coletou-se nesta região águas proveniente de diferentes fontes (nascente, açude e rio); solos e alfaces irrigados com essas águas, para realização de análises microbiológicas convencionais (contagens de bactérias heterotróficas totais e coliformes termotolerantes) e moleculares (PCR-DGGE). A contagem de coliformes fecais na alface sugere que existe influência da água de irrigação e do solo na contaminação desses vegetais. O agrupamento das comunidades bacterianas nas diferentes amostras obtido pela técnica de PCR-DGGE mostra que a água de irrigação tem influência maior na contaminação dessas hortaliças em relação ao solo onde são cultivadas. Esses resultados corroboram a necessidade de monitoramento de corpos d'água utilizados para irrigação e demonstram ser a técnica do PCR-DGGE de grande valia para o estudo das comunidades microbianas e, quando associada a iniciadores específicos podem ajudar na detecção de patógenos em alimentos.

**Palavras-chave:** irrigação por água; cultivo do solo; *Lactuca sativa*; coliformes fecais; eletroforese em gel com gradiente desnaturante.

## INTRODUCTION

Lettuce (*Lactuca sativa*) has been considered one of the most consumed vegetables in Brazil, mainly due to its organic characteristics, such as being low in calories and a rich fiber source, in addition to being a low

cost food, qualifying for different diets (GOMES NETO *et al.*, 2012). It is the most important vegetable with green leaves in Brazil's economy, with acreage of approximately 35,000 acres (KRINSKI; PELISSARI, 2012). Since it is consumed raw, this vegetable is widely studied in relation

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to its microbiological quality (PEREIRA; FREITAS; MACIEL, 2012), once it may suffer contamination from both the water used for irrigation and the soil where it is cultivated (ABREU *et al.*, 2010; GOMES NETO, 2012; URBANO *et al.*, 2017).

Water used in irrigation usually comes from rivers, streams, lakes or wells adjacent to cultivated areas, and the use of public water supply is rare, mainly due to its high cost. As this captured water does not undergo previous treatment, it can become a potential source of enteropathogens for the vegetable to be irrigated (MDLULI; THAMAGA-CHITJA; SCHMIDT, 2013). Several studies have identified a high degree of fecal contamination in vegetables from irrigation water and the cultivated soil. In many cultures, to increase productivity, the use of organic fertilizers made from animal manure, homemade bio-fertilizers and slurries made of organic compounds that didn't have time to complete composting lead to contamination of vegetables by potentially pathogenic bacteria. Such practices can lead to various issues with the quality of cultivated foods, especially those that grow close to the soil (SIMÕES *et al.*, 2001).

Methods for detection and enumeration of these pathogens in water, soil and food are usually based on the cultivation of these in culture medium in laboratories; but in the case of bacteria, only about 1% of the total bacterial community can be grown under these conditions (AHMADI; ROHANI; AYREMLOU, 2010). In addition, these methods take from 2 to 7 days to yield results (FUNG, 2002). Therefore, the application of molecular biology methods such as PCR (Polymerase Chain Reaction) and DGGE (Electrophoresis Denaturing Gradient Gel) may provide a fast, efficient and sensitive way of detecting microorganisms (ERCOLINI, 2004). Many studies have applied molecular techniques to study bacterial communities in samples of food, water and soil (BERRADA *et al.*, 2006; HANDSCHUR *et al.*, 2005).

Using molecular (PCR-DGGE) and conventional (coliform and total heterotrophic bacteria counts) microbiological techniques, this study aimed to investigate the influence of different sources of irrigation water and cultivated soil on the microbiological quality of lettuce (*Lactuca sativa*) grown in the Córrego Sujo Basin (53 km<sup>2</sup>), Teresópolis, RJ, an important agricultural pole whose production services the metropolitan region of Rio de Janeiro.

## MATERIALS AND METHODS

### Sample

Water used for irrigation, cultivated soil and lettuce were sampled in three private properties located in the Córrego Sujo region, in the city of Teresópolis, RJ, in August 2009, February, July and November 2010; each using a source of water for irrigation (spring, weir and river).

Water samples were collected with a sterile bottle placed on the surface of the body of water. Three lettuce samples were collected randomly in each plot and stored in sterile bags. Soil samples were taken near each lettuce collected, and assembled to make up a single sample for each collection point. All samples were kept in the dark and on ice until laboratory analysis (no more than 5 hours) (APHA, 2005).

### Microbiological analysis

In a laboratory, 25 g of each lettuce and soil sample were weighed. These samples were placed in sterile plastic bags, with the addition of 225 mL of 0.1% peptone water, and homogenized for 60 seconds. After this procedure, these samples and the water sample were submitted to microbiological tests (fecal coliforms, heterotrophic bacteria and *Salmonella* spp.) according to the methodology described in APHA (2005).

### Statistical analysis

The arithmetic average and standard deviation ( $\log_{10}$ ) of the counts of total heterotrophic bacteria and coliforms were calculated. The results were subjected to analysis of variance (ANOVA), followed by Tukey test at 5% level of significance using the GraphPad InStat software, version 3.0.

### Molecular analysis

A total of 500mL of water samples collected from different irrigation sources and 50 mL of lettuce wash-water previously filtered on an 0.8  $\mu$ m nitrocellulose membrane were then filtered on 0.22  $\mu$ m nitrocellulose membranes. These filters were stored in polypropylene tubes with a capacity of 1.5 mL and stored at -20°C for DNA extraction, which was carried out using the direct extraction method with a DNA extraction kit for soil (FastDNA<sup>®</sup> SPIN Kit for Soil) BIO 101 (California, USA). Half a gram (0.5 g) of the cultivated soil and half of the nitrocellulose membrane containing the filtrate from the water samples and the lettuce wash-water were used for DNA extraction. The quality of the DNA extracted was assessed by electrophoresis on 0.8% agarose gel.

The reactions for amplification of the 16S rDNA gene were performed with primers U968f-GC1 ("clip" + 5' AAC GCG AAG CTT AC3') and L1401r (5'GCG GTA TGT CAA GAC CC3') (NÜBEL *et al.*, 1996). Each reaction tube contained 50  $\mu$ L of 10x Taq polymerase, 2.5 mM MgCl<sub>2</sub> (Invitrogen), 200 $\mu$ Mol of each dNTP, 0.2  $\mu$ M of each primer (PROMICRO), 1% formamide, 5 $\mu$ g BSA (Sigma) buffer, 2.5U Taq polymerase (Invitrogen) and sterile milli-Q water. In each reaction tube, 2 $\mu$ L of DNA were applied. These mixes were transferred to the thermocycler (Perkin Elmer — DNA Thermal 480<sup>®</sup>) and submitted to a program of 5 min at 95°C followed by 35 thermal cycles of 1 min at 95°C, 1 min at 58°C and 1 min at 72°C, ending with 5 min at 72°C.

All PCR products were subjected to electrophoresis on 1.0% agarose gel and visualized with UV transilluminator and photographed with a Polaroid camera.

The DGGE experiments were performed with Dcode™ Universal Mutation Detection System equipment (Bio-Rad, Richmond, USA) as described by Muyzer, De Waal and Uitterlinden (1993). The PCR products obtained were applied in a volume of 30 µL, with 15 µL of dye (bromophenol blue) directly in polyacrylamide gel in 0.5× TAE buffer (20 mM Tris, 10 mM acetate, 05 mM Na 2 EDTA, pH 7.8) containing denaturing linear gradient of 40 to 70%. The gradient was formed by mixing solutions of polyacrylamide (6%) with another containing 0% to 70% of denaturing agents. The gel was left polymerizing for at least 2 hours and then coupled to DGGE device. Electrophoresis was developed with 75V / 60°C / 17h. After the run, the gel was stained for 40 minutes with Syber Green (Molecular Probes), observed in UV using a STORM (Amersham) imaging system.

The profiles of bands obtained in the DGGE gels were analyzed using the program Image Quant from the scanned by Storm 860 (Amersham Biosciences) imaging system, considering the presence or absence of bands in the DGGE profiles. Starting from these, matrices were built by dendograms in a Statistic Software using the coefficient of similarity method of Pearson and UPGA.

## RESULTS AND DISCUSSION

Irrigation water and the cultivated soil have been identified in several studies as potential sources of contamination of several crops, especially those that develop close to the soil (ALLENDE; MONAGHAN, 2015; GUIMARÃES *et al.*, 2003). Total heterotrophic bacteria, coliforms and detection of pathogens such as *Salmonella* in these substrates have been used to evaluate the microbiological quality of these samples and consequent contamination, using DGGE technique to verify the influence of different sources of irrigation and cultivated soil in the composition of bacterial communities present in vegetables such as lettuce (ERCOLINI, 2004).

### Microbial counts

Although it provides important information on the conditions of cultivation and storage, the Brazilian legislation does not impose any restriction on the count of total heterotrophic bacteria in foods, either in their fresh form or prepared for consumption in salads. However, some authors (ARBOS *et al.*, 2010; RODRIGUES *et al.*, 2014; SANTANA *et al.*, 2006) suggest that an acceptable count is up to  $10^6$  CFU/100g. The lettuce samples collected at the three sites studied had total counts equal to or greater than the limit of  $10^6$  in all four samples collected. According to Monteiro *et al.* (2013), when heterotrophic bacteria are present in larger amounts, vegetables begin to show

signs of deterioration. In addition, there isn't a standard established by law for the count of these microorganisms in relation to irrigation water or cultivated soil.

Adams, Hartley and Cox (1989) reported an increase of 2 or 3 log cycles in heterotrophic bacteria counts and visible leaf deterioration in lettuce after 2 days stored at 20°C, confirming the relevance of this count for the investigation of storage conditions.

Counts of heterotrophic bacteria (HB) obtained for samples of lettuce, water and soil, regardless of the source of irrigation (spring, weir or river), showed no significant difference ( $p > 0.0001$ ). Nevertheless, it should be noted that the lowest medium values were always found in the weir (Table 1). High levels of dissolved oxygen found in these waters (ARAUJO *et al.*, 2015) suggest a decrease in scores from HB totals, favoring the growth of cyanobacteria. According to Terra, Moura and Araujo (2008), counts found in irrigation waters resemble counts obtained in other water bodies in the same region, and are within the standard found in these types of environmental samples. Among the samples investigated, the cultivated soil had the highest average values for HB. These results suggest that the major bacterial load present in these waters can be due to the use of a "poultry litter" technique, which consists of spreading chicken manure into the soil in order to fertilize it.

The presence of total heterotrophic bacteria in water, soils and even in vegetables is not indicative of contamination, but high counts of these microorganisms indicate an input of organic matter in these environments. Although the presence of heterotrophic bacteria cannot be associated with risk to human health, it can alter these substrates, causing changes in the water's color and smell, increasing soil nutrients and decreasing storage time for vegetables, resulting in economic losses (PAULA *et al.*, 2003).

Fecal coliforms (FC) are a group of bacteria that are capable of fermenting lactose with gas production at 44.5°C. Their presence in food

**Table 1** - Minimum (Min), maximum (Max), medium (Med) values and standard deviation (SD) of total heterotrophic bacteria counts (HB) per 100 g of soil and lettuce, and 100 mL of irrigation water.

	Min	Max	Med	SD
H1	$5.2 \times 10^5$	$7.4 \times 10^6$	$1.9 \times 10^6$	$6.4 \times 10^0$
H2	$2.5 \times 10^5$	$8.3 \times 10^6$	$1.2 \times 10^6$	$4.4 \times 10^0$
H3	$5.4 \times 10^6$	$3.8 \times 10^7$	$1.2 \times 10^6$	$2.7 \times 10^0$
A1	$4.9 \times 10^4$	$4.3 \times 10^6$	$3.0 \times 10^5$	$7.5 \times 10^0$
A2	$1.1 \times 10^4$	$4.2 \times 10^5$	$6.9 \times 10^4$	$4.4 \times 10^0$
A3	$2.8 \times 10^4$	$1.7 \times 10^5$	$7.4 \times 10^4$	$1.9 \times 10^0$
S1	$5.3 \times 10^6$	$5.5 \times 10^7$	$1.0 \times 10^7$	$2.5 \times 10^0$
S2	$6.6 \times 10^6$	$1.1 \times 10^7$	$7.5 \times 10^6$	$1.2 \times 10^0$
S3	$7.6 \times 10^6$	$6.2 \times 10^7$	$1.3 \times 10^7$	$2.4 \times 10^0$

H: lettuce; A: water; S: soil; 1: spring; 2: weir; 3: river.

and water indicates unsatisfactory sanitary conditions, and the possible presence of pathogens (PEREIRA; FREITAS; MACIEL, 2012). ANVISA (National Health Surveillance Agency) Resolution No. 12 of January 2, 2001 determines the maximum limit of 10,000 FC/100g in fresh vegetables that are consumed raw (ANVISA, 2001), while Resolution 274 of the National Environmental Council (BRASIL, 2012) establishes a maximum limit of 200 FC/100 mL of water intended for irrigation of vegetables that are consumed raw.

In this study, contamination by FC was observed in all samples (Table 2). However, the lowest average values ( $1.1 \times 10^2$  NMP/100 mL), below the CONAMA's standard, were found in irrigation from spring water. These data were expected, since spring waters are clear of contamination sources, corroborating Terra, Moura and Araujo (2008), who studied various springs and rivers in Parque Nacional da Serra dos Orgãos, also in Teresopolis, RJ. The average values for water from the weir ( $2.2 \times 10^2$  NMP/100 mL) and river ( $5.4 \times 10^2$  NMP/100mL) agree with those obtained by Ribeiro *et al.* (2005), who analyzed waters used for irrigation of vegetable crops in Brazil, and also found higher values. These values, above the levels allowed by CONAMA, may be associated with the presence of residencies and animals surrounding the river and weir. Although the law forbids it, waters with high levels fecal contamination are often used to irrigate lettuce and other vegetables that are consumed raw.

The average values of FC found in water samples were significantly different from those found in soil and lettuce ( $p < 0.0001$ ). The latter only differ from lettuce irrigated with water from the river ( $p < 0.0001$ ).

The presence of FC in the soil samples ranged between  $2.0 \times 10^1$  to  $1.7 \times 10^4/100$  g with no significant difference, regardless of the source of water used ( $p > 0.0001$ ). These results are probably due to contamination from irrigation water and the use of the "poultry litter" technique. According to Simões *et al.* (2001), moisture due to irrigation and the presence of organic material in these environments maintain

**Table 2** - Minimum (Min), maximum (Max), medium (Med) values and standard deviation (SD) of fecal coliform (FC) counts per 100 g of soil and lettuce, and 100 mL of irrigation water.

	Min	Max	Med	SD
H1	$2.1 \times 10^4$	$2.3 \times 10^4$	$2.1 \times 10^4$	$1.0 \times 10^0$
H2	$1.6 \times 10^3$	$4.2 \times 10^4$	$1.0 \times 10^4$	$4.1 \times 10^0$
H3	$1.1 \times 10^3$	$5.0 \times 10^4$	$7.9 \times 10^3$	$6.7 \times 10^0$
A1	$4.5 \times 10^1$	$2.0 \times 10^2$	$1.1 \times 10^2$	$1.9 \times 10^0$
A2	$8.2 \times 10^1$	$7.8 \times 10^2$	$2.2 \times 10^2$	$2.9 \times 10^0$
A3	$2.0 \times 10^2$	$1.1 \times 10^3$	$5.4 \times 10^2$	$1.8 \times 10^0$
S1	$4.5 \times 10^1$	$1.7 \times 10^4$	$1.3 \times 10^3$	$1.1 \times 10^1$
S2	$2.0 \times 10^2$	$2.0 \times 10^3$	$6.4 \times 10^2$	$2.7 \times 10^0$
S3	$2.0 \times 10^1$	$1.1 \times 10^4$	$8.5 \times 10^2$	$1.5 \times 10^1$

H: lettuce; A: water; S: soil; 1: spring; 2: weir; 3: river.

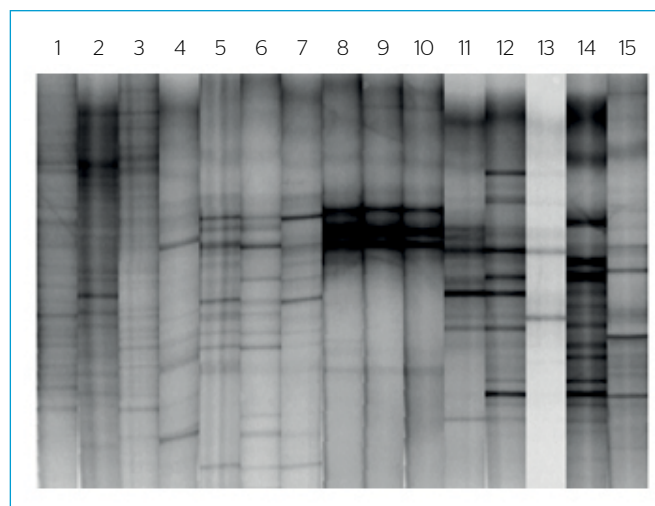
microbial populations more numerous and for longer periods when compared to those found in waters from different sources.

Lettuce samples showed the highest values of FC ranging from  $1.1 \times 10^3$  to  $5.0 \times 10^4$ . These results corroborate with most studies in the literature mentioning irrigation water as a major source of microbial contamination of vegetables during cultivation (GUIMARÃES *et al.*, 2003; ESTEVES; FIGUERÔA, 2009).

According to Ramos *et al.* (2014), in places where hygienic-sanitary practices are not adequate, the use of contaminated water to irrigate vegetables or even through the use of manure as fertilizer, the risk of contamination by pathogens such as *Salmonella* spp. is increased. Despite the presence of fecal bacteria in the waters analyzed, only one lettuce sample irrigated by weir waters showed the presence of *Salmonella*. Nevertheless, it is important to constantly monitor the presence of this microorganism in vegetables, especially in those consumed raw.

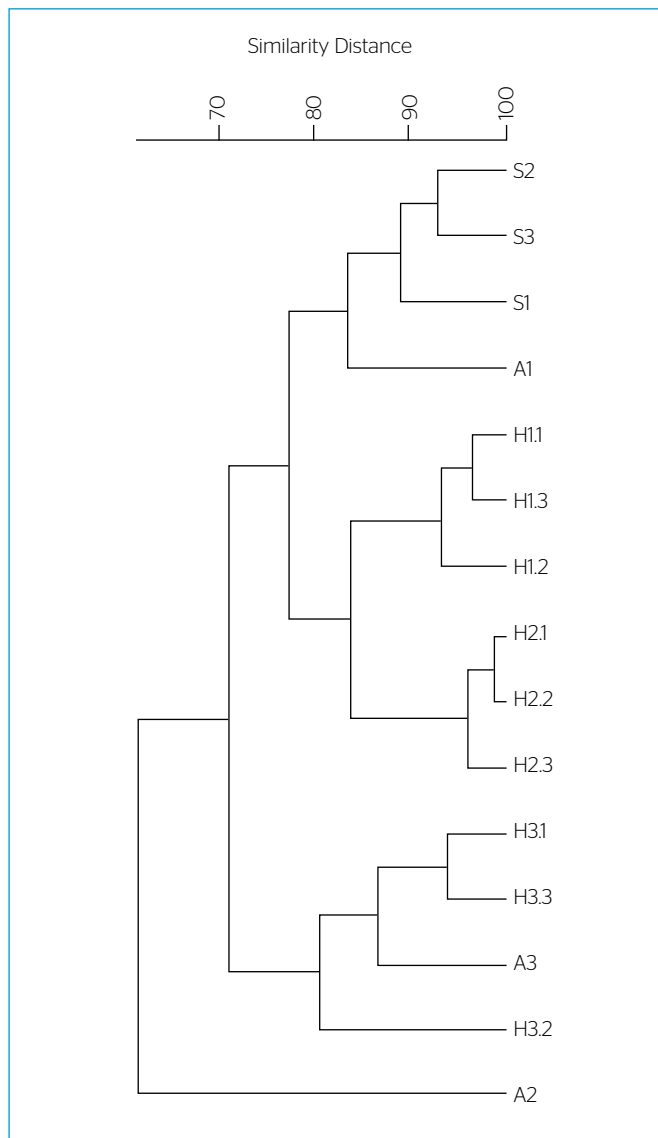
## The bacterial communities

Bacteria are distributed in different communities and environments. Their variety and amount can be determined by the physical, chemical and biological characteristics of these environments (YANNARELL; KENT, 2009). The study of the structure of these communities may reveal the influence that these environments have in their composition. Carmo *et al.* (2013), when studying bacterial communities in different species of tank bromeliads through the DGGE technique, noted that although these communities show specific bacterial populations, they are also heavily influenced by their surrounding environment. From the bands shown in DGGE gel, which represent different populations, we can observe a high diversity in lettuce, irrigation waters and cultivated soil (Figure 1).



**Figure 1** - DGGE for observation of the bacterial community present in irrigation water samples, cultivated soil and lettuce. Numbers 1 to 15 correspond to the following samples, respectively: 1-S2, 2-S3, 3-S1, 4-A1, 5-H1.2, 6-H1.3, 7-H1.1, 8-H2.1, 9-H2.2, 10-H2.3, 11-H3.1, 12-H3.3, 13-A3, 14-H3.2, 15-A2.

Analysis by UPGA performed from the profiles obtained in these DGGE bands shows the formation of three large groups (Figure 2). At first, we can observe clustering of samples of cultivated soil with spring irrigation water (S2, S3, S1 and A1). Such clustering of the soil samples is probably due to the physical and chemical characteristics that they share, determining the structure of bacterial communities found. Such results have been observed by Peixoto *et al.* (2002) while working with cultivated soil of tomato (*Lycopersicon esculentum*). The similarity between spring water and soils may be explained by the fact that these waters are largely influenced by the surrounding soil (DONADIO; GALBIATTI; DE PAULA, 2005).



**Figure 2** - Dendrogram generated by Statistica software (using the Pearson similarity coefficient and UPGA) to analyze the DGGE profiles of bands of irrigation water (A1 A2 and A3), soil (S1, S2 and S3) and lettuce samples (H1.1, H1.2, H1.3, H2.1, H2.2, H2.3, H3.1, H3.2, H3.3).

The second group clustered lettuce samples by dividing them regarding the source of irrigation water (H1.2, H1.3, H1.1 and H2.1, H2.2, H2.3); weir and river, respectively. Such clustering shows the presence of a characteristic microbiota in these vegetables that separate them from the communities present in soil and water, but also evidences the influence of the source of irrigation water in the separation into two subgroups. The third and last group is formed by vegetables irrigated by river water (H3.1, H3.2, H3.3, and A3), demonstrating, once again, the influence of the source of irrigation water on the composition of the microbiota in vegetables. The bacterial community sample A2 (weir water) is highlighted in the other groups in the dendrogram, which can be explained by the high degree of eutrophication found in these waters, characterized by a strong greenish color. The characteristics of highly eutrophic waters select specific populations to live in these conditions.

The analysis of the DGGE gel obtained showed that different soils have similar communities and suggests that the microbial communities present in lettuce are influenced by the communities present in different sources of the water used for irrigation.

## CONCLUSION

Although some sources had low microbial counts, such as those found in spring waters, the presence of fecal contamination in irrigation water is a serious public health problem, especially when used for irrigation of vegetables that are consumed raw. The influence of these and cultivated soil on the quality of lettuce can be observed by the high counts of fecal coliforms found in the latter. With PCR-DGGE, it is possible to observe that the bacterial communities on the cultivated soil are distinct from those found in vegetables, while irrigation waters, despite not group together with the vegetables — with the exception of river water — show relation with and influence over the communities present in lettuce. These results support the need for monitoring water bodies used for irrigation, and demonstrate that the PCR-DGGE technique is valuable for the detection of the source contamination in microbial communities.

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## REFERENCES

- ABREU, I.M.O.; JUNQUEIRA, A.M.R.; PEIXOTO, J.R.; OLIVEIRA, S.A. (2010) Microbiological quality and productivity of lettuce under chemical and organic fertilization. *Ciência e Tecnologia de Alimentos*, Campinas, v. 30, p. 108-118.
- ADAMS, M.R.; HARTLEY, A.D.; COX, A.D. (1989) Factors affecting the efficacy of washing procedures used in the production of prepared salads. *Food Microbiology*, v. 6, n. 2, p. 69-77. [https://doi.org/10.1016/S0740-0020\(89\)80039-5](https://doi.org/10.1016/S0740-0020(89)80039-5)
- AHMADI, M.; ROHANI, S.M.R.; AYREMLOU, N. (2010) Detection of *Staphylococcus aureus* in milk by PCR. *Comparative Clinical Pathology*, Germany, v. 19, p. 91-94. <https://doi.org/10.1007/s00580-009-0901-0>
- AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). (2001) Resolução RDC nº 12, de 2 de janeiro de 2001. ANVISA. Available at: <[http://www.anvisa.gov.br/legis/resol/12\\_01rdc.htm](http://www.anvisa.gov.br/legis/resol/12_01rdc.htm)>. Accessed on: Mar. 11, 2020.
- ALLENDE, A.; MONAGHAN, J. (2015) Irrigation Water Quality for Leafy Crops: A Perspective of Risks and Potential Solutions. *International Journal of Environmental Research and Public Health*, v. 12, n. 7, p. 7457-7477. <https://doi.org/10.3390/ijerph120707457>
- AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). (2005) *Standard methods for the examination of water and wastewater*. 21<sup>th</sup> ed. Washington, DC.: APHA.
- ARAUJO, F.V.; VIEIRA, L.; JAYME, M.M.A.; NUNES, M.C.; CORTÉS, M. (2015) Avaliação da qualidade da água utilizada para irrigação na Bacia do Córrego Sujo, Tereópolis, RJ. *Cadernos de Saúde Coletiva*, Rio de Janeiro, v. 23, n. 4, p. 380-385. <https://doi.org/10.1590/1414-462X201500040083>
- ARBOS, K.A.; SOSSELA DE FREITAS, R.J.; CACHOEIRA STERTZ, S.; CARVALHO, L.A. (2010) Segurança alimentar de hortaliças orgânicas: aspectos sanitários e nutricionais. *Ciência e Tecnologia de Alimentos*, v. 30, supl. 1, p. 215-220. <https://doi.org/10.1590/S0101-20612010000500033>
- BERRADA, H.; SORIANO, J.M.; PICÓ, Y.; MAÑES, J. (2006) Quantification of *Listeria monocytogenes* in salads by real PCR. *International Journal of Food Microbiology*, v. 107, n. 2, p. 202-206. <https://doi.org/10.1016/j.ijfoodmicro.2005.07.006>
- BRASIL. (2012) Conselho Nacional do Meio Ambiente. *Resoluções do Conama: Resoluções vigentes publicadas entre setembro de 1984 e janeiro de 2012*. Brasília: Ministério do Meio Ambiente. 1126 p.
- CARMO, F.L.; SANTOS, H.F.; PEIXOTO, R.S.; ROSADO, A.S.; ARAUJO, F.V. (2013) Tanks bromeliad waters from atlantic rain forest: a specific or distinct environments for the research of bioactives bacterial? *Brazilian Journal of Microbiology*, Brasil, v. 45, n. 1, p. 185-192. <https://doi.org/10.1590/S1517-83822014000100024>
- DONADIO, N.M.M.; GALBIATTI, J.A.; DE PAULA, R.C. (2005) Qualidade da água de nascentes com diferentes usos do solo na bacia hidrográfica do Córrego Rico, São Paulo, Brasil. *Engenharia Agrícola*, Jaboticabal, v. 25, n. 1, p. 115-125. <https://doi.org/10.1590/S0100-69162005000100013>
- ERCOLINI, D. (2004) PCR-DGGE fingerprinting: novel strategies for detection of microbes in food. *Journal of Microbiological Methods*, Amsterdam, v. 56, n. 3, p. 297-314. <https://doi.org/10.1016/j.mimet.2003.11.006>
- ESTEVES, F.A.M.; FIGUEIRÓA, E.O. (2009) Detection of intestinal parasites in green vegetable comerce in public street markets in Caruaru (PE). *Revista Baiana de Saúde Pública*, Brasil, v. 33, n. 2.
- FUNG, D.Y. (2002) Rapid Methods and Automation in Microbiology. *Comprehensive Reviews in Food Science and Food Safety*, v. 1, n. 1, p. 3-22. <https://doi.org/10.1111/j.1541-4337.2002.tb00003.x>
- GOMES NETO, N.J.; PESSOA, M.R.L.; QUEIROGA, I.M.B.N.; MAGNANI, M.; FREITAS, F.I.S.; SOUZA, E.L.; MACIEL, J.F. (2012) Bacterial counts and the occurrence of parasites in lettuce (*Lactuca sativa*) from different cropping systems in Brazil. *Food Control*, Guildford, v. 28, n. 1, p. 47-51. <https://doi.org/10.1016/j.foodcont.2012.04.033>
- GUIMARÃES, M.A.; ALVES, E.G.L.; FIGUEIREDO, H.C.P.; COSTA, G.M.; RODRIGUES, L.S. (2003) Frequência de enteroparasitas em amostras de alface (*Lactuca sativa*) comercializadas em Lavras, Minas Gerais. *Revista da Sociedade Brasileira de Medicina Tropical*, v. 36, n. 5, p. 621-623. <https://doi.org/10.1590/S0037-86822003000500014>
- HANDSCHUR, M.; PINAR, G.; GALLIST, B.; LUBITZ, W.; HASLBERGER, A.G. (2005) Culture free DGGE and cloning based monitoring of changes in bacterial communities of salad due to processing. *Food and Chemical Toxicology*, Oxford, v. 43, n. 11, p. 1595-1605. <https://doi.org/10.1016/j.fct.2005.05.009>
- KRINSKI, D.; PELISSARI, T.D. (2012) Occurrence of the stinkbug *Edessa mediatubunda* F. (Pentatomidae) in differents cultivars of lettuce *Lactuca sativa* L. (Asteraceae). *Bioscience Journal*, Uberlândia, v. 28, n. 4, p. 654-659.
- MDLULI, F.; THAMAGA-CHITJA, J.; SCHMIDT, S. (2013) Appraisal of Hygiene Indicators and Farming Practices in the Production of Leafy Vegetables by Organic Small-Scale Farmers in uMbumbulu (Rural KwaZulu-Natal, South Africa). *International Journal of Environmental Research Public Health*, v. 10, n. 9, p. 4323-4338. <https://doi.org/10.3390/ijerph10094323>
- MONTEIRO, M.L.G.; MÁRSICO, E.T.; MANO, S.B.; TEIXEIRA, C.E.; CANTO, A.C.V.C.S.; VITAL, H.C.; CONTE JÚNIOR, C.A. (2013) Influence of good manufacturing practices on the shelf life of refrigerated fillets of tilapia (*Oreochromis niloticus*) packed in modified atmosphere and gamma-irradiated. *Food Science & Nutrition*, Cleveland, v. 1, n. 4, p. 298-306. <https://doi.org/10.1002/fsn3.41>

- MUYZER, G.; DE WAAL, E.C.; UITTERLINDEN, A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied Environmental Microbiological*, Washington, v. 59, n. 3, p. 695-700.
- NÜBEL, U.; ENGELEN, B.; FELSKA, A.; SNAIDR, J.; WIESHUBER, A.; AMANN, R.I.; LUDWIG, W.; BACKHAUS, H. (1996) Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *Journal of Bacteriology*, v. 178, n. 19, p. 5636-5643. <https://doi.org/10.1128/jb.178.19.5636-5643.1996>
- PAULA, P.; RODRIGUES, P.S.S.; TÓRTORA, J.C.O.; UCHÔA, C.M.A.; FARAGE, S. (2003) Contaminação microbiológica e parasitológica em alfaces (*Lactuca sativa*) de restaurantes self-service, de Niterói, RJ. *Revista da Sociedade Brasileira de Medicina Tropical*, Brasil, v. 36, n. 4, p. 535-537.
- PEIXOTO, R.S.; DA COSTA COUTINHO, H.L.; RUMJANEK, N.G.; MACRAE, A.; ROSADO, A.S. (2002) Use of *epoB* and 16S rRNA genes to analyse bacterial diversity of a tropical soil using PCR and DGGE. *Letters in Applied Microbiology*, Washington, v. 35, n. 4, p. 316-320. <https://doi.org/10.1046/j.1472-765x.2002.01183.x>
- PEREIRA, J.A.; FREITAS, F.I.S.; MACIEL, J.F. (2012) Qualidade Microbiológica da Alface (*Lactuca sativa*) Comercializada em João Pessoa-PB. *Revista de Biologia e Farmácia*, João Pessoa, v. 8, n. 1, p. 125-131.
- RAMOS, M.O.; BEGOTTI, I.L.; ROSA, G.; VIEIRA, G.F.P.; MESSA, V.; MERLINI, L.S. (2014) Avaliação parasitológica de alfaces (*Lactuca sativa*) comercializadas no município de Umuarama, Paraná, Brasil. *Revista Brasileira de Higiene e Sanidade Animal*, v. 8, n. 3, p. 1-12. <http://dx.doi.org/10.5935/1981-2965.20140113>
- RIBEIRO, A.S.; GUERRA, R.M.S.N.C.; COSTA, F.N.; ALVES, L.M.C. (2005) Avaliação das condições higiênico-sanitárias de alfaces e águas de irrigação de hortaliças da Ilha de São Luís - Ma. *Revista Higiene Alimentar*, Brasil, v. 130, p. 20-23.
- RODRIGUES, R.Q.; LOIKO, M.R.; PAULA, C.M.D.; HESSEL, C.T.; JACXSENS, L.; UYTENDAELE, M.; BENDER, R.J.; TONDO, E.C. (2014) Microbiological contamination linked to implementation of good agricultural practices in the production of organic lettuce in Southern Brazil. *Food Control*, v. 42, p. 152-164. <https://doi.org/10.1016/j.foodcont.2014.01.043>
- SANTANA, L.R.R.; CARVALHO, R.D.S.; LEITE, C.C.; ALCÂNTARA, L.M.; OLIVEIRA, T.W.S.; RODRIGUES, B.M. (2006) Qualidade física, microbiológica e parasitológica de alfaces (*Lactuca sativa*) de diferentes sistema de cultivo. *Ciência e Tecnologia de Alimentos*, v. 26, n. 2, p. 264-269. <https://doi.org/10.1590/S0101-20612006000200006>
- SIMÕES, M.; PISANI, B.; MARQUES, E.G.L.; PRANDI, M.A.G.; MARTINI, M.H.M.; CHIARINI, P.F.T.; ANTUNES, J.L.F.; NOGUEIRA, A.P. (2001) Hygienic-sanitary conditions of vegetables and irrigation water from kitchen gardens in the municipality of Campinas, SP. *Brazilian Journal of Microbiology*, Brasil, v. 32, n. 4, p. 331-333. <https://doi.org/10.1590/S1517-83822001000400015>
- TERRA, E.; MOURA, L.R.; ARAUJO, F.V. (2008) Avaliação microbiológica das águas da travessia Petrópolis - Teresópolis (Parque Nacional da Serra dos Órgãos, RJ). *Espaço & Geografia*, Brasil, v. 11, n. 1, p. 17-33.
- URBANO, V.; MENDONÇA, T.; BASTOS, R.; FONSECA SOUZA, C. (2017) Effects of treated wastewater irrigation on soil properties and lettuce yield. *Agricultural Water Management*, v. 181, p. 108-115. <https://doi.org/10.1016/j.agwat.2016.12.001>
- YANNARELL, A.C.; KENT, A.D. (2009) Bacteria, Distribution and Community Structure. In: ENCYCLOPEDIA OF INLAND WATERS. Urbana: University of Illinois at Urbana-Champaign. v. 3. p. 201-210.