

## **Mapping of physical-chemical, microbiological, and chemical component characteristics of water samples from Nile tilapia slaughterhouses**

**Mapeamento das características físico-químicas, microbiológicas e dos componentes químicos de amostras de água de abatedouros de tilápia do Nilo**

**Mapeo de las características de los componentes físico-químicos, microbiológicos y químicos de las muestras de agua de los mataderos de tilapia del Nilo**

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### **Abstract**

The aim of this study was to analyze the physicochemical and microbiological characteristics of the water used in fish processing and tilapia fillets in slaughterhouses. The study analyzed the processing water from nine slaughterhouses. The water samples for analysis were collected at three points: process water (PW), it is the water used inside the slaughterhouses in direct contact with the fish; clean water entering the purification tank (EPT) and water leaving the purification tank (LPT). The processing waters of the nine tilapia slaughterhouses were analyzed and characterized according to their microbiological and physical-chemical characteristics. The results of microbiological analyzes meet the values indicated by legislation, in most cases. Regarding microbiological data, we can highlight that there was an increase in the total coliforms of the water entering the purification tank to the water leaving the purification tank in five slaughterhouses. Still, there was an increase in the aerobic mesophilic bacteria content observed in the outgoing water in relation to the inlet water of the purification tank in seven slaughterhouses. In relation to the physical chemical analyzes for the process water samples, the results show that the evaluated indices are in accordance with the values indicated by the legislation. The levels of Cd, Mg, Sc and Cd were below that detectable by the analysis in all

slaughterhouses. The levels of Al, Sc, As, Rb, Ba, Pb, Mn, As, Se, Rb, Ag, Sb, Ba and Pb were detected only in one or two slaughterhouses. It is concluded that although some abattoirs have water characteristics outside the limits indicated by the legislation, the observed changes were not significant and small adjustments are necessary for the adequacy.

**Keywords:** Fish meat; Microbiology; Water quality; Food safety; Sustainability.

### Resumo

O objetivo deste estudo foi analisar as características físico-químicas e microbiológicas da água utilizada no processamento do pescado e dos filés de tilápia em frigoríficos. O estudo analisou a água de processamento de nove frigoríficos. As amostras de água para análise foram coletadas em três pontos: água de processo (PW), é a água utilizada dentro dos frigoríficos em contato direto com os peixes; água limpa que entra no tanque de purificação (EPT) e água que sai do tanque de purificação (LPT). As águas de processamento dos nove frigoríficos de tilápia foram analisadas e caracterizadas quanto às suas características microbiológicas e físico-químicas. Os resultados das análises microbiológicas atendem aos valores indicados pela legislação, na maioria dos casos. Em relação aos dados microbiológicos, podemos destacar que houve um aumento no total de coliformes da água que entra no tanque de purificação para a água que sai do tanque de purificação em cinco frigoríficos. Ainda assim, houve aumento do teor de bactérias aeróbias mesófilas observado na água de saída em relação à água de entrada do tanque de purificação em sete frigoríficos. Em relação às análises físico-químicas das amostras de água de processo, os resultados mostram que os índices avaliados estão de acordo com os valores indicados pela legislação. Os teores de Cd, Mg, Sc e Cd ficaram abaixo dos detectáveis pela análise em todos os frigoríficos. Os níveis de Al, Sc, As, Rb, Ba, Pb, Mn, As, Se, Rb, Ag, Sb, Ba e Pb foram detectados apenas em um ou dois frigoríficos. Conclui-se que embora alguns matadouros tenham características hídricas fora dos limites indicados pela legislação, as alterações observadas não foram significativas e pequenos ajustes são necessários para a adequação.

**Palavras-chave:** Carne de pescado; Microbiologia; Qualidade da água; Segurança alimentar; Sustentabilidade.

### Resumen

El objetivo de este estudio fue analizar las características fisicoquímicas y microbiológicas del agua utilizada en el procesamiento de filetes de pescado y tilapia en mataderos. El estudio analizó el agua de procesamiento de nueve mataderos. Las muestras de agua para análisis se recolectaron en tres puntos: agua de proceso (PW) es el agua utilizada dentro de los mataderos en contacto directo con el pescado; agua limpia que ingresa al tanque de purificación (EPT) y agua que sale del tanque de purificación (LPT). Se analizaron y caracterizaron las aguas de procesamiento de los nueve mataderos de tilapia en cuanto a sus características microbiológicas y fisicoquímicas. Los resultados de los análisis microbiológicos cumplen los valores indicados por la legislación, en la mayoría de los casos. En cuanto a los datos microbiológicos, podemos destacar que hubo un aumento en el número total de coliformes desde el agua que ingresa al tanque de depuración hasta el agua que sale del tanque de depuración en cinco mataderos. Aun así, se observó un aumento en el nivel de bacterias aeróbicas mesófilas en el agua de salida en relación con el agua de entrada del tanque de depuración en siete mataderos. Con relación al análisis fisicoquímico de las muestras de agua de proceso, los resultados muestran que los índices evaluados están de acuerdo con los valores indicados por la legislación. Los contenidos de Cd, Mg, Sc y Cd estuvieron por debajo de los detectables por el análisis en todos los mataderos. Los niveles de Al, Sc, As, Rb, Ba, Pb, Mn, As, Se, Rb, Ag, Sb, Ba y Pb solo se detectaron en uno o dos mataderos. Se concluye que, aunque algunos mataderos tienen características de agua fuera de los límites indicados por la legislación, los cambios observados no fueron significativos y se requieren pequeños ajustes para su adecuación.

**Palabras clave:** Carne de pescado; Microbiología; Calidad del agua; Seguridad alimenticia; Sustentabilidad.

## 1. Introduction

Among foods of animal origin, fish are present the greatest conditions favorable to deterioration, and, therefore, are considered highly perishable. This is mainly since the fish have a pH close to neutrality, a high amount of nutrients and water content, which means that a wide range of biochemical reactions can be triggered, facilitating the entry and development of microorganisms (Gonçalves, 2011).

Thus, the control of water from the creation of the fish to the final product of the fish is essential for maintaining a low range of microorganisms present and of metal residues and other chemical elements in the meat of the fish at the end of the entire process. The large number of bacteria found in mucus, gills, and intestines of fish (González, et al., 1999) makes microbial contamination the most important concern in fish processing (Gram & Huss, 1996). These changes in association with intrinsic and extrinsic factors can increase the susceptibility to deterioration of the fish, directly influencing the original

organoleptic characteristics of the fish, facilitating the development of food-borne diseases (Huss, 1997; Massaguer, 2005; Jay, 2005; Boari, et al., 2008; Gonçalves, 2011), and compromise the product's useful life (Adebayo-Tayo, et al., 2012). As far as points of interest for microbial control in a slaughterhouse, the water in the purification tank, the processing of the fish at the time of processing and the water used in the ice used for the maintenance of the chilled or frozen fish, are the points that deserve attention and care.

In addition, the wastewater from both the fish purification and the water used in fish processing must be monitored, as the disposal of water without proper care and with some contamination, can harm the environment, being a potential focus of eutrophication of natural waters and artesian wells located close to the slaughterhouse. The control and regulation of the water quality used in all stages of fish processing is of paramount importance both to ensure food security and to minimize the possible environmental damage that can occur due to improper disposal and contaminated water to the environment.

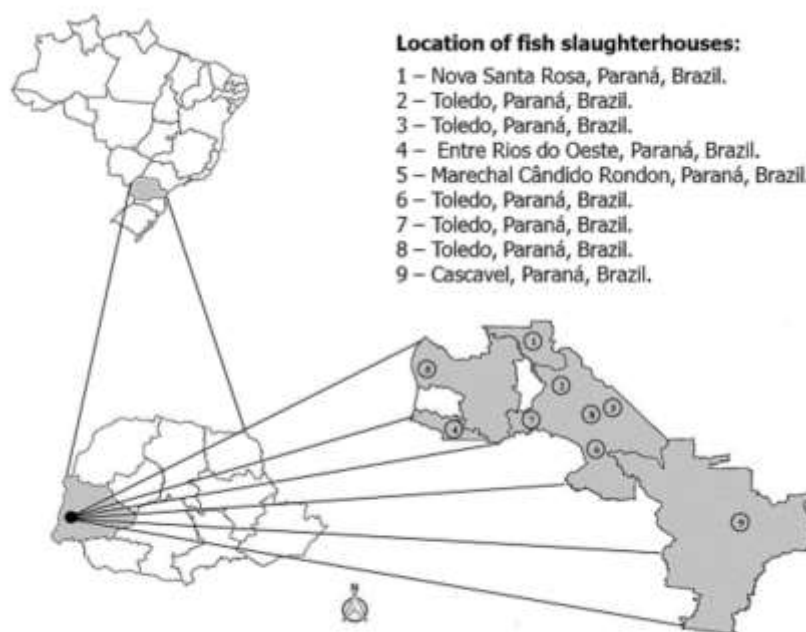
Thus, the study was carried out with the objective of analyzing the microbiological and chemical characteristics of the water used in processing fish, in Nile tilapia slaughterhouses.

## 2. Methodology

### 2.1 Study Area and sampling

The study was carried out in five cities in the western region of the state of Paraná, Brazil (Figure 1). The area of this study comprises the largest developed region in the Nile tilapia cultivation and slaughter segment in southern Brazil. Nine slaughterhouses were chosen to assess the quality of the water used in the animal purification and slaughtering internal processes, as well as to evaluate the quality in terms of physiochemical and microbiological aspects of the fillets produced. The slaughterhouses were identified as S1, S2, S3, S4, S5, S6, S7, S8, and S9. A collection of samples of fillets and water was carried out from December to March. The collections followed methodologies for sampling as recommended by ICMSF (2002); Alimentarius (2007); AOAC (2016), and APHA (2017).

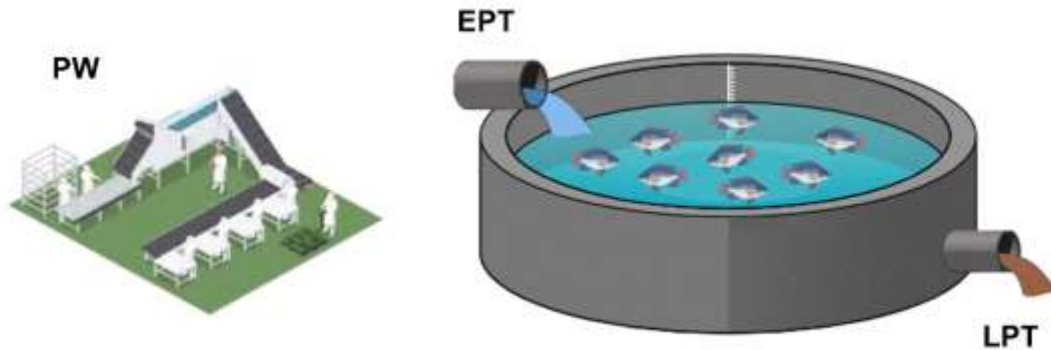
**Figure 1.** Map of the study region, indicating the location of the fish slaughterhouses, located in the state of Paraná, Brazil.



Source: Prepared by the authors (2020).

The water samples were identified as process water (PW), that is, it is the water used inside the slaughterhouses in direct contact with the fish; clean water entering the purification tank (EPT) and water leaving the purification tank (LPT) (Figure 2). All identifications were followed by the number of the slaughterhouses evaluated.

**Figure 2.** Places where water samples were collected for analysis.



Source: Prepared by the authors (2020).

## 2.2 Microbiological analysis

Aerobic mesophilic and psychotropic microorganism analyses were performed using a representative part of the samples (25 g or mL), where they were stored in the Stomacher bag and homogenized for 60 seconds in a Stomacher machine with 225 g of chilled peptone saline diluent (0.85 % NaCl with peptone a 0.1 %). Followed by an appropriate 10-fold dilution of the homogenate using saline peptone as the diluent. For each blank dilution, replicates were prepared. 0.1 mL of each appropriate dilution step was spread on the surface of dry media in Petri dishes. Mesophilic bacteria were determined by using deep plate count agar (35°C, 48 h) and psychotropic bacteria using surface plate count agar (20°C, 120 h). Procedures prescribed by ICMSF (2002); Alimentarius (2007), and APHA (2017).

Total coliforms and thermotolerant coliforms bacteriological tests were performed after sampling. The methods used to recover the bacterial load were the most likely number (NLP) with the presumptive test in tryptose with lauryl sulfate at 35°C for 24-48 h and confirmation in bright bile green broth at 2% (35°C, 24-48 h). For coliform bacteria and for thermotolerant coliforms, confirmation in CE broth and incubation at 44.5°C for 24 h was conducted (ICMSF, 2002; APHA, 2017).

For coagulase-positive *Staphylococcus aureus*, samples of 0.1 mL of serial dilutions of fish or water sample homogenates were spread on the surface of Baird Parker agar at a temperature of 35 to 37°C and incubated for 24 to 48 h. Suspicious colonies were identified by the coagulase test (ICMSF, 2002; APHA, 2017).

For *Salmonella* spp., pre-enrichment by incubating the sample dilutions at 37°C for 24 h was performed. Subsequently, 1.0 mL and 0.1 mL samples were inoculated into Selenite Cysteine and Rappaport-Vassiliadis broth, respectively, both containing 1.0 mL of novobiocin (0.4%) and left for incubation (37°C; 24 h). Then, the enrichment was spread on Brilliant Green and MacConkey agar followed by incubation (37°C; 24 h). Suspicious colonies were inoculated on TSI and TSA agar for serological tests (ICMSF, 2002; APHA, 2017).

### **2.3. Physicochemical analysis**

#### **2.3.1 Water analysis**

In all water collections, the following parameters were analyzed: water temperature ( $^{\circ}\text{C}$ ), dissolved oxygen ( $\text{mg L}^{-1}$ ), pH, and alkalinity ( $\text{mg L}^{-1}$ ) were evaluated in situ using a Hanna digital potentiometer. The other parameters were performed using the techniques suggested by APHA (2017), free residual chlorine (FRC  $\text{mg L}^{-1}$ ; 4500-Cl.G.3 method), total dissolved solids (TDS  $\text{mg L}^{-1}$ ; 2540 B method), sedimentable solids (SES  $\text{mg L}^{-1}$ ; 2540 F method), Alkalinity (ALKA  $\text{mg L}^{-1}$ ; 2320A/2320B methods), total nitrogen (TN  $\text{mg L}^{-1}$ ; 4500 C method), chemical oxygen demand (COD  $\text{mg L}^{-1}$ ; 5220 D method), and determination of biochemical oxygen demand (BOD  $\text{mg L}^{-1}$ ; 5210 B method).

The elementary chemical concentration of the water and fillet samples was analyzed using a portable benchtop TXRF instrument, featuring an air-cooled low power X-ray metal-ceramic tube with a molybdenum target, working at 20 keV of energy for 1000 s, and a liquid nitrogen-free Silicon Drift Detector (SSD) with ranging element from sodium (Na) to uranium (U) (Bruker, 2007). For this particular research, the following elements were analyzed: Na, K, Mg, Ca, Ba, Sc, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Nb, Ag, Cd, Pb, P, S, Cl, As, Se, and Br.

## **3. Results**

Next, the results obtained for the microbiological and physicochemical parameters of the water will be presented and discussed.

### **3.1 Microbiological parameters of process water**

Table 1 presents the results regarding the microbiological analysis of the water samples from the entrances and exits of the fish purification tanks and the water samples used in the tilapia slaughter process.

**Table 1.** Microbiological populations parameters of water samples utilized in: (A) inlet depuration tank; (B) outlet depuration tank; (C) industrial slaughter process.

Variables (A)	Water inlet depuration tank								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
TC (MPN mL <sup>-1</sup> )	23.00	>23.00	<1.10	>23.00	<1.10	16.00	1.10	>23.00	<1.10
TCL (MPN mL <sup>-1</sup> )	<1.10	<1.10	5.10	<1.10	<1.10	<1.10	<1.10	5.10	<1.10
<i>S. aureus</i> (CFU mL <sup>-1</sup> )	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00
<i>Salmonella</i> spp.	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
MAB (Log (CFU mL <sup>-1</sup> ))	2.36 ±0.04	3.38 ±0.05	3.49 ±0.05	<1.00	<1.00	2.15 ±0.04	<1.00	1.90 ±0.03	4.75 ±0.07
PB (Log (CFU mL <sup>-1</sup> ))	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	<1.00	4.68 ±0.07
Variables (B)	Water outlet depuration tank								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
TC (MPN mL <sup>-1</sup> )	>23.00	>23.00	6.90	9.20	5.10	>23.00	>23.00	>23.00	>23.00
TCL (MPN mL <sup>-1</sup> )	>23.00	>23.00	>23.00	>23.00	>23.00	>23.00	>23.00	>23.00	>23.00
<i>S. aureus</i> (CFU mL <sup>-1</sup> )	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00
<i>Salmonella</i> spp.	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
MAB (Log (CFU mL <sup>-1</sup> ))	4.47 ±0.07 <sup>b</sup>	4.27 ±0.06 <sup>c</sup>	3.19 ±0.05 <sup>d</sup>	2.90 ±0.04 <sup>e</sup>	2.13 ±0.03 <sup>f</sup>	4.41 ±0.07 <sup>bc</sup>	3.06 ±0.05 <sup>de</sup>	5.45 ±0.08 <sup>a</sup>	4.51 ±0.07 <sup>b</sup>
PB (Log (CFU mL <sup>-1</sup> ))	4.20 ±0.07 <sup>b</sup>	3.98 ±0.07 <sup>c</sup>	4.31 ±0.07 <sup>b</sup>	3.01 ±0.05 <sup>e</sup>	3.06 ±0.05 <sup>e</sup>	4.65 ±0.08 <sup>a</sup>	3.35 ±0.06 <sup>d</sup>	2.97 ±0.05 <sup>c</sup>	5.68 ±0.07 <sup>b</sup>
Variables (C)	Slaughterhouses process water								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
TC (MPN mL <sup>-1</sup> )	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10
TCL (MPN mL <sup>-1</sup> )	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10	<1.10
<i>S. aureus</i> (CFU mL <sup>-1</sup> )	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00
<i>Salmonella</i> spp.	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
MAB (Log (CFU mL <sup>-1</sup> ))	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	4.56 ±0.07
PB (Log (CFU mL <sup>-1</sup> ))	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	<10.00	5.68 ±0.09

\*Abs= Absent; MAB= Mesophilic aerobic bacterial; PB= Psycchrotrophic bacteria; TLC= Thermotolerant coliform; TC= Total coliform; CFU= Colony forming; S1 – S9 = number of slaughterhouse. Source: Prepared by the authors (2020).

For total coliforms (TC), values ranging from <1.10 to> 23 MPN mL<sup>-1</sup> were observed in the water inlet of the purification tank, from 5.10 to> 23 MPN mL<sup>-1</sup> in the water out of the purification tank, and < 1.10 MPN mL<sup>-1</sup> in the water used in the fish processing process in all slaughterhouses evaluated in this study. There was an increase in the TC of the water entering the purification tank to the water leaving the purification tank in slaughterhouses S3, S5, S6, S7 and S9.

In the analysis of thermotolerant coliforms (TCL), values <1.10 were observed in the inlet water of the tanks, with the exception of slaughterhouses S3 and S8, which presented values of 5.10 MPN mL<sup>-1</sup>. In the water leaving the purification tank, all slaughterhouses showed values >23 MPN mL<sup>-1</sup> of TCL, while in the process water, the value was <1.10 in all

slaughterhouses.

Regarding the parameters of *S. aureus* and *Salmonella* ssp., all samples expressed values of  $<10$  CFU mL<sup>-1</sup>, respectively.

The content of aerobic mesophilic bacteria (MAB) varied from  $<1.00$  to 4.75 CFU mL<sup>-1</sup> in the water entering the purification tank, while in the water leaving the purification tank the values ranged from 2.13 to 5.45 CFU mL<sup>-1</sup>. An increase in the MAB content was observed in the outgoing water in relation to the inlet water of the purification tank in all slaughterhouses, with the exception of S3 and S9. For process water, a value of  $<10$  was observed in all slaughterhouses, with the exception of S9 slaughterhouse, which had an exact value of 4.56 CFU mL<sup>-1</sup>. In addition, a significant difference was observed for the MAB content obtained in the water from the purification tank between the slaughterhouses evaluated, where S8 had the highest value and differed from the others, and S5 had the lowest value.

Regarding psychotropic bacteria (PB), with the exception of the S9 slaughterhouse (4.68), all presented results  $<1.00$  (Log CFU mL<sup>-1</sup>) in the water entering the purification tanks. In the water leaving the purification tanks, he observed results ranging from 2.97 to 4.65 (Log CFU mL<sup>-1</sup>), with the S6 slaughterhouse showing the highest value (4.65). For the water used in fish processing, a value of  $<10$  was observed for all slaughterhouses, with the exception of S9, which presented a value of 5.68 (Log CFU mL<sup>-1</sup>).

### 3.2 Chemical Analysis

Table 2 presents the results regarding the physical-chemical analysis of the water samples from the entrances and exits of the fish purification tanks and the water samples used in the process of processing the fish.

The results presented for total dissolved solids (TDS) in the inlet water of the purification tank and in the water used in the process, showed values lower than  $<0.10$  mg L<sup>-1</sup>. On the other hand, at the exit of the purification tank, this variable showed values from 80 to 2137 mg L<sup>-1</sup>, with slaughterhouse S8 having the lowest value and S2 the highest value.

For the data obtained from the sedimentable solids (SS) (mg L<sup>-1</sup>), it was observed both in the water in and out of the purification tank, and in the process water, a value below 1 mg L<sup>-1</sup>.

The results of the alkalinity (mg L<sup>-1</sup>) of the inlet water of the purification tank ranged from 2.61 in the S2 slaughterhouse to 37.50 in the S5. For the water leaving the purification tank, the alkalinity results ranged from 3.03 in S1 to 37.80 in S5. In the water used in fish processing, the variation was between 10.40 in S1 to 31.70 in S6.

The nitrogen (N) (mg L<sup>-1</sup>) content observed in the inlet water of the purification tank and in the water used in the processing of fish was  $<1.00$ , while, in the water out of the purification tank values ranging from 0.35 to 0.95 were observed.

For the Biochemical oxygen demand (BOD) variable (mg L<sup>-1</sup>), values below 2 were observed for the inlet water of the purification tank and the fish processing water, while for the outlet water of the purification, values ranging from 25 to 1470 were observed.

For the evaluation of the chemical oxygen demand (COD) (mg L<sup>-1</sup>), it was observed that the water of entry in the purification tank and the water used in the processing of the fish presented values below 2.00, while in the water of disposal of the purification tank the values varied from 156 (S9) to 3270 (S1).

For the water used in fish processing, the content of free residual chlorine (FRC) was also analyzed, as the water is treated with chlorine. Values ranging from 0.18 (S9) to 1.62 (S8) were observed.

**Table 2.** Physical-chemical parameters of water samples in: (A) inlet depuration tank; (B) outlet depuration tank; (C) industrial slaughter process

Variables (A)	Water inlet depuration tank								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
TDS (mg L <sup>-1</sup> )	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
SS (mg L <sup>-1</sup> )	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
ALK (mg L <sup>-1</sup> )	3.41 (4.00E <sup>-2</sup> )	2.61 (4.00E <sup>-2</sup> )	11.40 (8.12E <sup>-2</sup> )	16.60 (1.06)	37.50 (12.23)	20.40 (3.41)	16.20 (2.87)	3.61 (4.00E <sup>-2</sup> )	11.20 (2.88)
N (mg L <sup>-1</sup> )	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
BOD (mg L <sup>-1</sup> )	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
COD (mg L <sup>-1</sup> )	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
pH	6.00 (0.10)	7.00 (0.40)	7.50 (0.24)	7.20 (0.21)	8.20 (0.25)	7.00 (0.22)	7.80 (0.29)	7.00 (0.19)	6.80 (0.14)
Variables (B)	Water outlet depuration tank								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
TDS (mg L <sup>-1</sup> )	90.00 (10.32)	2137.00 (2.23E <sup>2</sup> )	130.00 (11.12)	81.00 (9.23)	325.00 (21.24)	174.00 (41.68)	164.00 (9.87)	80.00 (12.39)	265.00 (24.87)
SS (mg L <sup>-1</sup> )	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
ALK (mg L <sup>-1</sup> )	3.03	8.43 (1.45)	21.80 (2.98)	18.40 (1.62)	37.80 (4.79)	23.60 (2.13)	8.43 (1.09)	3.41 (3.03E <sup>-2</sup> )	10.70 (2.29)
N (mg L <sup>-1</sup> )	< 1.00	0.47 (2.03E <sup>-2</sup> )	0.35 (3.00E <sup>-2</sup> )	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	0.95 (5.00E <sup>-2</sup> )
BOD (mg L <sup>-1</sup> )	1470.00 (2.13E <sup>2</sup> )	283.00 (15.32)	252.00 (17.73)	292.00 (21.79)	236.00 (18.32)	198.00 (16.52)	25.00 (2.64)	213.00 (14.87)	106.00 (8.67)
COD (mg L <sup>-1</sup> )	3270.00 (5.83E <sup>2</sup> )	540.00 (62.41)	560.00 (58.12)	650.00 (72.69)	525.00 (48.79)	440.00 (39.63)	500.00 (47.87)	475.00 (40.09)	156.00 (9.74)
pH	6.30 (0.23)	6.70 (0.40)	7.10 (0.56)	7.10 (0.60)	7.80 (0.60)	7.20 (0.54)	7.20 (0.60)	6.90 (0.45)	6.80 (0.40)
Variables (C)	Slaughterhouses process water								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
FRC (mg L <sup>-1</sup> )	0.80 (2.00E <sup>-2</sup> )	0.68 (1.80E <sup>-2</sup> )	1.10 (3.20E <sup>-2</sup> )	0.29 (6.80E <sup>-3</sup> )	1.33 (2.10E <sup>-2</sup> )	0.67 (9.88E <sup>-3</sup> )	0.31 (2.88E <sup>-2</sup> )	1.62 (0.22)	0.18 (6.78E <sup>-3</sup> )
TDS (mg L <sup>-1</sup> )	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
SS (mg L <sup>-1</sup> )	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
ALK (mg L <sup>-1</sup> )	10.40 (0.88)	15.30 (1.33)	13.80 (0.99)	17.00 (2.14)	26.10 (2.96)	31.70 (3.66)	18.40 (1.41)	9.44 (0.78)	11.20 (2.29)
N (mg L <sup>-1</sup> )	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00	< 1.00
BOD (mg L <sup>-1</sup> )	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
COD (mg L <sup>-1</sup> )	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00	< 2.00
pH	6.90 (0.13)	6.90 (0.12)	7.40 (0.40)	7.10 (0.44)	7.60 (0.51)	7.00 (0.22)	7.40 (0.33)	7.00 (0.10)	7.00 (0.12)

\*FRC= Free residual chlorine TDS= Total dissolved solids; SS = sedimentable solids ALK= Alkalinity; N= Nitrogen; BOD= Biochemical oxygen demand; COD= Chemical oxygen demand. Source: Prepared by the authors (2020).



Table 3 shows the results obtained from the analysis of the chemical components of the inlet water of the purification tank. The levels of Mg, Sc, and Cd were below that detectable by the analysis in all slaughterhouses.

The levels of Mn, As, Se, Rb, Ag, Sb, Ba, and Pb were detected only in one or two slaughterhouses. The variations observed for the other chemical elements can be better observed in the table.

**Table 3.** Concentrations of elements in inlet water (InLW) from nine slaughterhouses.

Element	Mean $\pm$ SD (mg L <sup>-1</sup> )								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
Na	2.54 (0.23)	2.41 (0.55)	2.64 (0.67)	2.28 (0.89)	2.35 (0.15)	1.19 (5.00E <sup>-2</sup> )	2.39 (1.20)	1.73 (0.39)	1.51 (2.00E <sup>-2</sup> )
Mg	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Al	1.41 (0.58)	4.34 (0.62)	< LOD	< LOD	< LOD	6.86 (1.25)	< LOD	< LOD	< LOD
S	0.28 (0.06)	0.23 (2.00E <sup>-2</sup> )	0.18 (5.00E <sup>-2</sup> )	0.46 (5.00E <sup>-2</sup> )	0.35 (2.00E <sup>-2</sup> )	0.27 (1.00E <sup>-2</sup> )	0.74 (8.00E <sup>-2</sup> )	0.17 (5.00E <sup>-2</sup> )	0.14 (5.00E <sup>-2</sup> )
Cl	< LOD	9.90E <sup>-2</sup> (4.00E <sup>-2</sup> )	0.76 (4.00E <sup>-2</sup> )	8.00E <sup>-2</sup> (3.00E <sup>-2</sup> )	0.36 (4.30E <sup>-2</sup> )	0.34 (7.00E <sup>-2</sup> )	< LOD	< LOD	< LOD
K	1.17 (2.00E <sup>-2</sup> )	0.72 (2.00E <sup>-2</sup> )	0.68 (5.00E <sup>-2</sup> )	0.37 (3.00E <sup>-2</sup> )	0.46 (2.00E <sup>-2</sup> )	0.25 (2.00E <sup>-2</sup> )	0.15 (9.00E <sup>-2</sup> )	0.29 (6.00E <sup>-2</sup> )	0.50 (3.00E <sup>-3</sup> )
Ca	3.04 (3.00E <sup>-2</sup> )	1.83 (2.00E <sup>-2</sup> )	2.97 (2.00E <sup>-2</sup> )	2.62 (2.00E <sup>-2</sup> )	4.77 (3.00E <sup>-2</sup> )	3.81 (9.00E <sup>-2</sup> )	6.29 (4.00E <sup>-2</sup> )	2.27 (2.00E <sup>-2</sup> )	9.69 (4.00E <sup>-2</sup> )
Sc	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Ti	8.80E <sup>-2</sup> (6.00E <sup>-3</sup> )	0.60 (1.00E <sup>-2</sup> )	0.30 (7.00E <sup>-3</sup> )	0.30 (7.00E <sup>-3</sup> )	7.60E <sup>-2</sup> (6.00E <sup>-3</sup> )	3.00E <sup>-2</sup> (9.00E <sup>-3</sup> )	1.32 (1.00E <sup>-2</sup> )	0.14 (6.00E <sup>-3</sup> )	0.16 (6.00E <sup>-3</sup> )
V	< LOD	1.70E <sup>-2</sup> (0.005)	4.00E <sup>-2</sup> (4.00E <sup>-3</sup> )	4.10E <sup>-2</sup> (4.00E <sup>-3</sup> )	0.18 (6.00E <sup>-3</sup> )	< LOD	1.10E <sup>-2</sup> (5.00E <sup>-3</sup> )	0.17 (5.00E <sup>-3</sup> )	9.00E <sup>-3</sup> (3.00E <sup>-3</sup> )
Cr	< LOD	< LOD	9.00E <sup>-3</sup> (3.00E <sup>-3</sup> )	< LOD	1.70E <sup>-2</sup> (4.00E <sup>-3</sup> )	< LOD	1.10E <sup>-2</sup> (4.00E <sup>-3</sup> )	< LOD	< LOD
Mn	1.00E <sup>-2</sup> (3.00E <sup>-3</sup> )	< LOD	< LOD	< LOD	< LOD	< LOD	2.60E <sup>-2</sup> (3.00E <sup>-3</sup> )	< LOD	< LOD
Fe	7.30E <sup>-2</sup> (3.00E <sup>-3</sup> )	2.12 (1.00E <sup>-2</sup> )	0.23 (4.00E <sup>-3</sup> )	0.33 (4.00E <sup>-3</sup> )	9.20E <sup>-2</sup> (3.00E <sup>-3</sup> )	0.11 (4.00E <sup>-3</sup> )	1.56 (1.10E <sup>-3</sup> )	0.11 (3.00E <sup>-3</sup> )	0.14 (3.00E <sup>-3</sup> )
Co	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Ni	< LOD	< LOD	5.00E <sup>-3</sup> (2.00E <sup>-3</sup> )	4.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD	0.11 (3.00E <sup>-3</sup> )	< LOD	5.00E <sup>-2</sup> (2.00E <sup>-4</sup> )
Cu	< LOD	0.10 (3.00E <sup>-3</sup> )	1.80E <sup>-2</sup> (2.00E <sup>-3</sup> )	1.50E <sup>-2</sup> (2.00E <sup>-3</sup> )	6.00E <sup>-3</sup> (2.00E <sup>-3</sup> )	< LOD	1.90E <sup>-2</sup> (2.00E <sup>-3</sup> )	5.00E <sup>-2</sup> (2.00E <sup>-3</sup> )	7.00E <sup>-3</sup> (2.00E <sup>-3</sup> )
Zn	1.50E <sup>-2</sup> (2.00E <sup>-3</sup> )	4.10E <sup>-2</sup> (2.00E <sup>-3</sup> )	4.00E <sup>-2</sup> (2.00E <sup>-3</sup> )	1.20E <sup>-3</sup> (1.00E <sup>-3</sup> )	1.50E <sup>-2</sup> (2.00E <sup>-3</sup> )	2.20E <sup>-2</sup> (2.00E <sup>-3</sup> )	7.30E <sup>-2</sup> (3.00E <sup>-3</sup> )	1.80E <sup>-2</sup> (2.00E <sup>-3</sup> )	4.20E <sup>-2</sup> (2.00E <sup>-3</sup> )
As	< LOD	< LOD	< LOD	2.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	6.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD	< LOD	< LOD
Se	< LOD	< LOD	< LOD	1.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD	< LOD	1.00E <sup>-2</sup> (1.00E <sup>-3</sup> )	< LOD
Br	1.70E <sup>-3</sup> (1.00E <sup>-3</sup> )	7.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	3.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	9.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	2.90E <sup>-2</sup> (1.00E <sup>-3</sup> )	1.50E <sup>-2</sup> (1.00E <sup>-3</sup> )	8.00E <sup>-2</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD
Rb	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	1.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD

Sr	1.90E <sup>-2</sup> (1.00E <sup>-3</sup> )	1.50E <sup>-2</sup> (1.00E <sup>-3</sup> )	4.10E <sup>-2</sup> (1.00E <sup>-4</sup> )	1.90E <sup>-2</sup> (1.00E <sup>-3</sup> )	7.90E <sup>-2</sup> 0.24 (3.00E <sup>-3</sup> ) (1.00E <sup>-3</sup> )	4.30E <sup>-2</sup> (1.00E <sup>-3</sup> )	2.70E <sup>-2</sup> (1.00E <sup>-3</sup> )	4.80E <sup>-2</sup> (1.00E <sup>-3</sup> )
Ag	0.60 (5.00E <sup>-2</sup> )	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Cd	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Sb	< LOD	< LOD	0.10 (1.10E <sup>-2</sup> )	< LOD	< LOD	< LOD	< LOD	< LOD
Ba	< LOD	< LOD	< LOD	< LOD	2.80E <sup>-2</sup> (1.20E <sup>-2</sup> )	< LOD	< LOD	< LOD
Pb	3.00E <sup>-2</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

\*< LOD= Limit of detection; E= x10. Source: Prepared by the authors (2020).

Table 4 shows the results obtained from the analysis of the chemical components of the process water. The level of Cd was below that detectable by the analysis in all slaughterhouses.

The levels of Al, Sc, As, Rb, Ba, and Pb were detected only in one or two slaughterhouses. The variations observed for the other chemical elements can be better observed in the table.

**Table 4.** Concentrations of elements in process water (PW) from nine slaughterhouses.

Element	Mean ±SD (mg L <sup>-1</sup> )								
	S1	S2	S3	S4	S5	S6	S7	S8	S9
Na	5.39 (0.23)	3.98 (1.54)	2.73 (0.51)	2.16 (0.67)	0.96 (0.95)	1.84 (0.64)	1.52 (3.00E <sup>-2</sup> )	2.05 (0.44)	1.51 (2.00E <sup>-2</sup> )
Mg	13.93 (1.63)	8.48 (1.54)	< LOD	< LOD	< LOD	7.51 (1.60)	28.78 (1.98)	< LOD	< LOD
Al	20.65 (2.59)	< LOD	< LOD	< LOD	< LOD	< LOD	10.10 (0.57)	< LOD	< LOD
S	< LOD	0.54 (0.15)	0.38 (5.00E <sup>-2</sup> )	0.90 (0.06)	0.88 (9.00E <sup>-2</sup> )	< LOD	0.81 (0.38)	0.24 (5.00E <sup>-2</sup> )	1.35 (0.10)
Cl	0.31 (0.12)	< LOD	< LOD	< LOD	0.56 (5.00E <sup>-2</sup> )	2.38 (0.06)	< LOD	0.06 (3.00E <sup>-2</sup> )	4.61 (0.10)
K	0.45 (0.12)	0.29 (9.00E <sup>-2</sup> )	0.36 (0.29)	0.32 (0.25)	0.47 (0.42)	0.48 (0.18)	0.32 (0.27)	0.42 (8.00E <sup>-2</sup> )	0.50 (3.00E <sup>-2</sup> )
Ca	2.65 (4.00E <sup>-2</sup> )	8.87 (7.00E <sup>-2</sup> )	3.27 (3.00E <sup>-2</sup> )	2.90 (3.00E <sup>-2</sup> )	22.15 (9.00E <sup>-2</sup> )	50.10 (0.10)	12.32 (0.35)	2.23 (2.00E <sup>-2</sup> )	13.67 (7.00E <sup>-2</sup> )
Sc	< LOD	< LOD	< LOD	< LOD	< LOD	0.14 (1.00E <sup>-2</sup> )	< LOD	< LOD	< LOD
Ti	< LOD	1.17 (2.00E <sup>-2</sup> )	0.39 (1.00E <sup>-2</sup> )	0.12 (1.00E <sup>-2</sup> )	0.39 (1.00E <sup>-2</sup> )	0.22 (1.00E <sup>-2</sup> )	0.42 (5.00E <sup>-2</sup> )	7.50E <sup>-2</sup> 0.38 (1.00E <sup>-2</sup> ) (5.00E <sup>-3</sup> )	
V	< LOD	4.00E <sup>-2</sup> (1.00E <sup>-2</sup> )	4.20E <sup>-2</sup> 0.11 (1.00E <sup>-2</sup> ) (4.00E <sup>-3</sup> )		3.00E <sup>-2</sup> (6.00E <sup>-3</sup> )	< LOD	< LOD	0.18 (5.00E <sup>-3</sup> )	2.00E <sup>-2</sup> (5.00E <sup>-3</sup> )
Cr	< LOD	2.00E <sup>-2</sup> (9.00E <sup>-3</sup> )	7.00E <sup>-3</sup> (3.00E <sup>-3</sup> )	< LOD	9.00E <sup>-3</sup> (4.00E <sup>-3</sup> )	< LOD	< LOD	< LOD	< LOD
Fe	8.00E <sup>-2</sup> 1.19 (1.00E <sup>-2</sup> ) (7.00E <sup>-3</sup> )	0.28 (4.00E <sup>-3</sup> )	0.33 (3.00E <sup>-3</sup> )	0.27 (5.00E <sup>-3</sup> )	0.25 (4.00E <sup>-3</sup> )	0.46 (4.00E <sup>-2</sup> )	0.19 (4.00E <sup>-3</sup> )	0.38 (1.00E <sup>-2</sup> )	
Ni	< LOD	8.50E <sup>-2</sup> (5.00E <sup>-3</sup> )	< LOD	1.40E <sup>-2</sup> (2.00E <sup>-3</sup> )	< LOD	1.80E <sup>-2</sup> (2.00E <sup>-3</sup> )	< LOD	< LOD	5.90E <sup>-2</sup> (3.00E <sup>-3</sup> )
Cu	1.10E <sup>-2</sup> (4.00E <sup>-3</sup> )	< LOD	1.20E <sup>-2</sup> (2.00E <sup>-3</sup> )	1.20E <sup>-2</sup> (2.00E <sup>-3</sup> )	6.70E <sup>-2</sup> (3.00E <sup>-3</sup> )	1.40E <sup>-2</sup> (2.00E <sup>-3</sup> )	< LOD	4.00E <sup>-3</sup> 0.21 (4.00E <sup>-3</sup> ) (2.00E <sup>-3</sup> )	
Zn	< LOD	3.00E <sup>-2</sup> (5.00E <sup>-3</sup> )	1.90E <sup>-2</sup> (2.00E <sup>-3</sup> )	8.80E <sup>-2</sup> (2.00E <sup>-3</sup> )	1.30E <sup>-2</sup> (2.00E <sup>-3</sup> )	4.10E <sup>-2</sup> 0.18 (2.10E <sup>-2</sup> ) (2.00E <sup>-3</sup> )		1.10E <sup>-2</sup> 1.37 (8.00E <sup>-3</sup> ) (2.00E <sup>-3</sup> )	

As	< LOD	< LOD	< LOD	1.00E <sup>-2</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD	< LOD	< LOD	< LOD
Se	< LOD	< LOD	< LOD	4.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	< LOD	< LOD	1.00E <sup>-2</sup> (3.00E <sup>-3</sup> )	1.10E <sup>-2</sup> (1.00E <sup>-3</sup> )	< LOD
Br	< LOD	< LOD	< LOD	2.00E <sup>-3</sup> (1.00E <sup>-3</sup> )	< LOD	4.80E <sup>-2</sup> (1.00E <sup>-3</sup> )	2.70E <sup>-2</sup> (5.00E <sup>-3</sup> )	< LOD	1.30E <sup>-2</sup> (1.00E <sup>-3</sup> )
Rb	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	2.30E <sup>-2</sup> (1.00E <sup>-3</sup> )
Sr	< LOD	0.17 (3.00E <sup>-3</sup> )	4.20E <sup>-2</sup> (1.00E <sup>-3</sup> )	1.90E <sup>-2</sup> 0.11 (1.00E <sup>-3</sup> )	(2.00E <sup>-3</sup> )0.32	(1.00E <sup>-3</sup> )	0.30 (0.016)	2.80E <sup>-2</sup> (1.00E <sup>-3</sup> )	5.90E <sup>-2</sup> (1.00E <sup>-3</sup> )
Ag	0.69 (0.18)	< LOD	< LOD	0.18 (5.00E <sup>-2</sup> )	< LOD	< LOD	< LOD	< LOD	1.73 (0.32)
Cd	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD
Ba	< LOD	< LOD	< LOD	< LOD	6.50E <sup>-2</sup> (1.50E <sup>-2</sup> )	< LOD	0.56 (9.50E <sup>-2</sup> )	< LOD	< LOD
Pb	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD	5.10E <sup>-2</sup> (1.20E <sup>-2</sup> )	< LOD	< LOD

\*< LOD= Limit of detection; E= x10. Source: Prepared by the authors (2020).

#### 4. Discussion

The control of water quality for the handling of fish-based products is of great importance, since fish meat and its derivatives are excellent substrates for the development of microorganisms, including those of water transmission. All water that comes into contact with food must comply with the same microbiological standards as water for human consumption (Frazier & Westhoff, 2003).

In the food industry, water is essential and has high use and consumption, due to the various functions it plays in the processes and the need to sanitize establishments and equipment to guarantee the hygienic-sanitary quality of the final product (Massoud, et al., 2010). According to the World Health Organization (WHO) described in chapter 13 of the compiled manual Water Quality: Guidelines, Standards and Health (Ashbolt, et al., 2001) the microorganisms traditionally used to assess and monitor water quality belong to three classes, microorganisms that indicate sanitary deficiency, composed of heterotrophic bacteria and total coliforms; indicators of fecal contamination, which are part of the intestinal microbiota of man and warm-blooded animals, the main one being *E. coli*; also pathogenic microorganisms such as *Salmonella*, *Staphylococcus Aureus*, among others.

The fish purification tank in a slaughterhouse, is used to clean the fish that could be stored in polluted water, as well as to eliminate the contaminants contained in the intestinal tract of the animals that could interfere in the flavor and quality of the fish for human consumption. Studies show that tilapia submitted to a purification process for 8 h, show a significant improvement in quality and flavor, and the purification process during this time is capable of removing the earthy flavor from the fillet, and thus, increasing its acceptability of significantly by consumers (Rohani, et al., 2009).

Making the level of harmful contaminants and microorganisms after purification undetectable or non-existent, favoring human consumption and food security. Likewise, the water used during the process for bleeding, evisceration, and filleting of the fish must be clean and adequate, so that there is no contamination of the fish meat. In this sense, thermotolerant coliforms are the first-choice microorganisms for the assessment of pollution of fecal origin in the environment and in water, since they are predominantly constituted by the bacterium *Escherichia coli*, currently considered the most suitable indicator (Garcia-Armisen, et al., 2007).

Regarding the number of microorganisms present in the purification water (please see Table 1A, and 1B), there is no specific citation in legislation. However, animals must undergo procedures capable of cleaning and removing dirt, respecting the particularities of the species, before entering the slaughter process (Brasil, 2018). The levels observed in the microbiological analysis in these samples are higher, possibly due to the presence of transport water and the emptying of the animals' gastrointestinal tract in the purification tanks.

The increase in the content of total coliforms and of thermotolerant coliforms in the water collected from the purification tank outlet in relation to the inlet water, shows the importance of the fish purification process, since all this microbial load that remained in the outlet water of the purification, was in the fish itself, and was eliminated before the slaughter process.

The analyzed water samples (Table 1C), which come into direct contact with fish derivatives in the industrial process, are within the parameters established by the legislation (ANVISA, 2011), only the S9 slaughterhouse presented results above 2.7 (Log CFU mL<sup>-1</sup>) for MAB (4.56) and PB (5.68). Although, not being high values, the presence of these microorganisms above the recommended by the legislation, are already an indication of inappropriate water for the intended use. It is necessary to take actions that aim to reduce or totally remove the presence of these microorganisms, because values above what is allowed by the legislation must be investigated to identify the irregularity and take steps to reestablish these parameters within the appropriate range (ANVISA, 2011). According to Frazier and Westhoff (2003), all water that comes into contact with food must comply with the same microbiological standards as water for human consumption. For this reason, the control and monitoring of the characteristics of the water used in the fish processing is of paramount importance.

The chemical parameters of the water are the most important for characterizing the quality, as it allows the classification through its mineral content, determination of the degree of contamination and concentration of toxic pollutants or excess of some metal.

## 5. Conclusion

Thus, it is concluded that there is a similarity between the analyzes carried out in the slaughterhouses, in relation to the physical-chemical, microbiological, and component quality, with few points capable of influencing its quality, which can be corrected with training and improvement of techniques.

The information obtained in our research is extremely important for the evaluation of the characteristics of the water used in tilapia slaughterhouses. We suggest that future studies carry out, together with the evaluation of the physical-chemical, chemical and microbiological characteristics of the water, the evaluation of fish meat and processed fillet, to relate the effects of any problems in water quality in fish meat.

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