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## Evaluation of dissolved organic matter in bovine slaughterhouse wastewater using fluorescence spectroscopy associated with CP/PARAFAC and PCA methods

Murillo Cruz Matos<sup>a</sup>, Amanda Maria Tadini<sup>b</sup>, Fernando Rodrigues da Conceição<sup>a,c</sup>, Amílcar Machulek Junior<sup>a</sup>, Carlos Renato Menegatti<sup>d</sup>, Stéphane Mounier<sup>e</sup>, Anderson Rodrigues Lima Caires<sup>a</sup>, Gustavo Nicolodelli<sup>f\*</sup>

The present work aimed to evaluate the Dissolved Organic Matter (DOM) in effluents generated from a slaughterhouse industry at different stages of the effluent treatment process as well as in water body receiving effluent by using fluorescence spectroscopy technique associated with the canonical polyadic/parallel factor analysis (CP/PARAFAC) and Principal Component Analysis (PCA). Dissolved Organic Carbon (DOC), Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and humification index were also determined. The samples were collected in four sites: two effluent treatment plants, (i) entrance and (ii) exit of slaughterhouse wastewater; and two water bodies at Onça Stream, (iii) upstream and (iv) downstream. The DOM of the residual water at the entrance and exit sites of the slaughterhouse treatment system was shown to be composed mainly proteins, chlorophyll and in less extend of humic substance, while in the receiving water body it was mainly constituted humic substances. Particularly, the decrease of DOC, BOD and COD parameters at exit, compared to the entrance site, confirms the biodegradation of the organic matter by microorganisms in more humified compounds at the biological lagoons that corroborate with the results obtained by fluorescence spectroscopy. The biological and humification indices obtained by fluorescence spectra, for the Onça Stream, no show differentiation at the upstream and downstream sites, those results corroborate by the physical-chemical, PCA and CP/PARAFAC analyzes. Therefore, the fluorescence spectroscopy together with multivariate analysis (CP/PARAFAC and PCA) proved to be a viable tool for evaluating the quality of DOM in slaughterhouse wastewater.

### Introduction,

According to Organization for Economic Cooperation and Development (OECD) guidelines and Food and Agriculture Organization (FAO), the world beef production is projected to increase by 6 million tons carcasses from 2020 to 2029, where 81% of this increase will come from developing countries <sup>1</sup>. Currently, Brazil is the second-largest producer of beef in the world and the fifth largest exporter. In 2020, Brazil produced around 10 million tons of beef, exporting 2 million tons to dozens of countries worldwide under rigorous quality standards <sup>2</sup>, which generated US\$ 8.4 billions<sup>3</sup>.

Despite the excellent result from the economic point of view, it is necessary to slaughter about 222 million cattle to achieve this production <sup>2</sup>, which can cause environmental problems during the production process. In general, the industry is unable to take advantage of all the waste produced during the slaughter process, mainly from the washing processes of the industrial components, which generates wastewater for disposal. The effluents from all processing stages are collected

for biological treatment, usually by lagoons or anaerobic sludge blanket reactors <sup>4,5</sup>. After passing through a lagoon, or a series of stabilization lagoons, the effluent must reach the minimum condition to be released into the receiving water body, without causing damage to it. In order to guarantee the water quality, the receiving water body must be constantly monitored to attend the requirements of the standard established by the National Environment Council (CONAMA) in Brazil <sup>6</sup>.

Dissolved Organic Matter (DOM) is formed by soluble organic and complex structures dissolved in the aquatic system, originated from the decomposition of plant, and algae, tissues and microorganisms, and other compounds present in water <sup>7-9</sup>. Consequently, DOM may influence biogeochemical processes in aquatic environments <sup>10</sup>, being an important component for various chemical and biological processes in natural waters, such as phototransformation, biodegradation, mobilization and complexation of nutrients, metals and pollutants <sup>11</sup>. Due to the complex chemical nature of the DOM, rich in aromatic compounds, it is difficult to elucidate its molecular structure,

consisting of thousand of varieties of aliphatic and aromatic substructures, rich in functional groups, such as carbonyl, carboxyl, hydroxyl, phenolic and aromatic chains<sup>9,11</sup>. Nevertheless, despite its chemical complexity, DOM can be investigated and characterized by spectroscopic techniques, such as fluorescence spectroscopy<sup>12,13</sup>.

The advantage of analyzing organic matter (OM) by fluorescence spectroscopy is based on the high sensitivity of the technique, which also allows *in situ* analysis using portable devices<sup>12,13</sup>. In this scenario, fluorescence spectroscopy has been applied as a reliable technique to detect DOM, as recently reviewed in detail by Carstea et al<sup>14</sup>. Nevertheless, aiming to improve the understanding of the optical characteristics of the DOM by emission excitation matrix (EEM) signal, great attention has been given to develop algorithms to translate data from high dimensions into lower dimensions or to reduce the number of variables, such as principal component analysis (PCA) and canonical polyadic/parallel factor analysis (CP/PARAFAC)<sup>12,15-17</sup>. The CP/PARAFAC is a decomposition method based on the least squares, which decomposes the multidimensional signal of fluorescence into simple and independent components<sup>12,18</sup>. Li et al. (2020) have recently summarized the development of analytic tools, based on PCA and CP/PARAFAC, to characterize DOM in drinking water treatment processes<sup>12</sup>. Choi et al (2017) used EEM-CP/PARAFAC to characterize the chemical composition of municipal wastewater and to investigate the compositional change in its biodegradation<sup>19</sup>. Cohen et al. (2014) carried out a long-term examination of OM in wastewater along with the treatment by applying EEM-CP/PARAFAC<sup>20</sup>. EEM-CP/PARAFAC approach also has been used to reveals molecular size distributions of fluorescent components in DOM<sup>21</sup>. Ma and Li (2020) also demonstrated that the fluorescence spectroscopy can be applied for understanding the optical characteristics, composition and source of DOM in rivers of the Three Gorges Reservoir<sup>7</sup>. Furthermore, EEM-CP/PARAFAC technique has been used to ascertain the fluorophore group, indicating fecal pollution from the complex nature of DOM in surface water of Koshiro River Basin<sup>13</sup>. In general, DOM from slaughterhouse wastewater have been evaluated by electrochemical oxidation or coagulation, physico-chemical and biological treatment, advanced oxidation processes<sup>22-25</sup>, which can be possible to verify their concentration reduction of this material and other elements, as nitrogen and phosphorus<sup>22,24,25</sup>. Nevertheless, study realized by Louvet et al. (2013)<sup>23</sup> evaluated the effectiveness of slaughterhouse wastewater by biological treatment (active sludge), and the DOM was characterized by UV-Vis and fluorescence spectroscopy, but approaches in this study is completely different what is discussing in this work, that propose the use of CP/PARAFAC and PCA as complementary tools to fluorescence spectroscopy in the characterization of DOM in slaughterhouse wastewater. Rodríguez-Vidal et al (2020)<sup>26</sup> and Rodríguez-Vidal et al (2022)<sup>27</sup> characterized industrial wastewaters (including slaughterhouse effluents) searching for specific fluorescence fingerprints, but they did not perform a qualitative analysis using chemometrics methods.

In this context, the present study aimed to apply EEM fluorescence spectroscopy combined with multivariate methods to evaluate the DOM of effluents generated by the slaughterhouse industry. Furthermore, the optical results are compared to conventional water quality parameters to assess

the possible changes in the quality of the receiving water body caused by the release of the effluent residues.

## Materials and Methods

### A Cattle Slaughterhouse description:

The samples of this study were collected from Onça Stream (18° 30' 25.28"S / 54° 40' 35.83"W) and slaughterhouse (18° 30' 33.17"S / 54° 40' 33.78"W) called "Matadouro Esperança" located at Coxim, MS – Brazil, as shown in Figure 1. The daily wastewater volumetric production was (estimar a quantidade de água empregada, por 1000Kg de carne) for "Matadouro Esperança". The wastewater in bovine slaughterhouse is employed for cleaning the kill floor following described in literature<sup>28</sup>. Thus, this study focused on collecting the wastewater found at the entrance and exit of the effluent treatment of the cattle slaughterhouse, as shown in Figure S1.



Figure 1 - Location of the collection sites for the Onça stream sample and slaughterhouse residue represented by the points highlighted on the map (adapted from Google map).

### B Collection and preparation of samples

The sample collection procedures and cleaning of the used bottles for collecting the samples were carried out by following the recommendations described in CETESB (Companhia Ambiental do Estado de São Paulo)<sup>29</sup>. Twelve sets of samples were collected, three samplings for each of the four points described below. All samples were collected during the Brazilian summer, January, February and March (year 2021), in order to avoid seasonality issues. The surface water samples were obtained from site 1 – entrance of slaughterhouse wastewater; site 2 – exit slaughterhouse wastewater; site 3 – Onça Stream upstream; and site 4 – Onça Stream downstream (as can see in Figure 1). Then the samples were transported to the laboratory, filtered through a 0.2 mm membrane (Filter Pro) for the removal of small particles, and stored in the dark at 2°C until EEM analysis.

The treatment takes place in a four-Lagoon system, as described briefly below and the scheme of waste treatment generated by Slaughterhouse Esperança is showed in the Figure S1 (Electronic supplementary information). After Entrance (site 1), the effluent passes through an anaerobic lagoon, with a stabilization process taking place, without consumption of dissolved oxygen, resulting in a decrease in organic load. In the second stage, a facultative lagoon, it has aerobic and anaerobic zones, and during this process, the effluent biologically reacts to oxidize the organic matter. Finally, it passes through maturation lagoons 1 and 2, which aim to remove pathogenic organisms and nutrients. The treated effluent is collected at the Parshall gutter (site 2). The slaughterhouse has a release permit issued by IMASUL (state agency) where the maximum release flow is 3 cubic meters/hour. The discharges are continuous.

### C Physicochemical Analysis

DOC (dissolved organic carbon) was measured on a Shimadzu TOC-VCNS analyzer. The samples were filtered with a PES (Polyethersulfone) filter of 0.45  $\mu\text{m}$  porosity. Aliquots of 1 mL were added in TOC flasks containing 9 mL of deionized water, and 3 diluted samples were prepared from each sample received.

The 5-day test (SMWW 5210-B) was used to determine the Biochemical Oxygen Demand (BOD), which the sample was diluted with water and transferred to BOD, creating an environment conducive to bacteriological action since dilution water is saturated with oxygen and has nutrients that favor the metabolism and proliferation of bacteria. The equipment used to measure  $\text{O}_2$  consumption is an OD electrode with a luminescence sensor connected to a multiparameter (Hach, model HQ40d). For Chemical Oxygen Demand (COD), the closed reflux method with spectrophotometry (Hach-8000) was used. The COD values are defined in  $\text{mg L}^{-1}$  as  $\text{mg of O}_2$  consumed per liter of sample where the equipment used is the spectrophotometer (Hach, model DR-3900).

Phosphorus content was determined by the stannous chloride method (SMWW-4500D), which the sample is collected and transferred to a 250 mL Erlenmeyer flask, with a volume of 100 mL or diluted portion. Add 1.0 mL of strong acid solution and 0.4 g of ammonium persulfate. Placed on a hot plate for 40 minutes, until volume reduction (15-20 mL). The sample analysis was performed using a spectrophotometer (Hach, model DR-3900) by monitoring the absorption at 690 nm.

Nessler method (Hach Company-8038) was used to determine the total ammoniacal nitrogen, the ammoniacal determination is made in the range 0.02 to 2.5  $\text{mg L}^{-1}$  of  $\text{NH}_3\text{-N}$  by distillation. The sample analysis was performed using a spectrophotometer (Hach, model DR-3900) by monitoring the absorption at 425 nm.

In the case of Total Kjeldahl Nitrogen was used and validated by the Nessler method (Hach Company-8075). The pH of the samples was adjusted to 2.0 with sulfuric acid and cooled to 4  $^{\circ}\text{C}$ , requiring digestion and distillation. For reading it is used the spectrophotometer (Hach, model DR-3900) that idealized the method used in the quantification in the wavelength of 425nm.

Oil and grease content were determined using Soxhlet extraction SMWW-5520D method. Samples placed in glass flasks and acidified with concentrated  $\text{H}_2\text{SO}_4$  or concentrated HCl at  $\text{pH} \leq 2$ , refrigeration temperature between 4 $^{\circ}\text{C}$ . The samples are filtered on filter paper with a solution of diatomaceous silica, which the required material is retained and after a period of 4 h being distilled in the presence of the solvent n-hexane, the oil is extracted and quantified in  $\text{mg L}^{-1}$ .

The series of solids (totals, dissolved and suspended) were determined using the SMWW-2540 method, all gravimetric with some particularities. Total solids were quantified by 100 mL of sample from drying at 103-105 $^{\circ}\text{C}$  (SMWW-2540B). Total dissolved solids are quantified by SMWW-2540C method, which the material was filtered and dried in capsules. Finally, the suspended solids quantified by SMWW-2540D method in which the sample retained in the 2.0  $\mu\text{m}$  filter was considered. For the pH parameter determination is used the electrometric method (SMWW 4500-B) where the activity of  $\text{H}^+$  ions is determined. It uses the Thermo Scientific multiparameter portable meter, model Orion Star 5.

### D Fluorescence spectroscopy and statistical analysis

EEM spectra were obtained in a Fluorescence Spectrometer (SCINCO FS-2). The excitation and emission signals were recorded in the 220 – 620 nm and 250 – 800 nm ranges, respectively. The excitation and emission slits were set up at 5 nm, 600 nm/min scan speed and 20 ms integration time. A quartz cuvette with a 1.0 cm optical path and four polished faces was used. All measurements were performed at room temperature. Thirty-six EEM were measured, which three for each of the four collection sites during three months of analysis. Fluorescence indexes were calculated to further distinguish the composition and properties of DOM. Biological index (BIX) is defined by the ratio of the fluorescence intensity at  $\lambda_{\text{em}} 380$  nm divided by the fluorescence intensity at  $\lambda_{\text{em}} 430$  nm with excitation at 310 nm<sup>30</sup>. High values (BIX > 1) correspond to the presence of recently produced DOM of autochthonous origin (organic matter “fresh”) in water<sup>30,31</sup>. Humification index (HFIX) was adaptable for water samples, which it is determined as the total area under fluorescence emission spectrum in the 480 – 800 nm range with the excitation at 465 nm by Total Organic Carbon (TOC) ratio<sup>32</sup>.

The EEM were organized in a three-dimensional array and analyzed using the CP/PARAFAC multivariate method that decomposes it into three matrices, called loadings. The decomposition of EEM by CP/PARAFAC was performed using the “progMeef software” developed by Dr. Roland Redon from

MIO Laboratory of the University of Toulon, France. Three components were used in the decomposition, and the alternating least squares (ALS) algorithm was adopted as the trilinear decomposition method. The scatterings in the EEM were excluded using the Zeep procedure, using an interval of 15 nm<sup>33</sup>. The choice of components was based on the analysis of the consistency of the central tensor (core consistency diagnostics - CONCORDIA) and in the sum of squared estimate of errors (SSE) parameters<sup>18</sup>.

After the CP/PARAFAC decomposition of the EEM, 2D emission spectra contained in the EEM, referring to the components C1 ( $\lambda_{exc}= 320$  nm), C2 ( $\lambda_{exc}= 280$  nm), and C3 ( $\lambda_{exc}= 420$  nm), were employed at PCA. The spectral ranges used in the PCA were from 250 nm to 800 nm for all components. As we will see in the results and discussion item, these spectral bands were chosen because they present acceptable CONCORDIA values in the CP/PARAFAC decomposition<sup>18,33</sup>.

## Results and discussion

### A Physicochemical parameters of DOM

The physicochemical parameters of the samples, such as DOC, BOD, COD, Total Phosphorus, Ammoniacal Nitrogen, Total Nitrogen, total oils and greases, pH, and, total solids, are shown in Table S1 (Electronic supplementary information).

In Brazil, parameters such as BOD, COD, total nitrogen, ammonia nitrogen, oils and greases, total phosphorus, total solids and pH, are considered essential for the disposal of effluents in rivers, according to current legislation<sup>6,33</sup> and as described in the National Council of Environment (CONAMA) established by Resolutions 357/2005 and 430/2011. So, the results revealed that a smaller value of both BOD and COD was detected at the exit site, in relation to the entrance site (Table S1). This confirms the removal of organic matter during wastewater treatment (around 60% less of BOD and COD in the exit site).

Additionally, it was also observed that the punctual release of the effluent did not impact the BOD and COD values in the upstream and downstream sites (Table S1). Furthermore, the DOC, P, total oils and greases, and total solids values decreased in the exit point when compared with the entrance of the system because of the efficiency of the pre-treatment implantation system. In the upstream and downstream sites, the analyzed parameters did not show any difference between the locations and the determined values are in accordance with the national legislation. The ammoniacal nitrogen and total nitrogen values, in the mean of the three months analyzed, did not show statistical difference between the entrance and exit sites. At the exit site, several parameters have a high value in the mean standard deviation, it may be associated with the proliferation of algae noticed in the lagoons (which represent the nutrient conditions in a

eutrophic environment) at the collection time<sup>34,35</sup>. The pH remains within the limit recommended by the current legislation, which is between 6.5 and 9.0.

### 3.2. Fluorescence spectral characteristics of DOM

Figure 2 shows the EEM in which (a), (b), (c) and (d) corresponds to the entrance, exit, upstream and downstream samples (collected in February), respectively. The EEM for January and March samples are presented in the supplemental material Fig S2 and S3.

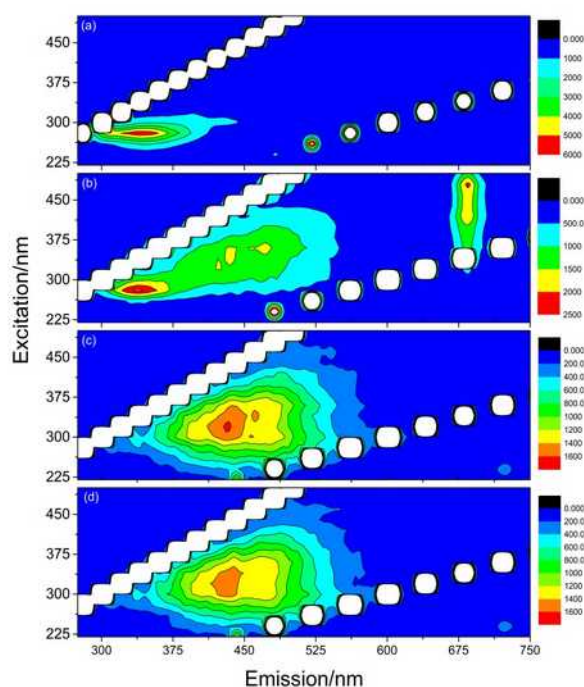


Figure 2 - Contour plots obtained by EEM fluorescence spectroscopy of the DOM from: slaughterhouse wastewater (Entrance (a) and Exit (b)) and Onça Stream (Upstream (c) and Downstream (d)).

The maximum emission at around 350 nm when excited in the 275 - 300 nm range (Fig.2a) refers to the autochthonous tryptophan-like (T)<sup>36-38</sup>. This component is considered as the protein-like comes from the microbial transformation of DOM terrestrial products<sup>39</sup>. The EEM referring to exit slaughterhouse wastewater presented three distinct emission regions, as shown in figure 2b. The first region is observed in the 290 - 370 nm range when excited between 250 and 320 nm. The second region of emission occurred between 400 and 550 nm when excited between 250 and 440 nm, which is strongly associated with different fluorophores related to the DOM materials<sup>40</sup>, including the presence of secondary metabolites such as ferulic acid, flavonoids, coumarin, and quercetin<sup>41</sup>. Besides, the DOM from Exit showed high fluorescence emission in the 680 to 710 nm range with maximum intensity at 690 nm, which is associated with the

chlorophyll emission, from the phytoplankton algae<sup>42</sup>. The emissions in the 425 to 475 nm range when excited between 280 and 325 nm are observed in the DOM from Onça Stream (Fig. 2c and 2d) and can be attributed to allochthonous humic-like that come from the land used and human activities<sup>37,43</sup>.

From Figures. 2a and 2b it can be seen that DOM, at the entrance, has protein composition, while a red-shift of the fluorescence groups was observed after the DOM treatment (exit slaughterhouse wastewater). This can be associated with the transformation of OM in which can be seen structural arrangements more complex and composed mainly by aromatic moieties<sup>38,44</sup>. During the transformation/degradation of organic matter by microorganisms, it can occur the preservation of compounds that are more resistant to chemical degradation, generating most chemically stable groups<sup>45</sup>, which can be confirmed by the increase of the fluorescence signal to red region. In addition, the fluorescence results indicate that the chemical composition of the DOM in the river was not significantly changed due to the waste released from the slaughterhouse, as can be seen in Figures 2c (Upstream) and 2d (Downstream), corroborating with the observed values for BOD and COD (Table S1).

To evaluate the contribution of OM in the set of samples, the EEM was decomposed via CP/PARAFAC. The decomposition method provides the fluorescence loadings, obtaining semi-quantitative information on the concentrations of fluorophores that make up the analyzed EEM, as well as the excitation and emission behavior of each component<sup>46</sup>. Through Figure S4, for a 5-component tested model, the SSE assumed lower values from 3 components. Additionally, from

3 components the explained variance does not increase significantly. Therefore, according to the recovered EEMs, the number of component best represents the experimental EEMs is 3, corroborating the high value of CONCORDIA. The EEM spectra were treated using the CP/PARAFAC, and it provided an EEM response model with components representing the independent contributions to the sample's fluorescence signal, as showed in Figure 3, (a) EEM components and (b) excitation and emission spectra.

The maximum peak of the excitation and emission bands were found centered on the following values of excitation ( $\lambda_{ex}$ ) and emission ( $\lambda_{em}$ ) wavelengths:  $\lambda_{ex}/\lambda_{em} = 320 \text{ nm}/440 \text{ nm}$ ,  $\lambda_{ex}/\lambda_{em} = 280 \text{ nm}/340 \text{ nm}$  and  $\lambda_{ex}/\lambda_{em} = 420 \text{ nm}/685 \text{ nm}$ , for components C1, C2 and C3, respectively. Humic substances compounds have an emission between 350 nm to 500 nm, with peaks centered on  $\lambda_{ex}/\lambda_{em} = 340 \text{ nm}/425 \text{ nm}$  and  $\lambda_{ex}/\lambda_{em} = 325 \text{ nm}/450 \text{ nm}$ , which are attributed to humic and fulvic acids, respectively<sup>47</sup>. Therefore, the emission signal of the C1 component can be correlated to the fluorescence signal of humic substances, such as humic and fulvic acids. Compounds such as proteins, revealed by the emission between 300 and 350 nm with maximum excitation and emission centered on  $\lambda_{ex}/\lambda_{em} = 280 \text{ nm}/340 \text{ nm}$ , which can be attributed to the amino acid tryptophan<sup>47</sup>. Consequently, the  $\lambda_{ex}/\lambda_{em}$  values of C2 component correspond to the excitation and emission of proteins. According to the values of  $\lambda_{ex}/\lambda_{em}$  and emission profile recovered from component C3, it is possible to confirm the presence of chlorophyll in the samples<sup>41,42,48</sup>. In addition to chlorophyll, it was also observed the contribution of secondary metabolisms, since the C3 component has an emission between 420 nm to 620 nm<sup>41</sup>.

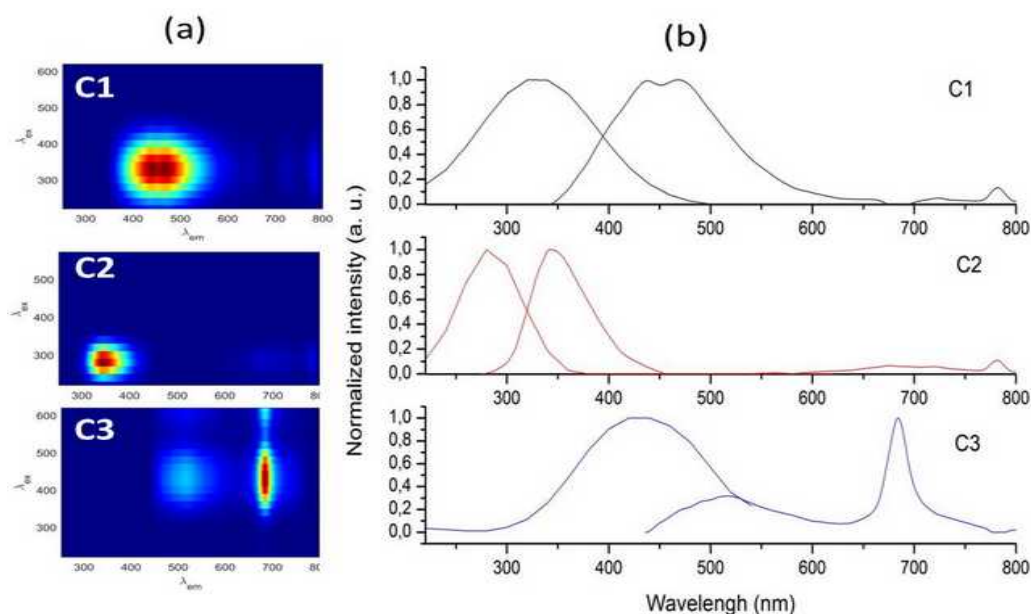


Figure 3 - (a) Components, C1, C2 and C3, obtained through EEM and CP/PARAFAC and (b) recovered excitation and emission spectra. We used three samples for each of the four points, in a single collection, a total of 36 EEM were analyzed.

Figure 4 presents the contribution results obtained through the CP/PARAFAC decomposition in which a three component model (C1, C2 and C3) was obtained with a core consistency diagnostics (CONCORDIA) of 95 %. Figures 4a, b, and c correspond to the average loadings of each component in January, February and March, respectively.

According to the characteristics attributed to the components C1, C2 and C3, it was possible to evaluate the transformation potential of the DOM in the slaughterhouse and in the aquatic body<sup>12,13</sup>. In Figure 4 (a, b and c), the contribution value of component C1 is lower at the entrance of the system, in relation to the exit site (30 to 52 %), demonstrating that at the entrance of the system there is a more biodegradable organic material ("fresh" materials) with a high potential for degradation, corroborating with that observed by BOD and COD (Table S1), since C1 (Figure 3a) refers to a more humified/transformed material (such as humic substances). As shown in Figure 4 (b and c), the contribution value of component C2 is much higher at the entrance, in relation to the other collection sites, about twice as high as the exit and more than eight times as compared to the stream samples. This can be associated with the fact that the entrance to the retention system is rich in protein, since this sector receives residues directly from the slaughterhouse without previous treatment. According to the observed in Figure 4 (a, b and c), the exit site presents a greater contribution from the C3 component than entrance site, indicating a high presence of chlorophyll and secondary metabolic compounds in the composition in this collection site. In relation to the upstream and downstream collection (Figure 4), both are predominantly made up of humic substances, with an insignificant presence of proteins and chlorophyll in the system, and did not show a significant increase of the components C1, C2 and C3, for three months, which indicating that the effluent generated in the slaughterhouse treatment system no changed the characteristics of the DOM in the Onça Stream.

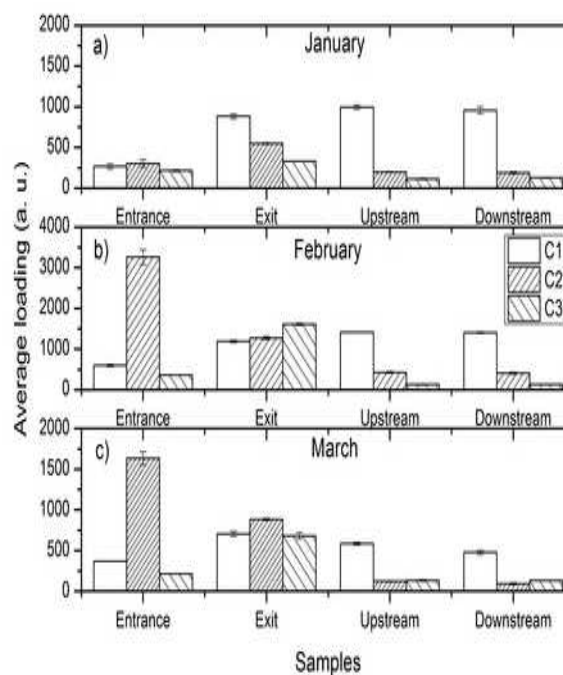


Figure 4 - Average of the loadings to the components C1, C2 and C3.

Additionally, the 2D scatter plots obtained by PCA treatment of components (a) C1, (b) C2 and (c) C3 are shown in Figure 5 (samples collected in February), respectively. The PCA for January and March samples are presented in the supplemental material Fig S5 and S6.

From Figures 5, S5 and S6, considering the C1, C2 and C3 components for the all months, the PCA results showed the upstream and downstream data appeared clustered separately from the entrance and exit data, where the entrance and exit are not clustered together. The PCA analysis confirmed the DOM present Onça Stream upstream and downstream has similar characteristics and it is not undergoing changes when receiving input from the effluent of the slaughterhouse.

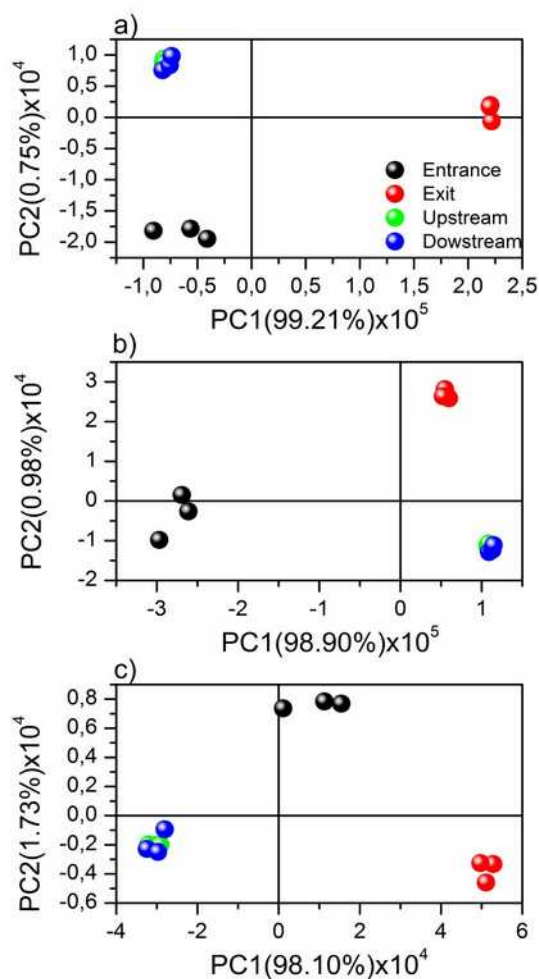


Figure 5 - 2D scatter plots obtained by PCA treatment of components: a) C1, b) C2 and c) C3. Entrance (Black), Exit (Red), Upstream (Green) and Downstream (Blue).

Table 1: Fluorescence indices for slaughterhouse wastewater and Onça Stream.

Samples	jan/21		fev/21		mar/21	
	BIX	HFIX	BIX	HFIX	BIX	HFIX
Entrance	1.12 ± 0.08	29,173 ± 925	1.2 ± 0.4	32,824 ± 510	1.65 ± 0.06	21,945 ± 430
Exit	0.96 ± 0.01	36,195 ± 1102	0.9 ± 0.2	97,330 ± 625	1.08 ± 0.03	45,627 ± 1857
Upstream	0.56 ± 0.02	17,529 ± 1102	0.63 ± 0.04	22,188 ± 1863	0.58 ± 0.00	17,100 ± 594
Downstream	0.58 ± 0.01	18,797 ± 990	0.67 ± 0.03	22,657 ± 370	0.59 ± 0.05	16,081 ± 516

In summary, the physicochemical characterization (Table S1) results showed a reduction in the content at the slaughterhouse wastewater exit compared to the entrance site for all parameters, especially DOC, BOD and COD, which confirms the biodegradation of OM by microorganisms in more humified compound at the biological lagoons (stabilization lagoons). This trend corroborates to the results obtained by fluorescence spectroscopy and subsequently confirmed by the

The fluorescent DOM compositional indicators were determined for the samples evaluated in this study as can see in Table 1. The humification index (HFIX) is an indicator of transformation and chemical stability of organic matter structure<sup>32,49</sup>, besides the biological index (BIX) is associated the presence of microbial-derived and "fresh" DOM<sup>30,31,50</sup>.

According to Table 1, it can be seen that the BIX decreased in the exit site of the system, corroborating with that observed by the BOD and COD (Table S1) for the slaughterhouse wastewater. These happened due to biodegradable organic matter transformation present in the entrance site that it was being consumed by the microorganisms at maturation lagoons in the exit site. Moreover, there was a greater amount of "fresh" organic matter of biological/microbial origin (BIX >1)<sup>30,31,50</sup> and a lower humification index (HFIX) in the entrance when compare to the exit site. However, the upstream and downstream show low BIX (0.56 to 0.67) that indicating more presence of organic matter from terrestrial sources. In addition, the humification indices, for the Onça Stream, showed no significant difference at the upstream and downstream sites to all collected samples, and these results corroborate by the physical-chemical and CP/PARAFAC analyzes, suggesting that the DOM from river does not received influence slaughterhouse wastewater.

biological and humification indices (Table 1). Besides, the slaughterhouse wastewater has a slightly alkaline pH of 8.2 at the exit site favoring the solubilization of more refractory DOM compounds, such as humic acids.

## Conclusion



The DOM of the residual water at the entrance and exit sites of the slaughterhouse treatment system was shown to be composed mainly proteins, chlorophyll and in less extent of humic substance (EEM detected low contribution), while in the receiving water body it was mainly constituted humic substances. Regarding the exit site comparing to entrance site the decrease of DOC, BOD and COD parameters (removed around 59-64%), confirms the biodegradation of organic matter by microorganisms in more humified compounds at the biological lagoons. This tendency corroborated with the results obtained by fluorescence spectroscopy and subsequently confirmed by the biological and humification indices. The humification indices for the Onça Stream no showed differentiation at the upstream and downstream sites, those results corroborate by the physical-chemical, PCA and CP/PARAFAC analyzes. So, the fluorescence spectroscopy together with multivariate analysis proved to be a viable tool for characterization of the quality of DOM in slaughterhouse wastewater. Moreover, as fluorescence spectroscopy is an optical tool for rapid analysis and having a low cost of analysis, it has a great potential for application for these analyses. In conclusion, the data from the sites downstream and upstream of the Onça stream were evaluated in the period of three months, and showed that the quality of the DOM in this stream was not altered with the discharge of wastewater from the cattle slaughterhouse in the summer period.

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## Author Contributions

Conceptualization - Matos and Nicolodelli; data curation - Mounier, FR da Conceição, Machulek Junior and Tadini; formal analysis - Mounier, FR da Conceição and Tadini; funding acquisition - Caires and Nicolodelli; investigation - Matos and Nicolodelli; methodology - Tadini, Matos, Menegatti and Nicolodelli; project administration - Nicolodelli; resources; software; supervision; validation - Mounier, FR da Conceição and Nicolodelli; visualization; roles/writing - original draft - Nicolodelli and Tadini; writing - review & editing - Mounier, Menegatti, Caires and Nicolodelli.

## Conflicts of interest

There are no conflicts to declare.

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