

# Physico-chemical and microbiological characterization of dairy effluents and water from the N'Djili River in the city of Kinshasa, Democratic Republic of the Congo

Bipendu, M. N.<sup>1</sup>, Lusasi, S. W.<sup>2</sup>, Tangou, T. T.<sup>3</sup>, Pwema, K. V.<sup>2</sup>, Mputu, K. J. N.<sup>4</sup>, & Mulaji, K. C.<sup>4</sup>

<sup>1</sup>Inorganic Chemistry Laboratory, Chemistry and Industry Department, Faculty of Science and Technology, University of Kinshasa, Kinshasa XI, DRC

<sup>2</sup>Limnology, Hydrobiology and Aquaculture Laboratory, Life Sciences Department, Faculty of Science and Technology, University of Kinshasa, Kinshasa XI, DRC

<sup>3</sup>Environmental Sciences and Management Department, Faculty of Science and Technology, University of Kinshasa, Kinshasa XI, DRC

<sup>4</sup>Laboratoire de Chimie Analytique et Environnement, Chemistry and Industry Department, Faculty of Science and Technology, University of Kinshasa, Kinshasa XI, DRC

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### Correspondence to:

Willy LUSASI SWANA

[willy.lusasi@unikin.ac.cd](mailto:willy.lusasi@unikin.ac.cd)

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## ABSTRACT

### Introduction

Dairy effluents essentially contain water and diluted milk or dairy product residues. They can also be sources of high levels of phosphorus and other major ions, which may lead to disturbances in the environments where they are discharged.

### Purpose

The aim of this study was to carry out a physico-chemical and microbiological characterization of dairy product effluents and water from the N'Djili River in the city of Kinshasa, Democratic Republic of Congo, in order to assess the possible hazards posed by these effluents to the environment, particularly at their point of discharge, the N'Djili River.

### Methods

Physical parameters were assessed *in situ* using an appropriate multi-parameter probe. Chemical parameters were determined spectrophotometrically, and microbiological parameters were determined by membrane filtration followed by culture of the samples to be analyzed.

### Results

The results obtained show that, with the exception of a few microbiological parameters, the highest concentrations of the parameters analyzed were found in dairy effluents compared with water from the N'Djili River. Average conductivity and turbidity values showed that dairy effluents (cleaning effluents:  $3998.7 \pm 0.95$   $\mu\text{S}/\text{cm}$  conductivity and  $1995.4 \pm 6.44$  ppm turbidity; raw effluents:  $896.05 \pm 0.95$   $\mu\text{S}/\text{cm}$  conductivity and  $464.5 \pm 86.7$  ppm turbidity) were more mineralized, saltier, and more turbid than N'Djili River water (site 2:  $273.33 \pm 13.42$   $\mu\text{S}/\text{cm}$  and site 1:  $124.25 \pm 4.74$   $\mu\text{S}/\text{cm}$ ). The biodegradability index values for dairy effluents indicated that they were biodegradable, with values ranging from 1.48 to 1.50. Generally speaking, high loads of total germs, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and moulds were reported in the dairy effluents examined.

### Conclusion

The results of this study are of great importance in biotechnology for the implementation of biological treatment techniques for the effluents studied. They highlight the need for reducing the load of organic and inorganic matter as well as microorganisms that can contribute to the degradation of receiving environments and their resources.

## INTRODUCTION

Dairy industry effluents are among the richest agri-food discharges in terms of organic matter (protein, lactose, vitamins, minerals) and various microorganisms. This formidable load makes these effluents a source of environmental pollution (Lhanafi et al., 2014). Like most agri-food industries, dairy processing plants generate large volumes of effluent loaded with organic matter (Bourbon, 2018). Indeed, industrial production increases from one year to the next, generating huge discharges, as the quantity of effluent produced depends on the quantity of milk processed (Araba et al., 2001). The nature and composition of this wastewater are largely determined by the manufacturing process and the nature of the products produced (Lhanafi et al., 2014). Effluents produced by milk and cheese production units are among the most polluting discharges to the environment, with concentrations exceeding normative values in most cases (Thierry & Marie-Laure, 2012).

The agri-food sector in general, and the dairy industries in particular, are very demanding in terms of water supply, both in quantity and quality, and consume large amounts of water (Messad, 2012). With regard to effluent management, several solutions can be envisaged by processors to treat effluent, including on-site treatment, spreading on nearby agricultural plots, or discharging effluent into the urban hydrographic network (Bourbon, 2018). These discharges are rich in nutrients (proteins, vitamins, phosphorus, and nitrates) due to their mixing with dairy product losses and whey rejected during manufacturing (Messad, 2012). Most of the water in processed milk is found in whey, which contains several soluble substances, the most important of which are lactose, proteins, and mineral salts (Bourdier & Luquet, 1980; Sottiez, 1990).

Moreover, wastewater quality is both an advantage, due to the presence of residual nutrients, and a risk of organic pollution when discharged into the environment without prior treatment (André, 2007). This can lead to contamination of surface or groundwater and asphyxiate aquatic ecosystems, with the likelihood of eutrophication (Messad, 2012).

In the Democratic Republic of Congo, numerous studies have focused on the degradation of aquatic ecosystems as a result of anthropogenic activities, notably mining effluents (Kashimbo et al., 2016; Unyumbe, 2021); water treatment effluents from REGIDESO (Bipendu et al., 2017); agricultural and pastoral activities (Kakundika et al., 2019); and domestic effluents (Vuni et al., 2021, 2024; Zamane et al., 2024). However, to our knowledge, information on the influence of effluents from the dairy industry – a branch characterized by high water consumption (Marchadier, 1985) and wastewater discharges very rich in microorganisms (Khoudir et al., 1997) and oxygen-consuming organic matter (Hamdani et al., 2001, 2005) – in the country's hydrosystems in general, and those of the city of Kinshasa in particular, is rare and fragmentary (Bokanga, 2018; Kambale & Tshibangu, 2019; Mokolo, 2020; Ngoy, 2021).

Dairy effluents, like the majority of liquid effluents, pose a rational management problem in many parts of DR Congo, particularly in Kinshasa (Bokanga, 2018). Furthermore, the physico-chemical quality of dairy effluents in the city of Kinshasa is an important topic, as it has implications for the environment and public health (Kambale & Tshibangu, 2019; Ngoy, 2021). Effluent from the dairy industry can contain various contaminants, such as organic matter, nutrients (like nitrogen and phosphorus), grease, and suspended solids (Ngoy, 2021). Parameters such as pH, chemical oxygen demand (COD), biological oxygen demand (BOD<sub>5</sub>), and heavy metal concentration are often measured to determine the quality of these effluents (Mokolo, 2020; Ngoy, 2021). Improvements are needed in the treatment of dairy effluent in Kinshasa to minimise its impact on the environment. This may include setting up more efficient wastewater treatment systems and educating dairy farmers on optimal waste management practices.

To achieve this, it is essential to analyse these effluents to assess their potential impact on aquatic and terrestrial ecosystems and their biodiversity. It is against this backdrop that this study focuses on the physico-chemical and microbiological characterisation of dairy effluents and the waters of the N'Djili River in the city of Kinshasa in the Democratic Republic of Congo, with the aim of assessing the possible dangers posed by these effluents to the

environment, particularly at their point of discharge – the N'Djili River.

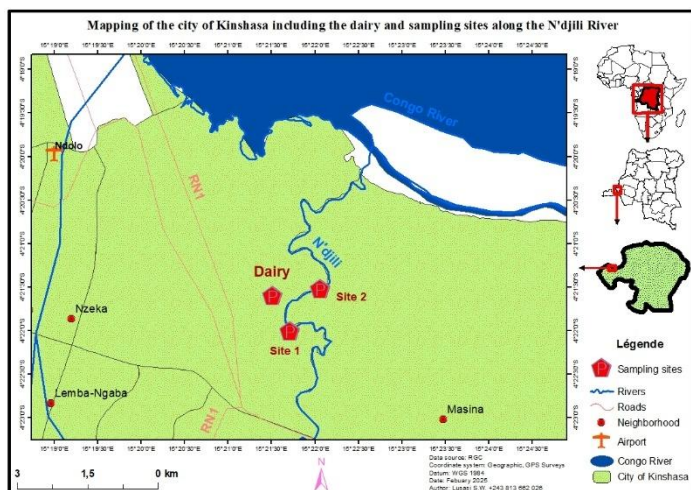
## METHODS

### Study environment

The dairy effluents used in this study were collected from a milk production unit located in the Ndanu district of the Limete commune and from a stretch of the N'djili River (Figure 1). The sampling section of the river lies between Avenue du Poids Lourds and the railway track in the Limete commune, city of Kinshasa, Democratic Republic of Congo.

Figure 1:

Map of the city of Kinshasa, showing the milk production unit considered in this study and the sampling sites along the N'djili River



The N'djili River was chosen because the dairy effluent produced by the plant is discharged into this river. Two sampling points were selected along the river: one site (site 1) upstream of the effluent discharge point, at a distance of approximately 300 metres, and a second site (site 2) downstream of the discharge point, also at about 300 metres.

### Dairy effluent and N'djili River water sampling

Sampling was conducted over a one-year period, from March 2022 to April 2023, during production periods to ensure the collection of effluents. Sludge forms of effluent were not considered; only raw liquid dairy effluents and cleaning effluents were sampled.

Samples were collected in 500 ml glass jars sterilised for 30 minutes at 100°C in a Memmert oven. Before sampling, the jars were rinsed several times with the effluent or water to

be analysed, sealed hermetically, and placed in a cooler for transport to the laboratory. The samples were stored in the cooler, protected from light, at 4°C in accordance with AFNOR standards (NF EN ISO 5667-1, NF EN ISO 5667-3, NF EN ISO 5667-10; AFNOR, 2020).

### Analysis of effluent and water physical parameters

Three physical parameters – temperature (°C), conductivity (µS/cm), and turbidity (ppm) – were measured *in situ* at the dairy effluent discharge point and at the two N'djili River sampling sites. A HANNA Combo HI9812-5 pH/°C/EC/TDS multiparameter probe was used. For each parameter, 12 successive measurements were taken per sample.

### Analysis of effluent and water chemical parameters

Chemical analyses were performed using a computer-assisted ED-XRF Xepos III spectrometer and a HACH DR/2400 spectrophotometer (UV). Six chemical parameters were analysed: chemical oxygen demand (COD, mg/L), biological oxygen demand over 5 days (BOD<sub>5</sub>, mg/L), magnesium ions (mg/L), phosphates (mg/L), ammonia (mg/L), and nitrates (mg/L). For each parameter, 12 samples were processed in the laboratory.

### Dairy effluent biodegradability index

The COD/BOD<sub>5</sub> ratio indicates the biodegradability of effluent. A ratio below 3 suggests that the effluent is readily biodegradable; a ratio above 5 indicates that the effluent is difficult to biodegrade. The COD value is always greater than or equal to the BOD<sub>5</sub> value (Grosclaude, 1999). Pollution is considered biodegradable if the ratio is below 2.5 (Bordet, 2007; Rodier et al., 2009). The biodegradability categories are as follows (Rodier et al., 2009; Bomba, 2022):

- COD/BOD<sub>5</sub> < 3: readily biodegradable effluent
- COD/BOD<sub>5</sub> > 3 and < 5: moderately biodegradable effluent
- COD/BOD<sub>5</sub> > 5: effluent difficult to biodegrade

### Microbiological analysis of effluent and water

Microorganisms were detected using the membrane filtration technique, suitable for counting bacteria present at low concentrations in water or other liquids (Anonymous, 2023). The study targeted five microorganisms: total germs, *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and microscopic moulds.

Bacteria were retained on a 0.45 µm pore-size membrane filter, which was then placed on an appropriate culture medium. After incubation, colony-forming units (CFUs) were counted to assess microbiological quality. The culture medium determined the type of microorganism revealed (Anonymous, 2023).

#### Hierarchical ascending classification of effluent similarity

Cluster analysis was used to group sampling units with similar characteristics (Piélou, 1984). The method is based on measuring similarity or dissimilarity distances between entities (Mergen, 2002). The result is presented as a dendrogram (Koumelan, 1999; Pwema, 2014).

In this study, ascending hierarchical clustering was applied to determine whether dairy effluents and N'djili River water samples were sufficiently similar to form distinct groups. Data included average concentrations of physical and chemical parameters, as well as microbiological characteristics.

#### Data analysis and statistical processing

Data were encoded in Excel 2013 to calculate means and standard deviations for each parameter. The normality of data was verified using Hartley's test for homogeneity of variances (Hartley, 1959; Dagnelie, 1975).

To test for significant differences in mean values of physico-chemical and microbiological parameters, one-way analysis of variance (ANOVA) was performed, followed by Fisher's Least Significant Difference (LSD) test at the 95% confidence level (Scherrer, 1984; Saville, 1990). Statistix software (version 10.8) was used for statistical analysis, Origin software (version 6.1) for graphing, and ArcGIS software (version 10.8) for mapping the dairy plant and sampling sites, based on coordinates recorded with a Garmin Etrex 64s GPS.

## RESULTS AND DISCUSSION

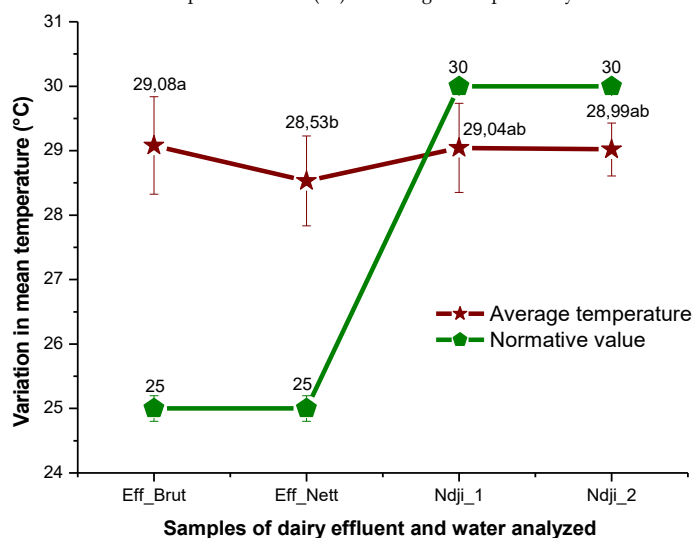
### Physical characteristics of effluents

#### Temperature

The dairy effluent and N'djili River water samples analysed in this study were warm, with temperature variation showing no significant difference ( $F = 1.85$ ,  $p = 0.1452$ ,  $LSD = 1.992$ ) between the different mean values, which ranged from  $28.53 \pm 0.69^\circ\text{C}$  (cleaning effluent) to  $29.08 \pm 0.75^\circ\text{C}$  (River Site 2 on the N'djili River). The mean temperature

values recorded in the N'djili River remain very close to the normative values set by the World Health Organization (WHO), which specifies a temperature of between  $20^\circ\text{C}$  and  $30^\circ\text{C}$  in aquatic ecosystems and  $25^\circ\text{C}$  for effluents (Tourette, 2002). Temperature influences the growth, reproduction, and nutrition of aquatic organisms; it also affects the duration of the lag phase, growth rate, and metabolism of microorganisms in a given environment (Tourette, 2002). For example, *Pseudomonas* release more proteases and lipases when the temperature is below  $10^\circ\text{C}$ , which leads to the spoilage of refrigerated milk (Tourette, 2002). Generally, the examined dairy effluents were warm. According to Hamdani et al. (2005), this temperature rise originates from waters coming from heat exchangers, barometric condensers, and hot washing waters. This was also the case for the examined cleaning effluents, which are discharged at a temperature of over  $100^\circ\text{C}$  before cooling. These temperature values fall within the range of the minimum threshold ( $5^\circ\text{C}$  to  $45^\circ\text{C}$ ) required for the maintenance and development of various microorganisms in a given medium (Tourette, 2002). The results of the present study are close to those obtained by Bipendu et al. (2017), where the overall average temperature of the water in the N'djili River was  $25^\circ\text{C}$ . Temperature therefore promotes different populations of germs; in milk or dairy effluents, lactic streptococci, enterobacteria, *Salmonella*, and *Shigella* are predominant at temperatures ranging from  $0^\circ\text{C}$  to  $10^\circ\text{C}$  (Tourette, 2002).

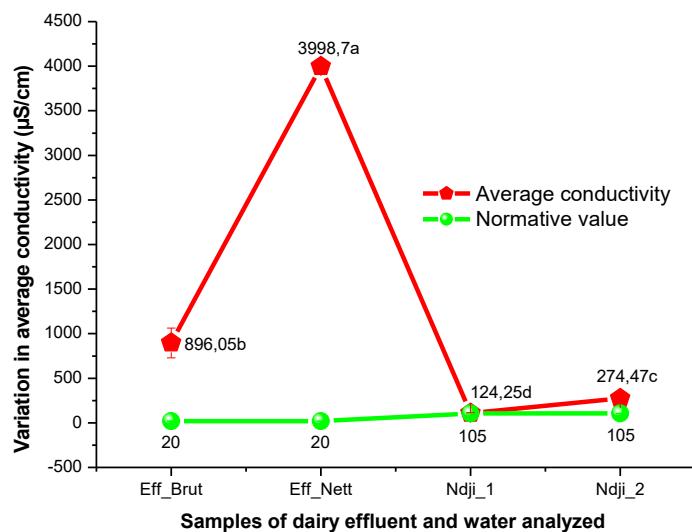
Figure 2: Variation in mean temperature value ( $^\circ\text{C}$ ) according to samples analysed



### Conductivity

The results (Figure 3) show that dairy effluents contain very high levels of suspended ions, with a highly significant statistical difference ( $F = 6896$ ,  $p = 0.0000$ ) between the mean values. With a critical comparison value of 1.992, the LSD test shows that the cleaning effluent, with  $3998.7 \pm 0.95 \mu\text{S}/\text{cm}$ , is more mineralised, followed by raw effluent with  $896.05 \pm 0.95 \mu\text{S}/\text{cm}$ , then N'djili River water at the second site with  $273.33 \pm 13.42 \mu\text{S}/\text{cm}$ , and finally N'djili River water at site 1 with  $124.25 \pm 4.74 \mu\text{S}/\text{cm}$ . Compared with the standard, the mean values found in dairy effluent and N'djili River water remain much higher. These results are close to those reported by Bipendu et al. (2017), who found conductivity values ranging between 186 and 202  $\mu\text{S}/\text{cm}$  in N'djili River water. The high conductivity values in the effluents are related to the mineralisation of salts accompanying the dairy manufacturing process (Milia & Chanez, 2018), while the conductivity values in the N'djili River water are linked to the natural mineralisation of freshwater (Franck, 2002). The increase in conductivity comes from salts such as sodium and chloride, resulting from the regeneration of ion exchange resins, washing chemicals, detergents, and disinfectants (Hamdani et al., 2005). The high conductivity of dairy effluents discharged into the N'djili River (where the water has a lower conductivity value compared to the dairy effluents) is likely to cause dysfunction in hydrosystems (Bipendu et al., 2017) due to excess nutrients in the water, which can lead to eutrophication (Yao et al., 2020).

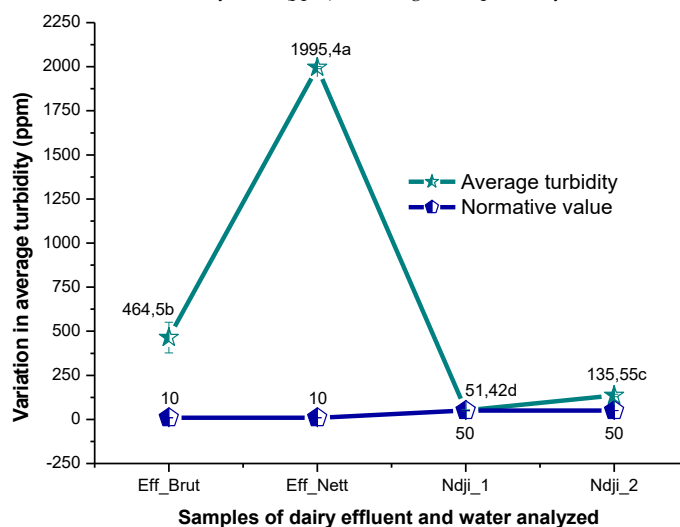
Figure 3: Average variation in conductivity ( $\mu\text{S}/\text{cm}$ ) according to samples analysed



### Turbidity

The one-way analysis of variance (ANOVA 1) applied to the data shows a highly significant difference ( $F = 6282$ ,  $p = 0.0000$ ) between the mean values. The critical comparison value ( $LSD = 1.992$ ) shows that the highest mean turbidity is found in the cleaning effluent at  $1995.4 \pm 6.44 \text{ ppm}$ , followed by the raw effluent at  $464.5 \pm 86.7 \text{ ppm}$ , then N'djili River at the second sampling point ( $135.42 \pm 7.17 \text{ ppm}$ ), and finally N'djili River at the first sampling point ( $51.45 \pm 3.26 \text{ ppm}$ ) (Figure 4). The average concentration of solid particles in the dairy effluent samples remains far higher than the values recorded in the river water, as well as normative values. According to Lamrini et al. (2005), the decrease in turbidity in effluents of organic compounds is due to the degradation of organic matter contained in raw water, whereas high turbidity levels are linked to the presence of undegraded organic matter (Milia & Chanez, 2018).

Figure 4: Variation in mean turbidity value (ppm) according to samples analyzed



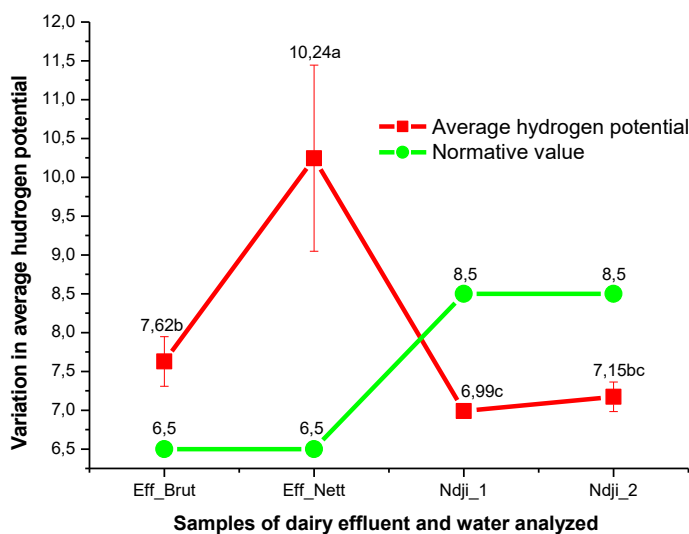
### Chemical characteristics of effluent

#### Hydrogen potential

The average pH concentration varies according to the sample considered and remains basic in dairy effluents, with a very highly significant statistical difference ( $F = 77.3$ ,  $p = 0.0000$ ). The LSD test (1.992) indicates that, with an average of  $10.24 \pm 1.19$ , the cleaning effluent is more basic than the raw effluent, which has an average pH of  $7.62 \pm 0.31$ . The water of the N'djili River is slightly acidic at site 1 ( $6.99 \pm 0.05$ ) and basic at site 2 ( $7.15 \pm 0.18$ ) (Figure 5).

Compared with normative values, the pH values found in dairy effluent remain well above the WHO standard. These results are close to those obtained by Hamdani et al. (2005) and Lhanafi et al. (2014). Hamdani et al. (2005) reported a pH ranging from 8.6 to 13.4 in dairy effluents, while Lhanafi et al. (2014) noted a pH range from 4.3 to 11.3 in raw, cleaning, and sludge forms of dairy effluents. Bipendu et al. (2017) reported a pH ranging from 6.35 in N'djili River water to 7.5 in drinking water treatment effluents by REGIDESO. According to Milia and Chanez (2018), the gradual addition of lime leads to an increase in pH, bringing the value to basicity. This observation was also noted in this study, where dairy effluents are limed in order to readjust the pH before discharge into the N'djili River.

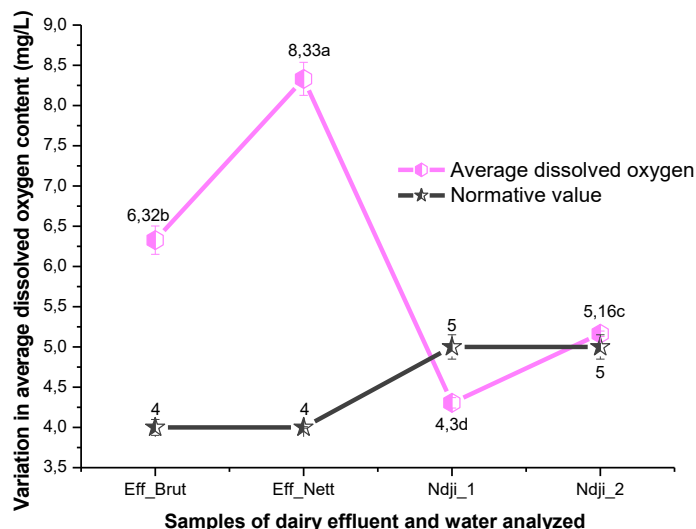
Figure 5: Variation in mean hydrogen potential value according to samples analyzed



### Dissolved oxygen

Dissolved oxygen content in dairy effluent and N'djili River water varies according to the samples considered (Figure 6). A one-way ANOVA applied to these data shows a highly significant difference ( $F = 1275$ ,  $p = .0000$ ;  $LSD = 0.1390$ ) between the averages, where cleaning effluent ( $8.33 \pm 0.2$  mg/L) and raw effluent ( $6.32 \pm 0.17$  mg/L) have higher average levels than N'djili River water, with  $5.16 \pm 0.03$  mg/L at site 2 and  $4.3 \pm 0.06$  mg/L at site 1. In general, effluent levels are far higher than the normative values set by the World Health Organization (WHO, 2017).

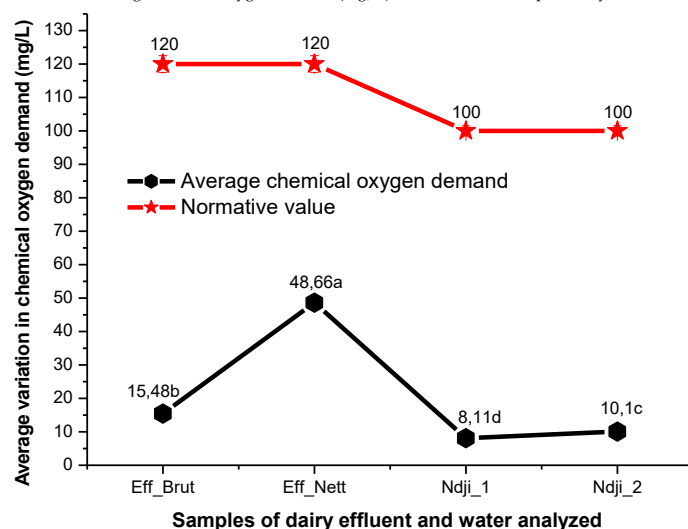
Figure 6: Variation in mean dissolved oxygen content (mg/L) according to samples analyzed



### Chemical oxygen demand

The statistical analysis of variance applied to the chemical oxygen demand data reveals a highly significant difference ( $F = 11,421$ ,  $p = .0000$ ) between the mean values recorded in the dairy effluent and N'djili River water. The LSD test's critical comparison value (0.0341) shows that cleaning effluent ( $48.66 \pm 0.62$  mg/L) has a higher mean value than raw effluent ( $15.48 \pm 0.23$  mg/L), followed by N'djili River water ( $10.1 \pm 0.08$  mg/L at site 2 and  $8.11 \pm 0.011$  mg/L at site 1; Figure 7). Compared with normative values, concentrations in both dairy effluent and river water remain below the standard (WHO, 2017).

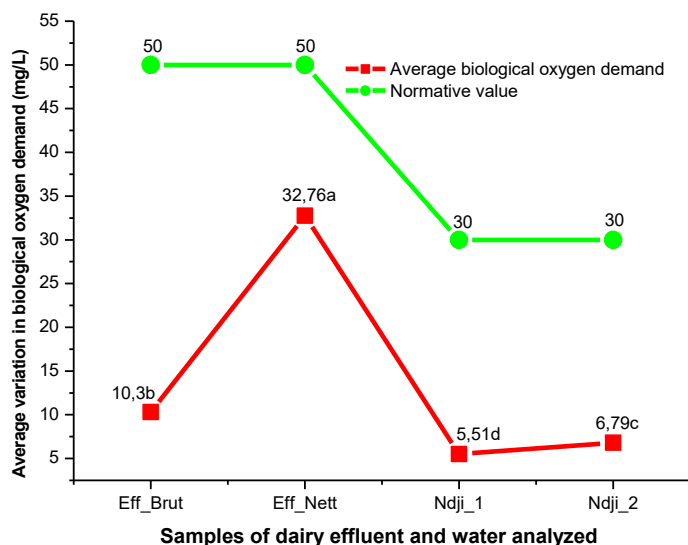
Figure 7: Variation in average chemical oxygen demand (mg/L) as a function of samples analyzed



### Biological oxygen demand

The results (Figure 8) show that the average biological oxygen demand concentration in dairy effluent and N'djili River water remains below WHO standards (WHO, 2017). One-way ANOVA indicates a highly significant difference ( $F = 23,645$ ,  $p = .0000$ ) between the concentrations found in the effluents and those in the river. With a critical comparison value of 0.3516, the LSD test shows that cleaning effluent ( $32.76 \pm 0.05$  mg/L) has a higher mean BOD than raw effluent ( $10.3 \pm 0.03$  mg/L) and N'djili River water. The high concentration of these parameters may be due to the occasional direct discharge of substandard milk or cream into sewers, and the presence of microorganisms that degrade organic matter, thereby affecting COD and BOD<sub>5</sub> levels (Hamdani et al., 2005). The regular influx of organic-rich effluents and wastewater with high COD and BOD<sub>5</sub> reduces dissolved oxygen in the receiving environment, negatively impacting aquatic ecosystems (Hamdani et al., 2005).

Figure 8: Variation in average biological oxygen demand (mg/L) according to samples analyzed

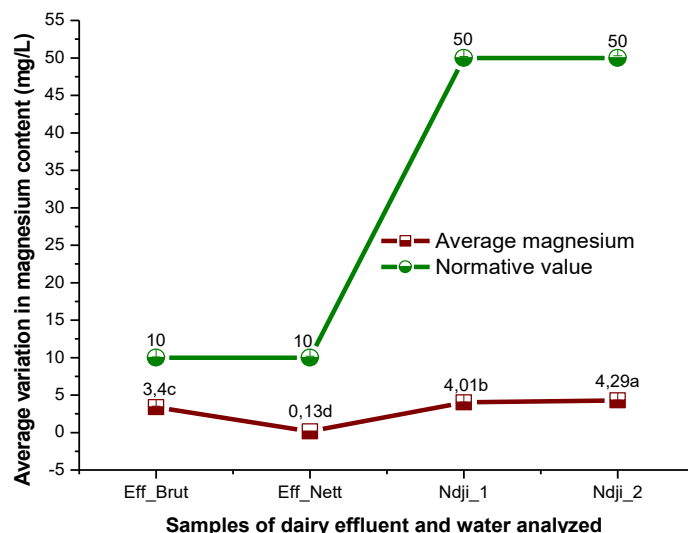


### Magnesium

Both dairy effluents and N'djili River water show very low average magnesium ion content compared with normative values (Figure 9). N'djili River water ( $4.01 \pm 0.03$  mg/L at site 1 and  $4.29 \pm 0.02$  mg/L at site 2) contains significantly more magnesium than dairy effluent ( $3.4 \pm 0.11$  mg/L for raw effluent and  $0.13 \pm 0.00$  mg/L for cleaning effluent;  $F = 7193$ ,  $p = .0000$ ,  $LSD = 0.0645$ ).

Figure 9:

Variation in average magnesium ion content (mg/L) according to samples analyzed

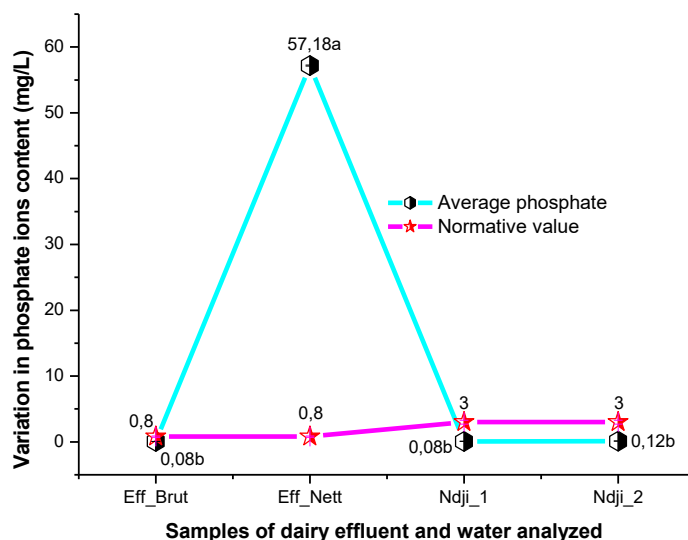


### Phosphate

A one-way ANOVA applied to the phosphate ion data reveals a highly significant difference ( $F = 24,938$ ,  $p = .0000$ ) between the mean values in the dairy effluent and river water (Figure 10). The LSD test (critical value = 0.0515) shows that cleaning effluent ( $57.18 \pm 0.08$  mg/L) has the highest phosphate levels, followed by N'djili River site 2 ( $0.12 \pm 0.00$  mg/L), raw effluent ( $0.08 \pm 0.00$  mg/L), and N'djili River site 1 ( $0.08 \pm 0.00$  mg/L). Only the cleaning effluent concentration exceeds the normative value (WHO, 2017).

Figure 10:

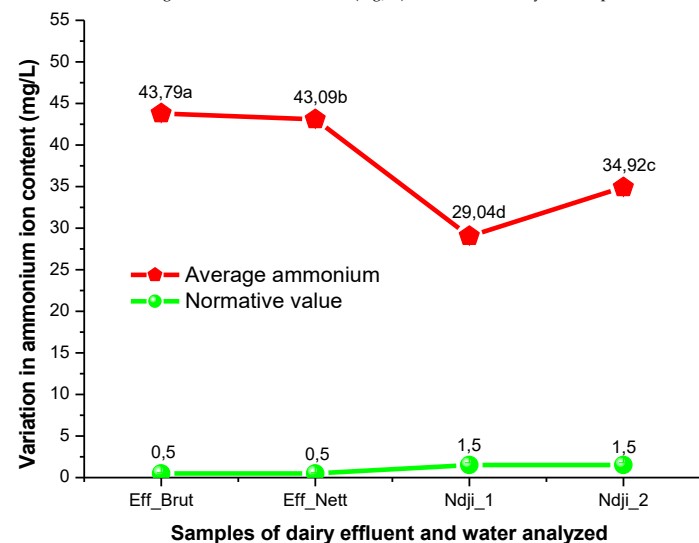
Variation in average phosphate ion content (mg/L) according to samples analyzed



### Ammonium

Ammonium ion concentration varies significantly ( $F = 53,470$ ,  $p = .0000$ ) across sample types (Figure 11). The LSD test (critical value = 0.0869) shows that dairy effluents are more concentrated in ammonium ions than N'djili River water. Raw effluent ( $43.79 \pm 0.15$  mg/L) has higher concentrations than cleaning effluent ( $43.09 \pm 0.02$  mg/L), followed by N'djili River site 2 ( $34.92 \pm 0.06$  mg/L) and site 1 ( $29.04 \pm 0.05$  mg/L). These concentrations are well above WHO standards (WHO, 2017).

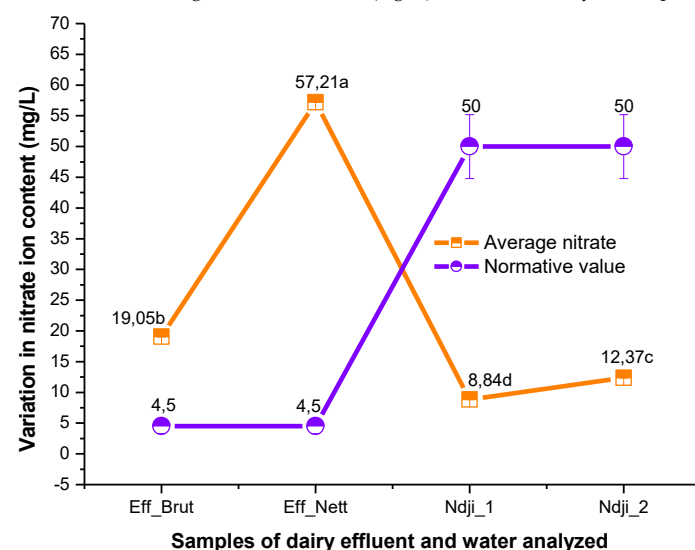
Figure 11: Variation of the average ammonium ion content (mg/L) based on the analyzed samples



### Nitrate

One-way ANOVA highlights a highly significant difference ( $F = 210,908$ ,  $p = .0000$ ) in nitrate concentrations (Figure 12). The LSD test (critical value = 0.0438) shows the highest nitrate concentrations in cleaning effluent ( $57.21 \pm 0.02$  mg/L), followed by raw effluent ( $19.05 \pm 0.04$  mg/L), N'djili River site 2 ( $12.37 \pm 0.02$  mg/L), and site 1 ( $8.84 \pm 0.04$  mg/L). Dairy effluents exceed WHO normative values (WHO, 2017).

Figure 12: Variation of the average nitrate ion content (mg/L) based on the analyzed samples



### Biodegradability index of effluents

Table 1 summarises the biodegradability index of dairy effluents and N'djili River water.

Table 1: Variation of the biodegradability index of the analyzed samples

Sample type	COD (mg/L)	BOD <sub>5</sub> (mg/L)	IBD
Raw effluent	15.49 ± 0.23	10.30 ± 0.03	1.5
Cleaning effluent	48.66 ± 0.62	32.77 ± 0.05	1.48
Site 1 (N'djili)	8.12 ± 0.11	5.52 ± 0.02	1.47
Site 2 (N'djili)	10.10 ± 0.08	6.79 ± 0.02	1.48

These IBD values (1.47 to 1.5) indicate that the effluents and river water are biodegradable. Similar findings were reported by Hamdani et al. (2005), who found an average COD/BOD<sub>5</sub> ratio of 2 in dairy effluents, suggesting treatability by biological means. Lhanafi et al. (2014) reported COD/BOD ratios ranging from 2 for raw effluents to 3 for cleaning effluents, attributing high values to the abundance of organic matter.

### Microbiological characteristics of dairy effluents and N'djili River water

Table 2 presents the microbiological characteristics of the samples.

**Table 2:**  
Variation in the microbiological characteristics of the analyzed samples

Sample type	Total germs (colonies/100 mL)	<i>E. coli</i> (colonies/100 mL)	<i>Enterococcus faecalis</i> (colonies/100 mL)	<i>Pseudomonas aeruginosa</i> (colonies/100 mL)	Moulds (colonies/100 mL)
Raw effluent	166.33 ± 5.39	467.83 ± 2.19	100.83 ± 2.5	520.5 ± 3.42	19.5 ± 1.58
Cleaning effluent	0.17 ± 0.28	0.25 ± 0.38	1.33 ± 1.06	33 ± 2.5	4.41 ± 1.08
Site 1 (N'djili)	169.83 ± 3.14	434.58 ± 2.25	100.58 ± 2.41	348 ± 2	529.75 ± 2.12
Site 2 (N'djili)	283.67 ± 4.5	397.5 ± 3	96.58 ± 2.11	299.16 ± 1.66	483.91 ± 3.41
WHO standard	100	0	0	0	0

Generally, significant bacterial and fungal loads are observed, with variations depending on sample type (WHO, 2017).

Generally, a significant bacterial and fungal load is observed in the effluents of dairy products as well as in the water of the N'djili River, with variations depending on the samples considered and the types of microorganisms sought. A very high microbial load above the normative values set by the WHO is observed in the analyzed samples.

It emerges that:

- The total germs are most numerous (i.e., 283.67 ± 4.5<sup>a</sup> colonies/100 mL) with a highly significant statistical difference (F = 4959; p = 0.0000; LSD = 4.7243) in the water of the N'djili River at site 2, followed respectively by the water of the N'djili River at site 1 (i.e., 169.83 ± 3.14<sup>b</sup> colonies/100 mL) and the raw effluent (i.e., 166.33 ± 5.39<sup>b</sup> colonies/100 mL), and finally the cleaning effluent (i.e., 0.17 ± 0.28<sup>c</sup> colonies/100 mL);
- One-way ANOVA shows that the load of *Escherichia coli* in the effluents of the analyzed dairy products varies highly significantly (F = 77311; p = 0.0000) between the different samples. The LSD test (2.2390) shows that the raw effluent carries a very high number (i.e., 467.83 ± 2.19<sup>a</sup> colonies/100 mL) of these microorganisms, followed by the water from the N'djili River at the first site (i.e., 434.58 ± 2.25<sup>b</sup> colonies/100 mL), then the water from the N'djili River at the second site (i.e., 397.5 ± 3<sup>c</sup>

colonies/100 mL), while the cleaning effluent presents a low proportion (i.e., 0.25 ± 0.38<sup>d</sup> colonies/100 mL);

- The contamination rate of dairy product effluents and waters from the N'djili River by *Enterococcus faecalis* varies depending on the samples considered, with a highly significant statistical difference (F = 4258; p = 0.0000) between the obtained average values. With a critical comparison value of 2.1418, the LSD test shows that raw dairy effluents carry a high number of germs (i.e., 100.83 ± 2.5<sup>a</sup> colonies/100 mL), followed by the waters of the N'djili River at the first site (i.e., 100.58 ± 2.41<sup>a</sup> colonies/100 mL), then the waters of the N'djili River at the second site (i.e., 96.58 ± 2.11<sup>b</sup> colonies/100 mL), and finally the cleaning dairy effluents with 1.33 ± 1.06<sup>c</sup> colonies/100 mL;
- The one-way analysis of variance applied to the data on the rate of *Pseudomonas aeruginosa* in the different samples analyzed reveals a highly significant statistical difference (F = 54226; p = 0.0000) between the mean values. The LSD test (2.4704) reveals that raw effluents with 520.5 ± 3.42<sup>a</sup> colonies/100 mL are more concentrated compared to the waters of the N'djili River at the first site (348 ± 2<sup>b</sup> colonies/100 mL) and then the second site of the N'djili River (299.16 ± 1.66<sup>c</sup> colonies/100 mL), while cleaning effluents are the least concentrated with 33 ± 2.5<sup>d</sup> colonies/100 mL;
- The fungal load in the analyzed samples varies very highly significantly (F = 114849; p = 0.0000; LSD = 2.4086), with the highest loads recorded in the water of the N'djili River (i.e., 529.75 ± 2.12<sup>a</sup> colonies/100 mL at site 1 and 483.91 ± 3.41<sup>b</sup> colonies/100 mL at site 2) compared to dairy effluents where the highest loads are recorded in raw dairy effluents (i.e., 19.5 ± 1.58<sup>c</sup> colonies/100 mL), followed by cleaning effluents (4.41 ± 1.08<sup>d</sup> colonies/100 mL).

The establishment of microorganisms at high concentrations accelerates environmental degradation. This process begins with the disappearance of good quality air, giving way to sour or acidic odours, then amine or

sulphurous, and finally ammoniacal or faecal in a putrid state (Eymard, 2003). Moreover, several other microbiological risks arise in environments where these microorganisms proliferate (Makengo, 2018). According to Hamdani et al. (2005), even if milk is processed or handled under the best hygiene conditions, in its raw state it still contains bacteria from the handler, from the animal carrying germs at the teat of the udder and skin, from the facilities and equipment used during processing, and finally from the surrounding environment of dairy cows or the production unit for manufactured dairy products (Javier, 1959). These results align with those obtained by Hamdi et al. (2005), Lhanafi et al. (2014), and Bipendu et al. (2017). Hamdi et al. (2005) highlighted that the presence of total coliforms in raw milk is linked to poor hygiene and processing conditions. A study conducted by Benachir (1985) showed a high abundance of total germs ranging from  $1.6 \times 10^4$  to  $1.3 \times 10^5$  CFU/ml. The evolution of the density or the rate of germs shows variation from one day to another. Lhanafi et al. (2014) reported a total germ count ranging from  $20.2 \times 10^3$  in chalky whey to  $49.6 \times 10^5$  in the liquid biological sludge of a dairy production unit in Morocco. Bipendu et al. (2017) reported a total germ count ranging from 15 to 50 CFU/100 ml at the water intake and treatment point on the N'djili River by REGIDESO. Bourbon (2018) reported that liquid effluents from dairy product processing carry high levels of *Escherichia coli* if they have not been treated under proper hygienic conditions. These observations are similar to those obtained by Tourette (2002), who highlighted the presence of several germs in dairy products and effluents, particularly *Escherichia coli* and *Enterococcus faecalis*. The presence of strains of these two germs in the effluents and the water of the N'djili River indicates contamination by faecal matter, consistent with observations made by Bonnefoy et al. (2002) and Mbadiko et al. (2019).

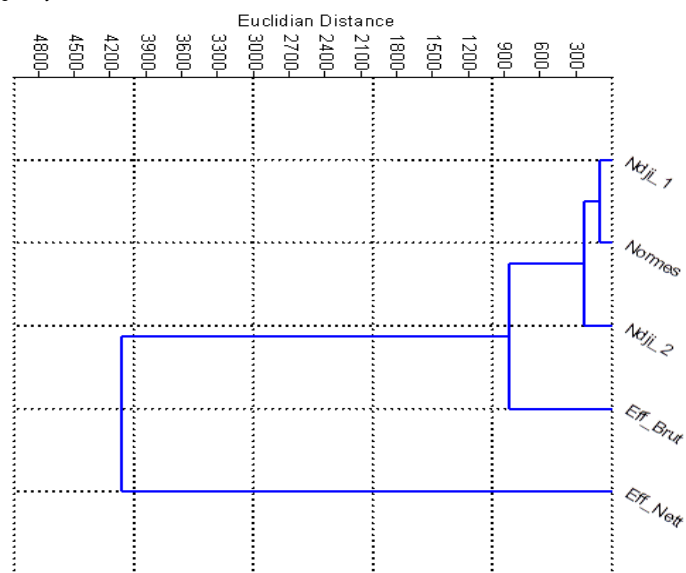
#### Hierarchical ascending classification of the quality of effluents and analyzed waters

##### Depending on the physical parameters

The similarity matrix of dairy product effluents as well as water samples from the N'djili River, established based on the analyzed physical parameters, highlights a very significant statistical difference ( $R^2 = 0.99$ ) where two main ecological entities of the analyzed samples are identified,

with three sub-groups in the first entity. The analysis of the information presented in Figure 13 shows that in the first entity, the waters of the N'djili River at the first site, as well as the WHO standard, are very close with almost similar physical quality and present a physicochemical quality very different from the waters of the N'djili River at the second sampling site, which alone form the second subgroup, different from the raw effluents of dairy products that present a third subgroup with physical quality very different from the other treatments. The second ecological entity is formed by a single batch: the cleaning effluents from dairy products, where the physical parameters differ significantly from the other samples mentioned above.

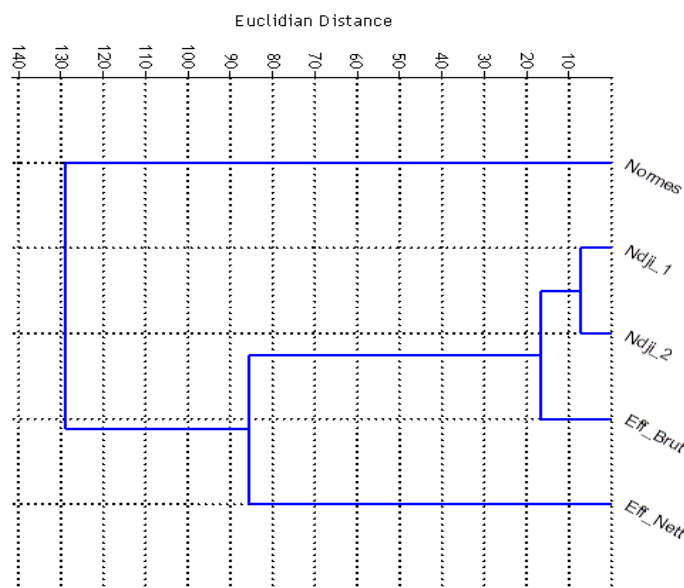
**Figure 13:** Similarity of dairy product effluents and N'djili River waters based on physical quality



##### Depending on the chemical parameters

Taking into account the chemical quality, two main ecological entities that are not similar, with a highly significant statistical difference ( $R^2 = 0.986$ ), are highlighted, with the second subdivided into three sub-groups (Figure 14). The normative values alone form the first entity and remain far from similar from a chemical point of view to the samples of the N'djili River water and the effluents of the analyzed dairy products. In the second entity, the two sites of the N'djili River show a very close similarity and together form the first subgroup compared to the raw effluents of dairy products, which differ and form the second subgroup alone; finally, the cleaning effluents are also dissimilar to the other samples and form the third subgroup alone.

**Figure 14:** Similarity of dairy product effluents and N'djili River waters based on chemical quality

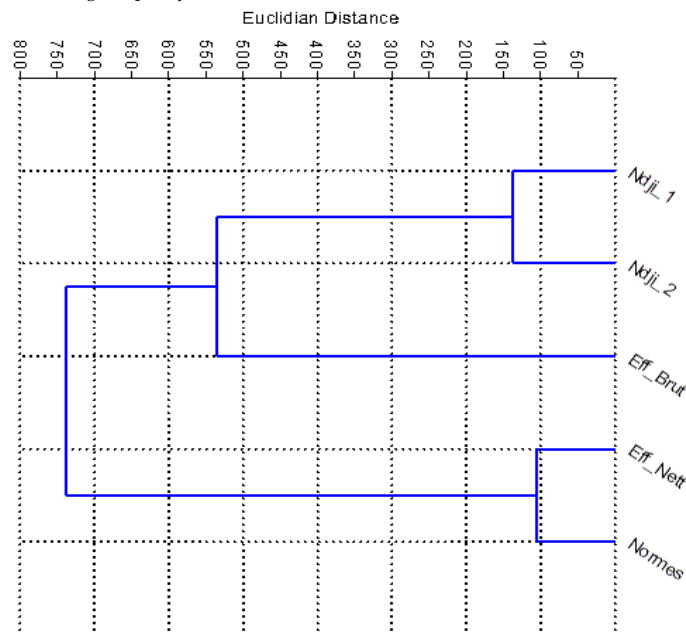


*Depending on the microbiological parameters*

The similarity dendrogram of dairy effluents and the water from the N'djili River highlights two statistically significant ecological entities ( $R^2 = 0.9954$ ) based on the microbiological quality of the considered samples (Figure 15). The first entity is subdivided into two dissimilar sub-groups, with the first consisting of the two sites of the N'djili River where the microbiological quality of these two sites is similar with high microbial loads, compared to the raw effluents of dairy products which alone form the second group. The second entity is formed by the cleaning effluents and the normative values, which show a very close similarity with low microbial loads. These results show that the analyzed dairy effluents and the water from the N'djili River exhibit significant faecal contamination. As for the water (especially runoff), it is likely to contain all bacterial species since it drains various types of waste, including faecal matter, and Gram-positive aerobic germs like *Pseudomonas* are common in freshwater (Tourette, 2002). In their studies, Bipendu et al. (2017) and Masua et al. (2023) indicate that the water of the N'djili River receives various organic and inorganic waste, particularly urine and faecal matter from sanitary installations whose drainage pipes are directly connected to the river. Contaminations of milk and dairy products are numerous from production to processing; these are secondary contaminations (Carlier et al., 1984). Humans are a major contamination agent: dirty hands and

clothes, sick individuals (respiratory, digestive, and skin conditions), and healthy carriers (Tourette, 2002).

**Figure 15:** Similarity of dairy product effluents and N'djili River waters based on microbiological quality



**CONCLUSION**

The aim of this study was to carry out a physico-chemical and microbiological characterisation of dairy product effluents and water from the N'djili River in Kinshasa, Democratic Republic of Congo, in order to assess the potential hazards posed by these effluents to the environment, particularly at their point of discharge into the N'djili River. The results showed marked variation in physico-chemical and microbiological quality between raw dairy effluent, cleaning effluent, and water from the N'djili River. The highest concentrations of the analysed parameters were observed in the dairy effluent. It was found that the physical, chemical, and microbiological concentrations of discharged dairy effluents exceeded the thresholds set by WHO normative values, posing a risk to the health of aquatic ecosystems and their resources. The relationship between the chemical and biochemical oxygen demand of the various dairy effluents analysed indicated that they are biodegradable, which reduces their hazardousness. Therefore, it is advisable that dairy product effluents be treated and stabilised before being discharged into receiving environments, particularly rivers, as was observed with the effluents analysed in this study.

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**Ethical Approval:** Data collection for this study was authorised by the University of Kinshasa, DRC.

**Conflicts of Interest:** None declared.

#### ORCID iDs:

Bipendu, M. N.<sup>1</sup>: <https://orcid.org/0009-0007-8213-1803>  
Lusasi, S. W.<sup>2</sup>: <https://orcid.org/0000-0002-2526-7903>  
Tangou, T. T.<sup>3</sup>: <https://orcid.org/0009-0003-6788-9684>  
Pwema, K. V.<sup>2</sup>: <https://orcid.org/0009-0002-2355-1668>  
Mputu, K. J. N.<sup>4</sup>: Nil identified  
Mulaji, K. C.<sup>4</sup>: <https://orcid.org/0000-0002-9406-3277>

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