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RESEARCH ARTICLE OPEN ACCESS

Efficiency of Urban Wastewater Treatment Plants (Messa and Cite Verte) in the Elimination of the Environmental Forms of Coccidian Protozoans in Relationship with the Organic Variables of the Ecosystems

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Abstract

In order to assess the purification performance of two purification stations in the city of Yaounde, a study was carried out from the long dry season to the short rainy season of the year 2021. The physico-chemical analyzes were carried out in the field and in the laboratory. The observation of the oocysts was made under the IVYMEN microscope with the 40X and 100X objective after concentration of the samples by the sedimentation method , the Faust and Ziehl-Neelsen modified experimentation. Biological analyzes show that the species of *Cryptosporidium* spp. was the highest (3228 ± 2203 oocysts/l) for the Messa station and (15469 ± 29405 oocysts/l) for the Cité verte station. The highest protozoan densities were recorded during the short rainy season (PSP). The least dense species was *Isospora* spp. with (83 ± 72 oocysts/l) at Messa and (65 ± 70 oocysts/l) at the Cité verte water purification station. The removal rates for these different species are quite satisfactory for ecological release of urban treated domestic wastewater into the adjoining streams.

Keywords: Wastewater treatment plants, Messa, Cité verte, oocystic load,purification efficiency

Introduction

Water cannot be considered as a simple commercial product, it must be classified as a universal heritage which must be protected, defended and treated as such. It is a vital resource for humans, their survival, their health and their food (Metahri, 2012). Although seemingly inexhaustible, water is very unevenly distributed on the planet. All countries will have, in the short or long term, to face the problem of its scarcity. The mobilization of surface water has always been a major concern of public authorities (Devaux, 1999; Scotland, 2001). After it's use, the water is charged with various elements modifying its physical, chemical and biological characteristics. Thus, the initially clear water turns into greyish and worn water (Niang, 1995). Wastewater is therefore defined as waste water emanating from a given community and which has probably been used for domestic, agricultural and industrial purposes (Nsom Zamo, 2003).

To respond to this situation of depletion of natural resources and to protect the environment, the use of urban wastewater treatment, often loaded with nutrients such as nitrogen and phosphorus, would represent a source of water and additional renewable and reliable fertilizers for agriculture on the one hand and on the other hand, would make it possible to alleviate the pressure on the conventional resources more adapted to the supply of drinking water to the populations. These are generally large volumes of water of which only a small percentage is treated (Landreau, 1982). These operations are carried out via wastewater treatment plants, which are facilities intended to purify domestic or industrial water and rainwater before discharge into the natural environment. The purpose of the treatment is to separate the

water from substances that are undesirable for the receiving environment. However, the treatment of waste in general and wastewater in particular is today a very worrying environmental issue. In fact, in developing countries, wastewater disposal and sanitation systems are dramatic (Montagero et al., 2002). This wastewater is often collected and discharged into nature without prior treatment, resulting in the contamination of aquatic ecosystems. This could further promote the dissemination of resistance forms of protozoa in the natural environment. However, many waterborne diseases are contracted during the consumption of poorly treated water, this due to the resistance of certain pathogens such as cysts and oocysts of intestinal protozoa. These parasites are responsible for numerous digestive parasitoses which pose a relatively significant morbidity problem (Cheikhrouhou et al., 2009). It is in this perspective that the present work aims to evaluate the purification performance of the aforementioned wastewater treatment plantsin the city of Yaounde, in relationship to ecological variables.

MATERIAL AND METHODS

a) Geographic framework

The city of Yaoundé is the political capital of Cameroon, the capital of the center region and the Mfoundi department. It is located on the edge of the South Cameroon plateau and in the interfluve of the Nyong and Sanaga rivers between 3°30' and 3°58' North latitude and between 11°20 and 11°40 East longitude. (Suchel, 1987) at an average altitude of 750 m. The climate that reigns in the city of Yaoundé is of the equatorial type (Yaoundéen), characterized by the alternation of two dry seasons and two rainy seasons; with an average temperature of 23.5°C contrasting between 16 and 31°C depending on the season and 1650 mm of water per year. The average humidity is 80% and varies during the day between 35 and 98%. The frequent winds are moist and blow in a southwesterly direction. Strong winds are directed to the northwest. The vegetation is of the intertropical type with a predominance of southern humid forest (Wéthé et al., 2003). On the geological level, the source rock which constitutes the geological substratum of the Yaoundé soils derives from a more or less micaceous quartzo-feldspathic material (Pelletier, 1969), hence the acidity of these soils with a pH fluctuating around 4.5 and 5.5 U.C in the superficial layers.

The samples were taken from February to May 2021 at two wastewater treatment plants in the city of Yaoundé. The water taken from the 1000 cc polyethylene bottles was brought back to the hydrobiology and environment laboratory of the University of Yaoundé 1 for analysis.

b) Measurement of physico-chemical parameters

The physico-chemical analyzes took place both in the field and in the laboratory following the recommendations of Rodier et al. (2009).

c) Field measurements

In the field parameters such as temperature (°C), dissolved oxygen (% saturation), Ph (UC) respectively using an electronic thermometer, an oximeter

d) Experimental protocol

Observation of resistance forms of protozoa

The samples will be collected using sterile 1 L polyethylene bottles and then transported to the laboratory. They will then be left to rest at ambient temperature for 24 hours for sedimentation, then the supernatant will be poured out and the volume of the pellet will be collected and assayed. Cysts and oocysts are observed under an optical microscope with a 40 X objective.

e) Sedimentation method

After homogenization of the pellet, 5ml of the sample (controls and tests) are taken and introduced into a test tube. The mixture obtained is brought to centrifugation at 1500 revolutions/min for 5 min using a centrifuge.

f) Zinc sulphate flotation method or modified Faust technique

This method allows the flotation of the cysts. After homogenization of the pellet, 5 ml of the sample are taken and introduced into a test tube. To this is added 3 ml of 33% zinc sulphate (specific density of 1.18) and the mixture obtained is brought to centrifugation at 500
revolutions/min for 4 to 5 min using a centrifuge. Next, the surface layer of the supernatant was removed using a pipette and spread between slide and cover slip.

g) Modified Ziehl–Neelsen method

It is a method which makes it possible to highlight the oocysts of protozoa. It consists of staining the slides. Indeed, a 10% zinc sulphate solution (allowing the oocysts to float) is added to the samples taken and distributed in the test tubes. The contents of these test tubes are then centrifuged at 500 revolutions/min for 5 min using a MINOR35 brand centrifuge to float the oocysts. The supernatant is removed using a micropipette and distributed on slides which are then air-dried to promote the adhesion of the sample to the slides. The slides are fixed in methanol and stained with basic fuchsin for 1 and 5 minutes respectively, rinsed with distilled water and 2% sulfuric acid (acting as a decolorizer for organisms other than oocysts) for 2 minutes. The slides are rinsed again and counterstained with 5% methylene blue (which stains other structures or organisms with the exception of oocysts. The preparation was placed on the stage of a YVIMEN brand microscope for observation.

h) Identification and counting of cysts and oocysts

Human parasitic intestinal cysts and oocysts will be identified using the WHO charts (1994). The measurements of the dimensions will be made using a micrometer carried by one of the eyepieces of the microscope. The number (X) of parasitic cysts and oocysts in 1 L of samples will be obtained using the formula of Ajeagah et al., 2014:

$$X = (y.Vx)/Vy$$

With: Vx= pellet volume in 1 L of samples, Vy= pellet volume used for observation, y= number of cysts observed in Vy.

i) Calculation of treatment efficiency

The performances of the STEP are expressed in terms of reduction rate on various parameters.
Each abatement rate is calculated using the following form
With :
R (%) is the abatement rate for a given parameter (COD, BOD5, TSS, Ptotal, etc.),
Pe is the value of the parameter at the input.
Ps is the value of the same parameter at the output.
In order to assess the performance of the STEP, it is necessary to compare the abatement rates x%, y% and z% with the standards (Khammar et al., 2013).

j) Comparison of means test

The spatial variation of the physicochemical and biological parameters measured was tested using the KruskalWallis test associated with the Mann-Whitney test. The KruskalWallis test thus made it possible to determine whether a parameter varies significantly from one station to another. The analyzes were performed using SPSS 20.0 software and the results assessed at the 95% safety threshold ($P < 0.05$).

K)Spearman's r-rank correlation test

The distribution not following a normal law according to the Kolmogorov and Smirnov test, the rank correlations of Spearman made it possible to evaluate the degree of connection between the physicochemical parameters on the one hand, and physicochemical and biological parameters on the other hand. . The analyzes were performed using SPSS 20.0 software and the results assessed at the safety threshold of 99% ($P < 0.01$) and 95% ($P < 0.05$).

Results and discussion

a) Physical Parameters

Temperature

The temperature values obtained during the study period vary between 25.95°C (at station CV1 (green city) during the short rainy season and 28°C at point MS4 (Messa) in the long dry season (Figure 1). Statistical tests show no spatially significant difference (p=0.488) but temporally significant (p=0.008).

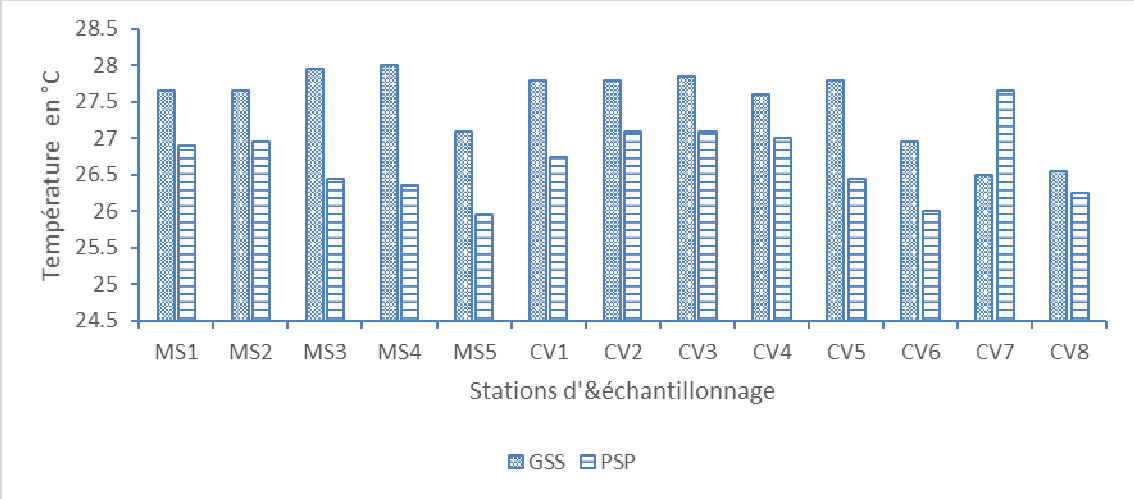

Figure1: Spatiotemporal variations in temperature during the study period

Suspended Solids (SS), Color, Turbidity, Total Dissolved Solids (TDS)

The values of Suspended Solids (SS) obtained vary with an average value of 351.55 ± 407.02 mg/L (Messa) and 545.40 ± 982.25 mg/L (Cité verte) (Figure 2 A) . Statistical tests show no significant difference in spatio-temporal terms (p=0.181 and p=0.209) and (p=0.072 and p=0.600).

The color variation profile shows that the values oscillate around an average of 554.41 ± 573.98 Pt.Co for Messa and 821.37 ± 792.84 Pt.Co (Figure 2B). As for the MES, the color, the tests show no significant difference on the spatio-temporal plan (p=0.080 and p=0.530) and (p=0.060 and p=0.600) (FIG. 2 B).

Turbidity values varied around an average of 44.85 ± 26.70 NTU (Messa) and 62.21 ± 40.75 NTU (Green City) (Figure 2C). The variation in turbidity is not significant on the spatio-temporal plan (p = 0.251 and p = 0.530) and (p = 0.074 and p = 0.248).

In general, the contents of Total Dissolved Solids (TDS) obtained during the study period vary around an average of 371.05 ± 189.62 mg/L (Messa) and 596.03 ± 136.44 mg/ The change in TDS content shows no significant difference in spatio-temporal (p=0.155 and p=0.455) and (p=0.423 and p=0.36) (Fig. 2D).

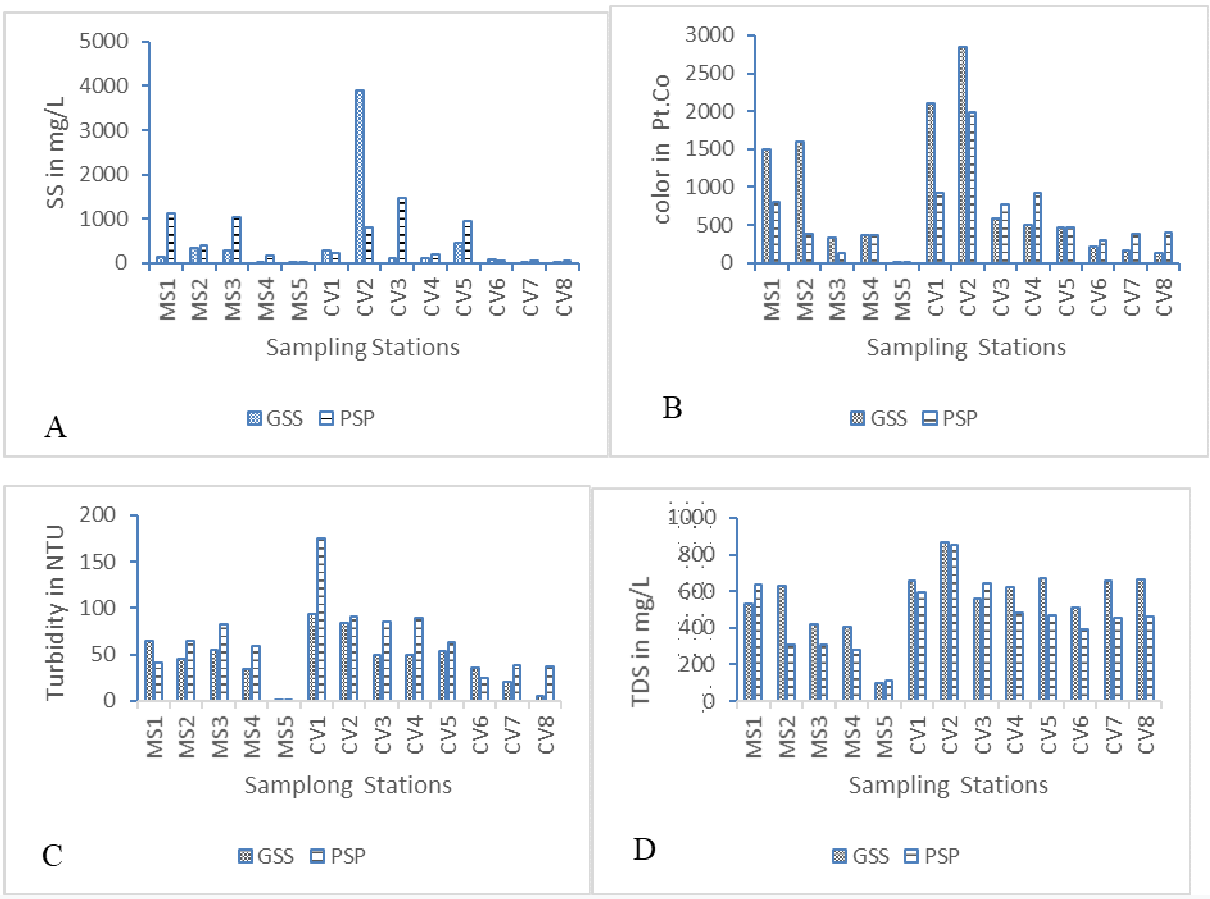

Figure 2: Spatiotemporal variations of SS (A), color (B), turbidity (B) and TDS (D) during the study period

b) Chemical parameters

Hydrogen Potential (pH)

The pH values obtained during the study period varied between 6.19 U.C at point MS5 (Messa) during the long dry season and 7.93 U.C MS2 during the short rainy season. For the Cité verte, the values varied from 6.94 U.C at point CV3 during the long dry season and 7.99 U.C at point CV2 during the short rainy season (Figure 3 A). The statistical tests show no significant difference on the spatial level ($p = 0.702$ and $p = 0.884$) but on the other hand the difference is significant on the temporal level ($p = 0.009$ and $p = 0.001$).

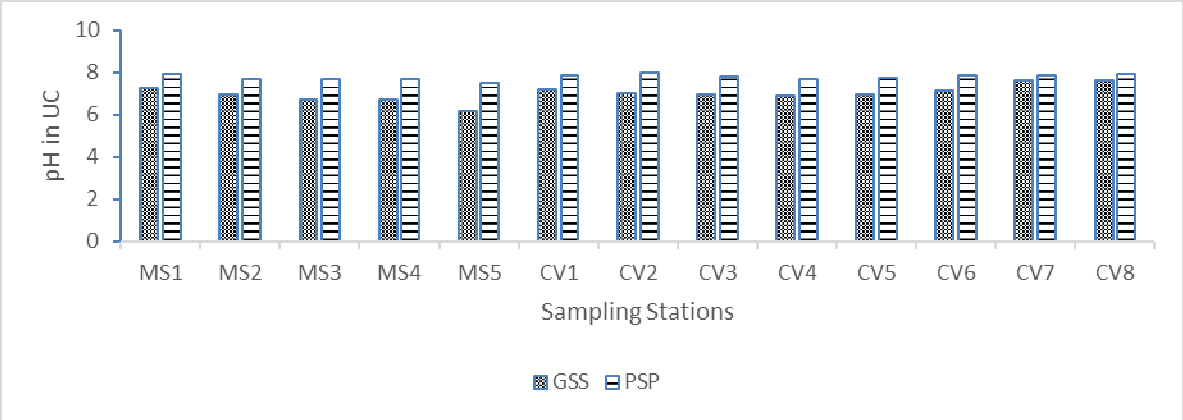

Figure 3: Variations in pH during the study period

Dissolved Carbon Dioxide, Dissolved Oxygen, Oxidability and Conductivity

The dissolved carbon dioxide values oscillate around an average of 76.23 ± 47.40 mg/L for Messa. Concerning the Cité verte, the values varied from 37.8 mg/L at point CV7 during the short rainy season and 139.05 mg/L at point CV1 during the long dry season (Figure 4 A). Statistical tests showed no spatio-temporal significant difference (p=0.321 and p=0.465) for Messa. While for the Green City there is no significant difference on the spatial level (p = 0.163) but it is significant on the temporal level (p = 0.46).

Overall, dissolved oxygen fluctuated around an average of 13.39 ± 18.43% at Messa. For the Cité verte, the values varied from 2.81 to 5.9% (Figure 4 B) respectively at points CV4 during the long dry season and CV1 during the short rainy season. Statistical tests showed no spatio-temporal significant difference (p=0.137 and p=0.917) for Messa. While for the Green City there is no significant difference on the spatial level (p = 0.619) but it is significant on the temporal level (p = 0.012). With regard to oxidizability, the average value for this parameter is 4.68 ± 2.13 mg/L of KMnO4 (Messa) and 4.75 ± 2.18 mg/L of KMnO4 (Cité verte) (Figure 4C). The differences observed are not significant in terms of space and time (p = 0.203 and p = 0.530) and (p = 0.236 and p = 0.793).The electrical conductivity varied from 176.5 µS/cm at the MS5 point in the long dry season to 1265.5 µS/cm at the MS1 point in the short rainy season (Messa) and between 778.4 µS/cm at the CV6 point in the short rainy season and 11618 µS/cm at point CV2 in the long dry season (Cité verte) (Figure 4 D). The difference in values for this parameter is neither spatially nor temporally significant at Messa (p = 0.143 and p = 0.465). On the other hand, in the green city, the difference is not significant on the spatial level (p = 0.427) but is on the temporal level (p = 0.036).

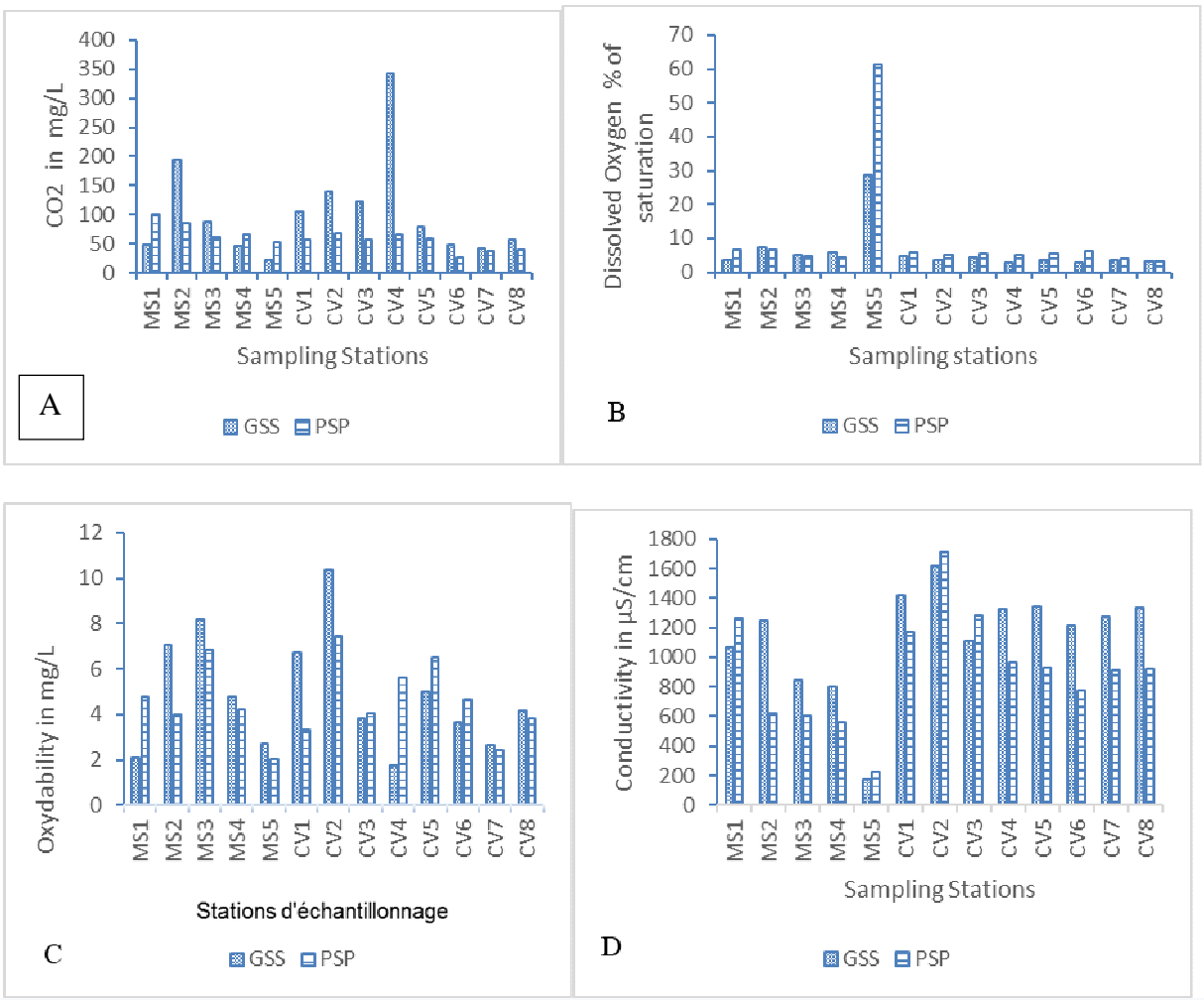

Figure 4: Variations of chemical parameters during the study period (CO2 (A), Dissolved oxygen (B), oxidability (C), electrical conductivity (D))

Nitrate, Orthophosphate and Ammonia Nitrogen

The nitrate content varied around an average of 106 ± 78.80 mg/L a (Figure 5 A). The differences observed for this parameter are not significant on the spatio-temporal level (p = 0.095 and p = 0.217).

The orthophosphate values oscillated around an average of 1.74 ± 1.14 mg/L of PO43- (FIG. 5 B). The statistical tests carried out show no significant difference in terms of space and time (p = 0.555 and p = 0.537).

For ammoniacal nitrogen, the contents fluctuate around an average of 1.67 ± 1.48 mg/L (Figure 5 C). Statistical tests show that the differences are not significant spatiotemporally (p = 0.555 and p = 0.051).

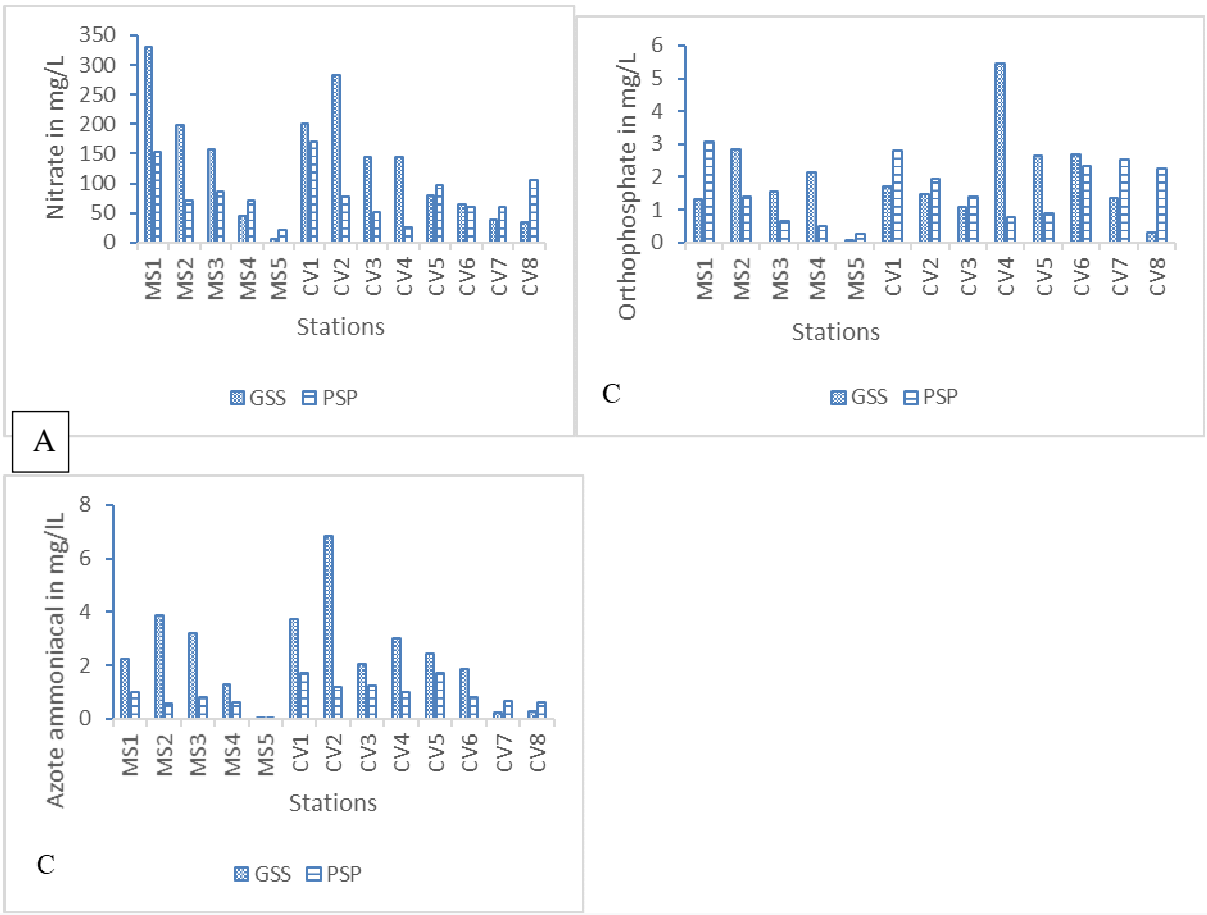

Figure 5A: Variations in nitrate (A) orthophosphate (B) and ammoniacal nitrogen (C) levels during the study period

c) Biological analyzes

Observations of protozoan oocysts made it possible to identify and count 294,421 protozoan oocysts belonging to 4 species. These are 277297 oocysts of Cryptosporidium spp., (95%) with 11102 oocysts of Cyclospora spp., (95%), 1968 oocysts of Isospora spp. oocysts, (0%) and 2054 oocysts of Sarcocystis spp., (1%) (Figure 5D).

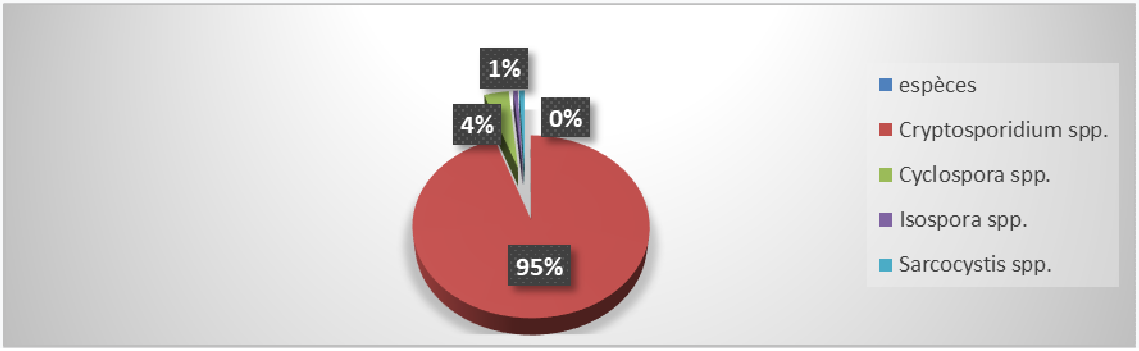

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Figure 5D: Relative abundance of different groups of protozoa during the study period
Spatiotemporal variation in oocyst densities of identified enteropathogenic protozoa

Spatially, the density varied from upstream to downstream of the different WWTPs with greater density of Cryptosporidium spp. with 16187 oocysts; for an average value of 3238 ± 2203 oocysts/L for Cryptosporium spp., (Messa). As for the Cité verte, the value of 123750 oocysts with an average density of 15469 ± 29405 oocysts/L was obtained. For the Cité verte station, the oocysts were denser at point CV2 with 93436 oocysts (23359 ± 46038 oocysts/L) and less dense at point CV8 with 813 oocysts (204 ± 262 oocysts/L). Concerning the Messa station (station with underground spreading), the densest point was MS2 6664 oocysts (1666 ± 2806 oocysts/L) and the least dense was MS5 734 oocysts for an average of 184 ± 305 oocysts/L. The least dense species was Isospora spp., with 663 oocysts for the Cité verte and an average of 83 ± 72 oocysts/L. Speaking of Messa, the value was 321 oocysts for an average of 65 ± 70 oocysts/L (figure. 6 A).

During the study period the density of species decreased from one station to another from upstream to downstream, the density of protozoan oocysts was higher during the short rainy season 63350 oocysts (5027 ± 12324 oocysts /L) lower during the long dry season with 9209 oocysts (709 ± 545 oocysts/L) (Figure 6 B). The densest species was *Cryptosporidium* spp. with 5,224 oocytes during the short rainy season for the Messa station and 58,058 oocytes for the Cité verte station. *Isospora* spp., was the least dense species with 140 oocysts for the Messa station and 150 oocytes for the green city.

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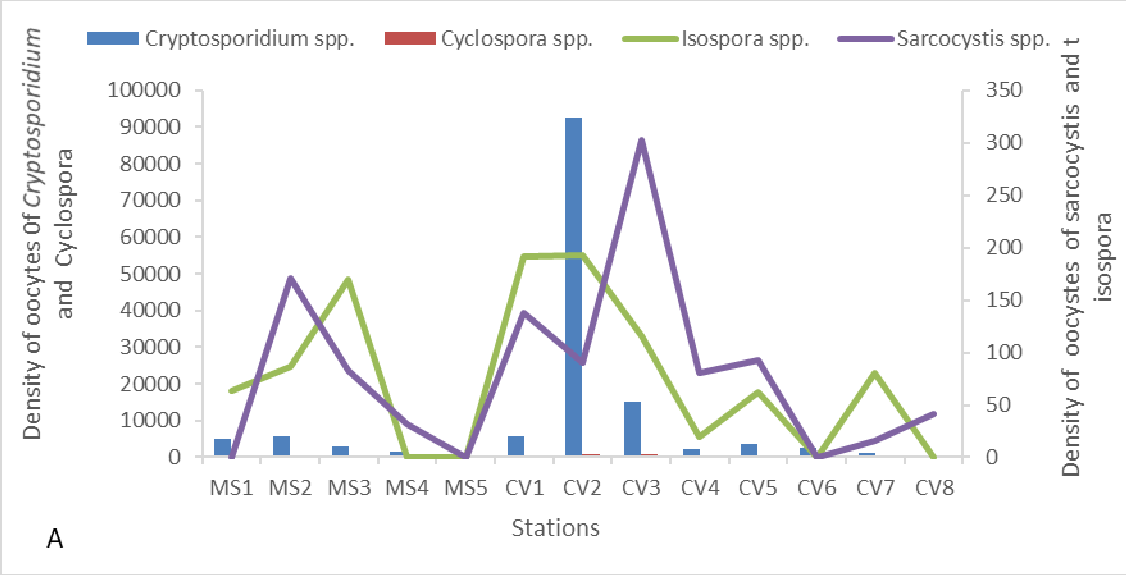

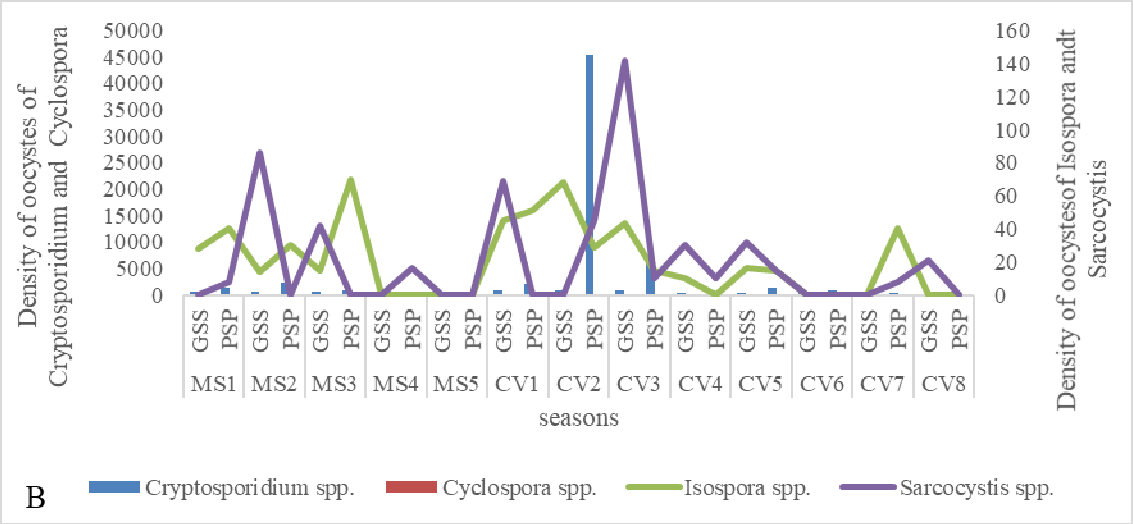

Figure 6: Spatial (A) and temporal (B) variation of protozoan species during the study period

Table 1: Correlations between certain physico-chemical parameters and oocyst density

Parameters	SS	Turbidity	Color	Nitrates	Ammonia
Cryptosporidium spp.	0,782**	0,775**	0,558**	0,322	0,256
Cyclospora spp.	0,699**	0,795**	0,610**	0,406*	0,395*
Isospora spp.	0,656**	0,655**	0,540**	0,673**	0,470*
Sarcocystis spp.	0,273	0,264	0,390*	0,275	0,455*

*= significant correlation 5 % **= significant correlation 1%

Table 2: Purification rate for certain physico-chemical and biological parameters in the treatment plants studied

Messa

Paramètres	**Entrance**		**Outlet**		**Efficiency in %**	
	GSS	**PSP**	**GSS**	**PSP**	**GSS**	**PSP**
Conductivity	1065	1265,5	176,5	222,5	83,42 %	82,43%
TDS	534	632,5	96,5	110	81,92%	82,60%
SS	132,5	1111	2,5	2,5	98,11%	99,77%
Turbidity	65	41,5	1	1	98,46%	97,59%
Color	1501,5	805	19	13,5	98,73%	98,32%
Orthophosphate	1,3	3,08	0,05	0,25	96,15%	91,88%
Oxydability	2,1	4,8	2,7	2,05	-28,57%	57,29%
Nitrate	330,5	152,5	5,2	21,7	98,42%	88,77%
Ammonia	2,25	1,005	0,03	0,05	98,66%	94,52%
Cryptosporidium	626	1216	218	100	65,18%	91,77%
Cyclospora	112	68	29	20	74,10%	70,58%
Isospora	28	40	0	0	100%	100%
Sarcocystis	0	86	0	0	0%	100%

Cité Verte						
	entrance		Outlet		Efficiency in %	
Parameters	GSS	PSP	GSS	PSP	GSS	PSP
Conductivity	1422,5	1175	1331	918	6,43%	21,87%
TDS	653,5	592	665,5	459,5	-1,83%	22,38%
MES	285	220,5	7,5	45	97,36%	97,95%
Turbidity	93	175	5,5	37,5	94,08%	78,57%
Color	2105	918,5	130	397,5	93,82%	56,75%
Orthophosphate	1,72	2,78	0,28	2,25	83,72%	19,06
Nitrate	201,5	170,5	34,25	104,65	83%	38,62%
Ammonia	3,75	1,7	0,29	0,62	92,66%	63,52%
Oxydability	6,75	3,35	4,17	3,85	38,22%	-14,92%
Cryptosporidium	763	2067	72	217	90,56%	89,50%
Cyclospora	138	135	39	58	71,73%	57,03%
Isospora	45	51	0	0	100%	100%
Sarcocystis	69	0	21	0	69,56%	0%

III.2. DISCUSSION

The biological analysis made it possible to identify the cysts and the oocysts of the protozoa divided into four species (*Cryptosporidium* spp., *Cyclospora* spp., *Isospora* spp., *Sarcocystis* spp.). We are dealing with two different purification systems, namely: an underground spreading station in Messa and a station with a hybrid filter and plant system in the green city. Cryptosporidium spp. was the densest species at both stations; this could be explained by the fact that this species has a double protective shell that allows it to resist environmental constraints. This explained the positive correlations obtained between the density of Cryptosporidium spp. and color (r=0.558), SS (r=0.785) and turbidity (r=0.775). Indeed, according to Medema et al. (1998), parasitic organisms are generally linked to suspended organic matter in the water. This bond caused by electrostatic interactions, those of Lifshitz-Van der Waals and the acid-bases of Lewis (Dai and Boll,2003) would facilitate their dissemination in the aquatic environment. The different species were denser during the short rainy season; this could be explained by the fact that the covers of the structures having been removed from the precipitation drained runoff water that could contain parasite oocysts into the various purification basins.

The effectiveness of the treatment on the forms of dissemination of protozoan parasites depends on the degree of initial water pollution. Similarly, the reductions in oocysts are a function of the initial physicochemical and microbiological characteristics of the raw water and proportional to the reduction in turbidity (98.46% for Messa and 94.08% for the green city) (Bratby, 2006). Indeed, given that the micro-organisms are attached to the particles in suspension, their sedimentation also involves that of the micro-organisms. These factors thus explain the deference observed in the reductions obtained. Thus, the more the clarification is perfect and the initial microbiological quality acceptable, the better the microbiological quality of the treated water (Kabore et al., 2013).

The abatement rates of the different species for the Messa underground spreading station shows a higher rate during the short dry season for all species with a yield of 91.77% for Cryptosporidium spp., 70.58% for *Cyclospora* spp., 100% for *Isospora* spp. and *Sarcocystis* spp. for an average of 90.58 ± 12.03%. On the other hand, for the station with a hybrid filter and plant system in the green city, it is the long dry season that marks the greatest reduction rate for the oocysts of protozoa rejected during the study with 90.56% Cryptosporidium spp., 71.73% *Cyclospora* spp., 100% *Isospora* spp. and 69.56% *Sarcocystis*

spp. for an average of 82.96 ± 12.84%. These results would be directly related to the nature of the wall of each oocyst. Indeed Cryptosporidium is very abundant in nature and very resistant to disinfectants compared to its small size and its double wall (Tsomené and Ajeagah, 2020; Health Canada, 2017). The oocysts of Cyclospora spp. are circular with a smooth double wall, inside these there is a greenish cluster called morula (Anses, 2014). Their size varies from 7 to 10 µm which makes them a little more resistant with a high knockdown rate of Isospora is characterized by a large size (25 to 35µm x11 to 16µm) and three forms in the environment. The immature ovoid form has one sporoplast, the mature ovoid form has two sporoplasts and the terminal ovoid form has two sporoplasts also that leave the center for the pole of the cell (Ajeagah et al, 2015). The nature of the form characterizes the resistance; sporocysts of Sarcocystis spp. is in an ovoid form and contains sporozoites. Their shell is chopped and their size fluctuates between 10 and 15 µm x 9 to 10 µm (Asi et al., 2021); reason why a large abatement was obtained for these species. We can say that these stations have a satisfactory purification capacity for these stations. The greatest decrease in oocysts was made at the level of primary treatment in the settling and desilting tanks for the two stations

Conclusion

In this work it was a question for us of evaluating the performances of the purification stations of the stations with underground spreading of Messa and hybrid system filter and plant of the Green city in the elimination of coccidia; we can say that in the general framework the various stations had a satisfactory biological performance in the elimination of protozoan coccidia and also for certain physico-chemical parameters measured. This performance could be even more effective with more efficient maintenance and sanitary engineering of the various structures of these stations.

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