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Physicochemical and microbiological characterization of effluents from the "Centre Hospitalier Universitaire de la Mère et de l'Enfant Lagune (CHU-MEL)" discharged in the Cotonou lagoon in Benin

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ABSTRACT

This study is carried out to characterize the CHU-MEL effluents discharged into the Cotonou lagoon to assess the potential hazards of these releases for this ecosystem. To achieve this goal, the effluents collected at the end of the spillways have been analyzed. The results obtained from the physicochemical and microbiological analysis made it possible to assess the quality of the effluents. The pH, temperature and conductivity measured *in-situ* are, on average, 6.65, 30.3 °C and 763 μS/Cm. The average contents of dissolved oxygen, nitrite, nitrate, ammonium and Nitrogen Total Kjedhal (NTK) are respectively 1.92 mg/L, 0.06 mg/L, 7.75 mg/L, 18.45 mg/L and 28.78 mg/L. The Biochemical Oxygen Demand (BOD$_5$) ranges from 38 mg/L to 53 mg/L, the Chemical Oxygen Demand (COD) from 97 mg/L to 274 mg/L, suspended solids (SS) belonging between 47 mg/L-398 mg/L and total phosphorus going from 0.37 mg/L to 13.02 mg/L. The microbiological parameters (total coliforms, faecal coliforms, *E. coli*, fecal streptococci and anaerobic sulphite reducers' spores) are generally out of the standard values. These effluents present therefor risks for the fauna and the flora of the Cotonou's lagoon and it is well indicated to assess them through ecotoxicity tests.
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Keywords: Hospital effluents, physicochemical parameters, microbiological parameters, pollution.

INTRODUCTION

The question of the presence of pollutants and the problems they generate in aquatic ecosystems is a major international concern because of environmental, health and economic issues. Among the different types of pollutants, those of hospital effluents must be paid a close attention. Effluents, generated by hospital activities, present a danger to human and his environment; due to their nature and the

importance of the specific substances they contain (drug residues, chemical reagents, antiseptics, detergents, etc.) (Boillot, 2008). Their management is becoming more and more important in public health concerns. This upsurge interest relates, on the one hand, to the importance of the risk linked to the production of waste raised from healthcare activities and, on the other hand, to the nuisances to both health and environment which can be caused by the treating technique of waste (DHSA, 2004). Indeed, hospitals generate a large quantity of liquid effluents containing many micropollutants discharged into urban networks without prior treatment, as well as conventional domestic wastewater (Boillot, 2008; Hisseien et al., 2015) that is poured in the natural environment without a clear idea of their effects on it (Emmanuel, 2004) mainly in developing countries.

Effluents, once released in the environment with their pathogen agents and pharmaceutical products, the local fauna and flora are permanently under risks. Indeed, the specificity of hospital effluents is related to its high degree of toxicity. Even at a very low concentration, substances contained in hospital effluents can affect animal lives: estrogens for example can cause fishes to be hermaphrodite (Qadouri et al., 2016).

Several studies on the characterization of hospital effluents have been conducted throughout the world (Leprat et al., 2002; Emmanuel, 2004; Hartemann et al., 2005; Zounkovà et al., 2006; Sadek et al., 2012; Bouzid et al., 2013; Touré et al., 2016). Some of these studies also highlight the intrinsic ecotoxicity of these hospital effluents, 5 to 15 times higher than that of urban effluents. This ecotoxicity is related to substances contained in these effluents, such as the case of bleach used in laboratories in order to eliminate pathogen germs (Philippot et al., 2016).

According to WHO (2016), Heath centers are not equipped and have not access to sanitation installations. When even in some extremely rare cases they have water connections, there are hardly reliable and not adapted to the needs of patients, sanitary personal and to visitors too. The fact is more severe in developing countries.

In Benin, very few characterization studies of hospital effluents have been realized. From the few that have been carried out, it comes out that the quality of the effluents is unsatisfactory when compared with the existed standard. These effluents, through the infiltration process, contaminate shallow groundwater (Makoutodé et al., 2000; Aissi et al., 2010). In Decree No 2006-087 of 08 March 2006 on the national policy of hospital hygiene of the Ministry of Health of Benin, it is stated that "most of health center do not dispose of appropriate structures for the liquid waste collection and treatment (activated sludge or bacterial bed treatment station). In some cases, liquid waste is discharged into the washing basin and drained to the septic tank. In other cases, they are discharged directly into the public sewer when it exists, and sometimes, they are even discharged into the environment. It is the case of CHU-MEL which effluents are oriented in Cotonou's lagoon. This practice contributes to environmental pollution and exposes the population to infections caused by multi-resistant germs ".

On the basis of these facts, it was considered useful to characterize the effluent discharged by CHU-MEL in the Cotonou lagoon in order to evaluate the potential dangers of these discharges on this ecosystem.

MATERIALS AND METHODS
Study area

The area study is subjected to subequatorial climate characterized by four seasons including a long dry season (LDS) from December to March, a long rainy season (LRS) from April to July, a short dry season (SDS) from August to September and a short rainy season (SRS) from October to November (Adam and Boko, 1993). The current study has been carried out on the effluents of the CHU-MEL. The CHU-MEL is specialized in the care of mother and child. It is located in the town and close to the edge of the Cotonou lagoon as showed on figure 1. Liquid effluents resulting from the various activities of the hospital

undergo a sort of treatment and are drained into the lagoon by underground pipe.

Water sampling

All sampling was carried out in the morning around 9 o'clock at the end of the pipes used as effluent dumps in the Cotonou lagoon, with the highest discharge rates obtained between 7 h et 10 h. All the samples were kept in cool box (4 °C) to the laboratory.

For the physicochemical and microbiological analysis, two sampling campaigns were carried out in 2015, one in October (E1) in rain season and the Second one four weeks later in November (E2) corresponding to the beginning of the dry season. Samples for physicochemical analysis were taken from 1.5 L plastic bottles rinsed three times with the effluent to be sampled. The bottles were fully filled. Those for microbiological analysis were taken from pre-sterilized 250 mL glass bottles. They were

filled to ¾ of their volume. All samplings were kept in icebox filled of ice to the laboratory for analysis purpose.

Effluents characterization

In order to determine the quality status of the CHU-MEL effluents, we focused on certain parameters allowing the assessment of water quality. These parameters are pH, conductivity, salinity, dissolved oxygen concentration, nitrogenous compounds (NH_4^+, NO_3^-, NO_2^-, NTK), total phosphorus, free chlorine, suspended solids (SS), BOD_5, COD and two heavy metals (lead and chromium). Table 1 lists the parameters measured on the effluent as well as the methods used. The microbiological characterization in this study was to search for total flora, total coliforms, presumptive Escherichia coli, faecal Streptococci, anaerobic sulphite reducers' spores spores according to the methods summarized in Table 2.

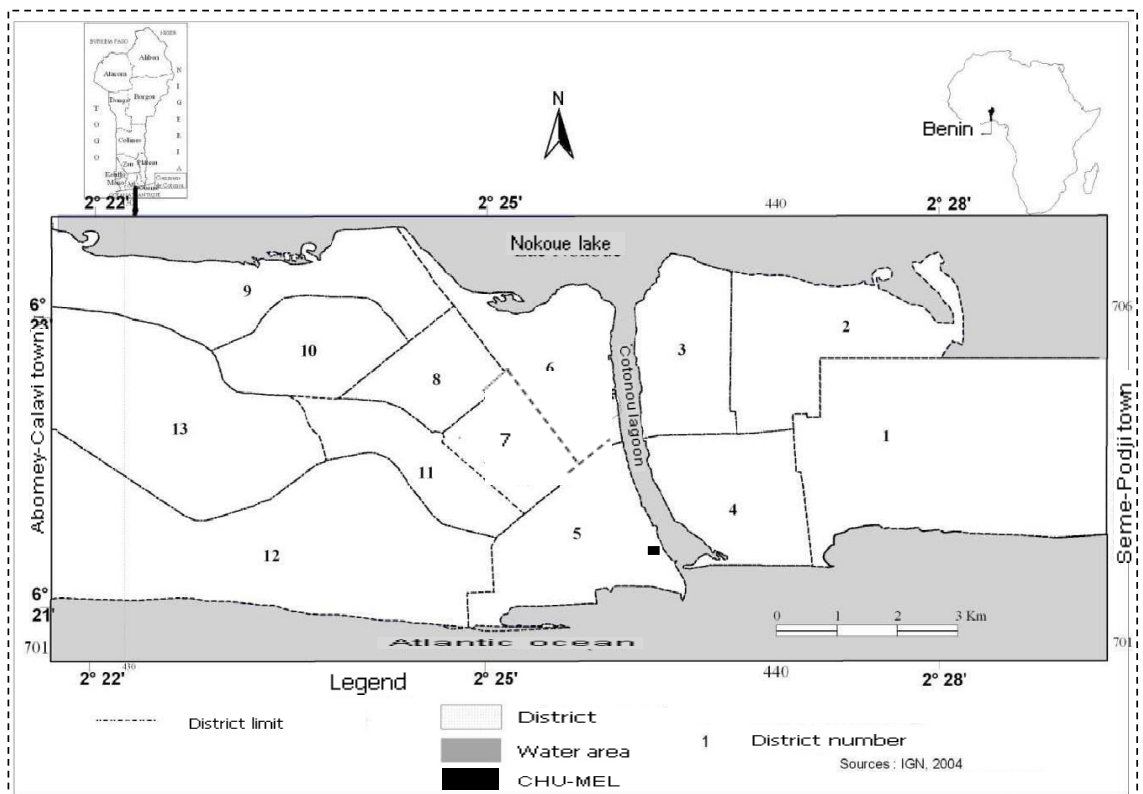**Figure 1:** Cotonou town map presenting the position of its Cotonou lagoon.

Table 1: Physico-chemical parameters and methods useds.

Monitored parameters	Materials and used methods	References
pH, Temperature, Conductivity, Salinity	Direct measurement by multi-parameter pH / Conductmeter WTW 340i	NF T 90 - 008) , NF T 90-029
Disolved Oxygen	Potentiometry. Electrochemical method	NF EN 25814
Suspension Solids	Colorimeter Method HACH DR 890	
Nitrate	Colorimeter. Cadmium reduction method	
Nitrite	Colorimeter. Diazotization method	NFT EN 26777
Ammonium,	The Nessler Method	
Nitrogen Total Kjedhal	The Nessler Method	
Biochemical Oxygen Demand (BOD5)	Oxytop Respirometric method in a thermostatic chamber	NF EN 1899-2
Chemical Oxygen Demand (COD)	Colorimeter. Potassium dichromate method	NF T90-101
Total Phosphorus	Acid ascorbic Method	NF EN ISO 15681-2
Hexavalent Chromium	Chromaver 3 method (HACH 8023)	NF EN ISO 18412
Lead (Pb)	Dithizone method (HACH 8033)	NF EN ISO 15586
Free chlorine	Colorimeter. DPD1 method	NF EN ISO 7393-2

Table 2: Microbiological parameters measured and methods used.

Microorganisms	Culture area	Seeding mode	Incubation duration	Particular observations	References
Total Coliforms	MacConkey sorbitol BCIG *E.coli*/coliform selective Agar (CM0981)	Seeding in the mass	24 h à 37°C	Red colony	NF EN ISO 9308-3 (1999)
Faecal Coliforms		Seeding in the mass	24 h à 37°C	Red colony	NF EN ISO 9308-3 (1999)
Escherichia coli		Seeding in the mass	24 h à 37°C	Blue colony	NF EN ISO 9308-3 (1999)
Faecal Streptococci	Slanetz and Bartley Agar of (Oxoid, CM 0377)	Seeding in the mass	48 h à 37°C	Red colony	NF ISO 7899-2
anaerobic sulphite reducers' spores	Meat-liver Agar (Oxoid, CM 0918)	Seeding in the mass	24 à 48 h à 37°C	Colony rounded by black halo	NF EN 26461-1– ISO 6461-1

RESULTS

Physicochemical characterization

The physicochemical characteristics of CHU-MEL effluents compared to certain standards are summarized in Table 3. These results are compared to the standard of WHO and to those adopted in Benin. They showed that the pH, the temperature, the conductivity and concentrations of nitrites, nitrates, lead and chromium are in line with standard. However ammonium, total nitrogen, total phosphorus, suspension substances concentrations, BOD5 and COD are higher than the standard (Table 3).

From the first to the second sampling, the values of certain parameters underwent remarkable variation. In fact, concentrations of NTK, total phosphorus, SS, BOD_5 and COD have significantly dropped. On the other hand, those of dissolved oxygen and ammonium nearly tripled (Table 3).

According to studies realized by Hartemann et al. (2005) and Mohee (2005), these fluctuations may depend on the origin and the effluent sampling moment. Indeed, they have proved in their work that the pollutant constituents vary according to the specificity of the hospitals, the place and even according to the moment of effluents sampling in the day.

Microbiological characterization

The identified bacterial species and counted in the CHU-MEL effluents are both total and faecal coliforms, faecal streptococci, clostridium spores and other germs. The obtained results are gathered in table 4. The values obtained for these different groups of microorganisms are significantly higher than the WHO standard (2012) of 10^3 UFC/100 mL for direct release into the environment.

Table 3: CHU-MEL effluents physicochemical parameters values along with sampling moments.

Parameters	Unities	Sampling E$_1$	Sampling E$_2$	mean values	Acceptable Standard (WHO Benin)
pH		6,76	6,54	6,65	6-9
Temperature	°C	29,6	31,00	30,3	< 30
Conductivity	µS/cm	738	788	763	< 2000
Dissolved Oxygen	mg/L	0,84	3,08	1,96	≥ 5
Nitrite (N-NO$_2^-$)	mg/L	0,043	0,077	0,06	< 3
Nitrate (N-NO$_3^-$)	mg/L	8,0	7,5	7,75	< 50
Ammonium (N-NH$_4^+$)	mg/L	14,4	32,5	23,45	< 0,2
Nitrogen Total Kjeldhal (NTK)	mg/L	57	0,56	28,78	< 15*
Total Phosphorus	mg/L	13,02	0,37	6,695	< 10
SS	mg/L	398	47	222,5	< 20
BOD$_5$	mg/L of O$_2$	53	38	45,5	< 25*
COD	mg/L of O$_2$	274	97	185,5	< 90
Lead	µg/L	0,112	0,187	0,159	< 1*
Chromium (Cr^{6+})	mg/L	0,032	0,092	0,06	< 0,1*
Free Chlorine (Cl$_2^-$)	mg/L	0,32	0,34	0,33	

* Benin standard

Table 4: Microbiological characteristics.

Parameter (UFC/100ml)	Sampling			Acceptable Standard (WHO)
	E1	E2	Moyenne	
Total flora	10100	20300	15200	
Total califorms	10690	12800	11745	
Faecal califorms	6930	8200	7565	2.10^3
Escherichia coli	6930	7380	7155	10^3
Fecal streptococci	1500	160	830	
anaerobic sulphite reducers' spores	60	35	47,5	

DISCUSSION

The average values of temperature and pH respectively equal to 30.3 °C and 6.7, are perfectly in line with the Benin standards of liquid waste discharge which recommends a temperature of 30 °C (with a maximum deviation of 1 °C from that of the discharging area), and a pH between 6 and 9 for domestic waste water. The measured temperature values are slightly higher than those measured by Makoutodé et al. (2000) on CNHU-HKM effluents, and those measured by Aissi et al., (2013) in health zones of Abomey-Calavi and So-Ava. The pH values obtained is in accordance with the WHO standard (2006), (Table 3). The trend is generally the same when comparing the measured parameters with those of other countries effluents. Thus, the pH values measured by Sadek et al (2012) on Sidi Kacem Provincial Hospital effluents range from 7.06 to 7.59, those measured by Bouzid et al (2013) on Mohamed V hospital of Meknes (Morocco) effluents vary from 7.39 to 8.83. They are in good agreement with those (5 to 8.8) reported by Boillot, (2008) on effluents of seventeen hospitals in France.

The electrical conductivity is used to estimate the overall mineralization and to estimate the total soluble salts in water (Rodier et al., 2009). The obtained values remain below the inferior limit of 2000 μS/cm (WHO, 2012). The CHU-MEL effluents have a lower conductivity values compared with those of Calavi and Sô-Ava sanitary zones effluents.

Indeed the conductivity value of these effluents varies between 3700 μS/cm and 4260 μS/cm (Aissi et al., 2013). For effluents from 17 hospitals in France, Boillot, (2008) reported conductivity values ranging from 11.2 to 313 μs /cm. Water with a conductivity between 600 and 1000 μS/cm being considered as a water with a high mineralization (SPE, 1997, Rodier et al., 2009), CHU-MEL effluents are strongly mineralized wastewater.

The mean dissolved oxygen level of 1.96 mg/L obtained is lower than the guideline (6 mg/L) set by the "L'ordonnance sur le déversement des eaux usées" (ODE) and also than the critical level (4 mg/L) below which the life of aerobic microorganisms is threatened (Cornaz, 2004). This low oxygenation of water may be either due to a high consumption of dissolved oxygen by the aerobic bacteria for the degradation of the organic matter or to a low photosynthetic activity of the phytoplankton (Hamaidi et al., 2009). It gives the effluents an anoxic and therefore reducing and asphyxiating character. Thus, this area could favor nitrite and sulfates reduction respectively into nitrites and sulfurs. Such modification may be responsible for the unpleasant odors of effluents (Rodier et al., 2009). This state of the environment justifies the high content of ammonia nitrogen (23.45 mg/L NH_4^+) compared with that of the oxidized forms of nitrogen (0.6 mg/L NO_2^- and 7.75 mg/L NO_3^-). This fact could be explained

by the oxidation of organic matters which in turn can give birth to ammoniacal nitrogen increasing (Gnagne et al., 2015).

As for Aissi et al. (2013) who obtained NTK values between 34.5 and 97.5 mg/L, the NTK value obtained for CHU-MEL effluents, on average equal to 28.73 mg/L, is higher than Benin standard which is 15 mg/L. Total phosphorus decreases from the first sample to the second (13.02 to 0.37 mg/L). The standard admitted in Benin is set a maximum of 2 mg/L. The discharge of CHU-MEL effluents into the Cotonou lagoon could lead to eutrophication. Indeed, the excess of nutrients nitrogenous salts (ammoniac, nitrate, nitrite) and phosphorus salts (phosphates) that are required for aquatic life can cause eutrophication phenomenon in the area, particularly in closed or semi-closed zones (Gnagne et al., 2015) such as lagoon of Cotonou.

The concentration of suspended solids (SS) decreases from the first sampling to the second one, and drops from 398 to 47 mg/L. The WHO standard for this parameter is 20 mg/L and is lower than we obtained. These results corroborate the low level of dissolved oxygen in the sense that their degradation consumes a significant amount of available oxygen. The high concentrations of SS in the studied effluents added to that originated from other pollution sources (Adjahouinou et al., 2012) could prevent light penetration in water. As consequence, the photosynthesis process by phytoplankton under water will decrease because of the limited dissolved oxygen in the aquatic area (Dongo et al. 2013). BOD_5 is a parameter that allows the assessment of biodegradable organic materials fraction. The results analysis show that BOD_5 concentrations are really high (53 and 38 mg/L) instead of 25 mg/L as recommended by the standard of domestic releases in Benin (Table 4).

According to the WHO standard, COD for effluents must be at least 90 mg/L. In CHU-MEL effluents, we recorded an average COD of 185.5 mg/L of O_2 compared with 125 mg/L of O_2 for the norm accepted in Benin. The COD/BOD_5 ratio (3.86) is greater than 2 showing that the CHU-MEL effluents are only moderately biodegradable (Rodier et al., 2009).

The quantities of lead and chromium found in these effluents are less than the Benin standard for industrial wastes set at 1 mg/L for lead and 0, 1 mg/L for hexavalent chromium. Giving that the recovered quantity of heavy metal are less than the fixed norms, the heavy metal pollution of the CHU-MEL effluents is then relatively low.

Total coliforms are present in effluents with an average of $11.745.10^5$ CFU/100 mL. The results obtained are consistent with those of the preliminary studies, which revealed much lower levels of concentration than urban classical effluents (average 10^4 and 10^6 CFU/mL). Indeed, the literature classifies hospital effluents as being less burdened with coliforms than urban wastewater, having a coliforms concentration of 10^{10} CFU/100mL (Boillot, 2008). The likely presence of antibiotic residus and of specific substances in effluents (disinfectants, antiseptic etc.) could explain this observation (Guessennd et al., 2013). These effluents contain a high concentration of E. coli with an average value of $7,155,10^5$ CFU/100 mL. The high density of E. coli bacteria in the waters analyzed clearly indicates their contamination by faecal germs (CEAEQ, 2000; Edberg et al., 2000). This state of facts therefore increases the epidemiological potential risk posed by their rejection without treatment (Servais et al., 2006).

The average number of streptococcal colonies per 100 ml of effluent is 8.3×10^4 CFU/100 mL. The presence of streptococci and anaerobic sulphite reducers' spores shows that the contamination is recent (Clausen et al., 1977). The anaerobic sulphite reducers' spores are present in the effluents in an average of $4.75.10^3$ CFU/100 mL. Indicating the effectiveness of the treatments, their presence shows a failure of the filtration (Rodier et al, 2009).

Most physicochemical and microbiological parameters exceed the norms admitted. This shows that those effluents present enormous risks for the living organisms of the lagoon. We proposed, based on all the above-mentioned results, that CHU-MEL effluent treatment system be reinforced and monitored.

Conclusion

This study was carried out to characterize the CHU-MEL effluents that are discharged into the Cotonou lagoon. The results show that effluents from CHU-MEL are in a state of undesirable pollution. In fact, the concentration of the various physicochemical and microbiological parameters analyzed exceeds the standards admitted for either industrial or domestic liquid waste.

Direct discharge of effluents without adequate treatments can have negative impacts on the trophic status of the lagoon's water and on the life of living organisms in this ecosystem. As with other sources of pollution, CHU-MEL effluents could play a capital role in the deterioration of the physicochemical and microbiological quality of the waters of the lagoon. Therefore, it would be benefic to assess the risk posed by the direct discharge of CHU-MEL effluents into the Cotonou lagoon.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

EASA: main author of the work; BB: Contribution in field work; CDA: Contribution in field work, advisory support and rereading of the manuscript; WGK: Advice, proofreading and translation of the manuscript in English; LK: Support and review of the manuscript; BCC: Support and review of the manuscript; EDF: Director of the Laboratory of Research on Wetlands (LRZH): Direction and supervision of a hydrobiological part of the work; PAE: Director of the Laboratory of Research in Biochemistry and Toxicology of the Environment (LaRBiTE): Supervisor of toxicological and ecotoxicological parts of the work, Thesis supervisor.

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