

PHYSICO-CHEMICAL AND BACTERIOLOGICAL CHARACTERIZATION OF SURFACE WATERS (WELL, POND AND LAGOON) AND INDUSTRIAL WASTEWATER IN COTONOU CITY (BENIN)

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Abstract: The reach of main objective of most sustainable development programs requires better water resource management. This study is part of this context and aims to assess the physico-chemical and bacteriological quality of four different types of water (well and pond of vegetables area of Cadjèhoun, lagoon of Cotonou and wastewater of SOEBBRA brewery) used by the population of Cotonou city for many uses. The physical parameters (temperature, pH, conductivity, turbidity, dissolved solids), chemical (phosphates, nitrates, nitrites, total nitrogen, dissolved oxygen, biological oxygen demand and chemical oxygen demand) and bacteriological (faecal Coliforms, fecal *Streptococcus*, *Escherichia coli*, Salmonella and Clostridium) were evaluated and compared with international standards. The main results are clearly shown a degradation of the quality of aquatic ecosystems studied. Except for the turbidity, the content of nitrites and total nitrogen, all other evaluated physico-chemical parameters did not respected the standards recommended for these waters. Strong organic pollution was recorded in these waters. These waters contained significant microbial communities including fecal pollution indicator bacteria (fecal streptococcus and faecal coliforms) and certain pathogens bacteria (*Escherichia coli*, Salmonella and Clostridium). The levels of these pollutants vary from one evaluated water to another. These different pollutions result from anthropogenic activities in the areas surrounding these water sources. Thus, the people using these waters are exposed to assured health risks. These waters must always be treated before any use.

Keywords: Surface water, wastewater, aquatic ecosystems, bacteriological quality, physico-chemical quality, pollution, contamination, Cotonou.

Introduction

Water is not a commercial product like any other but a heritage that must be protected, defended and treated as such. Unfortunately, over the last twenty years, human activities have caused huge changes in most terrestrial and aquatic ecosystems. Nowadays, the reach of major objective of sustainable development programs requires better water resource

management. One statistic illustrates the economic and social burden of polluted water: 80% of the diseases of the world population are caused by water (Cazalas and Gautron, 1993). Thus, the sustainable management of wastewaters, surface waters and underground waters is a global challenge for the XXI century.

In Benin, surface waters (lakes, lagoons, ponds, oceans, etc.) are often used as the receiving environment for wastes from residential areas, industries and manufacturing plants, which dump their untreated or poorly treated sewage. In urban or suburban areas, agriculture through agricultural inputs also helps to degrade the quality of groundwater. Thus, the indirect effects of ecosystem disruption due to continuous release of chemicals products in aquatic ecosystems are exposed the population to health, ecotoxicological and economic risk such as dystrophisation (hyper eutrophication).

Indeed, the excess nutrients (nitrates and phosphates) in aquatic ecosystems cause micro plankton or lake toxic outbursts inducing waterborne infections. Thus, the contamination of aquatic food by toxins and chemical pesticides has serious health damages and causes of low yields in agriculture. It is therefore essential to recognize and assess the hazards of accumulation of pollutants (nitrogen, phosphate and heavy metals) in aquatic areas by waste or polluted waters from agricultural, domestic and industrial activities.

Note that the nitrogen and phosphorus pollution are not limited to agricultural activities. Each inhabitant rejects per day 9-12 g of nitrogen (mainly associated with urine) and 3-4 g of phosphorus, mainly from detergents and washing powders where its use is to limit the disadvantages (scaling) induced by water hardness. We also find them in solid and dissolved forms in wastewater and surface waters (Miquel, 2003). Phosphorus and total nitrogen can thus be transformed into nutritive substances and accelerated the eutrophication phenomenon of aquatic systems.

Also, when the aquatic environment receives discharges of animal or human origin, the number and type of bacteria are capable of rendering the water unfit for human use (Errochdi, et al., 2012). In general, the development of microbial communities in surface waters and in groundwater's is influenced by weather-related factors and all the physico-chemical and biological characteristics of the biotope (Aguiza Abai, et al., 2014).

Currently, quantitative data of the chemical and bacteriological pollutions of Beninese watercourses are not available. In this context, this study aims to assess the physico-chemical and bacteriological quality of the waters collected from the lagoon of Cotonou, vegetables

production area of Cadjèhoun and the outlet of Beninese Society of Brewery in Cotonou city (West Africa).

1. Material and methods

1.1. Studied area and location of sampling sites

Cotonou city (economic capital) is located in south of Benin Republic in West Africa (Figure 1). It covers an area of approximately 79 km². It is located on the Benin Gulf on a sandy offshore bar between latitude 6°21' north and longitude 2° 26' south. It consists of a coastal plain containing wetlands and marshlands associated with major water systems (lake of Nokoué, lagoon of Cotonou and Atlantic ocean). It extends from either side of the lagoon of Cotonou connecting the lake of Nokoué and Atlantic Ocean. It is bounded by lake of Nokoué (north), the Atlantic ocean (south), the district of Seme-Kpodji (east) and the district of Abomey-Calavi (west). The city of Cotonou receives annual precipitation of 1.300 mm. The maximum rainfall occurs in June (300 mm to 500 mm). This city is characterized by two dry seasons (July-September and November-March) and two rainy seasons (March-July and September-November). The annual average temperatures are 26 °C (minimum) and 30 °C (maximum).

The city of Cotonou has several market gardens including the Cadjèhoun. This market garden is the most frequented by the population of Cotonou city. The Cotonou city is also home to several types of industries with Beninese brasserie of SOBEBRA whose rejects directly the wastewaters into lagoon of Cotonou. Finally, the studied lagoon of Cotonou is a channel of more than 4 km located at 49 m of altitude.

1.2. Types and waters sampling

Four different types of water have been investigated in this study (Figure 2). This is the waters collected from the lagoon of Cotonou (a), pond of market garden of Cadjèhoun (b), outlet of Beninese brewing industry of SOBEBRA (c) and well of market garden of Cadjèhoun (c).

The water samples were collected in 2014 during the dry season (November to March) to avoid the risk of interference with rainwater. Three sampling campaigns were conducted. During each campaign, four water samples were collected per site. A total of 48 water samples were collected. After homogenization of the water source, the water samples were taken using sterile bottles borosilicate (autoclaving at 180 °C for 1 h) according to the instant manual sampling method. The samples were immediately transported to the laboratory in a refrigerated cooler at 4 °C to reduce microbial growth. The different analyses were performed

in the laboratory of Hygiene and Basic Sanitation of the Department of Mines, Energy and Water (Benin).

Illustrations

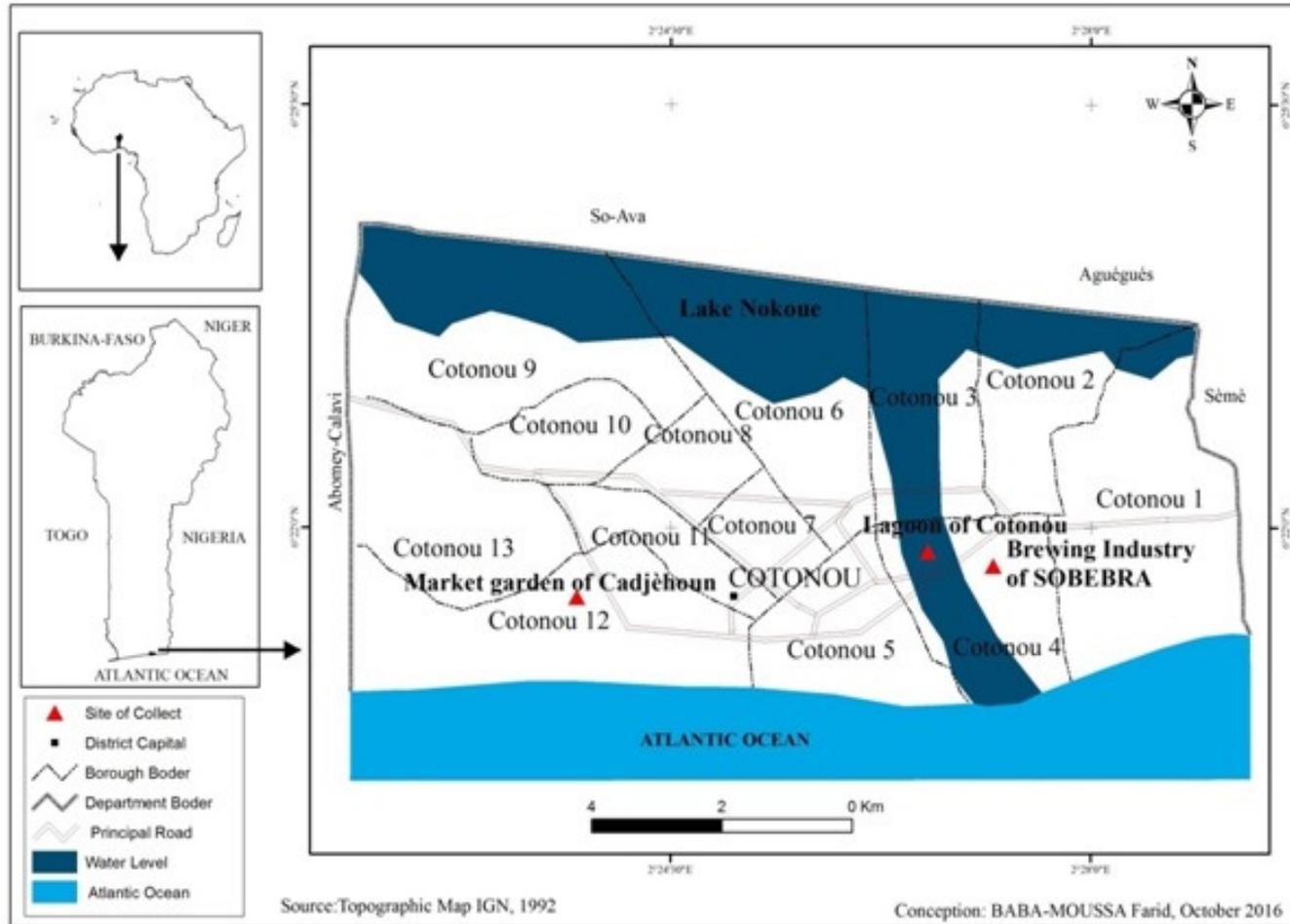


Figure 1: Geographical location of studied area and sampling sites

1.3. Physical parameters and methods of analysis.

The physical parameters such as temperature, pH and total dissolved solids, conductivity and turbidity were measured *in situ* by electrochemical method using respectively a pool thermometer (TFA T303033), a pH-multifunction meter (Multi line P4), a conductivity meter (WTW 330) and a portable turbidity meter (Wagtech).

2.4. Chemical parameters and methods of analysis.

The evaluation of chemical quality of water samples was performed essentially by colorimetry methods. The content of nitrates, nitrites and phosphates were determined by spectrophotometric method according to standard method of AFNOR NF T-90-10. Thus, nitrates, nitrites and phosphates were assayed respectively at 537 nm, 470 nm and 660 nm (spectrophotometer HACH DR 2000). Total nitrogen content was determined by the Kjeldahl method (1883). The dissolved oxygen was measured by the volumetric method using an oximeter (WTW). The Biochemical Oxygen Demand over five days (BOD₅) is performed according to the instrumental method described by Rodier, et al. (1996). The Chemical Oxygen Demand (COD) has been determined according to the standard method of AFNOR (T90-101).



Figure 2: Different types of sampled waters: (a) A public defecation point in Cotonou lagoon (b) The ladies was cleaning vegetables in Cadjèhoun pond, (c) SOBEBRA outlet rejecting waste water into Cotonou lagoon, (d) Well of Cadjèhoun.

2.5. Bacteriological parameters and methods of analysis.

Microbiological analyzes were performed under aseptic conditions according to standard method (ISO 7218) used in food microbiology. The water samples were prepared and the decimal dilutions were performed according to the guidelines of the ISO 6887 standard.

Enumeration of faecal coliforms and Escherichia coli: Fecal coliforms and *E. coli* were counted on Lactose agar containing TTC (Tetrazolium 2-3-5-tréphénylchloride) and Tergitol by the membrane filtration technique according to NF EN ISO 9308-1 (2000). These plates were incubated at 44 °C for 24 h. After incubation, the colonies of yellow, orange or brick red colors are assumed to be fecal coliforms, while orange colonies clearly are presumed *E. coli*. Confirmation of these species was realized using the oxidase and indole tests. Fecal coliforms are oxidase negative while *E. coli* are oxidase negative and positive for indole.

Enumeration of faecal Streptococcus: The enumeration of faecal streptococcus was performed according to the guidelines of French standard NF T 90-461 (2001). The water samples were inoculated onto Slanetz and Bartley medium and incubated for 48 h at 36 ± 2 °C. Streptococcus colonies (red or maroon) were confirmed by the catalase test (negative) and their growth in Esculin Bile Agar medium (presence of halo).

Research of Salmonella spp.: The research of *salmonella* spp. was conducted according to standard method of ISO 6579 (2002) using the simple method of pre-enrichment of nutrient broth (buffered peptone water, 37 °C for 16 h), followed by enrichment into broth Müller Kauffmann added to Tetrathionate (MKTTn). The bacterial suspensions were incubated at 37 °C for 21 h). The isolation was then performed on Xylose Lysine Deoxycholate (XLD) agar medium then on Salmonella-Shigella (SS) agar. Typical or suspect colonies of *Salmonella* spp. (red on XLD and colorless on SS) were confirmed by biochemical tests (urease and β-galactosidase).

Research of sulphite-reducing Clostridium: *Clostridium* spp. were searched according to the horizontal Method (ISO 7937, 2005). Each sample was transferred into tubes and heated for 10 mn at 80 °C to destroy the microbial vegetative forms and only keep the spore forms. Nine millimeter of Tryptone Sulfite Neomycin (TSN) agar medium are added, followed by 3 to 4 drops of paraffin oil (to create anaerobic conditions). This preparation was incubated during 24 h at 37 °C. The large black colonies are derived from sulphite-reducing anaerobic spores.

2.6. Statistical processing, statistical analysis and map realization

The collected data were processed with Microsoft Office 2013 spreadsheet. The figures and tables were also made using the same spreadsheet. Each evaluated parameter was subjected to

analysis of variance using R statistical software 3.03, to determine the nature of statistical difference of parameters between the four studied waters. The Geographical map showing the studied area and sampling sites was realized using the Arc Map software version 9.2.

2. Results and discussion

2.1. Physical characteristics of studied waters

For all physical parameters, the differences observed from one type of water to another were very highly significant ($p < 0.001$).

Temperature: The values of temperature ranged from 24.33 °C (well water) to 44.67 °C (wastewater from brewery) (Figure 3a). The temperature varied very little between the surface waters (wells and ponds of Cadjèhoun market gardener). The temperature was increasing on lagoon of Cotonou. According to the standard, we deduce that the temperature of surface waters was average. In contrary, according to temperature, the water of Cotonou lagoon is poor quality, while the waste water of brewing industry of SOBEBRA is extremely polluted. This high temperature this wastewater is due to the rejections of boilers and can cause depletion of Cotonou lagoon in Oxygen and biodiversity because this water is discharged into the Cotonou lagoon.

Acidity (pH): The pH values obtained are slightly basic into surface waters (7.67), basic into lagoon of Cotonou (8.72) and strongly basic (11.6) in the wastewater of SOBEBRA brewing industry (Figure 3b). Remember that when pH level is greater than seven, there is the predominance of the gaseous form of ammonia nitrogen (NH_3) which is very toxic especially for fish. A strong predominance of ammonia causes the death of certain fish species. Thus, acid-loving and neutrophil species could not resist to alkalinity recorded in lagoon water. Mara, (1980) report that at pH values lower than 5 or greater than 8.5 affect the growth and survival of many aquatic organisms.

Turbidity: Like previous parameters, turbidity was also varied between the types of water (Figure 3c). Turbidity assesses the quantity of suspended solids (organic debris, clay, microscopic organisms, etc.) in water. It is 0.75 NTU for well water, 10.24 NTU for pond water, 3.83 NTU for water of lagoon and finally 61.07 NTU for wastewater. According to SEEE standard (2008), both surface (well and pond) and lagoon waters have a high turbidity (<15). Contrary to precedent waters, the water of brewing industry is average quality (35-70). This average turbidity observed in the lagoon of Cotonou can be explained by the good flow of this watercourse (El Asslouj, et al. 2007).

Dissolved solids and electrical conductivity: Total Dissolved Solids (TDS) were about 600 mg/l in well water of market garden and lagoon of Cotonou, 3587.33 mg/l in wastewater of brewing industry and 17256.7 mg/l in pond water of market garden (Figure 3d). The quantity of dissolved solids in the water of market garden of Cadjèhoun pond is five times higher than that of the wastewater of brewing industry of SOBEBRA one hand and 28 times greater than that of the waters from well of market garden and lagoon of Cotonou.

The same trend was observed for the electrical conductivity parameter which shows a wide variation of chemical composition of waters (Figure 3d). Referring to guidelines of AGRBC (2011), none of the four waters didn't respect the requirements of the law relating to the conductivity threshold of surface water. According to the evaluation guidelines of SEEE (2008), the well water of market garden of Cadjehoun was good quality ($<1300 \mu\text{S}/\text{cm}$) and that of Cotonou lagoon was average ($<2700 \mu\text{S}/\text{cm}$). In opposite, the conductivity of wastewater of brewing industry was very poor quality ($<7000 \mu\text{S}/\text{cm}$) while that the pond water of market garden of Cadjèhoun is five times greater than that of the wastewater of SOBEBRA brewing industry. The very high values of electrical conductivity at different sampling sites indicate a significant mineralization of matters and confirm the presence of cations and anions such as nitrates and phosphate (Aguiza Abai, et al., 2014). The average values of electrical conductivity observed in waters of market garden (ponds and wells) respectively $34000 \mu\text{S}/\text{cm}$ and $1026 \mu\text{S}/\text{cm}$ are probably influenced by environmental conditions, including the use of chemical fertilizers and pesticides by market gardeners in this site. The water of ponds which the electrical conductivity is very much higher than the norm ($1500\text{-}3000 \mu\text{S}/\text{cm}$) would be inappropriate for irrigation of fields and for watching of vegetables (Figure 2b). The consumption of garden produces from market garden of Cadjehoun constitutes a health threat.

The lagoon of Cotonou in the vicinity of the outlet of brewing industry of SOBEBRA would be affected by it. Their electrical conductivities (respectively $1776 \mu\text{S}/\text{cm}$ and $6948 \mu\text{S}/\text{cm}$) and total dissolved solids ($694 \text{ mg}/\text{l}$ and $3587 \text{ mg}/\text{l}$) were relatively high. This result demonstrates the loads contained in the wastewater of brewing industry of SOBEBRA. This wastewater is continuously flowed into the lagoon of Cotonou (Figure 2c) would result in an advanced pollution latter thus constituting a danger to the public.

2.2. Chemical characteristics of studied waters

The difference of nitrites values between the different waters is no significant ($p > 0.05$). On the other hand, the difference of dissolved oxygen values between the different waters is

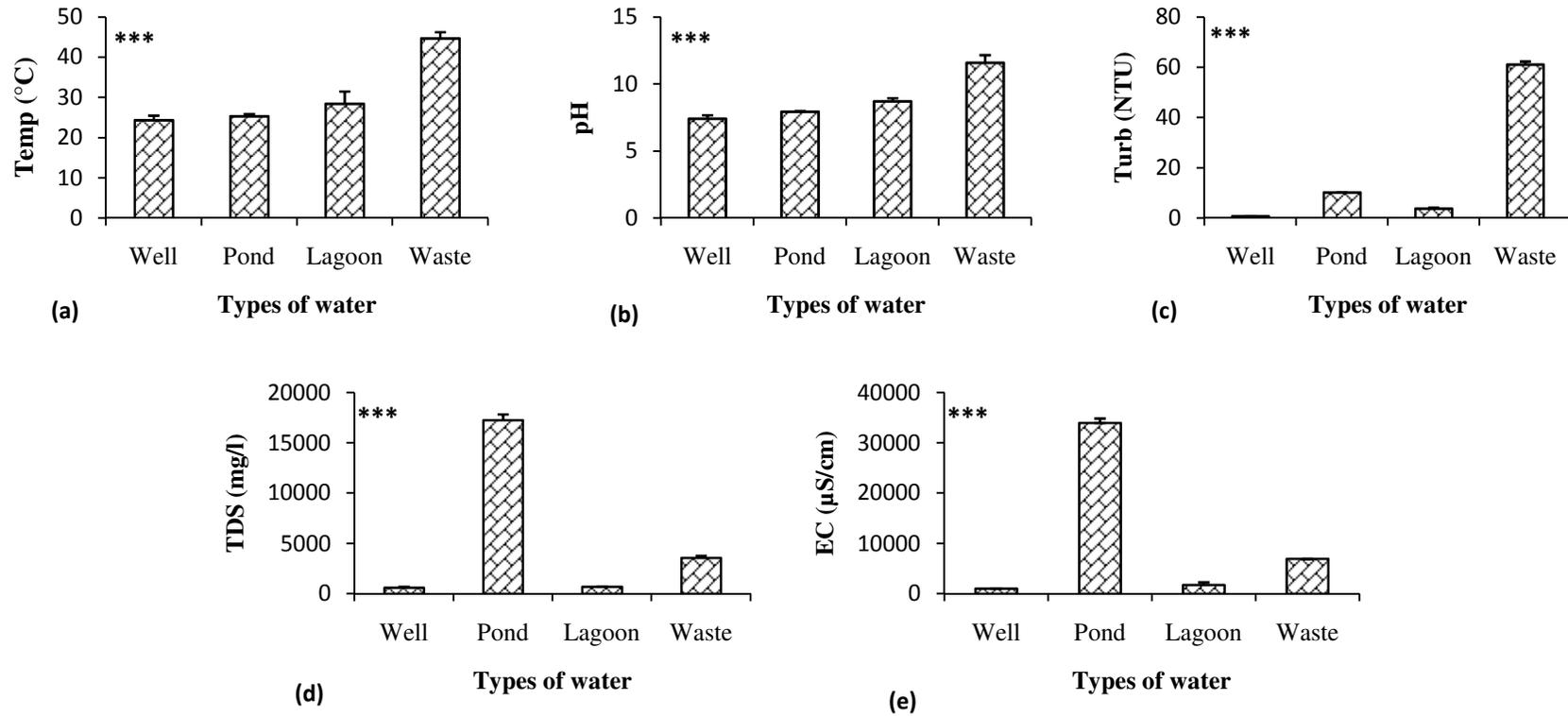


Figure 3: Physical characteristics of different studied waters. Temp: Temperature; Turb: Turbidity; TDS: Total dissolved solids; EC: Electric conductivity; nd = $p > 0.05$ (no difference); * = $p < 0.05$ (significant); ** = $p < 0.01$ (highly significant); *** = $p < 0.001$ (very highly significant).

significant ($p < 0.05$). For other chemical parameters, the difference is very highly significant ($p < 0.001$).

Levels of phosphates, nitrates, nitrites and total nitrogen: On all sites, nitrates values are varied from 5.56 to 32 mg/l (Figure 4b). These values highlight the moderate or net pollution of these waters. The concentrations of nitrates recorded in the lagoon of Cotonou (7.35 mg/l), wastewater of brewing industry of SOBEBRA (15.52 mg/l) and pond of market garden of Cadjehoun (5.56 mg/l) correspond to moderate pollution according to standard (5 to 25 mg/l). The highest concentration of nitrates (31.60 mg/l) obtained in wells of market garden of Cadjehoun shows a clear pollution; it is due to the seepage through the ground water of nitrogen fertilizers used by market gardeners (Errochdi, et al., 2012). Unfortunately, the market gardeners use the same well water to wash vegetables sold to the public. Through this practice, the nitrates are found in abundance in the food chain. Or the excess of nitrates in blood causes the methemoglobinemia due to abnormal accumulation of methemoglobin in hemoglobin. A high content of blood nitrates affects negatively the transport of oxygen to cells causing serious consequences (cancer or death).

The phosphate and nitrate level (Figure 4a) in wastewater of SOBEBRA brewing industry are very higher than those obtained in lagoon of Cotonou. Phosphorus is the major factor affecting the algal biomass in the eutrophication phenomenon. The danger of dystrophisation would spread into the lagoon of Cotonou. Contrary to lagoon, the contents of NO_3^- and PO_4^{3-} in ponds of market garden of Cadjehoun contribute to this phenomenon, which was also observed in the area, by the green color of the water, and abundant of green sheet (phytoplankton) lining the surface of ponds. The phosphorus content is due to the highly mineralization of organic matter (El Asslouj, et al., 2007).

The average value of phosphate (13,5mg/l) spilled at the lagoon is 7 times higher than the sustainable limit of aquatic ecosystems (2 mg/l) in reference to ABE and European Commission standards (1998). This situation may induce in the long run the dystrophisation in the lagoon when the PO_4^{3-} would have exceeded the allowable concentration in the lagoon due among others to the continuous flow of wastewater from the brewery SOBEBRA. This result is in agreement with that found by Dovonou (2011) on the risks of proliferation of aquatic plants by high eutrophication in the lake of Nokoue. It remembers that the Cotonou lagoon is known organic pollution from urban and industrial pollution from SOBEBRA brewing industry). We note the eutrophication phenomenon at the four sites, but this phenomenon is stronger in ponds water and wastewater rejected by brewing industry of SOBEBRA. The N/P

ratio (2) of the limiting factor of eutrophication of these two types of water was at least 3 times lower than 7 (Figure 4i).

Note however that the phosphorus, an essential element in the development of all living organisms, is naturally present in very small quantities in the soil and waters. But the case of most worrying is that pollution generated by phosphates.

Also would it note the highest concentration of PO_4^{3-} (5mg/l) in market garden wells that would be due to the filtration of phosphorus fertilizers used by market gardeners.

The lagoon of Cotonou being a transition area of watercourses from the river of Ouémé to Lake of Nokoue, it was found that there are often trade these waters respectively by the high or low tides generated by the rise levels of ocean. This finding is one of the consequences of global climate due to abuse use of fossil fuels (oil, coal and gas) by industrialized countries that still now the major worrying of the United Nations (Paris conference 2015 on climate, COP 21).

The nitrite contents recorded in the four types of water does not exceed critical thresholds. The nitrites contents were 0.03 mg/l in lagoon of Cotonou and well of Cadjehoun market garden, 0.08 mg/l in pond of market garden of Cadjehoun and 0.11 mg/l in wastewater of SOBEBRA brewing industry. According to SEEE standard (2008), the waters investigated in this study are a good quality in terms of their nitrites contents. The low concentrations of nitrites recorded could be explained by the fact that the nitrite ion (NO_2^-) is an intermediate compound and unstable in the presence of oxygen. The concentration of nitrite is generally much lower than that of the two other forms.

The significant organic load accompanied by a high consumption of dissolved oxygen by the main degradative agents such as heterotrophic bacteria, explain the low dissolved oxygen in these waters. Apart from the market garden wells, the condition of a50% of oxygen saturation (3 mg/l) required to maintain the autopurification is not satisfied in any of the waters. This situation is very marked in Cotonou lagoon which does not promote the rapid elimination of organic matter that is flowed every day there. But the risk of asphyxiation for benthic fauna and flora are related to lack of dissolved oxygen caused by the high demand for oxygen for the oxidation of this large amount of organic matter such as rotten vegetables at bottom of the lagoon, excreta thrown into the lagoon.

Summary assessment of anthropogenic eutrophication: Based on national and international standards, we conclude that the danger of dystrophisation bound phosphate is removed, but it remains effective consequently to nitrates contents. However, the phosphates contents that

avoid eutrophication are much lower. Nevertheless, the eutrophication phenomenon may indeed be triggered from 0.5 mg/l (Miquel, 2003). Similarly, a nitrates concentration of 10 mg/l constitutes an index of pollution in aquatic environments (Rodier, 1996). Indeed, the nitrate levels that avoid eutrophication of watercourses are fifteen times much lower than the allowable levels for drinking water (Miquel, 2003).

It is very difficult to fight against nitrates from agricultural practices. The only solution is to reduce the fertilizer inputs. To avoid eutrophication, the best solution is to reduce the phosphates content to fight against phosphorus inputs through the treatment of urban wastewater and the erosion in the land agricultural. Phosphorus is generally resulted in rivers adsorbed to form solid particles are eroded and washed away during floods. This leads to not let the bare soil in the rainy season, which again has the advantage of consuming a portion of nitrates in the soil. In some regions at risk, it is envisaged to subsidize these crops moist soil protecting soils and reducing the flow of nitrates (Miquel, 2003).

Table 1: Bacteriological characteristics of different studied waters

Microbiological parameters	Average microbial charge (CFU/100ml)			
	Well	Pond	Lagoon	Waste
Faecal Coliforms (FC)	00 (<10 ³)	4.1 x 10 ³ (<10 ³)	22 x 10 ² (< 10 ²)	00 (<10 ³)
Faecal Streptococques (FS)	3 x 10 ³ (<10 ³)	29 x 10 ³ (<10 ³)	9 x 10 ² (10 ²)	64.10 ³ (<10 ³)
Ratio FC/FS	0	0.14	2.44	0
<i>Escherichia coli</i>	3.7 x 10 ³ (<10 ³)	120 x 10 ³ (<10 ³)	19 x 10 ² (10 ²)	00 (<10 ³)
<i>Salmonella</i> spp.	00 (0 /1 1)	2 x 10 ³ (0 /1 1)	00 (0 /1 1)	00 (0 /1 1)
<i>Clostridium</i> spp.	0.3 x 10 ³ (<10 ³)	46 x 10 ³ (<10 ³)	00 (<10 ³)	3 x 10 ³ (<10 ³)
Signification	***	***	***	***

CFU: Colony Forming Units; The numbers in parenthesis represent the OMS standards (1989). nd = p > 0.05 (no difference); * = p < 0.05 (significant); ** = p < 0.01 (highly significant); *** = p < 0.001 (very highly significant).

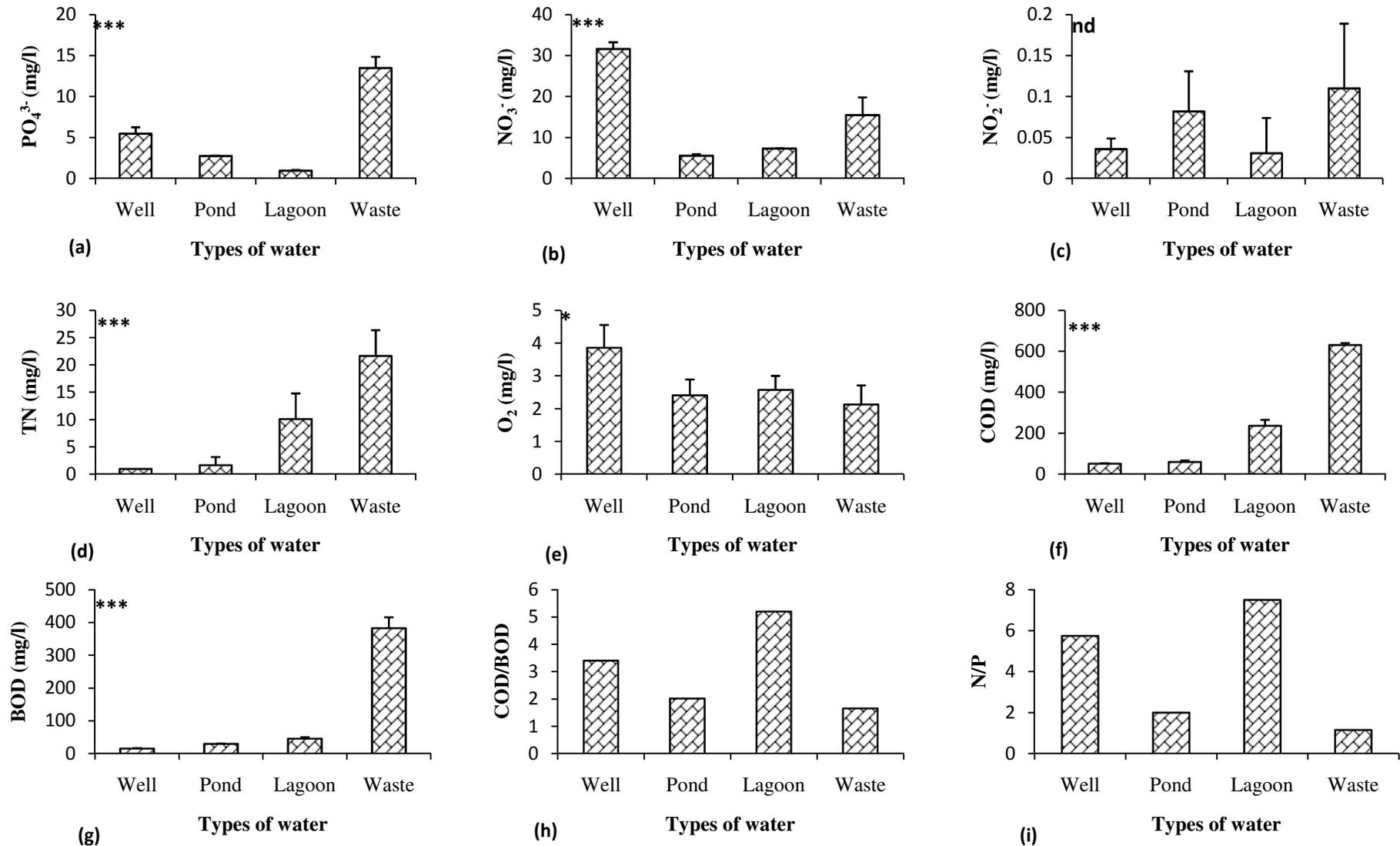


Figure 4: Physical characteristics of different studied waters. PO_4 : Phosphates; NO_3^- : Nitrates; NO_2^- : Nitrites; TN: Total Nitrogen; O_2 : Dissolved Oxygen; COD:Chemical Oxygen Demand;BOD: Biological Oxygen Demand. nd = $p > 0.05$ (no difference); * = $p < 0.05$ (significant); ** = $p < 0.01$ (highly significant); *** = $p < 0.001$ (very highly significant).

We can avoid eutrophication of the lagoon of Cotonou in banning the use of detergents contained the phosphates daily use at Cotonou or also treat urban or industrial wastewaters before discharge into lagoon. A sewer belt can be established around the lagoon of Cotonou to collect domestic wastewaters before they get to the lake, avoiding phosphate intake. Finally, note the need for dredging sediment which is one of eutrophication control procedures.

2.3. Bacteriological quality of various types of water

The difference of microbial density (Faecal Coliform, Faecal Streptococcus, *Escherichia coli*, *Salmonella* spp. and *Clostridium* spp.) between the different waters is very highly significant ($p < 0.001$) (Table 1).

Microbial density of faecal coliform and streptococcus: Well water from market garden of Cadjehoun and wastewater from the brewing industry of SOBEBRA were not contained by Faecal Coliform (FC). The absence of Faecal Coliform in wastewater of brewing industry can be explained by their harsh environmental conditions (45 °C and pH 11.6) affecting the viability of these germs. It is also due to physico-chemical pollution that does not promote the natural purification of oxidizable compounds (Figure 2) whose concentrations are higher in wastewater than the other studied waters. In other hand, the ponds water of market garden of Cadjehoun and those of Cotonou lagoon have contained respectively 4.1×10^3 CFU/ml and 22×10^2 CFU/ml of FC (Table 1). These microbial densities were higher than standard. Indeed, the density of FC must be less than 10^3 CFU/100 ml of ponds water and less than 10^2 CFU/100 ml of lagoon water (OMS, 1989). The microbial densities of Faecal Streptococcus (FS) recorded in the four types of water exceed the recommended standards. The wastewater from the brewing industry of SOBEBRA was the most contaminated by FS (64×10^3 CFU/100ml). This contamination observed in the outlet of brewing industry can be due to the contribution of manure stray animals along the drainage channel. FS are usually found in surface waters that undergoes the faecal pollution (Figure 2a). The fluctuations of faecal germs density are strongly linked to environment factors such as density of animal and human populations. Indeed, the study realized by Dovonou (2008) on Lake Nokoue revealed high contamination by faecal and total coliforms. The authors of the previous study explains this highly faecal pollution of the lake in that area by its high human density and the lack of sanitation provisions and the biggest dumps of garbage observed in the bank.

The ratio of FC/FS for the water of Cotonou lagoon was greater than 1 and indicates that the recorded faecal pollution is of human origin. Indeed, the populations located along the

Cotonou lagoon installing latrines on the lagoon (Figure 2a). The ratio of FC/FS of other types of water is less than 1 and indicates pollution from animal origin. Note that on the market garden of Cadjehoun, market gardeners regularly use animal droppings (chicken, bat, etc.) for crop fertilization. This contamination can come from these droppings.

Microbial density of E. coli, Salmonella spp. and Clostridium spp.: Apart from the wastewater from the brewing industry of SOBEBRA which did not contain *E. coli*, all other types of water were contaminated with *E. coli* at levels exceeding the standard. The water of Cotonou lagoon was 5 times more contaminated with *E. coli* (19×10^2 CFU/100 ml) than well water of market garden of Cadjehoun (3.7×10^3 CFU/100 ml). The highest contamination by *E. coli* was recorded in ponds water of market garden (120×10^3 CFU/100 ml). Note that *E. coli* is a enterobacteria very fears in the clinical area, because it responsible for severe gastroenteritis.

Only the pond water was contaminated with *Salmonella* spp. (2×10^3 CFU/100 ml). *Salmonella* have an important place in the human and animal pathology. It is the pathogen most searches in wastewater. Adjahouinou, et al. (2014) are revealed the contamination of collector water of Dantokpa (Cotonou) with *Salmonella*.

Concerning *Clostridium* spp., apart from the lagoon water of Cotonou, all other waters were contaminated with *Clostridium*. The microbial density of *Clostridium* spp. into well water was acceptable, while that wastewater of brewing industry of SOBEBRA and pond water of market garden of Cadjehoun are greater than the standard. Indeed, *Clostridium* spp. are faecal microorganisms whose spores are highly resistant. The very high density of *Clostridium* spp. recorded in wastewater of brewing industry of SOBEBRA would be justified by the low dissolved oxygen quantity (anoxia) and high temperature (44.67 °C) of these waters would promote the proliferation of *Clostridium* spp. (stuffy and hot channel). Periodic cleaning of the channels would be recommended to prevent the development of these germs. Still have to initially bring the industries to install pre-treatment facilities in their industries to respect the discharge standards in public watercourse. The discharges of industrial wastewater into receiving waters should be subject to environmental regulations.

4. Conclusion

The main physico-chemical and bacteriological results obtained in this study show clearly a degradation level of the quality of studied aquatic ecosystems (wells and ponds waters from the market garden of Cadjehoun, water from the lagoon of Cotonou and wastewater from the brewing industry of SOBEBRA). Apart from turbidity, content of nitrites and total nitrogen,

all other evaluated parameters did not respect the recommended standards for these types of water. The indicators parameters of organic pollution show a very high content of biodegradable organic substances. The ratio COD/BOD₅ of wastewater of SOBEBRA brewing industry indicates that the substances are biodegradable, characteristics of industrial effluents pollution. The ratio COD/BOD₅ of Cotonou lagoon indicates that these substances are mostly toxic and not biodegradable under. These waters have contained high densities of bacteria indicative of fecal contamination. The presence of pathogens such as *Salmonella* spp. and *Clostridium* spp. amplifies the epidemiological risk to the population. Different pollutions recorded come from anthropogenic activities realized in the surrounding stations namely the use of manures, droppings of animals and chemical fertilizers by market gardeners, daily discharge of human excreta in the Cotonou lagoon and along their bank, and finally the systematic discharge of untreated wastewater from the brewing industry of SOBEBRA in the lagoon of Cotonou. Thus, the people living in contact with these waters are therefore exposed to sure health risks. These waters must imperatively be treated before all other uses.

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