

Characterization of the chemical and bacteriological risks of the effluents from some services of the Hassan II Hospital Center in Fez

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Abstract

The Aim of our work was to evaluate the physicochemical and bacteriological risks as well as the concentration of the heavy metals on the liquid effluents of some services of the hospital Hassan II in Fez. The analyses were performed according to the international standards for wastewater discharge recommended by World Health Organization 1989 (WHO). The results showed that these effluents were very loaded with pollutants and constitute a threat to the environment and to the health. Indeed, the concentrations for COD and BOD₅, respectively, in 100% and 75% of the samples were very high and do not comply with the WHO standards as well as for conductivity in 58%, nitrates in 66%, ammonium and the suspended solids in 100% of the samples. While other physicochemical parameters such as pH, temperature and nitrites do not exceed current standards. From a bacteriological point of view, the presence of pathogenic germs was demonstrated, such as fecal coliforms, staphylococcus, fecal streptococci and clostridium at all the studied sampling sites, while the germ of salmonella was absent. The analysis results of the heavy metals comply with the wastewater discharge standards recommended by WHO. The results of this work showed that, the effluents from the Hassan II hospital in Fez require a pretreatment before evacuation in the communal sanitation sewers.

1. Introduction

Water is much more than just a human need; it is the most vital element for life. Unfortunately, its availability is not ensured everywhere and its quality often fails to regard the standards for waste water, which causes multiple risks to environment and human health [1]. The problems related to the depletion of groundwater have begun to be felt and the pollution generated by the effluents comes, among other things, from the activity of the hospital services, specially, that the latter are evacuated in the same way as conventional urban waste the municipal sewerage network without pretreatment.

These releases contain specific substances such as drug residues, chemical reagents, antiseptics, detergents, disinfectants and pathogens such as bacteria, viruses and fungi [2]. They are therefore a major source of emissions of liquid pollutants into the environment and pose a potential danger to human health [3]. The issue of the presence of pollutants in aquatic environments and particularly in water resources is a major international concern because of environmental, health, strategic and financial issues [4].

The aim of this work is to determine, through the analysis results of the various physicochemical and bacteriological parameters obtained, the pollution degree of the effluents for some services in HASSAN II hospital.

2. Materials and methods

2.1. Study area

The CHU is a health establishment created to meet the needs of the population in the regions, Fez-Boulemane, Meknès-Tafilalet and Taza-Al Hoceima-Taounate. Its objective is to improve the rate of medical coverage and to disencumber existing health facilities in these areas. It opened in 2007, but it was in January 2009 that the new hospital complex was inaugurated by His Majesty King Mohammed VI. It is located near the road of Séfrou, in the southeastern part of Fez, over a total area of 12 hectares, with a Covered area of 78 102 m². It is a suburban establishment, vertical of the tertiary level predominantly surgical:

- Surgical services;
- Medical services;
- Medical and technical services;

The hospital has a large activity, illustrated by a high days number of hospitalization (JH) which is 123285 days, for 2011, with an average occupancy rate (TOM) of 13.57% beds, one litter functional capacity (LFC) of 840 beds, an admission (ADM) of 20,661 patients and a large pathological diversification supported by the hospital services.[5]

The sanitation network of the CHU contains several collectors, draining the discharges of various services. It is a collective sanitation with a system allowing the separation of the rainwater from hospital effluents. Beyond the main collector, the system is unitary and all releases are discharged through a single network to the communal sewerage network which extends over a 1200 m path.

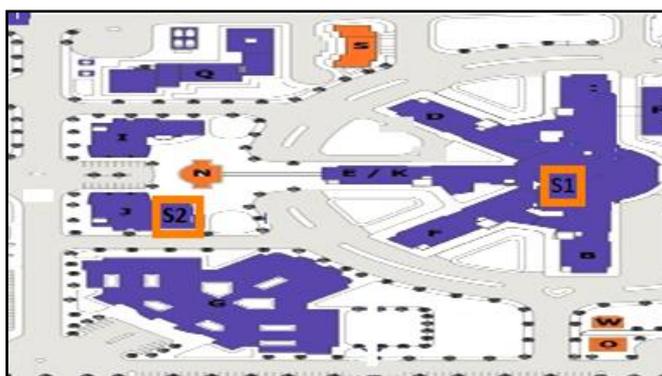


Figure 1: The sampling sites of the Hassan II Hospital Center in Fez

2.2. Sampling

The study was carried out over a six-month period from October 2015 to March 2016. The samples were taken every Wednesday at 11 am where the hospital activity is complete, at the level of two collecting sites. Each site connects one, two or three services: Site 1 and Site 2 (**S1 and S2**). The samples transportation to the laboratory was carried out in an enclosure maintained at a temperature close to 5 ° C to ensure their representativeness. All analysis were carried out within 24 hours of collection.

2.3. Experimental

2.3.1. Parameters and methods of wastewater analysis:

- Physicochemical analysis:

The physico-chemical parameters sought are: pH, temperature, electrical conductivity, Biochemical Oxygen Demand (BOD₅), Chemical Oxygen Demand (COD), Suspended Solids (SS), Nitrates (NO₃⁻), Nitrites (NO₂⁻) and ammonia (NH₄⁺). They are analyzed according to the protocols recommended by Rodier and al. [6]. These parameters were analyzed at the Laboratory of Biotechnology in the Faculty of Sciences of Fez.

The temperature and the pH were measured by the use of a "33100 Jenway" pH meter, and the electrical conductivity by a "wtw 82362 weilhein" conductivity meter. The BOD₅ was determined by the instrumental method by using the BOD meter enclosure adapted to the incubation's conditions and equipped with a WTW stirrer type TS606/3 (temperature maintained at 25 °C for 5 days). The COD was measured by acid oxidation with potassium dichromate in the presence of iron and ammonium sulfate at 120 °C for two hours. The suspended solids were produced by filtering a volume of waste water on a cellulosic filter (0.45 μm mesh). The other parameters were analyzed by colorimetric dosage of nitrites, nitrates and ammonia.

- Analysis of heavy metals:

Heavy metal analyses were performed by using the induced coupled plasma with emission spectrometry (ICP-AES) technique at the Innovation Center of Fez.

- Bacteriological analysis:

The bacteriological parameters were analyzed in the laboratory according to the analysis protocol of Rodier and al 2009 [7]. The used analytical methods are given in Table 1 and are concerned with the counts of fecal germs such as fecal coliforms, faecal streptococci, staphylococci and clostridium and also in the search for salmonella.

Table 1: Incubation conditions, culture media and protocol for the analysis of the seeds [7]

Microorganisms	Incubation	Culture medium	Volume	Method of analysis Rodier and al 2009 [7]
Fecal Coliforms	44°C during 18 à 24 h	Whey Agar TTC and With Tergitol	100 ml	Filtration technology on 0.45 µm filter membrane
Faecal streptococci	37°C/48h	Slanetz-Bartley	100 ml	Filtration technology on 0.45 µm filter membrane
Staphylococci	37°C 24h- 48h	Baird Parker	100 ml	Filtration technology on 0.45 µm filter membrane
Clostridium	24h-48h	TSC	20 ml	Incorporation into agar
Salmonella	Incubate 24h à 42°C then 24h à 37°C	Insemination (42°C) on DCL + hektoen (0.1 ml of culture to 37 °C)	Search for salmonella In 5 liters	Pre-enrichment For 24 hours then Seeding and incubation

3. Results and discussion:

3.1. Physicochemical parameters:

The results of the physicochemical analysis for each parameter are shown in Table 2. The values given represent the average of two samples taken during the same month.

Table 2: Measurement of the physical parameters of effluents: S1 and S2.

		pH	Temperature	Cond (µs/cm)
October	Site 1	7,8	24,9	1642
	Site 2	7,7	23,6	2353
November	Site 1	8,1	22,5	2795
	Site 2	7,4	22	4680
December	Site 1	7,7	19,8	4780
	Site 2	7,4	19	4665
January	Site 1	8,08	19,3	8940
	Site 2	8,1	17,7	5202
February	Site 1	7,65	17	2365
	Site 2	7,7	16	2185
March	Site 1	7,6	16,8	2340
	Site 2	7,5	15,5	2040
	Limit value*	5,5-8,5	30°C	2700(µs/cm)

Limit value * International standards for wastewater discharge recommended by WHO1989 [8]

The temperature is very important for the salts solubility, especially of the gases and the determination of pH. It acts as a physiological factor on the growth metabolism of microorganisms living in water. The average temperature values shown in Table 2 during the six-month study period range from 17.7°C to 23.5°C for the two sampling sites and are in line with the WHO (1989) standards which set the maximum temperature at 30°C. These values are consistent with those reported at the Provincial Hospital of Sidi Kacem with a temperature of 19.8 °C [9], also the El Ghassani hospital in Fez which is 17.11°C [3] and the hospital of Lyon with a Mean of 15.5 °C [11].

The mean values of the measurement of the hydrogen potential shown in Table 2 show that the pH of all the samples studied remains relatively neutral and varies between 7.4 and 8.1, which meets the WHO standards which the pH in the range [6.5-8.5]. These results are similar to those found for the hospital of Sidi Kacem with a pH of 7.27[9]; While the pH of the discharge from El Ghassani hospital in Fez is 8 [3] and the Mohamed V hospital varies between 7.39 and 8.83 [4].

The study of the evolution of the parameters, indicators of the salinity, showed that the values of the electrical conductivity (CE) shown in Table 2 present the very important mineralization of the effluents, with the highest value being 8940 $\mu\text{s/cm}$ for S1 and 5202 $\mu\text{s/cm}$ for S2. It exceeds the upper limit stipulated by the regulation which is of 2700 $\mu\text{s/cm}$. These results are in agreement with those found by Sadek and al [9], with an average of 3140 $\mu\text{s/cm}$ and by Berrada and al [12] with a value varying between 3340 $\mu\text{s/cm}$ and 3560 $\mu\text{s/cm}$. While Boillot and al [10] recorded, for 17 hospitals, a range of 11.2 to 313 $\mu\text{s/cm}$ below standard and Bouzid and al [4] with an average which varies between 846 $\mu\text{s/cm}$ and 1109 $\mu\text{s/cm}$. The suspended solids represent all the mineral and organic particles contained in the waste water. The Knowledge of the concentration of colloidal elements in wastewater is necessary for assessing the impact of pollution on the aquatic environment. The average quantity of the suspended solids is given in Table 3.

Table 3: Measurements of the medium value of the SS of S1 and S2

SS (mg/l)	October	November	December	January	February	March
Site 1	100	70	104	200	253	267
Site 2	114	88	79	120	133	140

All SS values far exceed those established by the WHO, which is 20 mg/l. The latter are much higher for S1 especially during the months of January, February and March. This result can be explained by the increase in the hospital activity of this department considered among the most important of the UHC. The same results were found at the Mohamed V hospital in Meknes [4] and the Sidi Kacem hospital with values of 297.67 mg/l and 233.33 mg/l respectively [9]. By against, the average value of the SS in the CHRU of Limoges [13] is relatively less and it is equal to 77 mg/l. The different nutrient concentrations are shown in Table 4.

Table 4: Measurements of the concentration of the nitrogen compounds contained in S1 and S2

Nitrogen compounds		October	November	December	January	February	March	Limit Values
NH_4^+	Site 1	2,493	1,885	0,935	3,718	3,095	4,03	<0,5mg/l
	Site 2	0,873	1,578	0,898	0,786	1,4	1,375	
NO_3^-	Site 1	0,391	0,308	1,148	5,886	6,535	6,732	<1 mg/l
	Site 2	0,147	0,844	1,719	3,586	3,470	4,46	
NO_2^-	Site 1	0,172	0,46	0,132	0,349	0,107	0,332	<1 mg/l
	Site 2	0,110	0,125	0,024	0,493	0,108	0,303	

The concentrations of ammonium NH_4^+ are very high compared to the WHO standard that is 0.5 mg/l. These values are more important for the sampling site S1 during the first three months of the year. This result is in perfect agreement with the previous result where the hospital activity appears to be more important during this period. The nitrate concentration values during the two months of October and November remain below the WHO standard that is 1mg/l, while they are experiencing a dramatic increase especially for S1: ranging from 1.148 mg/l in December to 6.732 mg/l during the month of March.

The presence of ammonia in large quantities is indicative of contamination by human-induced releases probably due to the speed transformation of urea into ammonia. These results are consistent with those of Boillot and al [10] and Bouzid and al [4]. However, all recorded nitrite concentrations are below the WHO standard which is 1 mg/l, regardless of the sampling site throughout the working period.

These concentrations could be explained by the fact that the nitrite ions are unstable in the presence of oxygen and easily transform into nitrate ions. This explains why its concentration is generally lower than that of the nitrate and ammonium ion. On the other hand, Bouzid and al [4] found values which are above the standard.

The parameters results of the BOD₅ and the COD are shown in figures 2 and 3. The biochemical demand for oxygen expresses the amount of oxygen necessary for the degradation of the biodegradable organic matter by the microorganisms during 5 days.

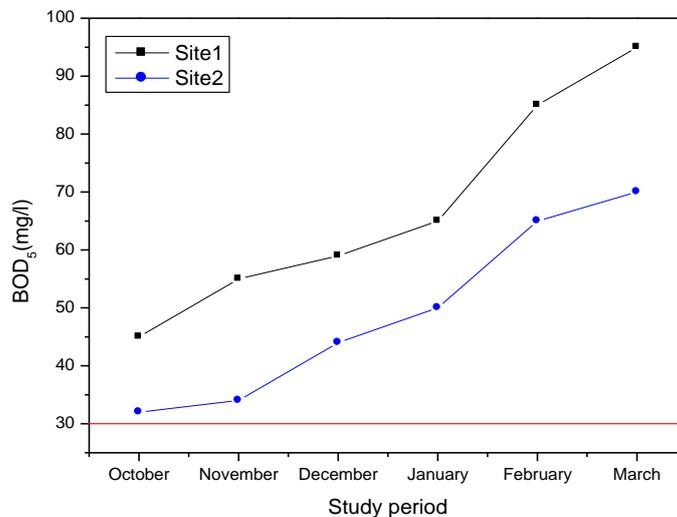


Figure 2: Evolution of the concentration of BOD₅ for S1 and S2

The BOD concentration values show a significant change from October to March with values from 45 mg/l to 95 mg/l for S1 and from 32 mg/l to 70 mg/l for S2. These values are higher than the WHO standard that is 30 mg/l. The mean BOD₅ over the six months was 72 mg/l for S1 and 54.5 mg/l for S2. A similar result was found by Sadek and al [9] with an average of 44.66 mg/l and by Bouzid and al with an average value of 1671, 5 mg/l [4]. The values of the COD measured during the six months are given in figure 3.

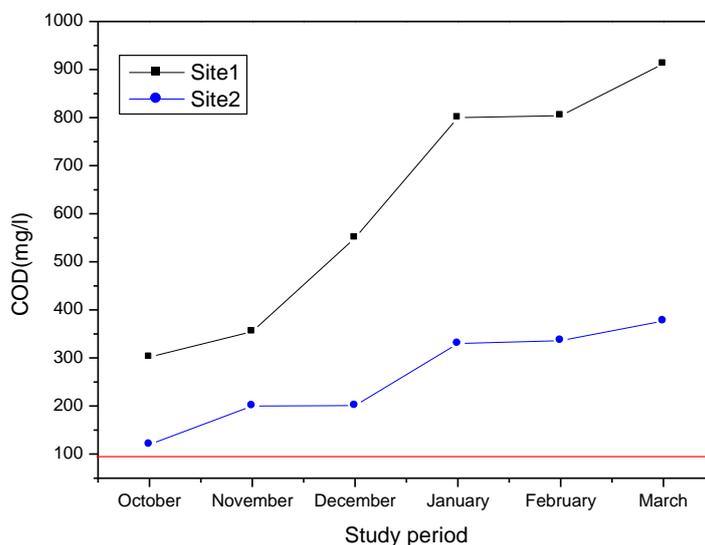


Figure 3: Evaluation of the concentration of COD for S1 and S2

The analysis of the two curves related to the two sampling sites S1 and S2 shows an evolution. This trend is more pronounced for S1. Indeed, the values range from 302 mg/l to 912 mg/l for S1 and from 120 mg/l to 377 mg/l for S2. These exceed the value of the WHO standard which is 90 mg/l. The results are in agreement with that of El Ghassani hospital in Fez, which varies between 115.2 mg / l and 617.5 mg / l [3] and 2709.25 mg/l at the Mohamed V hospital in Meknès [4].

This high increase in the COD and the BOD₅ is probably favored by the diversity of disinfectant and antiseptic products and the abundance of organic matter in the sewers of the hospital, which promotes microbial growth [2].

COD/BOD₅ report:

The COD/BOD₅ ratio allows estimating the biodegradability of organic matter, to determine the degree of pollution and to optimize the physicochemical parameters of a given effluent in order to propose a suitable treatment [14].

The COD/BOD₅ ratio recorded an average value of 10.85 mg/l for S1 and 5.7 mg/l for S2. These values which are in agreement to those obtained at the two hospitals in Rabat [15] are between 6.5 mg/l and 8.21 mg/l.

Therefore, the liquid effluents of the UHC have a high organic load and poor biodegradability due to the chemical nature of the hospital discharge since the COD/BOD₅ ratio is greater than 3 according to ONEP 1999 standards [16].

3.2 Results of heavy metals:

The analysis results of heavy metals by the (ICP-AES) are shown in Table 5.

Table 5: Average concentration of As, Cd, Cr, Cu and Pb.

	As(mg/l)	Cd (mg/l)	Cr(mg/l)	Cu(mg/l)	Pb(mg/l)
Site 1	<0.01	<0.145	<0.01	<0.038	<0.164
Site 2	<0.01	<0.147	<0.01	<0.01	<0.161
Limit Value	0.5	0.2	0.5	3	1

All the values show concentrations in accordance with the standards for the two sampling sites S1 and S2, which is given in the table for the five metals during the six months. This is in agreement with the results found by Tahiri and al [3].

3.3 Bacteriological results:

The seeds sought, identified and counted in the effluents of S1 and S2 are first faecal coliforms then fecal streptococci, staphylococci and clostridium. The relative results are shown in Table 6.

Table 6: Average concentrations of analyzed germs

Analyzed Germs	Prelevement Sites	October	November	December	January	February	March
Fecal ColiformsCFU/100ml	Site1	39,1.10 ⁴	28,7.10 ⁴	16,5.10 ⁴	13,6.10 ⁴	6,5.10 ⁴	4,5.10 ⁴
	Site 2	38,2.10 ⁴	18,9.10 ⁴	9,6.10 ⁴	7,3.10 ⁴	6,3.10 ⁴	7,6.10 ⁴
Fecal StreptococciCFU/100ml	Site 1	15,3.10 ⁴	18,5.10 ⁴	9,5.10 ⁴	8,4.10 ⁴	6,6.10 ⁴	7,4.10 ⁴
	Site 2	46,6.10 ⁴	21,7.10 ⁴	16,8.10 ⁴	11,4.10 ⁴	8,3.10 ⁴	9,7.10 ⁴
StaphylococciCFU/100ml	Site 1	26,9.10 ⁴	16,9.10 ⁴	17,7.10 ⁴	11,2.10 ⁴	4,8.10 ⁴	4,5.10 ⁴
	Site 2	28,4.10 ⁴	27,3.10 ⁴	23.10 ⁴	16,6.10 ⁴	10.10 ⁴	7,8.10 ⁴
ClostridiumCFU/20ml	Site 1	26.10 ⁴	16.10 ⁴	10.10 ⁴	4.10 ⁴	12.10 ⁴	7.10 ⁴
	Site 2	24.10 ⁴	25.10 ⁴	15.10 ⁴	8.10 ⁴	14.10 ⁴	14.10 ⁴

*CFU: Colony Forming Unit

▪ ***Fecal coliforms:***

The values of fecal coliform concentrations all remain above the current standard of 1000 CFU /100ml. In addition, the latter incur a progressive decrease along the period of the study. Indeed, for S1 this value increases from $39.1 \cdot 10^4$ to $4.5 \cdot 10^4$ CFU/100 ml and $38.2 \cdot 10^4$ CFU / 100 ml at $7.6 \cdot 10^4$ CFU / 100 ml for S2. The fecal coliforms could provide information on the eco-toxicity rate of the studied effluent. In addition, Emmanuel and al [11] suggested that the concentration of fecal coliforms may be an indicator of the degree of water pollution and also an indirect indicator of the presence or absence of antibiotics or disinfectants.

The average concentration of the fecal coliforms is $18.15 \cdot 10^4$ CFU/100 ml at S1 and $14.6 \cdot 10^4$ CFU/100 ml for S2. At El Ghassani hospital, the average is $1.9 \cdot 10^3$ CFU /100ml and $6.2 \cdot 10^2$ CFU /100 ml [3]. However, at Mohamed V hospital, a much higher average concentration of $7.44 \cdot 10^6$ CFU/100ml was recorded [17].

▪ ***Fecal streptococci:***

The count values of the faecal streptococci are higher at S2. In fact, they recorded a maximum value of $46.6 \cdot 10^4$ CFU / 100 ml and $15.3 \cdot 10^4$ CFU / 100 ml in October for S2 and S1 respectively; Then decreases during the sampling period to reach the value of $9.7 \cdot 10^4$ CFU / 100 ml for S2 and $7.4 \cdot 10^4$ CFU / 100ml for S1 in March.

The increase in the value of streptococci is justified by the nature of the services provided by the hospital department which deals with infectious diseases.

The average streptococcal count of $10.5 \cdot 10^4$ CFU / 100 ml for S1 and $19.08 \cdot 10^4$ CFU / 100 ml for S2. These results are similar to that found by Bouzid and al [4] which is $15 \cdot 10^4$ CFU / 100 ml and Ameziane and al [17] with an average of $2.28 \cdot 10^6$ CFU / 100 ml. But, they are higher than the results found by Tahiri and al [3] that showed streptococci with an average of $1.1 \cdot 10^3$ CFU / 100 ml and $2 \cdot 10^4$ CFU / 100 ml.

▪ ***Staphylococci:***

The results of the staphylococci count are almost identical at the two sampling sites and show that the samples taken are very loaded with staphylococci. It shows a decrease of $26.9 \cdot 10^4$ to $4.5 \cdot 10^4$ CFU / 100 ml for S1 and $28.4 \cdot 10^4$ to $7.8 \cdot 10^4$ CFU / 100 ml for S2. The mean staphylococcal count was $15.7 \cdot 10^4$ CFU / 100 ml for S1 and $18.1 \cdot 10^4$ CFU / 100 ml for S2. This is in agreement with the result found by Boillots and al [10] which is of the order of $6.08 \cdot 10^2$ CFU/ 100 ml. These results are much lower than the concentration recorded at Mohamed V hospital in Meknes [17] which is $3.6 \cdot 10^7$ CFU / 100 ml. This could be explained by the low consumption of water, cleaning products, disinfectants and detergents by the hospital.

▪ ***Clostridium:***

The microbiological analysis of samples S1 and S2 shows the presence of clostridium in almost identical proportions. The values show a decrease in the number during the study period from $26 \cdot 10^4$ CFU / 20 ml to $7 \cdot 10^4$ CFU / 20 ml at the S1 level and from $24 \cdot 10^4$ CFU / 20 ml to $14 \cdot 10^4$ CFU / 20 ml at the S2 level. The mean value of clostridium is $12 \cdot 10^4$ CFU / 20 ml for S1 and $16,6 \cdot 10^4$ CFU / 20 ml for S2. The same results were found at the Mohamed V hospital [17], which shows a variation ranging from $10 \cdot 10^4$ CFU / 20 ml to $60 \cdot 10^4$ CFU / 20 ml. The latter are recognized for their great resistance to disinfection and they can survive in water much longer than the coliforms. This resistance could explain their concentration in hospital effluents that exceeds the one of urban effluents [17]. The increase in the number of fecal coliforms, faecal streptococci, staphylococci and clostridium during October, November and December is probably related to the exceptional lack of precipitation this year, which contributes to the concentration of the organic load and promotes the development of bacteria. On the other hand, the decrease in temperature during the months of January, February and March further slowed down the growth of the germs, which explains the decrease in the number of germs during this period. In addition, the fecal coliforms could provide information on the eco-toxicity rate of the effluent studied. The results obtained are in agreement with the preliminary studies of Tahiri, Bouzid, Ameziane and al. Which revealed a significantly lower bacterial load than urban effluents, which is 10^4 and 10^6 CFU / ml [4]. Indeed, hospital effluents are classified by the literature as being the least burdened with the coliforms as in wastewater urban areas which are in order of 10^{10} FCU / 100 ml [18]. This low concentration of germs in hospital effluents is probably related to the use of high concentrations of antibiotics and disinfectants [3, 10].

▪ ***Salmonella :***

The search for germs in the effluents showed a total absence of salmonella for the two sampling sites and throughout the period of the study, which is in conformity with the standards in force. On the other hand, the results obtained at the two hospitals [15] revealed the presence of several types of salmonella: salmonella chester, enteritidis (multiresistant), and two multiresistant salmonella.

Conclusions

The analysis carried out during the six months showed that hospital effluents had a high level of pollutants of both physicochemical and bacteriological origin. Indeed, all average values of physicochemical parameters were higher than the international standards for wastewater discharge recommended by the WHO, especially for site S1, such as:

- Conductivity is 3810 $\mu\text{s} / \text{cm}$ For S1 and 3520 $\mu\text{s} / \text{cm}$ for S2.
- SS is 165 mg / l for S1 and 91 mg / l for S2.
- The ammonia content is 96 mg / l for S1 and 1.15 mg / l for S2, and nitrate is 3.55 mg / l for S1 and 2.37 mg / l for S2.
- The concentration of BOD₅ is 72mg / l for S1 and 54.5mg / l for S2 and for COD is of the order of 607 mg / l for S1 and 248mg / l for S2.

The analysis results of heavy metals showed compliance with the WHO standards.

The bacteriological enumeration showed the presence of the pathogenic germs in high concentration, the average number of germs is for:

- Fecal coliforms of 18.15 10⁴CFU / 100 ml for S1 and 14.65 10⁴CFU / 100 ml for S2.
- Fecal streptococci of 10.95 10⁴CFU / 100 ml for S1 and 19.08 10⁴CFU / 100 ml for S2.
- Staphylococci sp of 13.610⁴CFU / 100 ml for S1 and 18.8 10⁴CFU / 100 ml for S2.
- Clostridium spores are 12.5 10⁴CFU / 20 ml for S1 and 16.6 10⁴CFU / 20 ml for S2.

On the other hand, a total absence of salmonella was noticed throughout the study period for both sampling sites. The hospital effluents of the Hassan II hospital were less difficult to biodegrade because the COD / DBO₅ ratio was > 3. It is therefore more than necessary to seek a suitable chemical or biological treatment in order to reduce the organic matter and to improve the quality of the latter.

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