Evaluation of the bacteriological and physicochemical risk of hospital effluents: case of the Mohamed V hospital in Meknes

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ABSTRACT: Like other countries, Morocco is committed to protecting environment. One of the actions is Law 28 00 on waste because it is harmful to environment and to health. However, hospital effluents are getting out of hand. This study looked at those at Mohamed V Hospital, which serves a population of almost 2 million people. The methodology relates to physicochemical and bacteriological analyzes of representative samples of these effluents by monthly samples over a period of one year. These were carried out in front of the four wings of the hospital between 11 a.m. and noon (maximum activity period). The search for bacteria of medical interest was carried out at the medical analysis laboratory of the Mohamed V hospital. The bacterial count as well as the physicochemical analyzes were carried out at the drinking water & wastewater pollution control laboratory of the Autonomous Distribution Authority of Water and Electricity of Meknes and of the environment and health laboratory of the Faculty of Sciences of Meknes. The results showed that some parameters correlate with others and that these effluents pose a threat to the environment and to health. Indeed, the chemical pollution values are high (NH_4^+, NO_2^-, NO_3^-) compared to WHO standards. The presence of pathogenic germs is demonstrated such as pseudomonas, acinetobacter, clostridium, staphylococcus and streptococcus. On the other hand, these effluents are biodegradable since the COD / DBOS ratio varies between 1.1 and 2 (<2).

1. Introduction

Hospitals offer services of various kinds and complexity [1], from simple to more complex procedures such as invasive procedures (surgical procedures, endoscopy, etc.). Indeed, these services allow the care of patients at the level of different care units of the hospital. These generate waste of different kinds. In addition, technological advances in the medical field and the increasingly increased demand for care, lead to an increase in the quantities of waste produced, increasing the risks to human health and the environment [2]. This medical and pharmaceutical waste (D.M.P) is produced by hospitals structures responsible for preventing or reducing risks for patients, professionals and the ecosystem. D.M.P is divided into two types: liquid waste represented by hospital effluents and solid waste [3].

Waste management is unsatisfactory and does not respect the environment; this result has been confirmed by several studies [4]. However, to improve this management, there is no support for effective and appropriate actions; which thus favors an abundance and an increase in negative consequences on environment and on human health [4]. The problem of pollutants' presence in natural environments, especially in water resources, is an international irritation because of environmental, health, strategic, financial and economic issues. Indeed, the generated waste represents between 0.5 and 12 of gross domestic product, of which about one-third for the Healthcare Risk Waste (HCRW) (table I) [5, 6].

In this observation, it's interesting to carry out a study whose objective is to determine degree of physical-chemical and bacteriological pollution related to hospital effluents.

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Table I: Generation of waste from care activities according to Gross Domestic Product.

	All waste generated	Healthcare Risk Waste
High gross domestic product (GDP)	1,1 to 12	0,4 to 5,5
Average gross domestic product	0,8 to 6,6	0,3 to 0,4
Low gross domestic product	0,5 to 3	--

Source: WHO/Safety of Waste Management of Care Activities [6]

Mohamed V hospital of Meknes, given its history, it was built in 1953 and inaugurated on July 17, 1956, by the late His Majesty Mohamed V [8]. In 1987, it was established as Self-Managed State Service in accordance with the joint ordinance of Health Ministry and Finance Ministry N ° 6-87 of 11/27/1986. Its reputation makes it a reference center for care and consultations with regional or even interregional radiation. It is the largest hospital in Meknes /Tafilalt ancient region. According to "High Commission of Planning", it serves a population of around 2,317,000 [9]. It is a vertical hospital consisting of five floors, a ground floor and three subsoil. Its services are arranged in four wings in the shape of a star around a hall [8]. It is located on a constructed area of 3643 m2 [8]. It offers diagnostic and care in 22 medical specialties [10] with 16,059 admissions in 2014 [9], for a hospitalization rate of 0.69%. The functional Hospital Bed Capacity was 378 in 2013 [8] and in 2014 [9]. Since January 10, 2007, health ministry organizes annually the competition quality between the various structures of health [11]. Nationally, on all editions for hospitals in its class, Mohammed V hospital in Meknes is the most successful: he got the third prize in 2009 and the first prize two successive times in 2010 and 2011.

2. Materials and methods

Various physicochemical parameters must be monitored at regular intervals to check physicochemical pollution of discharges. Five-day biochemical oxygen demand, chemical oxygen demand, total suspended solids, total dissolved solids, pH, temperature, exposure time, total nitrogen and total phosphorus are examples of chemical parameters monitored as part of verification [7].

This study was carried out at Mohamed V hospital of Meknes. The frequency of sampling is monthly over a period of one year (March 2013-February 2014) in accordance with the joint ordinance of Equipment Minister and Minister of National Land Use Planning, Town Planning, Housing and Urban Policy n ° 1275-01 of 10 chaabane 1423 (17 October 2002) defining the surface water quality grid, in particular its article 4 which fixes the minimum number of samples on the basis of which the quality of water is assessed at 12 per year for places which are subject to the influence of pollution sources, at a rate of 1 per month [12]. The sampling time corresponds to the maximum activity period, it is between 11 am and 12 noon; P.Jehannin, in 1999 at the C.H. de Hyères, had set

the maximum period of care activities between 10 and 11 a.m [13]. The wastewater from each wing flows into its own manhole before reaching the main collector. The latter receives, in addition to the effluents from the four manholes, other wastewater and rainwater

Sample to be analyzed must be homogeneous and representative. Since contamination level is higher or lower depending on the place and time of sampling [14, 4]. The representative samples consist of manhole water corresponding to each of the four wing constituting the hospital's own care units and during hours corresponding to the maximum amount of care.

Samples are transported to laboratory in an enclosure maintained at an equal temperature close to 5°C (± 3) [15, 16]. The water for bacteriological analysis was sampled aseptically in a sterile 500 cc bottle.

Thus the points retained are:
• Point 1 (or station 1): Manhole collecting effluents from wing containing the laboratory, the medical imaging unit, the central operating room, the B1 Surgery departments. Cardiac ultrasound unit and the penitentiary service.
• Point 2 (or station 2): Manhole collecting effluents from wing containing the trauma, infantile surgery, neurology, cardiology and nest departments.
• Point 3 (or station 3): Manhole collecting effluents from wing containing the interim intensive care unit, neurosurgery, surgery A, pediatrics, endocrinology and gastrology departments.
• Point 4 (or station 4): Manhole collecting effluents from wing containing pharmacy services, intensive care unit, surgery B2, burns, pneumology, Medicine C1 and Infectiology.

The location of points (figure 1) is made on a sketch drawn up from a Google-Map that we have translated into a sketch that has been validated by the hospital administration. Samples are taken in subsoil 3 for points 1, 2 and 3, while those in point 4 are taken in subsoil 2.
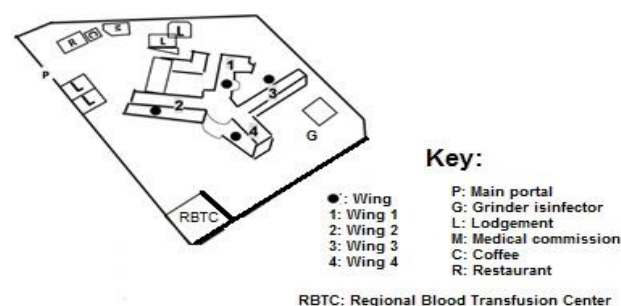

Figure 1: Layout of stations and wings at Mohamed V Hospital

A two liter plastic bottles are prepared to collect the samples. The samples are transported to the laboratory in two enclosures maintained at a temperature of almost 5 °C, one of which is transported to medical analysis laboratory at Mohamed V hospital; the other is sent to drinking water and wastewater pollution control laboratory of the Autonomous Water and Electricity Distribution Authority of Meknes (AWEDAM).

To ensure sample integrity, all analytical procedures are performed or started on the same day of collection or within 24 hours [17]. The arrangement of the four wings is shown in Figure 1.

Bacteriological analysis is divided into two parts: first, the search for and enumeration of bacteria indicative of faecal pollution, carried out at AWEDAM laboratory according to the Rodier 2009 protocol; then looking for bacteria of medical interest (pathogens), carried out in the medical analysis laboratory at Mohamed V Hospital using conventional methods.

Physico-chemical parameters sought are temperature, pH, conductivity, turbidity, SM, NH4 +, NO2-, NO3-, Kjeldehl nitrogen, PO4-, dissolved oxygen, COD and BOD5. They are analyzed according to the protocols recommended by Rodier et al. (2009). Some parameters were measured in situ (temperature, pH and dissolved oxygen), others in laboratory (Conductivity, turbidity, suspended matter, BOD5, COD, NTK, NO4-, NO2-, NH4 +, PO4).

Faecal microbiological contamination of water is a problem that has a major impact on water quality around the world. Pollution indicator bacteria include coliforms (total and fecal), Clostridium Sulfito-Reducers, faecal streptococci [18, 19]. Bacteria of medical interest sought are those that could be found in hospital wastewater. They are thought to be present in stool or in urine (Salmonella, Shigella, Coliforms, Vibrios, Streptococci, Enterobacteriaceae, etc.) [14] or bacteria responsible for infections associated with care (Staphylococci, Streptococci, Pseudomonas, etc.) [14,20].

3. Résultats:

3.1. Physicochemical parameters

Descriptive statistics of physicochemical parameters are shown in Table II.

Temperature: average temperature recorded is 23,265 ° C and oscillates between 18 ° C and 34 ° C. The maximum temperature, 34 ° C, was recorded at station 4 during the month of June. Thus, these effluents are classified of medium quality according to the Moroccan standard (class 2). High temperatures are recorded during the summer season (June, July and August) and low temperatures are recorded during the winter season.

Hydrogen potential (pH): Results show that hospital effluents comply with biological pH range which is between 6.5 and 8.5 [21] because their pH oscillates between 6 and 8.83 with an average of 7.48. We note that the only months that recorded an alkaline pH are April and September with 8.09 and 8 respectively and that in general effluents from Mohamed V hospital are basic.

Table II: Descriptive statistics of physicochemical analyzes

Variable	Min	Max	Moy	Standard deviation
t° (°C)	18,3	34	23,265	4,94
pH	6,5	8,83	7,504	0,47
Dissolved O2	0,16	8,1	2,339	2,16
Conductivity µS/cm	62	14586	2250,51	2793,05
Turbidity	2,3	718	136,77	186,59
Suspended solids	26,7	780	229,16	190,56
BOD5 (mg/l)	20	2890	580,16	706,57
COD (mg/l)	33	5080	1043,56	1079,07
NH4+	2	479	57,87	102,91
NTK	9,6	532,4	63,36	93,18
NO2-	0,01	19,7	3,26	4,76
PO4-	0,33	128,74	21,65	27,32
NO3-	0,01	92,92	37,24	33,34

Dissolved oxygen: Dissolved oxygen testifies to viability of bacterial species. In the present study, it varies from 0.16 mg / l to 12.7 with an average of 2,339 mg / l. The maximum dissolved oxygen value was recorded at station S2 in August, while low values were recorded at station S1 in March and November.

Conductivity: Study of evolution of parameters indicative of salinity showed that measurement of the average electrical conductivity reached a value of 2250,51 µs / cm with a very large variation ranging from 62 to 14586 µs / cm.

Turbidity (NTU): Turbidity recorded varies between 2.3 and 718 NTU (Nephelometric Turbidity Unit) with an average of almost 136,77 NTU.

Suspended matter (SM): SM vary between 26,7 and 780 mg / l with an average of 229,16 mg / l.

Biological oxygen demand (BOD5): Average BOD5 is 580,16 mg / l with a large variation, ranging from 20 to 2890 mg / l. High values are noted at station S4 in April and May while low values are recorded at station S1 in winter and summer.

Chemical oxygen demand (COD): At Mohamed V hospital in Meknes, COD varies dramatically from 33 to 5080 mg / l with an average value of 1043,56 mg / l. COD values are important at stations S3 and S4 during the spring season.

Nitrogen compounds: Table III shows the descriptive data relating to nitrogen compounds in effluents of Mohamed V Hospital.

Table III: Nitrogenous compounds contained in effluents of Mohamed V hospital in Meknes

	NH4+	NTK	NO2-	NO3-
Average	57,87	63,36	3,26	37,24
Minimal	2,00	9,6	0,01	0,01
Maximal	479,00	532,40	19,7	92,92
WHO standard	<0,5	< 4	1	<1

3.2. Bacteriological parameters

Clostridium is present with an average concentration of 164 CFU / 100ml (from 7 to 1407 CFU / 100ml). Total coliforms are present with an average of $4.5.10^6$ CFU / 100ml with a very significant variation ranging from 8.10^2 CFU / 100ml to 12.10^7 CFU / 100ml. Thermo-tolerant coliforms (feces) are identified at an average concentration of 10^5 CFU / 100ml. The enumeration of streptococci led to an average value of $1.35.10^8$ CFU / 100 ml. Bacteriological analysis identified and isolated *Pseudomonas putida*, *Acinetobacter baumanii*, *Staphylococcus aureus*, *Streptococcus sp.*, *Clostridium perfengens*. *Enterobacter aerogenes*, *Proteus sp*, *Serratia marcescens* and *Escherichia coli*.

4. Discussion

Temperature:

Temperature plays a very important role in solubility of salts and especially gases, and pH determination [22]. It also acts as a physiological factor on growth metabolism of microorganisms living in water. The average temperature recorded is 23,265° C, compared to 18.9 ° C at the same hospital in 2013 [23]. This increase could be explained, first by the fact that effluent pipe passes near boiler and kitchen just before being emptied into view and second by temperature influence of discharges in exit from laundry, from that of oils and fats from kitchen. This result is in accordance with the international standard established by WHO (1986) which sets the maximum temperature at 30 ° C. Thus, these effluents are classified of medium quality according to the Moroccan standard (class 2). These values agree with those recorded at Sidi Kacem hospital center with a temperature varying from 17 to 22 ° C with an average of 19.8 ° C [24] and those at Elghassani hospital in Fez which is 17.11 ° C [25].

Hydrogen potential (pH):

Hydrogen potential varies depending on nature of the basic or acidic effluents. It is an indicator of water pollution. The biological pH range is 6.5-8.5, lowering pH can increase toxicity [21]. Results show that effluents from Mohamed V Hospital comply with biological pH range which is between 6.5 and 8.5 [21] because their average pH is 7.48. This pH increased from 2013 when it was 6.9 [23]. We note that in general the effluents from Mohamed V Hospital are basic. According to Moroccan standards, these effluents switch between good and bad quality. In France, the interval is 5.5 to 8.5 [26]. The pH of wastewater from Sidi Kacem hospitals in 2012 was around 7.27 [24]. In Lyon, Boillot noted in 2008 a pH ranging from 5 to 8.8 [27].

Dissolved oxygen:

Dissolved oxygen testifies to the viability of bacterial species. In the present study, its average content of 2,339 mg/l increased by 2.03 mg/l since 2013 [23]. "The classes of aptitude for biology" of the 2nd version of the Evaluation Grids of the System for evaluating the quality of water in rivers classifies these effluents in the red zone and therefore of poor quality [28]. This wastewater is of poor to very poor quality according to Moroccan standards [29].

Conductivity:

Study of the evolution of parameters indicative of salinity has shown that measurement of average electrical conductivity has more than doubled since 2013 (969.67 µs/cm [23]) to reach a value of 2250,51 µs/cm with a very large variation ranging from 62 to 14586 µs/cm. The Moroccan standard classifies these effluents between class 3 and 5 that is to say from average to very poor quality. At Sidi Kacem Provincial Hospital, average conductivity is 3140 µs/cm [24] while Boillot, in Lyon, recorded, for 17 hospitals, an interval ranging from 11.2 to 313 µs / cm [27].

Turbidity (NTU):

Replication of microorganisms is noted by production of turbidity [30]. That, raised is on average 136,77 NTU. We can then say that these effluents are very cloudy since discharge standard is 5 to 30 NTU. This result is not in line with that of provincial hospital of Sidi Kacem in 2012 where turbidity is 14.76 NTU [24] and that of two large hospitals in region of Rabat Salé Zemmour Zaer in 2014 where it varies between 18 and 194 NTU [31].

Suspended matter (SS):

The average SS content (229,16 mg/l) has fallen by almost half compared to 2013 [23] but remains largely high compared to the WHO standard which is less than 20 mg/l. The suspended solids concentration must not exceed 100 mg/1 in the case of discharge into a collective sanitation network without a wastewater treatment plant [32] such as in the case of Mohamed V hospital. the latter comply with the Moroccan standard being set at a value between 200 and 1000 [29], but remain above wastewater discharge standards set at 50 mg/l [24]. These results are in line with those of Sidi Kacem hospital where SS content was 233.33 mg/l in 2012 [24], with those in Lyon in 2008 where they were 46 to 298 mg/l [27] and with those of CHRU in Limoges where average suspended solids was 77 mg/l in 2002 [14]. In United States of America, hospital wastewater is mainly domestic and characterized by a solids content of between 60 to 200 mg/l [27].

Biological oxygen demand (BOD5):

The average BOD5 of effluents from Mohamed V hospital decreased by approximately 25% from 2013 to now with 737.5 mg/l [23] and 580,16 mg/l respectively. Most often, it is far from within the WHO discharge standards set at less than 30 mg/l [21]. It must not exceed 100 mg/l (32) or must be between 50 and 400 mg/l [33]. The Moroccan standard classifies these effluents as being very bad since their BOD5 content exceeds 25 mg/l [29]. These results are not similar to those of provincial hospital of Sidi Kacem in which wastewater has a BOD5 content of 44.66 mg/l [24]. They are also not consistent with Darsy et al. at Limoges University Hospital in 2002 with a BOD5 measured at 177 mg/l on average and which reached 1095 mg/l as a maximum value [14]. United States-Environmental Protection Agency (US EPA) in 1989, points out that "wastewater from hospitals is essentially domestic and can be characterized by measuring global parameters within following limits: BOD5: 50 to 400 mg/l [27].

Chemical oxygen demand (COD):

At Mohamed V hospital, the COD, with an average value of 1043,56 mg/l, decreased from 1420 mg/l in 2013 [23]. The effluents are thus classified as being of very poor quality according to Moroccan standards (COD> 80mg/l). This content is well above WHO standard which is set at less than 90 mg/1. At Elghassani hospital in Fez in 2009, it is 115.2 mg/l to 617.5 mg/l [25]. At Limoges CHRU, it is on average equal to 539 mg/l [14]. In United States of America, United States-Environmental Protection Agency (US EPA) in 1989, indicates that the COD limits of hospital effluents are between 150 to 800 mg/l, classifying them as being mainly domestic [27].

Nitrogen compounds:

Nitrogen exists mainly in ionic form (ammonium NH_4^+, nitrite NO_2 and nitrate NO_3^-) as well as in gaseous form (N_2). Hospital wastewater contains organic nitrogen and ammoniacal nitrogen. The first is an element constituting living cells. The second (NH_4^+) comes from decomposition of organic nitrogen by bacteria and direct discharges (urine, feces). Ammonia and nitrogen dioxide pose problems for public health. They induce bacterial proliferation in water. The main source of concern is NO_3 nitrates. These ions are transformed in a weak acidic medium into nitrite ions which are toxic to human organism. Levels of ammonium, Kjeldahl nitrogen, nitrites and nitrates are all in excess of discharge standards set by WHO [28]. By comparing our results with Moroccan standards [29], we can advance that in terms of ammonium and Kjeldahl nitrogen, effluents from Mohamed V hospital are classified as very bad (NH4 +> 8mg/l and NTK> 3mg/l), while in terms of nitrates, they are classified as of medium quality (NO3- between 25 and 50 mg/l).

Orthophosphates:

The average phosphate levels (21,65 mg/l) increased since they were at a value of 10.65 mg/l in 2013 [23] and. They exceed WHO phosphate standard, which is set at a content of less than 2 mg/l [28]. While for Boillot, in Lyon, they are between 6.15 mg/l and 17.3 mg/l [27].

Results by parameter (analysis):

Descriptive statistics of physicochemical parameters are presented in Table II. The correlation matrix (Pearson (n)) tells us that there are four positive correlations. These are:
• The COD correlates positively with BOD5 (0.927) and SM (0.631)
• NH_4^+ correlates positively with BOD5 (0.567), COD (0.647)
• Kjeldahl nitrogen correlates positively with NH_4^+ (0.742), SM (0.663), BOD5 (0.718), COD (0.784)
• NO_2 positively correlates with NH_4^+ (0.509), SM (0.511), BOD5 (0.893), DCO (0.799), NTK (0.715)
Principal Component Analysis:
F1 and F2 axes have 53.88% (40,3 et 13,6) of information. Analysis of results shows that most of information is explained by the first two factor axes.

The formation of factorial axes F1 and F2 is characterized by the fact that no parameter contributes more than 20% (table IV). Based on the principle that only variables whose contribution is greater than the average contribution are retained for interpretation (Table IV), we can state that: BOD5, COD, nitrites, ammonium and Kjeldahl nitrogen are well represented on F1 axis; while temperature, conductivity, SM, NTK and orthophosphates are well represented on F2 axis. Nitrates are well represented on both axes.
Figure 2 shows that there are two well-distinguished correlation groups: the first consists of biological oxygen demand (BOD5), chemical oxygen demand (COD), nitrites (NO_2^-), ammonium (NH_4^+), suspended solids, turbidity, nitrate ions (NO_3^-), conductivity and hydrogen potential (pH) and Kjeldahl nitrogen (NTK). While the second group is made up of orthophosphates (PO_4) and dissolved oxygen.
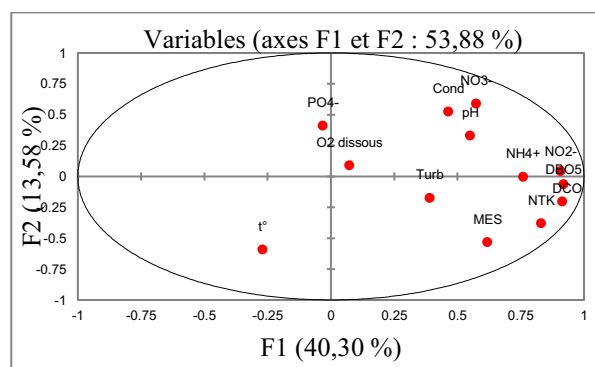

Figure 2 : Graphic representation of PCA

Organic and mineral pollution gradient starts from station S3, passes first through station S2 then station S4 and finally station S1 (figure 3). Orthophosphates are more important in S2 and S4 stations (figure 2 and figure 3)
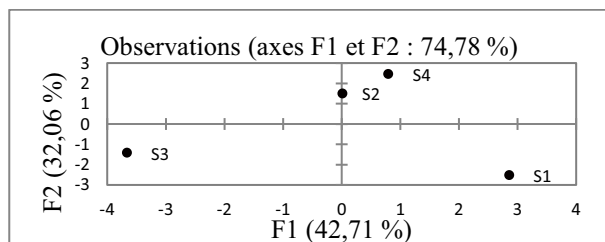
Figure 3: Principal Component Analysis of stations

Station S2 correlates positively with station S4 (Figure 3). Biological oxygen demand (BOD5), chemical oxygen demand (COD), nitrites NO2, ammonium NH_4^+ and Kjeldahl nitrogen NTK are well represented on F1 axis (table IV and figure 2). On the other hand, temperature, conductivity, suspended matter MES, Kjeldahl NTK nitrogen, orthophosphate PO4 + and nitrates NO3- are well represented on F2 axis (table IV and figure 2).

Table IV: Contributions of variables (%):

	F1	F2
t° (°C)	1,383	19,755
pH	5,790	6,212
Dissolved O2	0,103	0,462
Conductivity	4,105	15,670
Turbidity	2,917	1,724
Suspended matter	7,315	16,023
BOD5	16,134	0,224
COD	15,963	2,345
NH4+	11,026	0,000
NTK	13,198	8,145
NO2-	15,743	0,111
PO4-	0,019	9,616
NO3-	6,303	19,714
Average contributions	7,692	7,692

Physico-chemical analyzes of effluents from Mohamed V hospital concluded that these present both organic and mineral pollution with reference to national and WHO standards. Results showed that these effluents pose a threat to environment and health. Indeed, chemical pollution values are high (NH_4^+: avg = 59.6 mg/l against WHO standard of 0.5 mg/l; NO_2^-: 3.7 mg/l against WHO standard which is l mg/l; NO_3^-: 34.54 mg/l against WHO standard which is 1 mg/l; Kjeldahl nitrogen (NTK): 60.21; orthophosphates PO4: 18.56 mg/l). On other hand, Mohamed V hospital effluents are biodegradable since average COD / DBOS ratio is 1.76 (<2).

Evolution of physico-chemical parameters over the years:

Effluents of Mohamed V Hospital in Meknes change over time. To follow this evolution, we compared our results for certain parameters with those of 2013 (23) for the same hospital.
• Temperature: over years, effluents of Mohamed V hospital have gained almost 4 ° C between 2013 and 2016.
• The pH: it has evolved with + 0.4
• The conductivity rose from 969 to 2611, ie an increase of + 270%.
• The ratio between dissolved oxygen content of our study and that of 2013 is equal to 3.74.
• SM fell by almost half

• BOD5 and COD lost 25% and 32% respectively from 2013 currently.
• Nitrite concentration fell by 80%, going from 18.09 mg/l in 2013 to 3.7 mg/l now. On other hand, nitrate content increased more than ten times, going to 34,54 mg/l currently after having been at 3.19 mg/l.
• Orthophosphates increased by a coefficient of 1.74.

5. Conclusion

Results showed that some parameters correlate with others and that these effluents pose a threat to environment and to health. Indeed, chemical pollution values are high (NH4 +, NO2-, NO3-) compared to WHO standards. Presence of pathogenic germs is demonstrated such as pseudomonas, acinetobacter, clostridium, staphylococcus and streptococcus. On other hand, these effluents are biodegradable since the COD / DBOS ratio varies between 1.1 and 2 (<2).

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