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Bacteriological Quality of Landfill Leachates: Case of the Uncontrolled Landfill of the Koubia and Niamey 2000 in Niamey-Niger (West Africa)

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Abstract
The purpose of this study was to evaluate the microbiological quality of leachate from the Koubia and Niamey 2000 landfills. A total of twenty samples were collected in 500mL flasks. Temperature, pH, electrical conductivity and dissolved oxygen content were measured in situ. The enumeration of indicator bacteria of fecal contamination was carried out by the method of plating by incorporation in specific nutrient medium. Four bacteria were sought. The values noted for the physico-chemical parameters were also irregular compared to the standards of discharge but favorable for the most part to the good development of microorganisms. The concentrations found for the different bacteria counted were outside the norms except for the 200UFC/mL E. Coli content for the Niamey 2000 landfill. The respective concentrations of total coliforms were $2.9.10^5$ and $5.1.10^4$, CFU/mL for Koubia and Niamey 2000 respectively; those of fecal coliforms were $1.2.10^4$ and $7.02.10^3$ CFU/mL for Koubia and Niamey 2000 respectively. The Escherichia coli content for Koubia was 4600 CFU/mL. Salmonella, which should be absent in water, were found at concentrations of 850 and 400 CFU/mL for Koubia and Niamey 2000 respectively.
The results show that the leachates from the Niamey landfills are loaded with fecal germs. This indicates significant fecal contamination, a potential source of epidemics. It is therefore necessary to purify the leachate before releasing it into the environment in order to avoid the threat it poses to public health.

Keywords*: Fecal bacteria, Quality, Leachate, landfill, Koubia, Niamey 2000, Niamey*

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I. Introduction

Water, an essential commodity for life, can also be a source of disease when contaminated by potentially pathogenic agents [1, 2]. This contamination is generally the result of the consequences of various human activities (domestic, agricultural, industrial) that generate large quantities of waste. The bio-physical-chemical phenomena within the waste mass lead, together with meteoric waters, to the production of a liquid effluent called leachate. The leachate constitutes a favorable reservoir for the development of microbial flora, in particular the pathogens responsible for epidemics such as hepatitis, cholera, typhoid [3, 4]. On mostly uncontrolled landfills, releases can occur through advective transfers of leachate through defects in the lining structures [5]. A large number of waterborne human infections are caused by microorganisms whether viruses, bacteria or even protozoa [6]. Studies done on leachate from the Kenitra landfill in Morocco revealed high concentrations of total and fecal coliforms constituting a hazard to the receiving environment [7]. Works, dealing specifically with the impact of poor sanitation on the quality of water resources of cities in Asia and sub-Saharan Africa, have highlighted the bacteriological and nitrogenous pollution of surface water and rivers [8, 9]. Different groups of bacteria are used as indicators of water contamination by pathogenic microorganisms. Total coliforms and fecal coliforms are the most widely used and cited groups in the literature, but nowadays streptococci and Escherichia Coli are used in addition to coliforms [10]. Escherichia Coli is in fact considered today to be the best indicator of fecal contamination of surface waters [11]. Enumeration of bacteria is often based on their ability to secrete specific enzymes under certain conditions to transform certain compounds in their biological activity [12]. Their presence is a good although not exhaustive indicator of the intensity of fecal pollution and the presence of pathogenic microorganisms in waters.

The objective of this study is to evaluate the microbiological quality of leachate water from Niamey's landfills through the analysis of indicator bacteria (total and faecal coliforms, Escherichia coli and salmonella) present in these waters, in order to assess the risks of their untreated discharge into the environment.

II. Material and methods

2.1. Material and methods

2.1.1. Study area and sampling

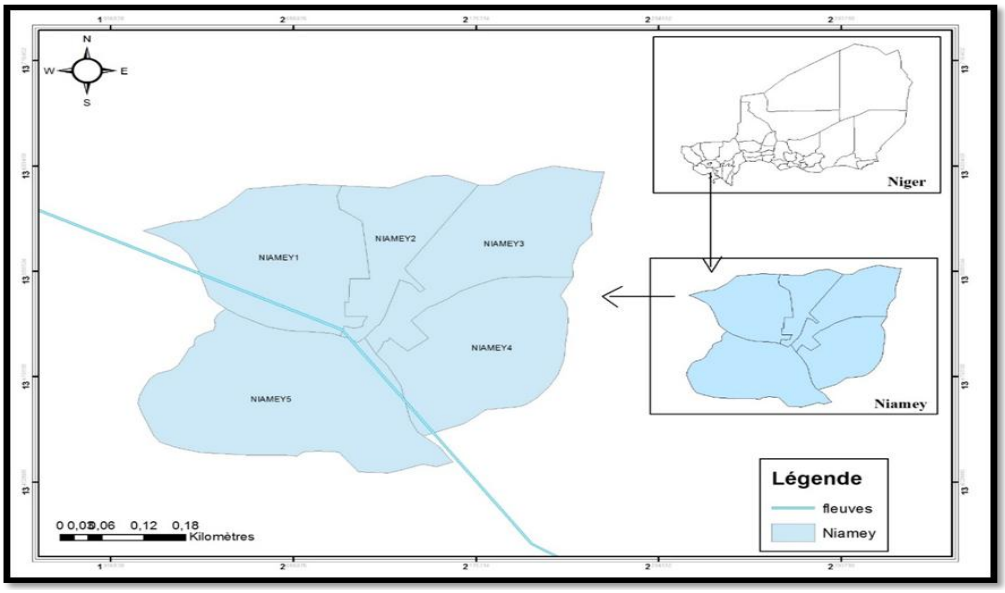

Figure no 1 : Geographical location of the city of Niamey

The city of Niamey is located in the southwestern part of Niger (Figure 1) between 13°28 and 13°35 north latitude and 2°03 and 2°10 east longitude. With an area of 275 km2, it is built on a plateau overlooking the left bank of the Niger River and on an alluvial plain on its right bank, between 180 and 240 m in altitude. Its administrative boundaries extend over 552.27 km², of which approximately 297.46 km² is urbanized. The river is the main source of drinking water for the city, although its constant degradation, caused by silting and pollution of its banks, is one of the consequences of many problems of sanitation.

The latest decentralization reforms, which still maintain a full communalization of the territory, erect the region into municipalities with special status. It is one of the regions with the lowest number of communes, with only five (5) communes (Figure 1).

2.1.2. For non-conservative parameters

The pH, dissolve oxygen and the electrical conductivity (EC) were measured by using a WTW 3430 multimeter.

2.1.3. Solutions used for the determination of microbiological parameters

Table 1 shows the different solutions used for the determination of fecal bacteria.

Table no 1: Solutions used for microbiology

Solutions	Concentration in g/L
Physiological water (NaCl)	9
Peptone water#	20
Mac Conkey	52
Eosin Methylen Blue (EMB)#	40
Rappaport Vassiliadis (RV)	26.6
Salmonella-Shigella SS#	63

: the preparation was made under heat

Mac Conkey media and EMB were autoclaved at 121°C for 15 minutes before running.

2.2. Methodology

2.2.1. Sample collection

A total of 10 samples were collected following the recommendations required for microbiological analysis [13]. Sterile bottles were used to collect representative samples. The samples collected were kept cool in a cooler until they were analyzed in the laboratory the same day they were collected.

2.2.2. In-situ parameters determinations

Temperature, PH, conductivity were determined in the field using the multi-parameter kit WTW 3430 SET F with three probes.

2.2.3. Determination of microbiological parameters

All manipulations were done under aseptic conditions. For all conditions, disposable consumables are sterile and reusable consumables are previously sterilized by autoclaving at 121°C (relative pressure of 1.02 bar) for 20 minutes for some and for others at 105°C in the oven.

2.2.3.1. Total coliforms (TC) and faecal coliforms (FC)

According to the International Organization for Standardization (ISO), the term "coliform" refers to rod-shaped, non-spore forming, Gram-negative, oxidase-negative, facultatively anaerobic organisms. The enumeration of these organisms at 35-37°C is often referred to as "total coliform enumeration, TC". Other organisms with the same characteristics but at 44°C are referred to as fecal coliforms, FC. Physiological water and Mac Conkey medium were used during the TC and FC enumeration process. The search and enumeration of coliforms were performed according to the AFNOR NF EN ISO 9308-1 standard [14]. The procedure is performed in three steps: dilution, incensing and incubation.

2.2.3.1.1. Preparation of the samples for the research of the TC and FC

The leachate being a waste water thus concentrated, it is necessary to proceed to dilutions and that, under aseptic conditions. To do this, 1 ml of each sample is introduced aseptically with a graduated pipette into a sterile test tube containing 9ml of sterilized physiological water. This corresponds to a 10^{-1} dilution. Introduce thereafter 1mL of the solution diluted to 10-1 in a sterile test tube containing 9 ml of physiological water which corresponds to a dilution to 10^{-2}. These dilutions continue progressively until 10^{-5}. For each dilution step, the pipette is changed. These different diluted solutions are used for the inoculation of the different culture media used for the research and the enumeration of germs.

2.2.3.1.2. Plating

The inoculation is done in petri dishes. Each petri dish is inoculated with 1 mL of the solution diluted in physiological water. Afterwards, the dishes are moved in the form of 8 and left to rest (1h).

2.2.3.1.3. Incubation

For CT the incubation is done at 37°C for 24 hours and for CF the incubation temperature is 44°C for 24 hours.

2.2.3.2. Search for Escherichia Coli (E. Coli)

The E. Coli detection process requires peptone water enrichment medium and eosin methyl blue (EMB) culture medium. The research and enumeration of E. Coli in leachates were carried out according to the AFNOR NF EN ISO 9308-1 [14] in three steps, namely enrichment, seeding and incubation.

2.2.3.2.1. Enrichment

1mL of the leachate sample is aseptically added to 9ml of peptone water (PWA) distributed in the tubes. PWA is a selective medium that is used for the enrichment of E. Coli. The objective of this step is to avoid the decrease of the pH and consequently to disfavour the growth of the other bacteria.

2.2.3.2.2. Isolation

Petri dishes were streaked with 1mL of the inoculum using a looped Pasteur pipette previously sterilized by flame.

2.2.3.2.3. Incubation

Petri dishes were incubated in the oven at 37°C for 24 hours. Escherichia coli: Colonies of 2 to 3 mm, flat, very dark purple with usually a metallic sheen.

2.2.3.3. Serchfor salmonelle

The bacteriological analysis of the leachate samples for Salmonella was done according to ISO 6579 [15]: pre-enrichment, enrichment, isolation and identification [13].

2.2.3.3.1. Pre-enrichlent

At the end of this non-selective phase, which uses a rich medium (EPT), all bacteria (including Salmonella) have multiplied.

The pre-enrichment step is based on the introduction of 1mL of leachate sample into each tube containing 10mL of 10g/L peptone water. The mixture is incubated at 37°C for 24 hours. Bacteria subjected to environmental conditions very different from those of their preferred environment (e.g. the digestive tract), recover their ability to multiply rapidly at the end of this phase.

2.2.3.3.2. Enrichment

Enrichment was performed with complete Rappaport Vassiliadis (RV) broth to minimize the growth of other bacteria associated with the sample and to continue selective multiplication of Salmonella. The RV solution was dispensed into tubes at a rate of 10 mL per tube. After, autoclaving the tubes, 1 mL of the pre-enriched solution was introduced into each of the broth tubes which were incubated at 37°C for 48 hours.

2.2.3.3.3. Isolation

The medium used was SS poured into Petri dishes. Isolation was done from the enrichment media. Petri dishes were inoculated with 1mL of the enriched media and incubated at 37°C for 24 hours. Characteristic Salmonella colonies (lactose negative) are opaque, translucent or transparent with a black center.

Figure 1 shows the media prepared and poured into Petri dishes.

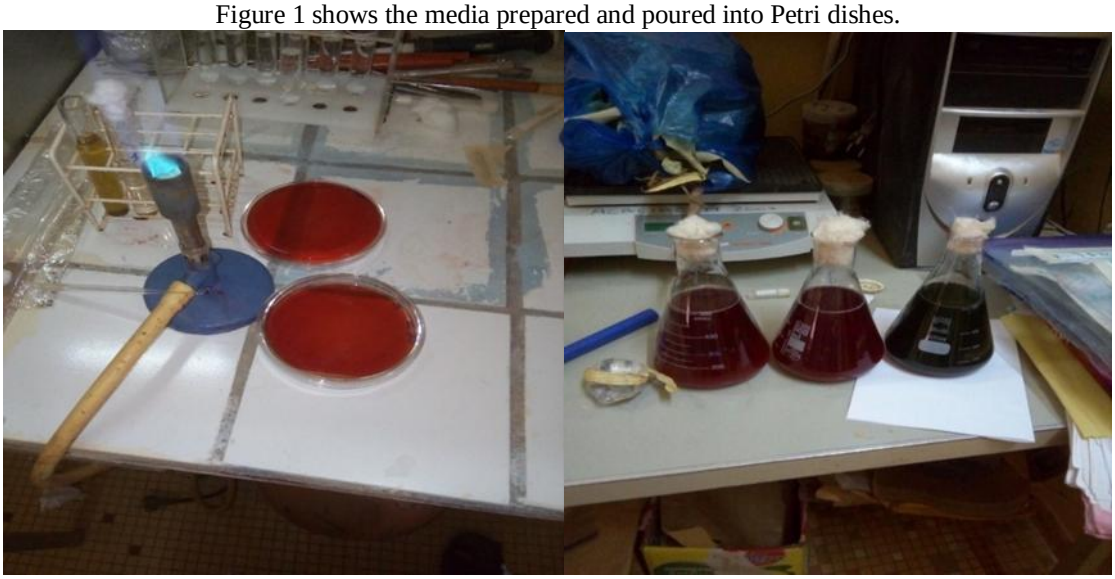

Figure no 2 : Prepared and cast media for bacteriological analysis

2.2.4. Expression of results

After the incubation period, the so-called usable plates were selected for the calculation. These boxes correspond to those coming from three successive dilutions and of which at least one box presents at least 15 colonies visible on the surface of the culture medium after incubation (i.e. a maximum of 6 boxes).

The results were read macroscopically by dividing each plate into four zones using a marker from which the colony of only one zone is read. Then, the set of colonies in a dish is multiplied by four and corresponds to the concentration C of CFU (Colony Forming Units).

Calculate the concentration "N" (CFU/mL) of culturable microorganisms in the sample using the following formula:

$$N = \frac{\sum C}{V(n1+0{,}1\ n2)d}$$

C : number of CFU (colony forming units) observed on all the selected and exploitable plates (plates coming from two successive dilutions and at least one of which contains 15 colonies; only the plates corresponding to a number of CFU lower or equal to 300 are considered in the calculation)

v : volume of the suspension spread on the surface of the media in mL.

n1 : number of boxes retained at the first dilution (the lowest).

n2 : number of plates retained at the second (highest) dilution

d : dilution rate corresponding to the lowest dilution retained (d = 1 for the undiluted sample; d = 0.01 for the 1/100 dilution etc.).

III. Results

2.1.In-situ parameters

These are the first indicators of the level of pollution of surface waters. The most commonly used ones, measured in the framework of our work, are gathered in table 2.

Table no 2: Non-conservative leachate parameters

Paramètres Echantillon	T°C	pH	Turbidity (NTU)	DO (mg/L)	EC (µS/cm)
Leachate 1	31,5 ±3,2	9,7 ± 1,4	443 ± 37,2	2,06 ± 0,75	4250 ± 1213
Leachate 2	32,7± 3,02	8,8 ± 0,86	406 ± 23	1,47 ± 0,97	2232 ± 1130

Legend : DO (DO : dissolved oxygen ; EC : electical conductivity)

Naturally, pure water is odorless, colorless and tasteless. With the slightest change in its composition, its characteristics follow the same dynamics.

The temperature of water is an important factor that governs almost all physical, chemical and biological reactions [16, 17]. The average temperatures of 31.5 and 32.7°C for Leachate 1 and Leachate 2, respectively, slightly exceed the 30°C temperature limit set by WHO [18]. The pH is considered as one of the most important parameters, which influence the leachate pollutant load of household and similar waste landfills [19]. The studied leachates are characterized by alkaline pH 9.7 and 8.8 for leachate 1 and leachate 2 respectively. These pH values are more or less within the discharge range set by the Nigerian discharge standard.

The electrical conductivity of a water represents a data allowing to evaluate in an approximate way, the global mineralization of a water and to estimate the total dissolved salts [13, 16, 20]. The leachates studied have conductivities of 4250 and 2232 μS/cm for leachate 1 and 2, respectively. These conductivities are well above the WHO [18] discharge standard (400 S/cm).

Dissolved oxygen concentration is a function of the rate of oxygen depletion in the environment by the activity of living organisms in the water and the decomposition processes of the organic matter present in the waters [12]. The dissolved oxygen (DO) values of 2.06 and 1.47mg/L for Leachate 1 and Leachate 2, respectively, are low and characterize highly loaded water. As for turbidity, the values obtained (Table 2) are significantly high compared to the 50NTU limit value set by WHO [18].

2.2.Microbiological parameters

This part consisted of the search for information on the level of microbiological pollution of the environment of the Koubia and Niamey 2000 landfills. For this purpose, we quantitatively analyzed the pollution germs in the leachates. The bacteria determined were total coliforms, fecal coliforms, Escherichia Coli and Salmonella. The results of their enumeration are shown in Table 3.

Table no 3: Results of TC, FC, E. Coli and Salmonella analyses

Bacteria Samples	Total Coliforms UFC/mL	Fecal coliforms UFC/mL	E. Coli UFC/mL	Salmonella UFC/mL
Koubia leachate	$2{,}9.10^5$	$1{,}2.10^4$	4600	850
Niamey 2000 leachate	$5{,}1.10^4$	$7{,}02.10^3$	200	400

2.2.1.Total coliforms and fecal coliforms

Examination of the results (Table 3) of the microbiological characterization of raw leachate from the uncontrolled landfills of Koubia and Niamey 2000 shows very poor bacteriological quality given the high levels of bacteria indicating overall pollution.

It can be seen for both landfills that TCs are more present than FCs, which confirms the work done on the commune of Ouésse-Ouidah (Cotonou) which reveals that in general FCs are always lower than TCs [21]. The analysis of fecal pollution germs (Table 3) showed the presence of high concentrations of total coliforms (TC) 2.9. 10^5 CFU/mL, and 5.10^4 while the average concentrations of fecal coliforms (FC) are 1.2. 10^4 and 7.02. 10^3 CFU/mL respectively for the Koubia and Niamey 2000 dumpsites. These bacterial loads are well above the WHO standard of 103 CFU/mL [22]. Total coliforms are a very frequently measured pathogenic bacterial community whose presence in a water is an environmental hazard [4].

2.2.2.Escherichia coli

Escherichia coli (E. Coli), called colibacillus which means colon bacillus, is the typical species of the genus Escherichia of enterobacteria. The appearance of this bacterium in water indicates the possible presence of pathogenic microorganisms. E. coli is an opportunistic pathogen capable of causing poisoning and diarrhea, as well as infections [4]. The E. coli levels obtained (Table 3) at the Koubia and Niamey 2000 sites were $4.6.10^3$ and 2.10^2 CFU/mL, respectively. The E. Coli value of the Niamey 2000 leachate is below the wastewater discharge limit of 1000 CFU/mL [18].

2.2.3. Salmonella

Salmonellae are aerobic gram-negative bacteria belonging to the Enterobacteriaceae family [23]. They are generally considered pathogenic although their virulence and pathogenicity vary greatly: typhoid fevers, systemic salmonellosis. In animals, Salmonella are responsible for abortions in sheep and mares [24]. They can be considered as a ubiquitous resistant germ very present in the environment [25] and they can also survive for weeks in dry conditions and up to several months in water constituting a favorable environment for their multiplication [26]. Concentrations of 850 and 400 CFU/mL (Table 3) are obtained for the Koubia and Niamey 2000 landfill respectively. Salmonella being highly present in feces. Environmental regulations exclude the presence of Salmonella in any effluent.

IV. Discussion

4.1.In situ parameters

From a quantitative point of view, the contents of the different parameters vary from one sampling point to another. The composition of the leachate, at different points of the landfill, sometimes shows important differences. This could be due either to the composition of the waste or to the duration of the landfill. Moreover, the burial of the waste being disordered, i.e. not following a precise pattern, it results in a constant disturbance of the physico-chemical and biological phenomena which govern the leaching.

The temperature of an effluent depends on the sunshine and the exchanges with the atmosphere. The temperatures obtained are higher than the limits set by the WHO [18], would be favorable to the maintenance of colonies of microorganisms "mesophilic" that develop at a temperature between 20 ° C and 40 ° C [27] and oxidation reactions and hydrolysis to finally enrich leachates in mineral elements such as chlorides [17]. This stipulates that the leachates of the Niamey landfills are excessively polluted. Similar results have been obtained on the Akouédo landfill in Côte d'Ivoire [28] and Ouésse-Ouidah in Benin [29]. The high temperatures may be due to the climatic conditions in the city of Niamey.

The pH obtained are of the same order of magnitude as those obtained on the Akouédo landfill in Ivory Coast (Kouassi et al., 2014) and on the Larache landfill (North Morocco) [30]. The pH of leachate can vary depending on the stage of maturation of the site and the seasons. Alkaline pH values can be explained by the biodegradation processes of organic matter within the waste mass, producing a significant amount of carbon dioxide. The dioxide once dissolved, could be transformed into bicarbonate which may be responsible for this alkalinity.

The conductivities obtained but remain low compared to the values of 14050 and 7805 S/cm obtained respectively on the Ouésse-Ouidah [29] and Akouédo [28] landfills. The differences observed from one dump to another, could be explained by the dilution phenomenon. Indeed, during the rainy season, the leachates receive a significant amount of water leading to a considerable dilution of the chemical elements present. A high conductivity leads to an increase in the mineral load of the leachates and especially in the salinity.

The low DO contents of the studied leachates may be related to the recorded high temperatures. Indeed low DO contents of waters go hand in hand with warm periods because cold water contains a greater amount of dissolved oxygen than water of high temperature [31]. A DO level below 3mg O2 / L of a water effluent indicates that it is highly polluted and the predominance of anaerobic process [12, 29]. Similarly very low DO levels cause an increase in the solubility of toxic elements that are released from the sediment. Oxygen content is therefore the result of a large number of biotic and abiotic factors [17]. This explains the complex interpretation of this parameter.

Turbidity is a parameter proportional to the state of degradation of waste on a landfill. Apart from the evolution, the composition of leachate obviously depends on the nature of the landfilled waste, the presence or absence of fermentable organic matter and the climatic conditions combined with the mode of operation of the site [17, 32].

4.2. Microbiological parameters

4.2.1. TC and FC

The differences in TC and FC concentrations between the two landfills could be due to the composition of the waste. The excessive concentrations of TC and FC in the Koubia landfill may be due to the composition of the waste present, but especially to the proximity of the landfill to the sewage sludge treatment plant. This proximity could actively increase the level of microbiological organisms via drainage or leaching from the area. At the Etueffont site in France, very low concentrations are obtained [4]. The low concentrations recorded at the Etueffont site are due to the fact that waste disposal was stopped for 4 years before the study was undertaken. The closure decreases the animal population at the site. This indicates that the level of microorganisms is largely related to waste inputs. The Koubia and Niamey 2000 dumps are located on the outskirts of Niamey. They are prime areas for stray domestic animals that spend hours a day grazing and are characterized by the presence of rodents. These two points, combined with the waste inputs to the site, could be the answer to the high TC and FC values found in Niamey's landfills.

4.2.2. E. Coli

E. Coli is the most specific indicator bacterium of fecal pollution, it is the most common and the most resistant to extreme environmental conditions [4, 13]. The presence of E. Coli in leachate water can come from baby diapers, animal and human waste. The high E. Coli content at the Koubia site compared to Niamey 2000 may be due to the proximity of the site to the sewage sludge treatment plant.

4.2.3. Salmonella

The results of some work show that the number of Salmonella in untreated urban effluent varies from a few bacteria per 100 ml to concentrations of the same order of magnitude as those of fecal indicators [33, 34, 35]. The presence of salmonella in the studied leachate samples is not in accordance with the WHO [18] regulations that recommend their total absence in a water. Researchers explained that temperature and nutrients are parameters determining the rate of germs in a water [35]. Average recorded temperatures of about 30° C are an optimal parameter to microbiological activity. These temperatures are factors determining the huge presence of salmonella in all samples compared to the low rates of presence in domestic wastewater of low temperature (21°C) from the city of Fez in Morocco [35]. The results of the work done in Lorraine, France, showed that the presence of Salmonella depends mainly on the temperature and then, to a lesser extent, on the specific rate of oxygen demand [36].

V. Conclusion

Microbiological analyses of leachates from the Koubia and Niamey 2000 dumpsites revealed the existence of total and thermotolerant coliforms or fecal coliforms, Escherichia Coli bacteria and Salmonella. The results gave very high concentrations:

Total coliforms of 2.9 10^5 and 5.1 10^4 CFU/mL respectively for the Koubia and Niamey 2000 landfill;

Fecal coliforms follow the same dynamics: 1.2 10^4 and 7.02 10^3 CFU/mL respectively for the Koubia and Niamey 2000 landfills;

Escherichia coli with 4.6 10^3 and 2 10^2 CFU/mL respectively for the Koubia and Niamey 2000 landfills.

Salmonella with 850 and 400 CFU/mL respectively for the Koubia and Niamey 2000 landfills.

These results show that the leachates from the two landfills studied are highly contaminated and constitute a potential threat to the receiving environment. It can also be seen that the Koubia landfill is more polluted than the Niamey landfill for all types of bacteria. Therefore, these effluents must be treated before discharge for good environmental safety.

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