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Assessment of Risk Connected to the Management of Effluents from Abomey-Calavi and So-Ava Laboratories

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Authors' contributions

This work was carried out in collaboration between all authors. Author AKA designed the study and contribute actively to all stages of this work, authors PAE, FL and MB assisted with the protocol, and the first draft of the manuscript; author CD performed the statistical analysis; authors PG, JS and ASYH managed the analyses of the study and the desk study. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This study was conducted to assess the risks connected to effluents released by laboratories performing biomedical analyses in four sanitary training units of Abomey Calavi and Sô-Ava area in Benin.
Study Design: It is a transversal and analytical study.

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Duration of Study: March 2nd to August 31st, 2010.
Methodology: The data were obtained based on an observation guide, by interviewing the participants and by analyzing the discharged liquid of laboratories as well as the ground waters (wells, drillings) situated near pits and other places used for discharging biomedical effluents.
Results: The results revealed a mismanagement of biomedical effluents. The mean values of pH ranged from 7.17 to 8.83. The conductivity exceeded the acceptable limits in 75% of the cases (2000 to 4260 µS / cm). The COD of sewage rose from 201 to 3400 mg/L and showed strong correlation with the BOD_5 (r^2=0.998) whose values ranged between 150 and 1700 mg/L. The contents in nitrogen (38.4 - 97.5 mg/L) and in phosphates (11.8 to 30 mg/L) exceeded the standards. Besides, significant concentrations of faecal Coliforms exceeding $100x10^3$ UFC / 100mL were recorded in 75 % of the analyzed effluents samples. This explains the ineffectiveness of the decontamination procedure carried out by the laboratory staff. The examination of well waters and water from the drillings situated near pits serving laboratories showed high turbidity (20 to 91,1 NTU), low rates of dissolved oxygen (< 5mg/L) and indications of bacterial contamination (160 UFC of *Escherichia coli* / 100mL).
Conclusion: This study suggests a connection between biomedical activities and groundwater pollution. This issue poses a real problem of public health because of the infectious, toxic and eco-toxic risks for the population and it is important to find an effective solution.

Keywords: Laboratories; effluents; risk; environment; health.

DEFINITIONS, ACRONYMS, ABBREVIATIONS

AFNOR : French Association of Standardization;
AOX : Adsorbable Organic Halogen
BOD_5 : Biochemical Oxygen Demand for five days
CFU : Colony Forming Unit
COD : Chemical Oxygen Demand
DHBP : Direction of Hygiene and Basic Purification
GPS : Geographic Position System
ISO : International Standardization Organisation
HIV : Human immunodeficiency Virus
NSI : Normalization Senegalese Institute
NTK : Kjedahl Nitrogen
NTU : Nephelometric Turbidity Unit
NUHC : National Universitary Hospital Centre
pH : Hydrogen Potential
WHO : World Health Organization
ºC : Celsius degree
WTW : Wissenschaftlich Technische Werkstutten.

1. INTRODUCTION

The management of biomedical effluents constitutes one of the challenges for the Sustainable Development. In Benin, the comparative analysis of the effluents at the inlet and outlet of the Purge Station of the biggest national hospital showed the inefficiency of the chemical and biological treatment [1]. According to Kummerer et al. [2] and Verlicchi et al. [3]

certain compounds such as organo-halogenated and medicinal residues are often released directly from purge systems without pretreatment. Indeed, if done without precaution, the discharges of products stemming from treatment activities, from radiology or biomedical analyses pollute the soil, ground water and nearby surface water. This pollution poses toxicological and infectious risks for human health [4]. Approximately 2 in 9 % of seroconversions in hepatitis B, C and in HIV could be connected to contact with blood residues or soiled trash cans [5]. Infections with Mycobacterium tuberculosis [6], enterovirus, and Legionella pneumophila, with prion [7], with Salmonella or endotoxins [8], were found in employees or animals that were in direct contact with the waste. As a rule, according to the Regulations referring to rational management of biomedical waste "any hospital effluent must be pre-processed before being released in the environment or poured in the public network of purification [9]. Regrettably, following the example of most of the sanitary structures in West Africa [10], these Regulations are not respected in Abomey-Calavi and Sô-Ava area in the South of Benin Republic. Therefore the present study aims at assessing the risks connected to the management of the biomedical effluents from laboratories of Abomey-Calavi and Sô-Ava

2. MATERIALS AND METHODS

2.1 Study Area

This study was led in the Sanitary Area (operational decentralized entity of health system) of Abomey-Calavi and Sô-Ava from March 2nd to August 31st, 2010 for a period of 6 months. The health system of Benin is organized as a pyramid scheme on three levels. The central level or the apex of the scheme belongs to the Health Ministry with hospital complexes of National Reference. The side levels include health departmental offices (six in number at the rate of one office for two departments) whereas the base of the scheme consists in 34 sanitary areas gathered into 2 or 3 districts of the country. The sanitary area is the operational decentralized entity of health system. It coordinates the most accessible sanitary units (health centers, clinics, maternity hospitals, isolated community clinic, village health units) depending on a top reference health centre or on a regional hospital.

Thus, Abomey-Calavi and Sô-Ava (two districts) form a Sanitary Area situated in the South of Benin (Fig. 1). Its area measures 868 km^2 with a cosmopolitan population of 513160 inhabitants. It accounts 18 public sanitary points and more than 200 private ones [11]. The relief is uneven in Calavi and the climate of subequatorial type. Grounds are ferruginous, sandy or hydromorphic by place. 82 % of Sô-Ava territory is lakeside. Recent studies in its main lake (Nokoué) report a strong contamination by heavy metals (lead, cadmium…) whose sources were yet to be identified [12,13]. In Calavi, the majority of the domestic well-water is polluted [14].

2.2 Water Sampling and Data Collection

Four laboratories were selected on the basis of important criteria such as frequentation and registration of the centers on the list of the Statistical Service. The techniques used were direct observation through regular visits of the sites and interviews through questionnaires of 26 personnel chosen according to their implications in production and management of effluents. They are laboratory staff, laboratory technicians, auxiliary nurses, maintenance agents involved in laboratory activities, technicians in charge of hygiene, unit head doctors, human and material resources managers, inhabitant delegates having a well located at less than 75 m from the pits and discharge channels of laboratory effluents.

Geographical coordinates were taken with the help of GPS Garmin at several levels.

A pre-inspection allowed us to assess precisely the drainage network in each sanitary unit, particularly the evacuative circuit from laboratories. This inspection proved that the effluents released in the sinks (Fig. 2) go through an underground pipe and cross a manhole behind the building (Fig. 3) to end in a rounded shaped catch basin (Fig. 4) located close to rectangular shaped septic tanks. Considering the fact that liquids draining from the sink cross no purge system before infiltrating into the ground through the catch basins not made of concrete, these are the true samples of laboratory effluent.

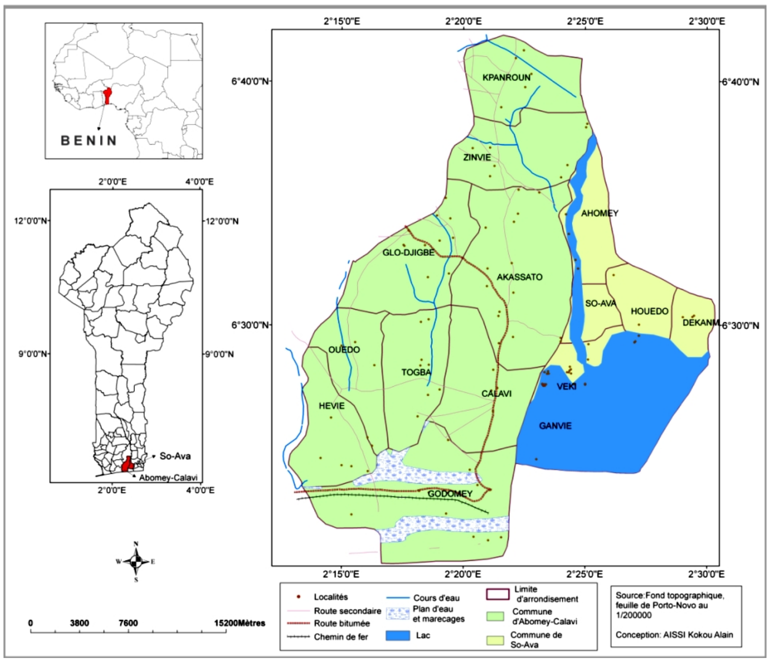

Fig. 1. Location Map of the Sanitary Area of Abomey-Calavi and Sô-Ava

Fig. 2. Sinks through which effluents are drained in the laboratory of the hospital of Abomey-Calavi

Fig. 3. Fix manhole under which the sink pipe passes to end in the catch basin

Fig. 4. Catch basin serving as a receptacle of laboratory effluents

Since these effluents stored in the catch basins are inaccessible because the catch basins are permanent and hermetically closed, we proceeded in the following manners. At first, sinks were blocked from 8 a.m in order to gradually collect the drained liquids after a chemical treatment carried out by the laboratory personnel. Later, every 2 hours, we collected samples of the composite liquid using a plastic jar of 1.5 L (Figs. 5,6).

Fig. 5. Discharge of soaking liquids from washing materials into the sink that was blocked beforehand

Fig. 6. collection of laboratory effluents samples from the sink with a plastic jar

Besides, well-waters from nearby pits and from other places of biomedical waste disposals were collected. Composite sampled waters were carefully labeled, preserved in iceboxes and immediately transported to the national hygiene and basic purification laboratory (NHBP) and analysis started within four hours.

2.3 Methods of Analysis

2.3.1 Physico-chemical analyses

The French Standards (NF) published by the French Association of Standardization [15,16]. served as reference for the analyses of water and effluents. pH, temperature and dissolved oxygen were measured with the help of a multimeter WTW pH / oxi 340i. The references are NF T 90 - 008) for pH and NF EN 25814 for dissolved oxygen. The conductivity was measured by means of a conductimeter WTW Cond 340i (NF EN 27888). The values of turbidity were recorded by means of a turbidimeter HACH 2100 P using the NF - EN 27027) guidelines. The COD, Kjeldahl nitrogen, total phosphorus, nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), orthophosphate (PO_4^{3-}), were measured after acidic mineralization by Molecular Absorption Spectrophotometer (HACH DR 2800) according to the AFNOR standards. The BOD_5 was measured by respirometric method (NF EN 1899-1) and the oxydability by determination of permanganate index with $KMnO_4$. (NF- EN 8467).

2.3.2 Bacteriological analyses

The enumeration of micro organisms was carried out by counting the colonies obtained in 24 hours after culture at 44ºC on Rapid *E. Coli* (NF V - 08 - 05) agar. This agar is selective and allows the distinction of thermotolerant *Escherichia Coli* among all the faecal coliforms. Its preparation was carried out in accordance with the manufacturer guidelines and maintained melted at 47ºC until the moment of its use. The culture was realized by mixing 5 ml of each sample (not diluted) followed by successive dilutions (ten factors) of these samples into different Petri dishes carefully labeled. It is important to state that all this was performed close to the flame of Bunsen gaslamp to avoid all kinds of contamination. 20 minutes later, 15 - 20 ml of prepared agar was spilled on the innocula and carefully mixed. The mixture was left on the laboratory bench top under laminar flow hood until solidification. Finally, the dishes were placed in an incubator at 44ºC. After 24 hours, the pink (*Escherichia Coli*) and blue (other Coliform) colonies were counted. The results were expressed in CFU/100mL.

2.4 Data Processing

Statistical analyses were carried out using SPSS version 16.0. The comparisons of the different means with standards were performed using students test. The difference is significant if $p < 0.05$. The maps were drawn using Arc-Gis software.

3. RESULTS AND DISCUSSION

3.1 Typology of the Chemical and Biological Products Observed in Laboratories during Our Inspection

Blood, urines and human stools were the most common substances analyzed. The examination of pits was carried out only in Calavi health centre and in the Hospital of Sô-Thanhoué. The bleach was the most commonly disinfectant used.

The colouring agents of Gram and Giemsa existed everywhere whereas Zielh A and B were used only in Calavi health centre and in the Hospital of Sô-Thanhoue. According to their indications on the specification sheet, the kits of reagent contained a variety of chemical substances such as: conservatives (azide of sodium), enzymes (oxidases, peroxidases, phosphatase), amino or phosphated elements (nitrite, diethylamine, 4-amino-antipyrine, phosphates tampon), acids, base and others (cyanomethemoglobin, cyanide, dimethylsulfoxide, etc.). All these items were observed in the laboratories during our inspectoral investigation. We specify that no specific analyse was done in this study to look for these chemical substances in the samples.

3.2 Opinion Poll about the Management of the Effluents from Laboratories

Eleven staff out of 17 i.e. 64.7 % were used to throwing back the blood products in sinks. 88.2 % acted the same way to eliminate liquids stemming from chemical reactions. At the Regional Hospital and at Calavi health centre, the final destination of the blood products was a pit lost in the far end of a corner in the courtyard (Table 1). The incompletely used, expired or spoilt reagents were incinerated, buried, or simply poured on the garbage dumps of general waste. Till now, no laboratory has set written procedures on biomedical wastes management. The directives of sorting and separation of the wastes at the source were not actually respected. The dilutions of the bleach were made by guesswork and the period of contact needed for ensuring a complete decontamination was not standardized. Moreover, it is observed that in many cases, the bleach containers are not immediately closed after use, thus exposed to ambient temperature and sun light. That can cause the decrease of free chlorine concentration with time therefore weakening the bactericide potential of the bleach. Six agents out of 25 questioned, i.e. 24 % stated they were dissatisfied with the management of the liquid waste in their institution.

Table 1. Final destination of the liquid discharges by laboratory

Types of waste	Regional hospital of calavi	Health centre of calavi	Hospital of Sô-tchanhoué	Health centre of Sô-Ava
Blood products	- bottomless pit - pit via sink	- bottomless pit - pit via sink	pit via sink	pit via sink
Saddles, spit, rest of reagent that are damaged or made obsolete	incinerator or garbage dump	Bottomless pit	incinerator or garbage dump	Bottomless pit
Urines, the other biological liquids, residual liquids of the reactive processes, coloring, detergent, etc.,	pit via sink	pit via sink	pit via sink	pit via sink

Source: onsite investigation.

3.3 Exposure and Prevention Measures of the Risks in the Laboratories

The agents were aware of infectious (17/17), toxic (15/17), ecotoxic and carcinogenic (14/17) risks that the laboratory effluents posed. The most frequent modalities of exposure were: the spatter and the inhalation during the carrying out of the laboratory analyses or during the discharge of soiled liquids (9/17). The vaccination of the staff exposed to hospital-borne infections (viral B hepatitis in particular) and the trainings on the management of risks connected to the wastes did not follow the guidelines set by the World Health Organization [17].

3.4 Physico-chemical and Bacteriological Characteristics of the Effluents

3.4.1 Results of the parameters measured in situ

The results are presented in Table 2. The average pH of the effluents measured in laboratories ranged from 7.17 to 8.83. The average temperatures ranged between 28.0 and 29.7ºC. The average conductivities at the Regional Hospital (3700 µS/cm) and in Calavi health centre of (4260 µS/cm) exceeded the acceptable standards. The average contents of dissolved oxygen were lower than 5 mg/L in all locations.

3.4.2 Results of the parameters measured in laboratory

The mean concentrations of the BOD_5 ranged from 150 to 1700 mg/L and were all higher than the standards fixed to 50 mg/L (Table 3). The average COD ranged between 201 to 3400 mg/L. The average content of nitrogen (34.5 mg/L) and of total phosphorus (97.5 mg/L) exceeded the limits fixed which are respectively 11.88 mg/L and 30 mg/L (Table 3). The pool of effluents analyzed in Calavi health centre did not contain faecal coliforms. At the Hospital of Sô-Tchanhoue, the effluents were strongly contaminated with *Escherichia coli* ($6.88x10^5$ UFC/100mL) whereas at the Regional hospital and at the health centre of Sô-Ava, colonies of the other types of coliforms faecal were $1.28x10^5$ and $1.02x10^5$ CFU/100mL respectively).

Table 2. Comparison of the parameters measured in situ with the acceptable standards for effluents samples obtained from sink

Parameters		Regional hospital of calavi	Health centre of calavi	Hospital of Sô-tchanhoue	Health centre of Sô-Ava	Acceptable standard
Hydrogen Potential (pH)	pH 1	9.12	9.17	8.00	7.87	
	pH 2	10.55	6.09	7.18	9.21	
	pH 3	7.48	9.54	10.09	7.66	
	pH 4	8.17	9.32	3.41	8.74	
	Mean pH	**8.83**	**8.53**	**7.17**	**8.00**	**6 – 9**
	Std	1.33	1.63	2.79	0.73	
Temperature (T) in ºC	T° 1	28.0	28.7	28.0	28.0	
	T° 2	28.9	28.9	30.0	27.0	
	T° 3	30.0	28.0	28.7	28.0	
	T° 4	29.9	33.2	25.3	29.8	
	Mean T	**29.2**	**29.7**	**28.0**	**28.2**	**< 30**
	Std	0.94	2.36	1.98	1.17	
Conductivity (Cond) *in µS/cm*	Cond 1	3100	3300	500	1960	
	Cond 2	3760	4100	400	1380	
	Cond 3	3880	4900	102	3480	
	Cond 4	4060	4740	586	3100	
	Mean Cond	**3700**	**4260**	**397**	**2480**	**< 2000**
	Std	210.84	977.20	418.56	727.36	
Dissolved oxygen (O_2) in mg/L	O_2 1	4.77	3.07	2.43	4.30	
	O_2 3	5.00	4.10	4.51	3.73	
	O_2 3	3.93	4.43	2.00	4.03	
	O_2 4	4.02	5.00	3.46	4.74	
	Mean O_2	**4.43**	**4.15**	**3.1**	**4.2**	**> 5**
	Std	0.53	0.81	1.12	0.42	

Std = Standard deviation
Source: onsite investigation.

Table 3. Comparison between mean values of each parameter with the standards

Parameters	Unit	Regional Hospital of Calavi	Health centre of Calavi	Hospital of Sô-Tchanhoué	Health centre of Sô-Ava	Acceptable Standard
BOD_5	mg/L	**1700**	**330**	**150**	**430**	**< 50**
COD	mg/L	**3400**	**764**	**201**	**876**	**< 200**
Nitrogen NTK	mg/L	**97.5**	**78.7**	**38.4**	**34.5**	**< 30**
Total Phosphorus	mg/L	**30**	**11,8**	**30**	**20**	**< 10**
Faecal Coliforms	UFC /100mL	**1.28×10^5**	0	**6.88×10^5**	**1.02×10^5**	**$< 2.10^3$**
E. Coli	UFC /100mL	0	0	**6.88×10^5**	0	**< 1**

Source: onsite investigation

3.4.3 Physico-chemical and bacteriological Characteristics of the surrounding groundwater nearby the inspected laboratories

The turbidity of waters from the well (20 NTU) and from drilling (91.1 NTU) was highly above the standards of potability (5 NTU). The content of dissolved oxygen was low in samples. Nitrite, nitrate, ammonium and orthophosphate concentrations were in accordance with the WHO limits. The water of the drilling had a higher oxydability (8 mg/l of 0_2) than the standard but was exempt from faecal coliforms contrary to the water of the well which contained $3.92.10^3$ UFC/100mL among which 0.160. 10^3 *Escherichia coli* (Table 4).

Table 4. Comparison of the averages of the physico-chemical and bacteriological parameters of waters from the well and from drilling with the standards of drinkability

Parameters	Unit	Well in Health Centre of Calavi	Drilling in Hospital of Sô-Tchanhoué	Acceptable Standard (WHO)
pH	**-**	**4.7**	**6.53**	7- 8,5
Temperature	ºC	26	25	< 30
Conductivity	µS/cm	202	218	< 2000
Turbidity	NTU	**20**	**91.1**	< **5**
Salinity	‰	0	0	0
Dissolved oxygen	mg/L	**3.28**	**3.82**	**≥ 5**
Robust dissolved totals	mg/L	203	209	< 1000
Oxydability	mg/L of 0_2	0,54	**8**	**< 5**
Nitrite NO_2^-	mg/L	0.052	0.072	< 3
Nitrate NO_3^-	mg/L	30.48	13	< 50
Ammonium NH_4^+	mg/L	0.13	0.15	< 0,2
Orthophosphate PO_4^{3-}	mg/L	0.26	0.37	< 5
Faecal Coliforms	UFC /100mL	**$3{,}92x10^3$**	0	< 2
Escherichia coli	UFC /100mL	**$0{,}160x10^3$**	0	0

Source: onsite investigation

3.5 Discussion

3.5.1 Management of the liquids effluents in laboratories

The directives of sorting and separation of the effluents at the source were not properly respected in the laboratories of the Sanitary Area of Abomey-Calavi and Sô-Ava contrary to the laboratories of Abidjan [5]. One of the causes of this negligence was lack of harmonization in the practices of waste management which should comply with 18189 ISO guidelines [18]. Most laboratories have the habit to throw the effluents in pits via sinks or directly in holes dug in a corner which triggers the risk of transfer of biomedical pollutants into aquifers [19]. The outdoor incineration of the biomedical wastes is also a bad process [20,21]. If the bactericidal and virucidal potential of bleach is worldly renowned [22], its efficiency in the decontamination of effluents depends on several factors such as: rate of dilution, the duration of conservation of the diluted liquid and the period of contact with the material to be disinfected [21,23]. It was observed in most of the laboratories that the staff made random dilutions and rarely observed suitable wait-time for the disinfection to be efficient resulting in triggering potential risks of infection. Besides, the decrease in free chlorine concentration caused by bad practices of the staff can explain the inefficiency of the bleach.

3.5.2 Risk management in the inspected laboratories

Though most of the workers were aware of the threat posed by the biomedical effluents, they did not comply with safety instructions. Those who did not regularly wear gloves justified their behavior either by lack of subsidies in terms of protection, lack of ideal behavior from their immediate superiors who also did not comply with safety instructions, or discomfort caused by the wearing of gloves. These reports showed the social influence of the individual psychological perceptions and of the social background on the attitude of the individuals facing the daily professional danger. That is why Coppieters et al. [24] suggest the integration of a psycho-sociological approach in the assessment of risks. The viral hepatitis B and C are classified among the first five professional diseases in a hospital environment [25] and the vaccination recommended for the staff at risk [23] was not organized in spite of the high rates of accident of exposure to blood (29% found in the visited laboratories) following the example of prevalence (39%) reported by Zannou et al. [26].

3.5.3 Quality of the biomedical effluents stemming from laboratories

The temperature of the effluents (lower than 30°C) showed that there was no thermal pollution. The high values of conductivity (> 2000 µS / cm) in 75 % of the effluents proved the abundance of mineral elements as reported by Emmanuel [27] and Belokda [28]. In the National University Hospital Centre (NUHC) of Cotonou, Makoutodé et al. [1] had also found high conductivities ranging from 3200 to 5320 µS/cm with average pH of 7 and temperatures lower than 30°C in effluents. In respect of the oxidizing materials, 75% of the COD exceeded the limit of 200 mg/L fixed in Senegal [29] and in Morocco [28]. The recorded COD values exceeded the American standard (150 - 800 mg/L) fixed by the Environmental Protection Agency [30]. High values (450 mg/L, 1095 mg/L, 510 mg/L and 1940 mg/L) were respectively found in the NUHC of Cotonou [1], in the Regional University Hospital Centre of Limoges [31], at the hospital of Port-au-Prince [27] and at Mohamed V's hospital of Safi [28]. The high values above NSI standards [29] in terms of nitrogen and phosphorus could be a threat especially to the lakeside villages (Sô-Ava and Sô-Tchanhoué). In fact nitrites are dangerous poisons for the aquatic organisms, even in low concentrations [32]. By degrading haemoglobin, which becomes inapt of transporting the oxygen they can cause the death of fish by asphyxia [33]. Sometimes, nitrites are transformed into nitrates which contribute to the eutrophication [27,32]. The proliferation of faecal coliforms during the bacteria culture in 75% of the effluents put in doubt the efficacy of the bleach decontamination carried out in laboratories. This ineffectiveness of treatment by chlorination was also noticed by Makoutodé et al. [1] in the NUHC who observed more than 103 UFC colonies of faecal coliforms in 100 ml of effluent after treatment.

3.5.4 Analyses of the impact of the biomedical effluents on groundwater

According to the model of rise in the ground (Fig. 7), the shallow depth of groundwater at the level of Sô-Ava (4 m) and Sô-Tchanhoué (5 m) makes them more vulnerable to biomedical pollutants compared to Calavi (13 m of depth at the level of the health centre and about 19 m in the local hospital). The pollution of the groundwater in Calavi was in agreement with the findings of [14] who stated that 100% of wells in this locality contain germs (*Escherichia coli,* faecal Streptococci, Salmonellas, Shigellae, *Clostridium perfringens*, Staphylococci) as well as nitrites, iron and manganese. Among the potential sources of this pollution and in spite of the complexity of the mechanisms of circulation of pollutants in aquifers [27], the strong contribution of biomedical activities could be suspected.

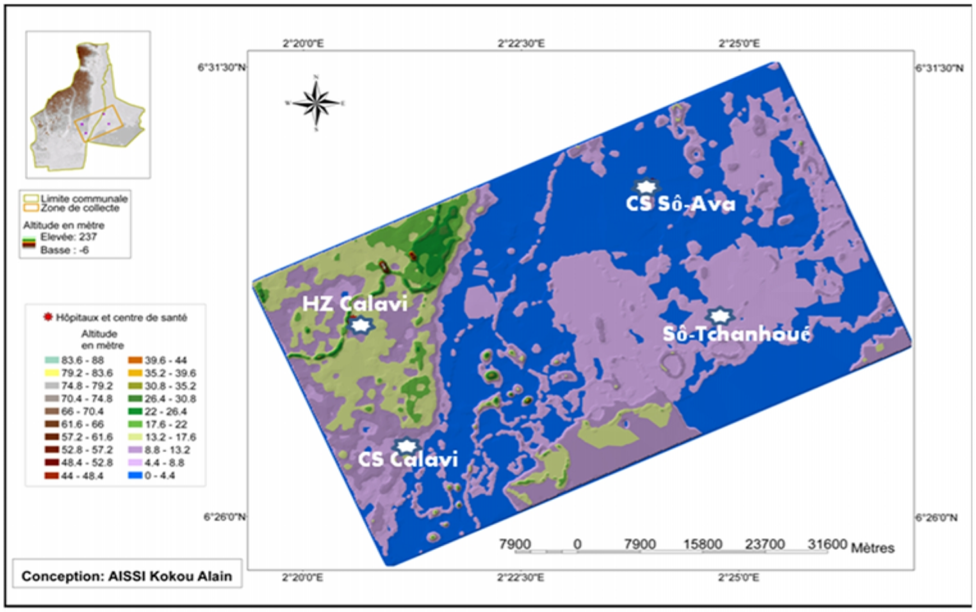

Fig. 7. Model of rise in the ground of the study area
HZ = Regional Hospital ; CS = Health Center
Source: onsite investigation

4. CONCLUSION

The present study allowed us to assess the risks connected to the management of effluent stemming from laboratory. The results revealed that the most used product in the treatment was the bleach. However, the lack of training and knowledge of the effective use of that product caused its inefficiency. Besides, the results obtained prove that effluents stemming from biomedical laboratories of Abomey-Calavi/Sô-Ava Sanitary Zone present infectious, toxic and eco-toxic risks. Consequently, their management could be better ensured particularly by an eco-systemic approach to human health. This risk assessment could be deepened by epidemiological inquiries (calculation of risk index within the staff and the exposed populations) and specific toxicity tests as well. The mechanisms of transfer of persistent pollutants (heavy metals, residues of AOX, etc.) should be analyzed and preventive actions such as public means of raising awareness, a better training of the staff and a frequent survey of mitigating measures should be carried out.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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