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Characterization and Cytogenotoxicity of Birnin Kebbi Central Abattoir Wastewater

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Abstract
There is a growing suspicion on the toxicity of abattoir wastewaters that necessitates its worldwide safety evaluation. This study determined the properties and cytogenotoxicity of Birnin Kebbi Central Abattoir Wastewaters. Selected physicochemical and microbiological characteristics of the wastewaters were assessed using standard protocols, after which its cytogenotoxicity was determined using the Allium cepa toxicity test. Fifty (50) A. cepa bulbs were distributed equally into 5 groups and grown over tap water (control) and 25, 50, 75, and 100 % (v/v) of the wastewaters, respectively for 72 hours. The root-tips of the bulbs were then examined for chromosomal aberrations. The physicochemical analysis showed that the levels of cadmium (Cd), chromium (Cr), iron (Fe), and nickel (Ni) were within the World Health Organization (WHO) permissible limits for wastewaters, but not lead (Pb), nitrate, sulphate, total suspended solids (TSS), turbidity, chemical oxygen demand (COD), biochemical oxygen demand (BOD), and dissolved oxygen (DO). The microbiological characterization indicated that the viable bacteria and fungi counts were above the WHO limits (<400 CFU/ml), while coliform was not detected. The cytogenetic test showed that the root length increase and mitotic index (MI) of the exposed samples were insignificant (p>0.05), while the control samples were significant. Chromosomal abnormalities, including chromosomal bridges as well as sticky and vagrant chromosomes were observed in the root cells of the exposed samples, but not the control. The results infer that the wastewaters could be toxic and pose public health threat. Therefore, there is a need for proper wastewater management at the abattoir.

Keywords: *BOD, Coliform, Nitrate, Physicochemical, Vagrant chromosome*

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1. Introduction

Wastewater pollution has become a global burden due to increasing industrialization, urbanization and population growth (Akpor *et al*., 2014). The burden is more in developing countries due to poverty, ignorance and poor policy enforcement. On average, individuals living in cities in developing countries generate between 30 and 70 mm^3 of wastewater per year (Edokpayi *et al*., 2017). These wastewaters are often discharged into the environment untreated, resulting in water and land pollution (Edokpayi *et al*., 2017). Wastewater deteriorates river water quality and contaminates the land with both short- and long-term consequences on human health and the environment (Morrison *et al*., 2004; Edokpayi *et al*., 2017). Wastewaters contain a variety of health-deteriorating pollutants such as heavy metals, pathogens, and nutrients (Baloyi *et al*., 2014). The nature and concentrations of these pollutants depend on sources as well as technologies in place (Vysokomornaya *et al*., 2015). As such, the source and nature of pollutants in a wastewater need to be identified to manage the wastewater effectively.

Abattoirs are one of the largest consumers of water in many cities, producing the corresponding amount of wastewater (Ayoola *et al*., 2019). In developing countries, direct discharges of untreated wastewater from abattoirs into the surrounding environment are a common practice, which results in public health hazards. Abattoir wastewater may contain several types of pollutants such as harmful microorganisms, organic and inorganic compounds as well as odor-producing elements (Alfonso-Muniozguren *et al*., 2018). Findings by Elemile *et al*. (2019) revealed that pollutants in abattoir wastewater may cause enormous environmental and public health hazards, particularly it may affect the quality of nearby groundwater. According to USEIP (2018), when humans release untreated wastewater from the abattoir into water bodies in large quantities and high concentrations, it can stimulate high algae growth. This can deplete oxygen and suffocate aquatic organisms. These show that abattoir wastewater can pose a significant public and environmental health threat. Therefore, the safety of wastewaters emanating from all metropolitan abattoirs needs to be ascertained.

Birnin Kebbi in northwestern Nigeria is thriving, noticeably after it became the capital of Kebbi State about three decades ago. Successive governments have built many structures and facilities in the city among which is the central abattoir, constructed about 12 years ago. The abattoir discharges its wastewaters directly into the surroundings and, to the best of our knowledge, no study has been conducted on the health and environmental safety of the wastewater emanating from the facility, particularly its cytogenetic effects. Cytogenotoxity is often done with *Allium cepa* toxicity test to investigate the genetic effects of a suspected toxicant. *A. cepa* test is cheap, simple to conduct, and effective for evaluating both plant and animal toxicants (Leme and Marin-Morales, 2009; Olorunfemi *et al*., 2012). Accordingly, this study employed *A. cepa* toxicity test to determine the cytogenotoxicity of wastewaters obtained from the Birnin Kebbi central abattoir.

2. Materials and methods

2.1 Description of the study area

The Birnin Kebbi Central Abattoir is situated along Birnin Kebbi-Jega Road. Birnin Kebbi is the capital of Kebbi State in the Northwestern Nigeria on latitude 12°27′57.8808″ N and longitude 4°11′58.2864″ E (Figure 1). Kebbi State has a landmass of about 36,800 km^2 and, as of 2006, around 3,238,628 people lived in the state (Eme and Idike, 2015). The people of Birnin Kebbi are predominantly cereal farmers and animal breeders. The population of Birnin Kebbi has been thriving since it became the capital of the state, which necessitated the establishment of the abattoir to cater for the increasing demand for meat. The abattoir consumes a large volume of water daily and discharges same as wastewater into the surroundings.

The climate of the state is semi-arid, characterized by alternating wet and dry seasons. The Sudan-Guinea Savanna natural vegetation of the state has been reduced to pure Sudan by long-term anthropogenic activities. The average monthly and annual rainfalls are 112.21 mm and 787.53 mm, respectively, with the rainy season spanning April through October (Ismail and Oke, 2012). The average temperature is 26 ^{0}C, but it could rise above 40 ^{0}C during the hot season and fall to 21 ^{0}C during the cold season (Gulma, 2013).

2.2 Collection of the wastewater

Duplicate samples of wastewater from the abattoir were collected in sterilized plastic bottles from the discharge site of the facility in March 2019. The samples were preserved with 10% HNO_3, covered tightly, and moved to the laboratory where they were stored in a refrigerator at about 4 ° C prior to analysis.

2.3 Physicochemical characterization of the wastewater

All the reagents used in the physicochemical analysis and other tests in this study were prepared from chemicals of high analytical grades (AnalaR). The container for each reagent was washed with a detergent solution, after which it was rinsed properly with the reagent.

Physicochemical characteristics that change with time, such as pH and temperature were measured on-site using a digital pH meter (Pye Unicam pH and conductivity meter model 292) and a thermometer (mercury-in-glass thermometer), respectively. Other characteristics, including the total suspended solids (TSS), turbidity, dissolved oxygen (DO), chemical oxygen demand (COD), biochemical oxygen demand (BOD), nitrate, and phosphate were conducted in the laboratory as described by Aniyikaiye *et al.* (2019).

The concentrations of copper (Cu), iron (Fe), cadmium (Cd), chromium (Cr), lead (Pb), and nickel (Ni) in the wastewater were determined by atomic absorption spectroscopy (AAS) as described by Yahaya *et al*. (2019). About 0.5 ml of the wastewater was mixed with 25 ml aqua-regia solution in the digestion tube and then digested at 120 ^{o}C for 3 hours. The digested material was filtered into 100 ml beaker, and the solution was analyzed for the selected metals using Atomic Absorption Spectrometer (UNICAM model 969).

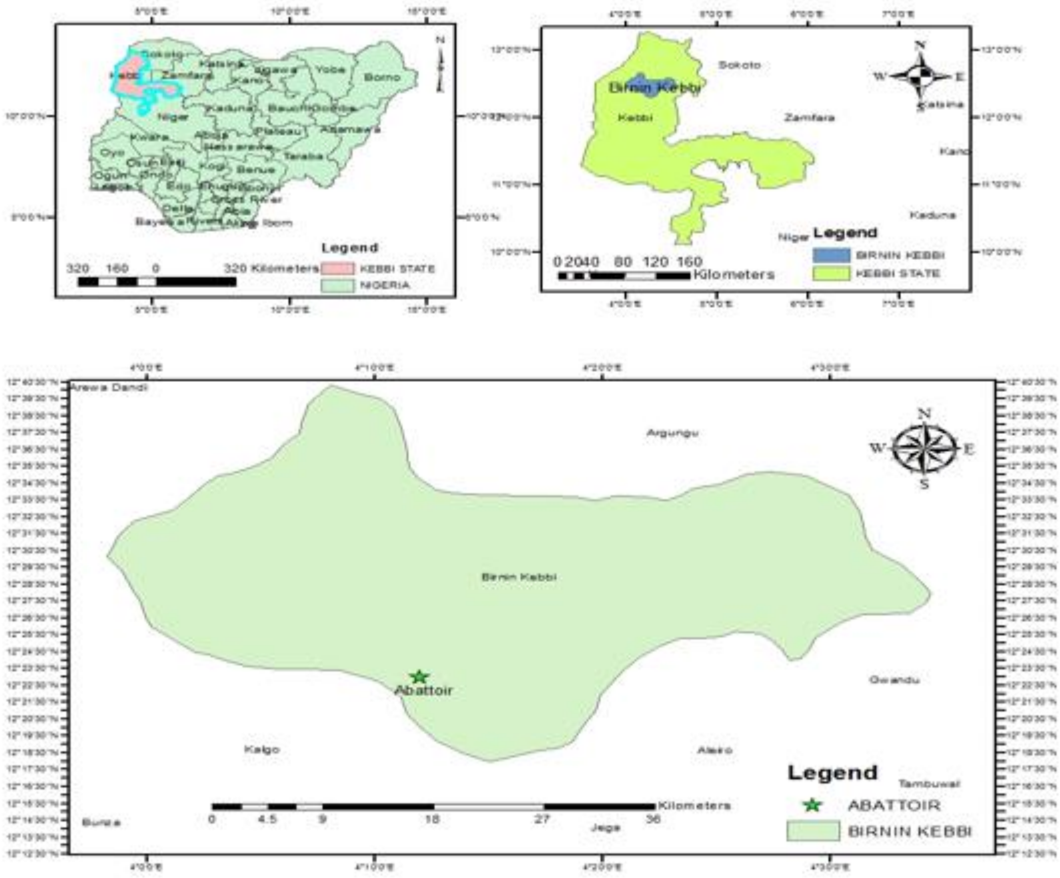


Fig. 1: Location of Birnin Kebbi Central Abattoir

2.4 Microbiological characterization of the wastewater

The bacteria in the wastewater, including the total bacteria and coliform counts, were determined using the membrane filtration technique described by Brock (1983). One hundred (100) ml of the sample was passed through a sterile cellulose filter, after which the filter was placed on a nutrient agar plate and incubated for about 24 hours at 35 ^{0}C. The bacteria colonies formed on the plate were then counted with a colony counter. The coliform colonies were estimated using the two-step enrichment procedure in which the filters containing bacteria were placed on an absorbent pad saturated with lauryl tryptose broth and incubated at 35 ^{0}C for 2 hours. The filters were then transferred to an absorbent pad saturated with M-Endo media and incubated for 22 hours at 35 ^{0}C. Sheen colonies were seen and then estimated with a colony counter. The same filtration technique was used to estimate the fungi colonies, but the agar nutrient was supplemented with an antibiotic to prevent bacterial growth (Babič *et al*., 2017).

2.5 Collection of *A. cepa* bulbs and cytogenetic evaluation

The cytogenetic effects of the wastewater were determined using the *A. cepa* toxicity test described by Fiskesjo (1988). Eighty (80) healthy-looking *A. cepa* bulbs weighing about 31 g each were purchased from Birnin Kebbi Central Market in March 2019. The bulbs were air-dried for 14 days, after which 50 viable bulbs were selected and subjected to surface sterilization before being randomly distributed equally into 5 groups. Group 1 contained the control samples and was grown over beakers containing 100 ml tap water at room temperature and humidity for 72 hours. The test samples in groups 2 through 5 were grown over beakers containing 25, 50, 75, and 100 % of the wastewater, respectively under the same condition and duration as the control. The root length of the bulbs was monitored during the experiment, after which about 3 cm of the roots was cut and fixed immediately in aceto-alcohol in the ratio 1:3. Each root tip was macerated in drops of 1 N HCl at 60° C for 3 minutes, followed by staining in Carbol Fuchsin stain (Koa, 1975). The root tips were afterward squashed in a 2 % aceto-orcein in 45 % acetic acid. Permanent slides were made and the number of dividing cells and chromosomal aberrations were determined by examining 1000 cells per slide under a microscope.

2.6 Data analysis

Data analysis was carried out with the Statistical Package for Social Sciences (SPSS) version 20 for Windows. Comparison of data among the test and control groups was done using the Student's *t*- test. The $p \leq 0.05$ was considered statistically significant.

3. Results

3.1 Physicochemical parameters of the wastewater

Table 1 shows the mean values of the levels of the tested physicochemical characteristics of the wastewater. The temperature, pH, Ni, Cr, Fe, Cu, and Cd were detected within the WHO limits for wastewater. However, other tested parameters such as turbidity, TSS, Pb, DO, BOD, COD, and nitrate were recorded at levels above (or below, in the case of DO) WHO limits.

3.2 Microbial counts of the wastewater

The microbial counts of the wastewater are shown in Table 2. Bacteria and fungi colonies were detected in the wastewater above the respective WHO limits for wastewater, however, coliform colonies were not found.

3.3 Daily root length increase of *A. cepa*

The daily root length increase of the exposed and unexposed *A. cepa* is shown in Table 3. A significant ($p<0.05$) growth increment was observed in the control samples, while the test samples showed growth inhibition.

Table 1: Physicochemical characteristics of the Birnin Kebbi Central Abattoir Wastewater

Parameter	Detected Level	WHO Limit
Temp (^{O}C)	24.31 ± 0.128	25
pH (unit)	6.70 ± 0.033	6.0 - 9.0
TSS (mg/L)	2501.17 ± 50.23	2000
Turbidity (NTU)	AL	5.0
Cu (mg/L)	0.732	2.0
Fe (mg/L)	0.34 ± 0.01	5.0
Cd (mg/L)	0.009 ± 0.0012	0.01
Cr (mg/L)	0.033 ± 0.001	0.05
Pb (mg/L)	0.033 ± 0.011	0.02
Ni (mg/L)	0.012 ± 0.009	0.2
DO (mg/L)	0.060 ± 0.013	1.0
BOD (mg/L)	201.10 ± 15.13	60.0
COD (mg/L)	925.10 ± 50.36	150.0
Nitrate (mg/L)	9.71 ± 2.01	1.0
Sulphate (mg/L)	460 ± 50	750

Data were expressed as mean ± SE (n = 3); AL = above limit; WHO = World Health Organization, 2011

Table 2: Microbial counts of the Birnin Kebbi Central Abattoir Wastewater

Microbe	Detected Level (CFU/ml)	WHO Limit
Bacteria	16200 ± 100	< 1000
Coliform	0.000 ± 0.000	< 400
Fungi	12000 ± 200	< 1000

Data were expressed as mean ± SE (n =3); WHO = World Health Organization, 2011

Table 3: Daily root length increase of the *A. cepa* exposed to the Birnin Kebbi Central Abattoir Wastewater

Treatment Conc. (%)	Day 1 (cm)	Day 2 (cm)	Day 3 (cm)
Control	$0.34^{a} \pm 0.01$	$0.55^{b} \pm 0.02$	$0.73^{c} \pm 0.03$
25	$0.13^{a} \pm 0.03$	$0.20^{a} \pm 0.01$	$0.22^{a} \pm 0.06$
50	$0.12^{a} \pm 0.04$	$0.14^{a} \pm 0.05$	$0.13^{a} \pm 0.03$
75	$0.12^{a} \pm 0.03$	$0.11^{a} \pm 0.01$	$0.12^{a} \pm 0.01$
100	$0.11^{a} \pm 0.01$	$0.11^{a} \pm 0.04$	$0.10^{a} \pm 0.02$

Values were expressed as mean ± SE (n = 3); values with different superscripts 'a', 'b' and 'c' along the same row are statistically different at $p<0.05$ (student's *t*-test).

3.4 Chromosomal aberrations induced by the wastewater

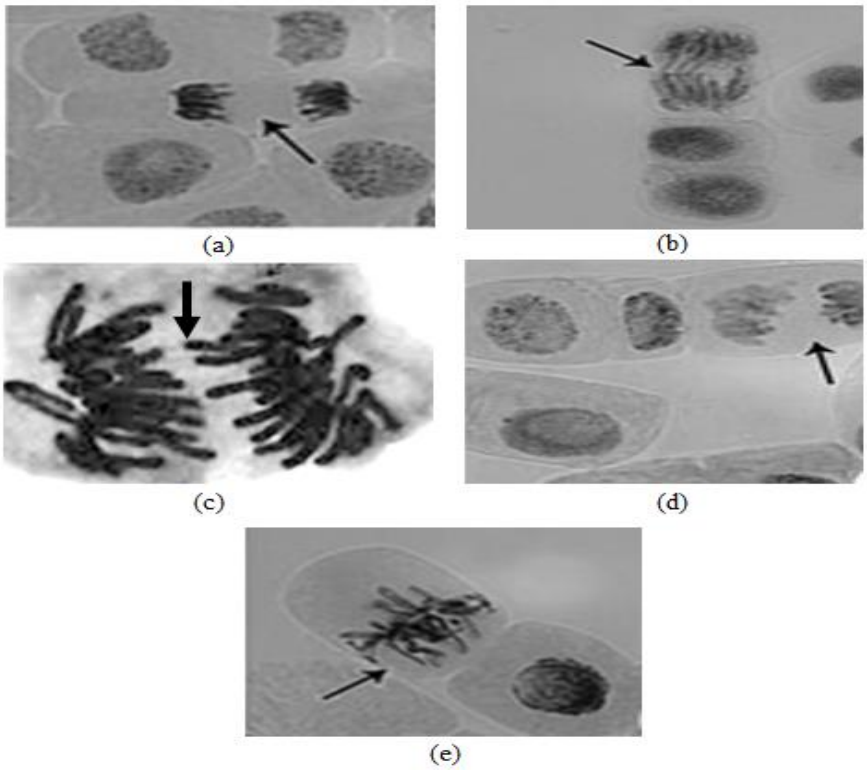
Table 4 reveals the number of dividing cells, the mitotic index and chromosomal aberrations detected in the exposed *A. cepa*. While 38 dividing cells were recorded in the control samples, 24, 22, 23 and 19 dividing cells were observed in the 25, 50, 75 and 100 % of the wastewater, respectively. Compared with the control, the mitotic index of the wastewater was significantly reduced ($p<0.05$) with 100 % concentration being the most reduced followed by 50 %, 75 % and 25 %, respectively. The number of chromosomal abnormalities induced in the exposed *A. cepa* increased with the increasing concentrations of the wastewater (25 % < 50 % 75 < 100).

Figure 2 (a-e) shows the chromosomal aberrations observed in the cells of the root tips of the control and exposed *A. cepa*. Normal chromosomes were observed in the anaphase of the cells of the control samples (a), while chromosomal bridges and vagrant chromosomes were seen in the samples exposed to 25 and 50 % of the wastewater, respectively (b and c). Sticky chromosomes were seen in the samples exposed to 75% (d), while delayed anaphase and vagrant chromosomes were observed in samples exposed to 100% wastewater (e).

Table 4: Chromosomal aberrations induced by the Birnin Kebbi Central Abattoir Wastewater

Treatment Conc. (%)	TCN	ND	ST	CM	BF	VG	LG	MC	TA (%)	MI	MI ± SEM
Control	1000	38($P_8M_{14}A_9T_7$)	0	0	0	0	0	0	-	3.80	3.80±0.34
25	1000	24($P_0M_0A_1T_{23}$)	9	0	0	2	0	0	45.83	2.40	2.40±0.50*
50	1000	22($P_4M_9A_6T_3$)	7	0	3	1	1	0	54.55	2.20	2.20±0.43*
75	1000	23($P_3M_9A_{10}T_1$)	10	0	0	2	3	0	65.22	2.30	2.30±0.38*
100	1000	19 ($P_4M_6A_8T_1$)	6	2	0	5	0	0	68.42	1.90	1.90±0.26*

Values were expressed as mean ± SE (n = 3); values with an asterisk (*) are statistically different from control at $P<0.05$ (student's *t*-test); TCN = total cell number; ND = number of dividing cells; ST= stickiness; CM = c-mitosis; BF = bridge fragment; VG = vagrant; LG = laggard; MC= micronuclei cell; TA = total aberrations; SEM = standard error of the mean; MI = mitotic index; P = prophase; M = metaphase; A = anaphase;T= telophase.



a = Normal anaphase observed in the control *A. cepa* (x 40), b = Chromosomal bridge at telophase observed in the *A. cepa* grown over 25 % of the wastewater (x 40), c = Vagrant chromosomes observed in the *A. cepa* grown over 50 % of the wastewater (x 40), d = Sticky chromosomes observed in the *A. cepa* grown over 75 % of the wastewater (x 40), and e = Delayed anaphase and vagrant observed in *A. cepa* grown over 100 % of the wastewater (x 40)

Fig. 2 (a-e): Chromosomal aberrations detected in the root cells of *A. cepa* bulbs

4. Discussion

The physicochemical and biological characteristics of Birnin Kebbi Central Abattoir Wastewater, as well as its cytogenetic effects, were determined in this study. In the physicochemical characterization (Table 1), the concentrations of the Pb, nitrate, turbidity, TSS, BOD and COD were higher than the WHO limits for wastewaters, while DO was lower. Similar findings were reported by Dauda *et al*. (2016), Shukri *et al*. (2017) and Oyekunle and Lateef (2017). The wastewater evaluated in this study also contained permissible levels of some other tested parameters, including Ni, Cu, Cr and Fe. Akharame *et al*. (2017) and Adesina *et al*. (2018) also reported permissible levels of Ni, Cu, Cr and Fe in wastewaters obtained from abattoirs. Though some of these metals recorded at permissible levels are considered safe at minute quantities, they can accumulate gradually to toxic levels in the environment and biological systems. According to Akpor and Muchie (2010), some metals are persistent in the environment and can bio-accumulate to toxic levels along the food chain. The microbial characterization of the wastewater (Table 2) detected colonies of bacteria and fungi above the permissible limits, while coliform colony was not detected. This finding agrees with Adesemoye *et al*. (2006), Rabah *et al*. (2010) and Nafarnda *et al*. (2012), who detected bacteria and fungi colonies above the permissible levels in soil and water contaminated with abattoir wastewater. The high microbial populations of the wastewater could be due to its high nutrient content, particularly nitrate, Fe and Cu. Additions of Fe (ferrous sulfate) to water has been shown to increase the number of *Escherichia coli* (Appenzeller *et al*., 2005). Similarly, addition of Cu to contaminated soil has been demonstrated to increase fungal growth (Rajapaksha *et al*., 2004). Xia *et al*. (2007) also reported an increase in the growth of bacteria following an addition of nitrate to contaminated soil.

The observed characteristics of the studied wastewater showed that Birnin Kebbi Central Abattoir may be toxic. This is evident in the insignificant daily root increase of the treated *A. cepa* (Table 3) compared with the control. Heavy metals as well as high BOD and low DO detected in the wastewater might have contributed to the reduced root growth of the exposed samples. Akpor *et al*. (2014) linked heavy metal pollution in water, soil, and foods to reduced physiological and metabolic activities, culminating in reduced growth and development in plants and animals. High BOD and depleted DO in wastewaters have also been reported to affect seed germination and seedling growth (Chhonkar *et al*., 2000). The high bacterial populations noticed in the wastewater could also contribute to the growth retardation observed. According to Ivanov *et al*. (2017), bacterial infections can induce oxidative stress, affecting cell growth and development. Fungi live in or on plant tissues and can disrupt physiological functions of plants, including growth (Abdulkhair and Alghuthaymi, 2016). The wastewater also contained high TSS and turbidity, both of which could reduce light penetration and photosynthesis, resulting in reduced growth. The toxicological potential of Birnin Kebbi abattoir wastewater is also evident in the reduced mitotic index and chromosomal abnormalities observed in the cells of the root tips of the exposed *A. cepa*. Some heavy metals detected in the wastewater have been linked with cellular and genetic damage (Bitto *et al*., 2014; Fuchs *et al*., 2018). Bacterial and fungal colonies detected in the wastewater can also compromise the immune system, causing inflammation and genetic disorders. Genetic Science Learning Center (2014) and Shreiner *et al*. (2015) reported that harmful microorganisms can disrupt the defense mechanism of an organism, setting off inflammations, autoimmunity, and genetic disorders.

5. Conclusions

The results showed that the wastewater contained high concentrations of Pb and appreciable levels of other tested water parameters such as Cd, Ni, Cu and Ni. The wastewater also had abnormal levels of TSS, turbidity, nitrate, phosphate, BOD, COD, DO as well as bacteria and fungi colonies. Consequently, the wastewater can be considered toxic, which is evident in the retarded root growth and mitotic index of *A. cepa* bulbs grown in varying concentrations of the wastewater. The wastewater also induced chromosomal aberrations in the cells of the root tips of the exposed samples. Since DNA and cellular components are nearly the same in plants and animals, these findings indicate that Birnin Kebbi Central Abattoir Wastewater can induce heritable and non-heritable genetic changes in humans. While we recommend more studies to verify our claims, the agencies in charge of public and environmental health in the city should keep a close monitor of the facility.

Conflict of interest

The authors have no interest to declare.

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