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Original Research Article

Concentration and Cytogenotoxicity of Heavy Metals and Microorganisms in Labana Rice Mills Wastewater Birnin Kebbi, Northwestern Nigeria

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ABSTRACT

Nigeria's rice milling industry is undergoing expansion to diversify the nation's economy and boost food sufficiency. However, there is a dearth of information on the safety of wastewater emanating from the industry. This study determined the physicochemical and microbiological characteristics as well as cytogenotoxicity of wastewater from a rice mill in Birnin Kebbi, Kebbi State, Nigeria. The water characteristics were determined using standard protocols, after which the wastewater was subjected to cytogenetoxicity test using Allium cepa toxicity test. Fifty Allium cepa bulbs divided equally into 5 groups were grown in beakers containing tap water (control) and 25, 50, 75, and 100 % (v/v) of the wastewater, respectively for 3 days. The root-tips of the bulbs were then examined for chromosomal aberrations. The physicochemical analysis showed that the levels of nickel (Ni), chromium (Cr), iron (Fe), pH, sulphate, and temperature were within the World Health Organization (WHO) permissible limits. However, lead (Pb), nitrate, turbidity, total suspended solids (TSS), dissolved oxygen (DO), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) were above the permissible limits. The microbiological analysis showed normal coliform counts (< 400 CFU/ml), while bacteria and yeast colonies were above the permissible limits (> 1000 CFU/ml)). In the cytogenetic test, the exposed A. cepa showed dose-dependent sticky and vagrant chromosomes and anaphase bridges as well as significantly reduced (p < 0.05) mitotic index (MI) and root length growth. These findings suggest that the wastewaters could pose some health hazards unless treated.

1. INTRODUCTION

Nigeria is increasing rice (*Oriza sativa* L) production to diversify the nation's economy and boost food sufficiency (Tobias and Castro, 2020). However, concerns are rife on the health and environmental consequences of wastewater emanating from rice milling processes, particularly the parboiling of rice. Parboiling of rice consumes large volumes of water and releases enormous quantities of wastewater

(Sayanthan and Thusyanthy, 2018). Parboiling 1 kg of paddy rice would require between 1 to 1.5 L of water, generating 0.4 to 0.52 L of wastewater (Sayanthan and Thusyanthy, 2018). These wastewaters are laden with potentially toxic organic and inorganic matters which are released into the surroundings, water bodies, and agricultural fields, thus contaminating them (Choudhary *et al.*, 2015). The organic and inorganic compounds in the wastewaters cause eutrophication of the waterways, thus compromising aquatic ecosystems and reducing their biodiversity (Choudhary *et al.*, 2015). The wastewaters also elicit a foul smell and when left for a long time may percolate down the soil and pollute the groundwater such as wells and boreholes, predisposing the consumers in the environment to health hazards (Shrivastava, 2011).

Despite the health and environmental consequences of rice milling, rice production continued to increase worldwide and rice is consumed as a staple food by over half of the world's population (Kumar *et al.*, 2017). Yearly, over 490 million metric tons of husked rice is produced globally (Shahbandeh, 2020). In Africa, Nigeria is currently the largest rice producer, accounting for about 0.8% of global rice production (Udemezue, 2018). As of 2018, Nigeria's rice production capacity had reached 15 million metric tons, with an annual growth increase of 5% (Udemezue, 2018). The rapid increase in the global population could be responsible for the continuous expansion in the rice industry in order to meet the food demands (Kumar *et al.*, 2017). Compared to other staple foods like maize and wheat, rice provides more energy per unit area of cultivated land, and hence, it can support more population (Lim *et al.*, 2014). Rice is also nutritionally rich and contributes about 21% of global human per capita energy as well as 15% of per capita protein (Oli *et al.*, 2014). Additionally, rice production creates huge employment opportunities worldwide. In Nigeria, people now earn a living by cultivating or working on rice farms, working in rice processing industries, and marketing rice grains.

Collectively, the above underscored the need for efficient wastewater treatment strategies in rice milling industries, to create a sustainable development between rice milling industries and host communities. But to achieve this, the nature and concentrations of contaminants in the wastewater discharge by rice milling industries need to be established. One of the rice milling industries established in the last decade in Nigeria is Labana Rice Mills Limited, Birnin Kebbi. The rice industry has two plants with a total installed capacity of forty metric tons of rice per day. Thus, the industry uses large volumes of water and discharges large amounts of wastewater daily. Unfortunately, there is a dearth of information on the safety of these wastewaters, particularly its cytogenetic effects which may be passed on to many generations. Cytogenotoxity testing of a suspected toxicant is often done with *Allium cepa* toxicity test. The test is cheap, simple to conduct, and effective for evaluating both plant and animal toxicants (Yahaya *et al.*, 2020). Therefore, this study characterized and employed *Allium cepa* toxicity test to determine the cytogenotoxicity of wastewaters obtained from Labana Rice Mills, Birnin Kebbi. The findings of the study are expected to form preliminary data towards effective wastewater management in the industry.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

The Labana Rice Mills Limited is located along the Kebbi-Argungu Motorway, Birnin Kebbi. Birnin Kebbi is the capital city of Kebbi State in northwestern Nigeria on latitude 12°27'57.880" North and longitude 4°11'58.2864" East (Figure 1). The State has boundaries with Zamfara State in the east, Sokoto State in the north, Niger State in the south, and the Niger Republic in the west. The climate of the state is semi-arid and characterized by a long dry season and short wet season with an annual rainfall of about 787 mm (Yahaya *et al.*, 2020). The average daily temperature is 26 °C but could fall below 21 °C during the cold season and rise above 40 °C during the hot season. The vegetation of the state is dominated by grasses and sparse trees. The indigenous people of Kebbi are predominantly farmers and animal breeders and are particularly known for rice cultivation. The Labana rice mills employs over a thousand workers and serves as a source of income to rice farmers in the state. The mill consumes large volumes of water daily, thus releasing enormous amounts of wastewater whose safety needs to be monitored periodically to prevent unintended hazards to workers and the host communities.

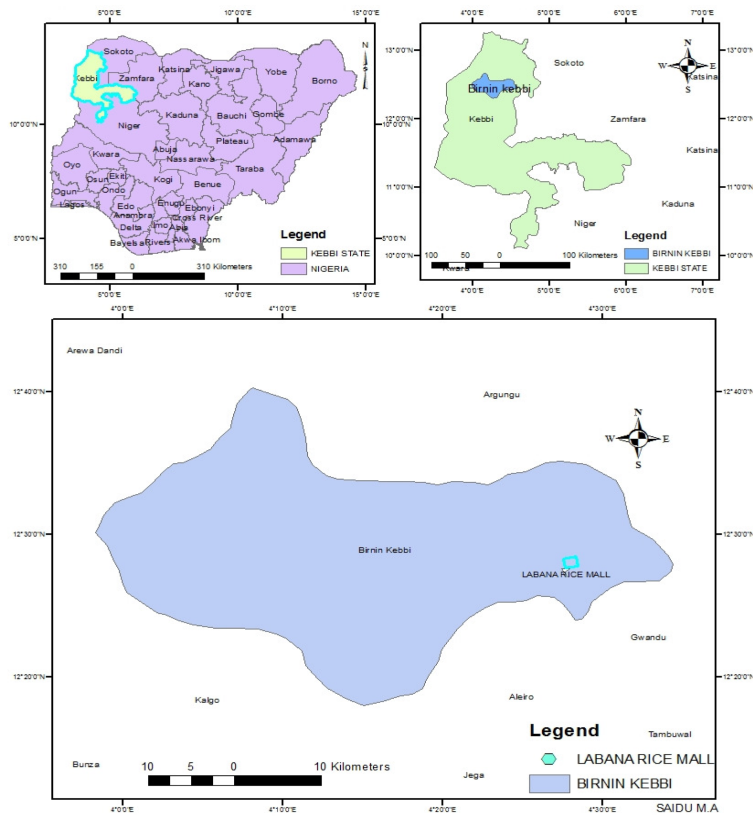


Figure 1: Locations of Labana Rice Mills, Birnin Kebbi (drawn using ArcGIS 10.3 software)

2.2. Collection of Wastewater

Samples of the wastewater were collected in duplicate at the discharge site and around the mills at different times of the day between October 2019 and April 2020. The wastewaters were collected in sterilized plastic containers, covered tightly, and moved to the laboratory where they were stored in a refrigerator at about 4 °C before analysis.

2.3. Physicochemical Analysis

Guidelines for measuring water quality parameters as described by WHO (2008) were used to determine the physicochemical parameters of the wastewater. To ensure accurate results, time-sensitive parameters, such as temperature, pH, and total suspended solids (TSS) were measured on-site with a mercury-in-glass thermometer, Pye Unicam pH, and HM Digital TSS meter model TSS-4, respectively. Other parameters, including turbidity, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD) as well as sulphate and nitrate concentrations were determined in the laboratory. These parameters were measured using TU5 Series Turbidimeter model 400sc, Clark DO Sensors model 5500, HACH BOD instrument model 25638018, as well as Perkin Elmer UV spectrophotometer model lambda 45 HACH, respectively. The concentrations of iron (Fe), 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 °C for 3 hours. The digested material was filtered into a 100 ml beaker, and the solution was analyzed for the selected metals using Atomic Absorption Spectrometer (UNICAM model 969).

2.4. Microbial Analysis

The total bacteria, coliform, and yeast species in the wastewater were quantified using the membrane filtration technique as outlined by Yahaya *et al.* (2020). One hundred (100) ml of the wastewater was passed through a sterile cellulose filter and then placed on a nutrient agar plate and incubated for 24 hours at 35 °C. The total bacteria growth on the plate was then counted using a colony counter. The technique was also used to quantify the coliform colonies. However, two-step enrichment was used to grow the microbes in which the filters containing bacteria were placed on an absorbent pad saturated with lauryl tryptose broth and incubated at 35 °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 °C. Sheen colonies were observed and then estimated with a colony counter. The nutrient agar was also used to estimate the yeast populations, but the agar nutrient was supplemented with an antibiotic to prevent bacterial growth.

2.5. Collection of *Allium cepa* Bulbs and Cytogenotoxicity Test

The cytogenetic effects of the wastewater were determined using the *A. cepa* toxicity test described by Fiskesjo (1988). Eighty (80) *A. cepa* bulbs with a mean weight 30±2 g were purchased from the Birnin Kebbi Central Market in October 2019. The *A. cepa* bulbs were air-dried for two weeks, after which fifty viable bulbs were selected for the study. The bulbs were sterilized and then divided into 5 groups of 10 each. Group 1 contained the control samples and was grown in beakers containing 100 ml tap water under ambient temperature and humidity for 72 hours. Groups 2-5 contained the test samples and were grown under the same conditions as the control over 25, 50, 75, and 100 % (v/v) of the wastewater. The root lengths of the *A. cepa* bulbs were recorded during the experiment, after which the roots were cut and fixed instantly in Aceto-alcohol in the ratio 1:3. The root tips of each bulb were macerated in drops of 1 N HCl at 60 °C for 3 minutes and then stained with Carbol Fuchsin (Koa, 1975). The mixture was later squashed in a 2% Aceto-orcein in 45% acetic acid. Permanent slides were made and mounted on Canadian turpentine for the identification of chromosomal abnormalities. Chromosomal abnormalities were determined and calculated by examining one thousand cells in each slide.

2.6. Quality Assurance and Control

All the reagents used for the heavy metal 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 and then rinsed properly with water and the reagent. Background contamination of the samples was checked to ensure the accuracy of the data. Blank samples were analyzed after five samples and all the analyses were replicated three times. The reproducibility of the values was found to be at the 95% confidence level. Therefore, the mean value of each heavy metal was used for further interpretations. The precision and accuracy of the analyzed metals were checked against standard reference materials for every heavy metal.

2.7. Data Analysis

The Statistical Package for Social Sciences (SPSS) version 20 for Windows was used for all analyses. The comparison of data between the test and control groups was done using the Student's *t*-test. The $p \leq 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Physicochemical Characteristics of the Wastewater

Table 1 shows the levels of the heavy metals in the wastewater in which Fe, Ni, Cr, pH, temperature, and sulphate were detected within the World Health Organization (WHO) permissible limits. However, Pb, TSS, turbidity, nitrate, DO, COD, and BOD were abnormal. This result is similar to those of Mukherjee *et al.* (2014) and Choudhary *et al.* (2015) who detected low DO as well as high COD, BOD, TDS, and nitrate in rice wastewater. The result also agrees with David *et al.* (2020) who noticed increased concentrations of Pb and reduced concentrations of Ni and Cr in rice following parboiling. However, the result contradicts those of Gerber *et al.* (2016) who reported abnormal concentrations of Fe in untreated rice wastewater. Padhan and Sahu (2011) and Mahananda *et al.* (2015) also reported high pH (alkaline) in rice wastewater as against the slightly acidic pH (6.97) observed in the present study.

Overall, the results suggest that the wastewater could pose some environmental and health risks unless treated. The high TSS of the wastewater shows that the wastewater contained high inorganic and organic materials, resulting in high turbidity. Additionally, the high organic and inorganic contents of the wastewater could be responsible for its high BOD and COD, depleting the DO. If the wastewater is continuously discharged into a water body, its high organic contents, particularly nutrients such as nitrate and sulphate, may lead to algae boom, culminating in eutrophication and low DO (USEPA, 2012). This may lead to the suffocation of aquatic lives, such as fish, crabs, and lobsters, among others. Moreover, the high TSS and turbidity of the wastewater may block sunlight penetration to some photosynthetic plants, affecting their survival. Prolonged exposure to heavy metals in the wastewater, particularly Pb detected at abnormal concentrations may predispose humans to health hazards through dermal contact as well as oral ingestion via drinking water. The possible sources of heavy metals in the wastewater are agrochemicals such as fertilizers, pesticides, herbicides, among others. The soils on which the rice plants were raised and the water used for parboiling could also contain heavy metals naturally.

Table 1: Physicochemical properties of the Labana rice mills wastewater

Properties	Detected levels	Permissible limits (WHO, 2011)
Temp (^{O}C)	22.90 ± 0.11	≤ 25
pH (unit)	6.79 ± 0.004	6.0 - 9.0
TSS (mg/L)	2301 ± 120.00	≤ 2000
Turbidity (NTU)	AL	≤ 5.0
Fe (mg /L)	2.02 ± 0.311	≤ 5.0
Cr (mg/L)	0.039 ± 0.0051	≤ 0.05
Pb (mg/L)	0.056 ± 0.003	≤ 0.02
Ni (mg/L)	0.013± 0.0031	≤ 0.2
DO (mg/L)	0.05 ± 0.0021	≥ 1.0
BOD (mg/L)	200.20 ± 21.23	≤ 60.0
COD (mg/L)	1200.10 ± 123.00	≤ 150.0
Nitrate (mg/L)	9.813± 1.23	≤ 1.0
Sulphate (mg/L)	203.00 ± 50.30	≤ 750

Values were expressed as mean ± SD (n = 3); WHO = World Health Organization; AL = above limit

3.2. Microbial Loads of the Wastewater

Table 2 shows that the bacteria and yeast colonies in the wastewater were above the WHO permissible limits for wastewater (≤ 1000 CFU/mL). However, the coliform colonies were within the permissible limits (≤ 400 CFU/mL). This result is similar to those of Mukherjee *et al.* (2016) who reported high bacteria and algae colonies in parboiled rice wastewater. Malik *et al.* (2011) also detected heavy bacterial isolates in rice wastewater. Additionally, Fatokun and Alikali (2006) reported permissible levels of coliform colonies in rice wastewater in Nigeria. The high inorganic and organic contents of the wastewater in the current study, particularly nutrients such as nitrate could be responsible for its heavy bacteria and yeast colonies. According to Amanidaz *et al.* (2015), organic carbon (though not assessed in the current study), and nutrients, are necessary for the growth and survival of all microorganisms. Some of the microorganisms could have also been introduced from the rice fields. In an experiment that investigated the microbial pollution of floodwater from rice fields, abnormal populations of bacteria were detected (Pittol *et al.*, 2018). In a related study, Xu *et al.* (2012) demonstrated that sewage irrigation increases soil bacteria, ammonia oxidizing bacteria, and aerobic cellulose decomposing bacteria. The heavy microbial species detected in the present study could be responsible for the high BOD of the wastewater, thus depleting the DO. This again shows that the wastewater could be toxic to aquatic lives as well as humans if the microorganisms are ingested through dermal or oral ingestions. Though coliform was detected in the wastewater within the permissible limits, its detection shows that the wastewater contained faecal contaminants, which may compromise the environment. It may also compromise the suitability of groundwater in the surroundings for drinking and domestic uses.

Table 2: Microbial loads of the Labana Rice Mills wastewater

Microbe	Detected levels (CFU/mL)	Permissible limits (WHO, 2011)
Bacteria	401201 ± 3365	≤ 1000
Coliform	150.00± 30.00	≤ 400
Yeast	1306 ± 90.00	≤ 1000

Values were expressed as mean ± SD (n = 3); WHO = World Health Organization

3.3. Root Length Growth of *Allium cepa*

Table 3 reveals the daily root length increase of the exposed *A. cepa* and control. The control samples showed a significant ($p < 0.05$) growth increase, while the treated samples had growth inhibition. An earlier study by Kumar *et al.* (2016) also showed that rice wastewater retarded seed germination as well as hindered root and shoot growth. In a related study, untreated rice wastewater hindered the growth of water lettuce (Mukherjee *et al*., 2014). This result again proved that the wastewater was toxic and could cause environmental and health hazards. Heavy metals in the wastewater, particularly Pb detected at abnormal levels, could be responsible for the growth inhibition. According to Nas and Ali (2018), Pb can retard growth and photosynthesis, disrupt mineral uptake, water balance, and hormonal status in plants. Pb exposure has also been shown to cause weight loss and retard development in mice (Donald *et al*., 1987). Though Fe, Cr, and Ni were detected within the permissible limits in the wastewater, strictly speaking, there are no safe levels for heavy metals. Heavy metals are non-biodegradable and so can bioaccumulate to toxic levels in biological systems, thus predisposing animals and plants to health hazards. Excessive Cr exposure inhibits plant growth and development (Wakeel and Xu, 2020). Exposure of plants to Ni may alter seed germination as well as growth of roots, stems and leaves (Sreekanth *et al*., 2013). Some of the investigated heavy metals and others in the wastewater may also interact and additively induce growth inhibition. For example, Gardner *et al.* (2013) and Zeng *et al.* (2019) observed growth retardation in some children exposed to a number of heavy metals. Furthermore, the wastewater was slightly acidic which could have contributed to the observed growth inhibition. According to Shrivastava *et al.* (2011), the acidic nature of rice wastewater may affect the alkalinity of the soil and over time causes irreversible changes in the soil and underground water sources. This may ultimately affect the growth and development of plants and animals (Shrivastava *et al*., 2011). The wastewater was also turbid, which could block photosynthetic organisms from sunlight, thus affecting their growth and development. Both plants and animals need oxygen for optimum growth and development, thus the low DO of the wastewater could also contribute to the growth inhibition.

Table 3: Daily root length increase of the *Alium cepa* exposed to Labana Rice wastewater

Treatment concentration (%)	Day 1 (cm)	Day 2 (cm)	Day 3 (cm)
Control	$1.24^a \pm 0.15$	$2.15^b \pm 0.53$	$3.32^c \pm 0.33$
25	$0.41^a \pm 0.15$	$1.71^a \pm 0.22$	$2.37^b \pm 0.26$
50	$0.21^a \pm 0.02$	$0.71^a \pm 0.25$	$1.01^a \pm 0.31$
75	$0.19^a \pm 0.01$	$0.21^a \pm 0.02$	$0.57^a \pm 0.15$
100	$0.19^a \pm 0.03$	$0.20^a \pm 0.05$	$0.31^a \pm 0.12$

Values were expressed as mean ± SD (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*)

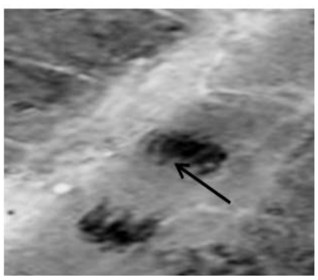
3.4. Chromosomal Aberrations in the *Allium cepa*

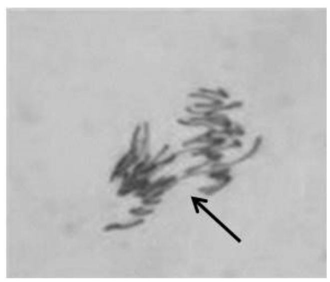
Table 4 reveals the number of dividing cells, mitotic index (MI), and chromosomal aberrations in the exposed and control *A. cepa*. The control samples had 38 dividing cells, while the samples exposed to 25, 50, 75, and 100% (v/v) of the wastewater contained 27, 25, 18, and 16 dividing cells, respectively. Compared to the control, the MI of the exposed samples showed a significant reduction ($p < 0.05$) in the order of 100% < 75% < 50% < 25%. Similar trends were also observed in the number of chromosomal abnormalities induced by the varied concentrations of the wastewater. Moreover, Figures (2a-e) describe the chromosomal abnormalities observed in the root-tip cells of the control and exposed samples. The cells of the control samples had normal chromosomes at telophase (Figure 2a), while the samples treated with 25 and 75% had vagrant chromosomes (Figures 2b and 2d). Bridges fragments and sticky chromosomes were seen in the samples treated with 50 and 100% of the wastewater (Figures 2c and 2e), respectively. There is a dearth of study on the cytogenotoxicty of rice wastewater. However, rice crop water was demonstrated by Wandscheer *et al.* (2017) to induce chromosomal abnormalities, including anaphasic and telophasic bridges, laggard chromosomes, and insignificantly reduced MI.

Table 4: Chromosomal aberrations in the root-tip of *A. cepa* exposed to Labana Rice wastewater

Treatment conc. (%)	TCN	ND	ST	CM	BF	VG	LG	TA (%)	MI	MI ± SEM
Control	1000	38 ($P_8M_{14}A_9T_7$)	0	0	0	0	0	-	3.80	3.80±0.34
25	1000	27($P_4M_{10}A_{10}T_3$)	5	0	0	3	1	33.33	2.70	2.70±0.32*
50	1000	25($P_5M_9A_7T_4$)	8	0	1	2	0	44.00	2.50	2.50±0.30*
75	1000	18($P_6M_3A_6T_3$)	0	0	1	1	8	62.50	1.80	1.80±0.28*
100	1000	16($P_6M_5A_3T_2$)	8	0	0	0	4	75.00	1.60	1.60±0.19*

Values were expressed as mean ± SD (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 = bridges fragment; VG = vagrant; LG = laggard; TA = total aberrations; MI = mitotic index; SEM = standard error of the mean; P = prophase; M = metaphase; A = anaphase; T = telophase


(a)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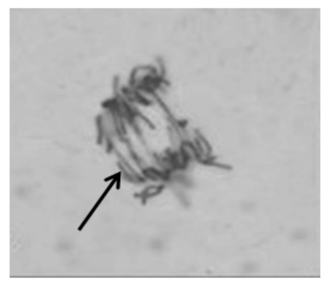
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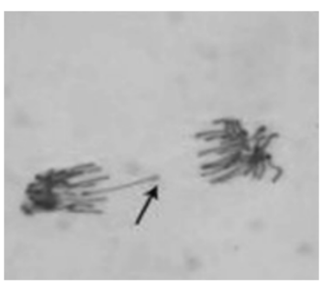
(d)


(e)

Figure 2 (a-e): Chromosomal aberrations detected in the root cells of the *A. cepa* bulbs
a = normal telophase observed in the control *A. cepa* (x 40), b = vagrant chromosomes observed in the *A. cepa* grown over 25% of the wastewater (x 40), c = bridges fragment observed in the *A. cepa* grown over 50% of the wastewater (x 40), d = vagrant chromosomes observed in the *A. cepa* grown over 75 % of the wastewater (x 40), and e = sticky chromosome observed in *A. cepa* grown over 100% of the wastewater (x 40).

The results of the current study further proved the toxicity of the wastewater. The heavy metals detected in the wastewater could be responsible for the observed cytogenetic effects. According to Jan *et al.* (2015), heavy metals can generate free radicals, causing cellular and DNA damage. In an experiment in which some tilapia fish were exposed to dietary Pb, dose-dependent oxidative stress and DNA damage were observed (Dai *et al*., 2012). Pb was also demonstrated in *A. cepa* to induce cytogenetic effects, including root damage, decrease in cell division, DNA fragmentation and micronucleus frequency (Arya *et al*., 2013). Elevated oxidative stress and DNA damage were reported in people exposed to Cr (VI) for a long time (Xu *et al*., 2018). Cr was also shown in *A. cepa* to induce DNA damage, micronuclei, chromosomal fragmentation and bridging (Patnaik *et al*., 2013). Ni was demonstrated in *Escherichia coli* to disrupt DNA replication and cause DNA damage (Kumar *et al*., 2017). In the same vein, the observed cytogenetic problems might also be caused by microorganisms detected in the wastewater, particularly pathogenic microbes. According to Žgur-Bertok (2013) and Weitzman and Weitzman (2014), some microbes may induce DNA damage or inhibit DNA repair mechanisms by compromising the host defense mechanism. The authors stated further that some microbes may cause an inflammatory response, disrupting host cellular architecture and epigenome. Furthermore, the DO of the wastewater was low which might elicit oxidative stress and contribute to the observed cytogenetic effects as demonstrated in fish by Copatti *et al*. (2019).

4. CONCLUSION

The results established that the wastewater contained abnormal concentrations of Pb and appreciable levels of Ni, Fe, and Cr. The wastewater also had non-permissible levels of TSS, turbidity, nitrate, BOD, COD, and DO as well as bacteria and yeast colonies. Consequently, the wastewater can be considered toxic, which is evident in the retarded root growth and mitotic index of the exposed *A. cepa*. The exposed samples also expressed some chromosomal abnormalities, including sticky and vagrant chromosomes as well as chromosomal bridges. These suggest that the wastewater can induce heritable and non-heritable genetic changes in biological systems. While we recommend more studies to verify our claims, the management of the industry should consider treating the wastewater prior to its discharge into the environment.

5. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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