Introduction

Meat is a commonly consumed food worldwide. Preferences for a type of meat, methods of dehairing slaughtered animals, and of processing dehaired meat for consumption differ from country to country and from culture to culture. In Nigeria and many other parts of Africa, in addition to the regular consumption of red meat, the habit of eating dehaired cow skin is common. This is despite the fact that governments, concerned organizations and individuals have mounted campaigns against the habit, since it poses a potential danger to the health of consumers and potentially threatens the leather industry.

In the southwestern part of Nigeria especially, dehaired cowhide meat is popularly referred to as ponmo. Ponmo is cowhide that has been processed, first by dehairing, to look like meat. There are two types; the first type is dehaired by shaving, called white ponmo, the other is dehaired by singeing, called brown ponmo. The names ‘white ponmo’ and ‘brown ponmo’ are reflections of their respective colors after dehairing and processing. Traditionally, singeing of cow skin to brown ponmo uses firewood for singeing, but due to a number of factors such as the unavailability of firewood, increasing number of cowhides for singeing, expanding ponmo market, urbanization, the urge to maximize profit etc, it became practically impossible for those in the ponmo business to continue to use firewood for singeing. As a result, various substances such as used plastics, scrapped tires, kerosene, and spent engine oil are used as fire sources, either alone or in combination with wood, to singe cowhide to brown ponmo. The singed cowhide is thereafter washed several times and

Objectives.

In the present study, physicochemical and genotoxic assessments of wastewater used to rinse ready-to-cook singed cowhide meat were carried out.

Methods.

Physicochemical analyses were carried out using the American Public Health Association procedures, while genotoxic assessment was carried out using Allium cepa chromosome assay.

Results.

The results of the physicochemical analyses indicated that some of the parameters, especially metals, were within the threshold of the limits set by the country’s regulatory agencies, but some parameters like phosphate, chloride, nitrate, biological oxygen demand, and chemical oxygen demand were higher in concentration. The wastewater inhibited the growth of A. cepa roots and caused a decrease in its mitotic index relative to the control onions exposed to water only. The highest root growth inhibition of 72.14% was induced by a 10% wastewater concentration, while the lowest (53.57%) was induced by a 5% wastewater concentration. In addition, the wastewater induced bi-nucleated, attached, vagrant, C-mitosis, sticky and bridged chromosomal aberrations. Wastewater at a 5% concentration induced the highest significant (P < 0.05) percentage chromosome aberration of 36.62% at 48 hours of exposure. Sticky chromosomes had the highest significant frequency (P <0.01) at the end of the 72-hour exposure period. No chromosomal aberration was observed in the control.

Conclusions.

These results indicate that singed cowhide meat wastewater is potentially genotoxic and environmentally harmful. Governments, public health practitioners, and relevant stakeholders should work in synergy to discourage the habit of processing cowhide into cowhide meat.

Competing Interests.

The authors declare no competing financial interests.

Keywords: allium assay, genotoxicity, ponmo, singed cow skin, wastewater.

Received February 23, 2018. Accepted October 16, 2018.

J Health Pollution 20: (181207) 2018 © Pure Earth
then boiled in water for several hours to bring about the initial softening of the hide. The boiled, singed hide is subjected to final softening by soaking in water until it is tender enough for cooking and looks appealing to consumers. Cow skin or hides that are dehaired by singeing are potentially exposed to contaminants like toxic organic compounds (polycyclic aromatic hydrocarbons, dioxins, furans, benzene) and trace/toxic metals which may be deposited by the singeing substrate. Studies have confirmed that cowhides processed for consumption by singeing have a higher contaminant load, especially metals, relative to those processed by other methods like shaving. In the present study, the wastewater in which singed cowhide meat had been soaked was first analyzed for physicochemical parameters, and thereafter tested for genotoxicity.

Methods

The common purple onion bulbs of average size (diameter: 5 to 6 cm) used in the present study were bought from Bariga market, Lagos (latitude 6°54’N and longitude 3°39’E). The wastewater in which singed cowhide meat received a final soak was obtained from a vendor at Ketu Market, in Lagos, Nigeria (latitude 6°58’N and longitude 3°34’E). A picture of singed cowhide made ready for processing by cutting into pieces is shown in Figure 1. Figure 2 shows pieces of processed, singed ready-to-cook cowhide meat soaking in the wastewater used for the present study.

Physicochemical analyses of singed cowhide meat wastewater

The physicochemical analyses of the singed cowhide meat (ponmo) wastewater were carried out in the laboratory of the Lagos State, Nigeria Environmental Protection Agency (LASEPA) using standard American Public Health Association (APHA) procedures as described in Okiei et al. The physicochemical properties tested included appearance, pH, total solids, total dissolved solids, dissolved oxygen (DO), 5-day biochemical oxygen demand (BOD5), chemical oxygen demand (COD), lead (Pb), zinc (Zn), cadmium (Cd), copper (Cu), and chromium (Cr).

Allium cepa genotoxicity test

The Allium assay test was adapted from Fiskesjo, Bakare et al., and Olorunfemi et al. The outer scales and brownish bottom plate of sun-dried onion bulbs were carefully removed, leaving the ring of primordial root intact. The peeled bulbs were placed in dechlorinated tap water during the cleaning procedure to prevent the primordial root from drying up.

The onion bulbs were grown in tap water at room temperature (25-30°C) for 24 hours. When the roots were 2-3 cm long, the bulbs were transferred to the ponmo wastewater of concentrations 5%, 10%, 20% and 100% (v/v, effluent/water). The test substrates were changed daily. Five onion bulbs were set up for each concentration including the control, out of which the best four were selected for evaluation. The root lengths of the onions were measured from 24 hours to 72 hours using a meter rule and expressed in centimeters (cm) as described by Fiskesjo.

Abbreviations

| BOD | Biochemical oxygen demand | COD | Chemical oxygen demand |

Figures 1 & 2 — (Figure 1) Singed cowhide made ready for processing by cutting into pieces; (Figure 2) Pieces of processed, singed ready-to-cook cowhide meat soaking in wastewater
percentage root length inhibition were calculated using Equations 1, 2, and 3 respectively.

Equation 1
Mean root length (cm) = (Summation of root lengths) / (Total number of root lengths counted)

Equation 2
Percentage root length = (Root length in test solution) / (Root length in control) × 100

Equation 3
Percentage (%) root length inhibition = (Root length in control-root length in test solution) / (Root length in control (water)) × 100

The squash technique for onion root as described by Adegbite and Olorode was used for the chromosomal investigation. Chromosome samples were taken from the root tip meristem containing actively growing cells. One root tip was squashed on each slide and stained with acetocarmine for 10 minutes. Cover slips were carefully lowered onto the slide to exclude air bubbles. To prevent the possible drying out of the preparation, the cover slips were sealed on the slides with clear fingernail polish. The slides were observed under the light microscope (Leica 2000 phase contrast microscope). Data on total cells, total dividing cells, and cells carrying chromosomal aberrations were taken from the slides prepared for each of the different concentrations and the control.

The mitotic index and percentage mitotic index were calculated using Equations 4 and 5.

Equation 4
Mitotic index = (Number of dividing cells) / (Total number of cells) × 100

Equation 5
Percentage mitotic inhibition = (Mitotic index in control-mitotic index in test solution) / (Mitotic index in control) × 100

The frequency of chromosomal aberrations was calculated by expressing the number of aberrant cells as a percentage of total dividing cells for each treatment (Equation 6). Scoring of chromosomal aberrations was taken from five microscopic fields for each of the different test solutions.

Equation 6
Percentage aberration = (Number of total chromosomal aberrations) / (Total number of dividing cells) × 100

Statistical data analyses
Data on the number of different aberrant chromosome types at the end of 72 hours were processed and compared by general linear model univariate analysis of variance. The mean values were compared for level of significance using the least significant difference test. All statistical analyses were carried out with IBM Statistical Package for the Social Sciences (SPSS) version 20 software.

Results
The results of the physicochemical analyses carried out on the cowhide meat wastewater indicated that some of the parameters, especially trace/toxic metals, were below or within the threshold of the limits set by the country’s regulatory agencies, Lagos State Environmental Protection Agency (LASEPA), Nigeria; and National Environmental Standards and Regulations Enforcement Agency (NESREA). However, some chemical parameters like phosphate, chloride, nitrate, BOD, and COD were higher in concentration (Table 1).

Effect of cowhide meat wastewater on the root growth of A. cepa
Cowhide wastewater inhibited the growth of A. cepa roots (Table 2). The highest root growth inhibitions produced by 5%, 10%, 20% and 100% ponmo effluent occurred at 72 hours of exposure. The highest root growth inhibition of 72.14% was induced by 10% wastewater concentration, while the lowest (53.57%) was induced by 5% wastewater concentration.

Mitotic activity and mitotic inhibition induced in A. cepa exposed to cowhide meat wastewater
The mitotic activity in the roots of A. cepa exposed to ponmo wastewater indicated that the wastewater caused a decrease in the mitotic index relative to the control onions exposed to water only. While the highest mitotic index (50%) was recorded in control onions exposed to water only, the lowest (3.33%) was recorded in the roots of onions exposed to a 100% ponmo wastewater concentration. No mitotic inhibition was recorded in onions exposed to water only, while the highest mitotic inhibition (93.34%) was recorded in those exposed to a 100% wastewater concentration. It was observed that the highest mitotic inhibitions induced by 5%, 10%, 20% and 100% ponmo wastewater concentrations all occurred at 72 hours of exposure (Table 3).
Chromosome aberrations induced by cow skin meat wastewater

Cow skin meat wastewater induced chromosome aberrations in the roots of A. cepa (Table 4). No chromosome aberration was observed in onions exposed to water (control). The chromosome aberrations observed were bi-nucleated, attached, vagrant, C-mitosis, sticky and bridged chromosomes. At 24 and 48 hours of exposure, chromosome aberrations were highest in 5% ponmo wastewater, while at 72 hours, chromosome aberrations were highest in 10% wastewater. Sticky chromosomes had the highest significant frequency (P <0.01) at the end of the 72-hour exposure period. Representative samples of aberrant chromosomes are shown in Figures 3 to 8.

Discussion

Results from the present study revealed that some physicochemical properties, especially metal concentrations, of the cowhide meat wastewater used in this study were within the threshold of the limits set by the Nigerian regulatory agencies. However, many parameters like phosphate, chloride, nitrate, BOD, and COD were higher.
### Table 3 — Mitotic Index and Mitotic Inhibitions in *A. cepa* Exposed to Different Concentrations of Cowhide Meat (Ponmo) Wastewater

| Wastewater concentration | Cells counted | Number of dividing cells | Mitotic index (%) | Mitotic inhibition (%) |
|--------------------------|--------------|--------------------------|-------------------|-----------------------|
|                          | 24 hrs 48 hrs 72 hrs | 24 hrs 48 hrs 72 hrs | 24 hrs 48 hrs 72 hrs | 24 hrs 48 hrs 72 hrs |
| Control                  | 1000 1000 1000 | 500 500 500 | 50.00 50.00 50.00 | 0.00 0.00 0.00 |
| 5%                       | 972 942 923 | 84 71 64 | 8.54 7.54 6.93 | 82.92 84.92 86.14 |
| 10%                      | 958 905 881 | 71 63 51 | 7.41 6.96 5.79 | 85.18 86.08 88.42 |
| 20%                      | 914 891 869 | 58 42 33 | 6.35 4.71 3.80 | 87.30 90.58 92.40 |
| 100%                     | 883 868 842 | 46 37 28 | 5.21 4.26 3.33 | 89.58 91.48 93.34 |

### Table 4 — Chromosome Aberrations Induced in *A. cepa* Root Cells Exposed to Cowhide Meat (Ponmo) Wastewater

| Wastewater concentration | Bnc | Att | Vag | C-mit | Stk | Brg | Total aberrations % aberrations |
|--------------------------|-----|-----|-----|-------|-----|-----|-------------------------------|
|                          |     |     |     |       |     |     |                               |
| 24 hours                 |     |     |     |       |     |     |                               |
| 5%                       | 3   | 4   | 8   | 3     | 1   | 7   | 26 30.95                     |
| 10%                      | 8   | 0   | 4   | 1     | 4   | 4   | 21 29.58                     |
| 20%                      | 1   | 1   | 1   | 0     | 6   | 5   | 14 24.14                     |
| 100%                     | 0   | 0   | 0   | 12    | 0   | 12 26.09                     |
| 48 hours                 |     |     |     |       |     |     |                               |
| 5%                       | 4   | 4   | 6   | 2     | 7   | 3   | 26 36.62                     |
| 10%                      | 2   | 1   | 5   | 0     | 3   | 2   | 13 20.63                     |
| 20%                      | 1   | 1   | 3   | 1     | 2   | 3   | 11 26.19                     |
| 100%                     | 0   | 0   | 0   | 8     | 0   | 8   | 21 21.62                     |
| 72 hours                 |     |     |     |       |     |     |                               |
| 5%                       | 3   | 0   | 2   | 1     | 1   | 1   | 8 12.50                      |
| 10%                      | 2   | 2   | 3   | 0     | 4   | 2   | 13 25.49                     |
| 20%                      | 0   | 0   | 6   | 0     | 7   | 0   | 13 39.39                     |
| 100%                     | 0   | 0   | 0   | 3     | 0   | 3   | 10 10.71                     |
| Total                    | 24b | 13be | 38c | 8be  | 58a | 27b | 168                          |

Abbreviations: a, significantly different (higher or lower) from b (P < 0.01); c, significantly higher than e (P < 0.05); Bnc, bi-nucleated; Att, attached; Vag, vagrant; C-mit, C-mitosis; Stk, sticky; Brg, bridged

### Table 4 — Chromosome Aberrations Induced in *A. cepa* Root Cells Exposed to Cowhide Meat (Ponmo) Wastewater
in concentration. The high level of total suspended solids most likely resulted from the crumbs of processed cowhide meat as evidenced by the appearance of the wastewater, which was brownish yellow with suspended particles. In addition, the organic nature of cowhide meat and its crumbs were likely responsible for the elevated levels of COD and BOD in the wastewater. The high BOD level was also likely responsible for the very low DO in the cowhide meat wastewater as the presence of high BOD levels in a water body will always bring about accelerated bacterial growth accompanied by correspondingly high oxygen consumption. Although past studies that directly measured the quality of cowhide wastewater could not be found in the literature search, other studies have confirmed that cowhide processed into meat by singeing are particularly prone to harboring relatively higher levels of metals and other contaminants deposited by the various substances used as a flame treatment. It can therefore be assumed that the bulk of the metal loads observed in the wastewater was released by the singed cowhide meat.

In general, the operators of cowhide meat businesses dispose of their wastewater in drainage canals that eventually empty into the lagoon, thereby increasing the pollution load of lagoon water and resident organisms like fish, prawns, and crabs, some of which serve as food for humans. A persistently low DO in a water body may be lethal for most fish and other aquatic animals, while chronic and cumulative exposure to metals like Cd, Cu, Pb may cause a variety of problems including disruption in normal vitamin uptake. Cadmium is a cumulative contaminant that is toxic to both humans and fish. Excessive suspended solids in aquatic habitats can contribute to increased turbidity leading to reduced light penetration and primary productivity. This suggests that singed cowhide meat (brown ponmo) is not only potentially harmful to the health of those who consume it, but the wastewater used to process it also constitutes a possible pollution threat to the environment.
especially the aquatic environment.\textsuperscript{2,4,16} Therefore, the discharge of this wastewater directly into waterways should be addressed.

The present study did not assess cowhide meat genotoxicity directly, rather, it evaluated the wastewater used in its processing as the \textit{A. cepa} assay requires a liquid medium. Not only did the wastewater inhibit \textit{A. cepa} root growth, it was also mitodepressive and genotoxic, inducing chromosomal aberrations such as binucleated, attached, C-mitosis, sticky and bridged chromosomes. This lends further credence to the assumption that singed cowhide meat wastewater is potentially harmful to the aquatic environment. The decreased mitotic index and the chromosomal aberrations produced in \textit{A. cepa} roots were likely due to the presence of metals and other parameters in the wastewater. The decreased mitotic index is believed to result from either a disturbance in the cell cycle or chromatin dysfunction induced by metal-DNA interactions.\textsuperscript{18} Sticky chromosomes, which were the most frequent aberrations recorded, indicate disruption of chromatin organization by contaminants.\textsuperscript{19} Sticky chromosomes have been considered a common sign of chromosomal toxicity that may lead to cell death.\textsuperscript{20} Since studies have correlated chromosome abnormalities and mutagenic activity found in root-tip systems with those found in mammalian cell systems, the genotoxic effects produced by cowhide meat wastewater in the root cells of \textit{A. cepa} in the present study can be extended to mammals.\textsuperscript{11,21} Since the effects of cowhide meat wastewater observed in this study were likely induced by the singed cowhide itself, the physicochemical characteristics and genotoxic effects of the wastewater observed in this study can equally be considered indirect effects of singed cowhide meat. Also worthy of note is the fact that singeing cowhide with wood, used tires, spent engine oil and other materials, as practiced by \textit{ponmo} operators presents potential air pollution problems.

**Conclusions**

The results of the present study indicate that singed cowhide meat wastewater is potentially genotoxic and environmentally harmful. Governments, public health practitioners, and relevant stakeholders should work in synergy to discourage the habit of processing cowhide into cowhide meat. This will help to protect human health, block a potential environmental pollution source, and at the same time save the hide and leather industry from the indirect competition it currently faces from cowhide meat.

**References**

1. Dada EO, Soares MT, Okunribido FG, Akinola MO. Groundwater quality in the neighbourhood of a medium-sized abattoir in Lagos, Nigeria. FUNAJ J Sci Technol. 2017; 3(2): 91-102.

2. Okiei W, Ogunlesi M, Alabi F, Osinghwe B, Sojinrin A. Determination of toxic metal concentrations in flame treated meat products, \textit{ponmo}. Afr J Biochem Res [Internet]. 2009 Oct [cited 2016 Dec 12]; 3(10): 332-9. Available from: https://academicjournals.org/article/article1380105382_Okiei%20et%20al.pdf

3. Leita I, Enne G, De Nobili M, Baldini M, Sequi P. Heavy metal bioaccumulation in lamb and sheep bred in smelting and mining areas of S.W. Sardinia, Italy. Bull Environ Contam Toxicol [Internet]. 1991 Jun [cited 2017 Mar 17]; 46(6):487-93. Available from: https://doi.org/10.1007/BF01689734 Subscription required to view.

4. Ekenma K, Anelon NJ, Ottah AA. Determination of the presence and concentration of heavy metal in cattle hides singed in Nsukka abattoir. J Vet Med Anim Health [Internet]. 2015 Jan [cited 2017 Mar 21];7(1):9-17. Available from:https://doi.org/10.5897/ JVMAH2014.0283

5. Clottery JA. Manual of the slaughter of small ruminants in developing countries [Internet]. Rome: Food and Agriculture Organization; 1985 [cited 2017 Jul 21]. Chapter 6, Slaughtering practices and techniques. Available from: http://www.fao.org/docrep/003/x6552e/ x6552e00.htm

6. Essumang DK, Dodoo DK, Adokoh KC, Koka V, Nkrumah BN, Nuer AC. Heavy metal levels in singed cattle hide (wele) and its human health implications. Proceedings of the First International Conference on Environmental Research, Technology and Policy; 2007 Jul 17-19; La Palm Royal Beach Hotel, Accra, Ghana. Ontario, Canada: Environmental Research, Technology and Policy; 2007. 19 p.

7. Obiri-Danso K, Hogarh JN, Antwi-Agyei P. Assessment of contamination of singed hides from cattle and goats by heavy metals in Ghana. Afr J Environ Sci Technol [Internet]. 2008 [cited 2016 Dec 12]; 2(8): 217-21. Available from: https://www.ajol.info/index.php/ajesj/article/view/15599

8. Standard methods for examinations of water and wastewater. 19th ed. Washington, D.C.: American Public Health Association; 1995.

9. Bakare AA, Okonola AA, Adetunji OA, Jenmi HB. Genotoxicity assessment of a pharmaceutical effluent using four bioassays. Genet Mol Biol [Internet]. 2009 [cited 2017 Feb 18]; 32(2):373-81. Available from: www.scielo.br/pdf/gmb/v32n2/a26v32n2.pdf [cited 2017 Feb 18]; 32(2):373-81. Available from: www.scielo.br/pdf/gmb/v32n2/a26v32n2.pdf

10. Fikesjo G. The allium test in wastewater monitoring. Environ Toxicol Water Qual [Internet]. 1993 Aug [cited 2017 Jun 17]; 8(3): 291-8. Available from: https://doi.org/10.1002/tox.2530080306 Subscription required to view.

11. Olorunfemi DI, Ogieseri UM, Akinbore A. Genotoxicity screening of industrial effluents using onion bulbs (\textit{Allium cepa} L.). J Appl Environ Manag [Internet]. 2011 Mar [cited 2017 Jan 15]; 15(1):211-6. Available from: https://www.ajol.info/index.php/jaesm/article/download/65700/53388

12. Adegbite AE, Olorode O. Karyotype studies of three species of \textit{Aspiliathouar}. (Heliantheae – Asteraceae) in Nigeria. Plant Sci Res Comm. 2002;3:11-26.

13. Federal Republic of Nigeria Official Gazette. No. 68, Vol. 96. Abuja, Nigeria: National Environmental Standards and Regulations Enforcement Agency of Nigeria; 2009. Government Notice No. 289. 44 pages.
14. Zhang C, Zhang W, Huang Y, Gao X. Analysing the correlations of long-term seasonal water quality parameters, suspended solids and total dissolved solids in a shallow reservoir with meteorological factors. Environ Sci Pollut Res Int [Internet]. 2017 Mar [cited 2017 Mar 27];24(7):6746-56. Available from: https://doi.org/10.1007/s11356-017-8402-1 Subscription required to view.

15. A guidebook for local governments for developing regional watershed protection plan [Internet]. Athens, GA: Northeast Georgia Regional Development Center; 2001 May [cited 2016 Oct 5]. 71 p. Available from: https://epd.georgia.gov/sites/epd.georgia.gov/files/related_files/site_page/devwtrplan.pdf

16. Oghonna II, Donatus EI. Effects of various methods of singeing on the heavy metal proximate and sensory properties of singed cowhide (Ponmo). Arch Appl Sci Res. 2015; 7(4):44-50.

17. Berry W, Rubinstein N, Melzian B, Hill B. The biological effects of suspended and bedded sediment (SABS) in aquatic systems: a review [Internet]. Washington, D.C.: United States Environmental Protection Agency; 2003 Aug [cited 2018 Oct 4]. 58 p. Available from: https://www.epa.gov/sites/production/files/2015-10/documents/sediment-appendix1.pdf

18. Glinska S, Bartczak M, Oleksiaik S, Wolska A, Gabara B, Posmyk M, Janas K. Effects of anthocyanin-rich extract from red cabbage leaves on meristematic cells of Allium cepa L. roots treated with heavy metals. Ecotoxicol Environ Saf [Internet]. 2007 Nov [cited 2017 Mar 16]; 68(3):343-50. Available from: https://doi.org/10.1016/j.ecoenv.2007.02.004 Subscription required to view.

19. Kuras L. Characterization of protein-DNA association in vivo by chromatin immuno-precipitation. In: Dickson RC, Mendenhall MD, editors. Signal transduction protocols: methods in molecular biology [Internet]. Vol. 284. New York: Humana Press; 2004 [cited 2017 Mar 16]. p. 147-62. Available from: https://doi.org/10.1385/1-59259-816-1:147 Subscription required to view.

20. Fiskejo G. Allium test for screening chemicals; evaluation of cytological parameters. In: Wang W, Gorsuch JW, Lower WR, Gorsuch JW, Hughes JS, editors. Plants for Environmental Studies. Boca Raton, FL: CRC Press; 1997 May. p. 307-33.

21. Majer BJ, Grummt T, Uhl M, Knasmuller S. Use of plants bioassays for the detection of genotoxins in the aquatic environment. Acta Hydrochim Hydrobiol [Internet]. 2005 Apr [cited 2017 Jun 27];33(1):45-55. Available from: https://doi.org/10.1002/ahhe.200300557 Subscription required to view.