Uniport Journal of Engineering and Scientific Research (UJESR)

Vol. 4, Issue 1, 2019, Page 34-42
ISSN: 2616-1192

(www.ujesr.org)

Characterization and Toxicological Evaluation of Labana Rice Mill Wastewater in Kebbi State Nigeria

Yahaya, T.O.[1], Anyebe, D.A.[2], Adebayo, A.O.[1], Adebisi, M.A.[1] and Muhammad, S.S.[1]
[1]Department of Biology, Federal University Birnin Kebbi, Nigeria
[2]Department of Biochemistry and Molecular Biology, Federal University Birnin Kebbi, Nigeria
*Corresponding author's email: yahayatajudeen@gmail.com and yahaya.tajudeen@fubk.edu.ng

Abstract
Nigeria is increasing rice production to boost food sufficiency, however, the nature and health risks of pollutants in rice milling wastewater need to be investigated for effective waste management. This study characterized and determined toxicity of Labana Rice Mill wastewater in Birnin Kebbi, Nigeria. After microbiological and physicochemical characterizations using standard protocols, 0, 25, 50 and 100% (v/v) of the wastewater were administered, respectively, to 24 rats (Rattus norvegicus L) equally divided into 4 groups for 28 days. Thereafter, blood parameters and liver enzymes as well as histopathologic changes in the livers and kidneys of the rats were estimated using standard protocols. Compared with World Health Organization (WHO) Standards, the microbiological characterization showed abnormal bacteria and yeast counts (>1000 cfu ml^{-1}). The physicochemical characterizations also showed abnormal levels of calcium (Ca), lead (Pb), total dissolved solids (TDS), dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), turbidity, and nitrate. The blood parameters of the treated rats revealed a reduced ($p<0.05$) concentration-dependent packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBC), and lymphocytes. Similar trend was also observed in the liver enzymes, namely alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), and albumin (ALB). There was no histologic change in the nontreated rats, while the treated rats' kidneys and livers revealed concentration-dependent cell degeneration and tubular atrophy as well as karyolysis and necrosis, respectively. The results obtained showed that the wastewater is toxic and could pose public and environmental health risks.

Keywords: *Anemia, Biochemical Oxygen Demand, Karyolysis, Lymphocytes, Necrosis*
Received: 17th November, 2019 *Accepted: 27th December, 2019*

1. Introduction

Rice (*Oryza sativa* L.) is a cereal crop of the grass family whose seeds are eaten as a staple food by more than half of the world's population (Lili *et al*., 2019). According to FAO (2001), most people in developing worlds, mainly in Africa and Asia, obtain about 75 % of their daily energy requirements from rice consumption. In Asia, a large proportion of the populace eats rice in every meal, providing up to 50 % of the dietary caloric supply (Muthayya *et al*., 2014). In West Africa, rice consumption among people living in cities has increased more than twofold since 1970 (IRR1, 2013). Rice is also the main source of income and employment for over 200 million households in developing countries (FAO, 2004).

The main reason for the increasing consumption of rice compared to other cereals is the convenience of its preparation, which fits the increasing tight schedule of modern humans. The industrialization which started in the 1840s has changed the role of women in that they now form part of the workforce, which gives them a limited time to cook or take care of their homes (Srinivasan and Shende, 2015). Hence, foods such as rice that can be prepared within a short time became the most popular sought after. Moreover, men work far away from homes nowadays, so much of their food, mostly rice, is consumed around their workplace. These behavioral changes have made rice the main source of energy and dietary requirements for all classes of urban dwellers (Frimpong, 2013). Unfortunately, in Africa and some other parts of the world, rice production has never met the demand of the increasing population (Roy and Shiina, 2010).

Worldwide, efforts are being made to increase rice production and, in Nigeria in particular, there is an increase in rice production to stem food shortages and diversify the economy. However, elsewhere, concerns are rife about the pollution potentials of rice processing plants (Behl, 2016; Zaman *et al*., 2006). Rice processing activities consume a huge volume of water thus, a large amount of wastewater containing toxic organic and inorganic substances is released into the environment (Paul *et al*., 2015). In particular, the Labana Rice Mill in Birnin Kebbi has two independent plants with an installed rice processing capacity of eight (8) metric tons per hour. Thus, the rice mill consumes a large volume of water daily and discharges the same as wastewater into the environment. The management of the mill and the residents use the wastewater for irrigation, yet to the best of our knowledge, the nature of pollutants in the wastewater and its toxicity have not been determined. A study in this direction is necessary to prevent unintended fatalities. Hence, this study characterized and evaluated the toxicity of the wastewater samples obtained from the rice mill.

2. Materials and methods

2.1. Study area

The Labana Rice Mill Limited is along Kebbi-Argungu motorway, Birnin Kebbi, Kebbi State, Nigeria (Fig. 1). Birnin Kebbi is in the northwest on latitude 12° 27' 57.8808" N and longitude 4° 11' 58.2864" E (Fig. 1). The state is bordered by Katsina and Zamfara State in the west, Sokoto State in the north, and Niger State in the south. The people of the state are predominantly farmers and cattle breeders and are famous for rice farming.

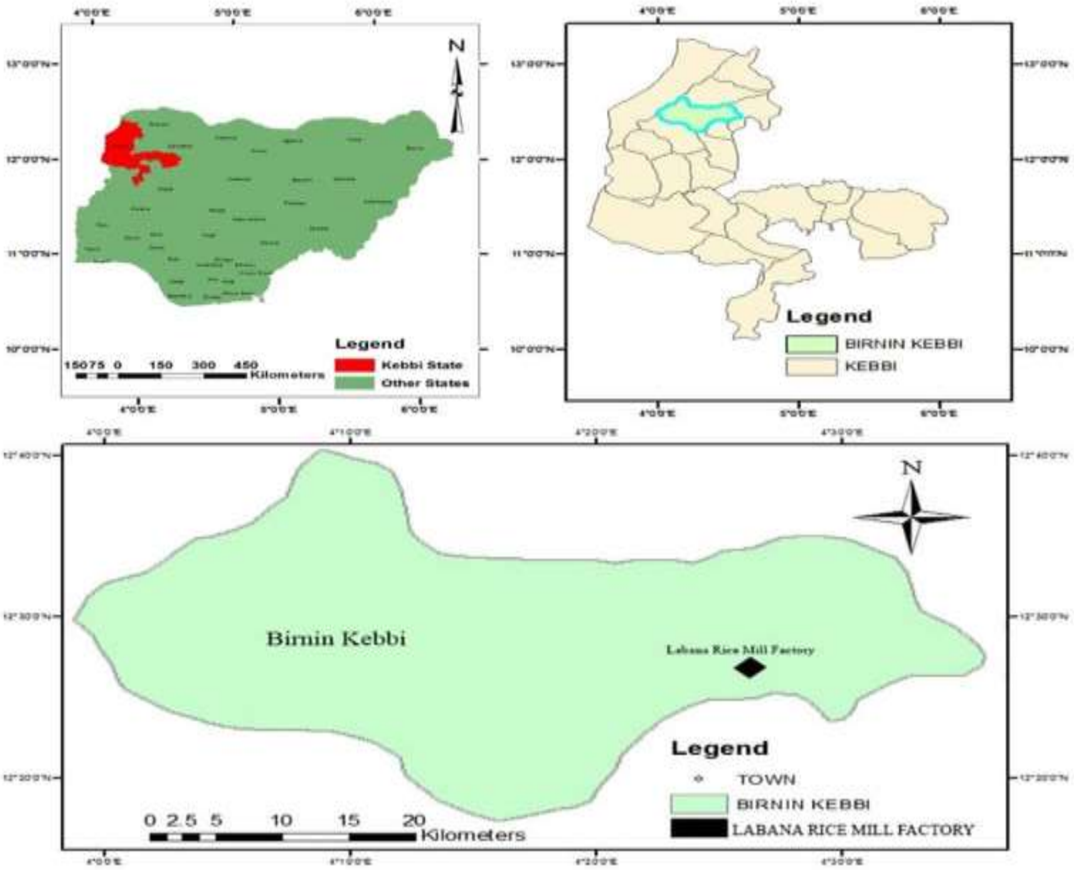

Fig. 1: Maps of Nigeria and Kebbi State showing Labana Rice Mill

2.2. Animal material and management

Twenty-four (24) experimental rats (*R. norvegicus*) of both sexes, aged 60 days and mean weight 230±10g were used for this study. The rats were kept in well-ventilated metal cages and allowed to acclimatize to the ambient environment before commencing the study. The rats had free access to water and pellet feeds from the Premier Feed Mills, Ibadan.

2.3. Wastewater collection and preparation

One (1) litre volume of wastewater was collected from the point of discharge of the mill in a clean and sterilized airtight bottle and moved into the laboratory immediately. Three concentrations (25, 50 and 100 % v/v) were prepared from the wastewater by dilution.

2.4. Physicochemical characterization of the wastewater

Guidelines for measuring water quality, as described by APHA (2012) were employed in the determination of the physicochemical properties of the wastewater. For accuracy, some water properties that change with time were taken *in situ*. These properties are temperature, pH, electrical conductivity (EC), and total dissolved solids (TDS) and were measured using a thermometer, pH meter, conductivity meter, and a handheld TDS meter, respectively. Other properties were measured in the laboratory and include hardness, turbidity, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), sulphate, and nitrate. These properties were measured using a spectrophotometer, turbidimeter, HACH DO analyzer, BOD incubator, HACH spectrophotometer, and UV spectrophotometer, respectively. The heavy metals in the wastewater, which are the magnesium (Mg), iron (Fe), cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni), calcium (Ca), and manganese (Mn), were determined using an atomic absorption spectrophotometer (AAS) (UNICAM Model 969).

2.5. Microbiological characterization of the wastewater

Heterotrophic plate technique, as described by Jayalakshmi and Lakshmiwas (2014) was used to determine the bacterial, coliform and fungal loads of the wastewater. One hundred (100) ml of the wastewater was passed through a sterile cellulose filter, after which the filter was placed on a nutrient agar plate and incubated at 37^0 C for 24-48 hours. Colonies of bacteria, coliform, and fungi were formed and counted.

2.6. Study design

Th**e** rats were divided into four (4) groups comprising 6 rats each. Group 1 was made the control and were fed normal feeds and water, while groups 2, 3, and 4 were daily administered 25, 50, and 100 % (v/v) of the wastewater, respectively for 28 days. At the end of the administration, the rats were sacrificed by cervical dislocation after sedating them with chloroform in a tightly covered bell-shaped glass jar. Blood samples were collected for hematological and liver function tests, and the livers and kidneys excised for histopathologic examination.

2.7. Analysis of blood parameters and liver enzymes

The micro - hematocrit method described by Bull and Hay (2001) was used to determine the packed cell volume (PVC), while hemoglobin (Hb) was estimated using the Sahli-Hellige Hemoglobin method described by Whitby and Britton (1935). The red blood cell count (RBC) was calculated using the Neubauer chamber method (Hesser, 1960), and the lymphocyte count was estimated based on the method of Heather and Tim (2016). The liver enzymes, including alanine amino transferase (ALT), aspartate amnion transferase (AST), and alkaline phosphate activity (ALP) were estimated by ultraviolet, colorimetric and spectrophotometric methods, as described by Bergneyer and Bernt (1974). The albumin and protein content were calculated using the Burt method described by Hoff (2000) and Layne (1957), respectively.

2.8. Histopathologic examination

The kidneys and livers of the rats were prepared for histopathological examination as described by Taylor *et al.* (2003). The tissues were processed using hematoxylin and eosin staining techniques, and qualitative methods with emphasis on the morphology, architectures, and cytology were used to analyze the tissues.

2.9. Data analysis

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

3. Results and discussion

3.1. Physicochemical properties of the wastewater

Table 1 shows the physicochemical properties of the wastewater in which the temperature, pH, sulphate, Mg, Fe, Mn, Cr, Ni, and Cd were within the WHO acceptable limits. However, Ca, Pb, turbidity, DO, BOD, COD, EC, TDS, and nitrate concentrations were not within WHO acceptable limits. The metals found could emanate from the chemicals used, or generated, during the milling processes. The high TDS could result from farm inputs and debris, resulting in the poor EC, high turbidity, COD and BOD of the wastewater. These findings show that the wastewater is toxic and can induce adverse effects. The heavy metals detected can bioaccumulate in biological systems and the environment, resulting in health and environmental hazards (Ali *et al*., 2019). Padhan and Sahu (2011) and Mahananda *et al*. (2015) reported similar findings in rice mill wastewaters, particularly high BOD, COD, chloride, and sulphate, among others.

Table 1: Physicochemical properties of Labana Rice Mill wastewater

Parameter	Level detected	Min. value	Max. value	WHO limit
Temp. (ºC)	22.300±0.2646	21.90	22.80	25º
pH (-)	5.900±0.0577	5.80	6.00	6.0 - 9.0
EC (µs/C^3)	11.533±0.1764	11.20	11.80	1000
TDS (mg/l)	8273.30±875	8110	8360	2000
Turbidity (NTU)	AL	AL	AL	5.0
Hard (mmol/l)	2.4267±0.1634	2.10	2.60	
Ca (mg/l)	1.1667±0.0882	1.00	1.30	0.01
Mg (mg/l)	0.3567±0.0318	0.30	0.41	60.0
Fe (mg/l)	1.3033±0.0545	1.21	1.40	5.0
Cd (mg/l)	ND	ND	ND	0.01
Cr (mg/l)	0.0300±0.0115	0.01	0.05	0.05
Pb (mg/l)	0.0500±0.0058	0.04	0.06	0.02
Ni (mg/l)	0.0300±0.0058	0.02	0.04	0.2
Mn (mg/l)	0.0300±0.0058	0.02	0.04	0.2
DO (mg/l)	0.185±0.040	0. 182	0.196	1.0
BOD (mg/l)	119.906±0.8556	118.30	121.22	60.0
COD (mg/l)	1467.676±3.650	1460.52	1472.31	150.0
Nitrate (mg/l)	9.873±0.3712	9.80	9.92	1
Sulphate (mg/l)	191.8400±2.0883	189.00	195.72	750

Values are expressed as Mean±SD; ND = Not Detected; AL = Above Limit

3.2. Microbiological properties of the wastewater

The microorganisms isolated from the wastewater and their counts are shown in Table 2. The bacteria and yeast counts were above the WHO limit of 1000 cfu ml^{-1}, while the coliform loads were within the permissible limit of 400 cfu ml^{-1}. The heavy microbial colonies could result from the water used for milling. The nutrients in the wastewater, particularly Ca and nitrate, could also contribute to heavy microbial growth. The heavy microbial colonies could contribute to the low DO of the wastewater reported earlier. According to Narragansett Bay Estuary Program (2017), high concentrations of nutrients in water can cause algae blooms whose die-off and decomposition may cause high BOD and low DO. These findings again show that the wastewater is toxic and can cause public and environmental health hazards. The microbial populations may contaminate food and public water supply causing waterborne diseases (Akpor and Muchie, 2011; Pirsaheb *et al*., 2017). A similar study by Pittol *et al*. (2017) also isolated some microorganisms in rice floodwaters.

Table 2: Microbial loads (cfu ml^{-1}) of Labana Rice Mill wastewater

Microbe	Level detected	Min. value	Max. value	WHO limit
Bacteria	318333±4409	310001	325321	< 1000
Coliform	145±20	140	150	< 400
Yeast	1516±101	1450	1500	< 1000

Values are expressed as Mean±SD and in cfu ml^{-1}

3.3. Effects of the wastewater on the blood parameters of the rats

Table 3 reveals the blood parameters of the treated and control rats. The blood parameters of the test rats show a concentration-dependent reduction and were statistically different ($p< 0.05$) from the control except for the RBC. An insignificant reduction was also noticed in the Hb levels of the rats treated with 25% of the wastewater. These observations show that the treated rats were anaemic, indicating that cytotoxic interactions occurred between the toxic substances in the wastewater and the rats' cells. Some heavy metals detected in the wastewater can induce several health problems, including haematological damage. Lead (Pb) and Ni, detected in high amounts in the wastewater, can cause anaemia (Millaku *et al*., 2015; ATSDR, 2017). The microbial species in the wastewater could also contribute to the anaemic condition of the treated rats (Alsaid *et al*., 2015).

Table 3: Blood parameters of the rats treated with Labana Mill wastewater

Blood parameters	Control	25%	50%	100%
PCV ($l\ l^{-1}$)	0.40±2.8^a	0.37±2.2^b	0.32±1.7^b	0.31±2.5^b
Hb ($g\ dl^{-1}$)	13.3±1.3^a	12.4±1.2^a	10.7±1.1^b	10.3±1.5^b
RBC ($mc\ mm^{-3}$)	5.61±0.8^a	5.31±0.9^a	5.24±1.0^a	5.12±0.7^a
Lymphocytes ($c\ \mu l^{-1}$)	2311±100^a	2121±114^b	2091±150^b	2018±120^b

Values are expressed as Mean ± SD (n = 6); mean values with different subscript 'a' and 'b' along the same row are statistically different from control at p< 0.05 (student's *t* –test).

3.4. Effects of the wastewater on the liver enzymes of the rats

Table 4 compares the levels of the liver enzymes of the control and treated rats. Except for the TP and ALB of rats administered 25% of the wastewater, the liver enzymes of the treated rats, including the ALT, ALP, and AST reveal a significant (p<0.05) concentration-dependent reduction**.** This suggests oxidative stress-induced cell death from the toxic substances in the wastewater. According to Moosavi and Shamushaki (2015), Nickel toxicity can deplete liver enzymes. Velma and Tchounwou (2010) also reported alterations in the liver enzymes of goldfish due to chromium (VI) toxicity.

Table 4: Liver enzymes of the rats treated with Labana Rice Mill wastewater

Liver enzymes	Control	25%	50%	100%
ALT (U^{-l})	33.3±2.61^a	50.00±3.20^b	114.67±5.52^b	134.67^a±6.20^b
AST (U^{-l})	13.67±1.45^a	134.67±25.31^b	206.33±12.02^b	239.67±10.27^b
ALP (U^{-l})	46.10±1.52^a	50.00±9.29^b	114.62±8.21^b	134.67±6.57^b
TP (g^{-dl})	7.03^a±0.88^a	6.90^a±1.06^a	6.53±1.23^b	6.50±1.16^b
ALB (g^{-l})	47.33±0.88^a	46.60±1.33^a	39.00±2.08^b	32.33±1.88^b

Values are expressed as Mean ± SD (n = 6); mean values with different subscript 'a' and 'b' along the same row are statistically different at p< 0.05 (student's *t* –test).

3.5. Histopathological effects of the wastewater

Plates 1-4 show the effects of the wastewater in the kidney tissues of the treated rats. The kidney tissues of the control rats showed no histological changes as normal glomeruli were observed (Plate 1). Cortical congestion and inflammations were observed in the tissues of the rats administered 25% of the wastewater (Plate 2). In Plate 3, the kidney tissues of the rats treated with 50% of the wastewater had epithelial cell degeneration, interstitial fibrosis with tubular atrophy, and congestion. Inflammatory changes and tubular atrophy were observed in the kidney tissues of the rats administered 100% of the wastewater (Plate 4).

Plates 5-8 reveal the liver histology of the treated and control rats. The control rats had no histological changes (Plate 5), while a congested central vein and karyolysis were observed in the rats treated 25% of the wastewater (Plate 6). Karyolysis, congestion within the vein and cellular infiltration were observed in the livers of the rats administered 50% of the wastewater (Plate 7). The rats dosed with 100 % of the wastewater had partial hepatic necrosis and lymphatic infiltration (Plate 8).

The histological changes observed in the treated rats further proved the toxicological potential of the wastewater. The toxic substances and the microbial populations in the wastewater could induce oxidative stress and inflammations in the tissues, resulting in histological damage. According to Javed *et al*. (2015), heavy metals can induce oxidative stress in tissues by generating free radicals, resulting in multi-organ damage. Microbial infections can disrupt the defense mechanism, stimulating inflammation and tissue damage. Some microorganisms may also produce toxins, which may damage several tissues. For instance, some forms of algae (blue-green) may produce toxins that are harmful if ingested by animals, including humans (MPCA, 2008).

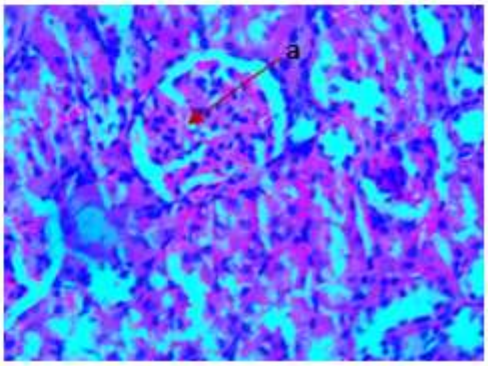

Plate 1: Photomicrograph of the kidney tissues of the control rats (x 400)

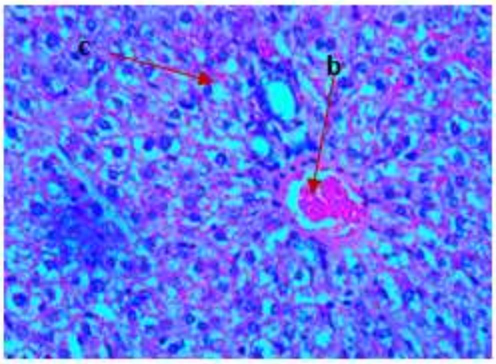

Plate 2: Photomicrograph of the kidney tissues of the rats treated with 25 % of the effluent (x 400)

a = Normal glomerulus, b = Cortical congestion, and c = Inflammatory changes

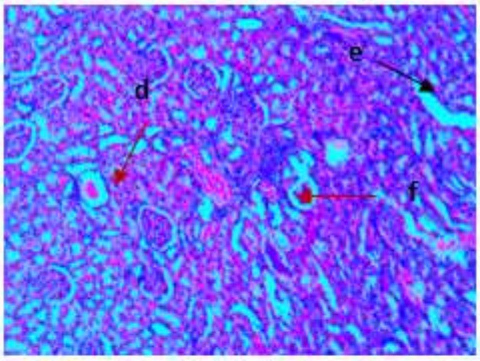

Plate 3: Photomicrograph of the kidney tissues of the rats treated with 50 % of the effluent (x 400)

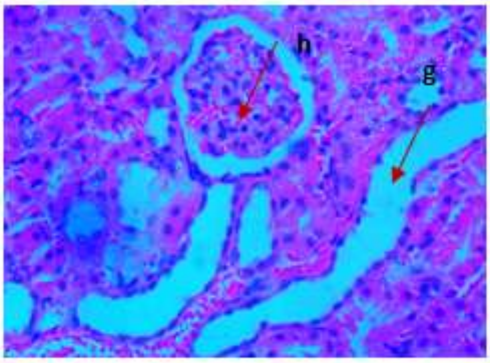

Plate 4: Photomicrograph of the kidney tissues of the rats treated with 100 % of the effluent (x 400)

d = Epithelial cell degeneration, e = Interstitial fibrosis with tubular atrophy, f = Congestion, g = Inflammatory changes, and h = Tubular atrophy

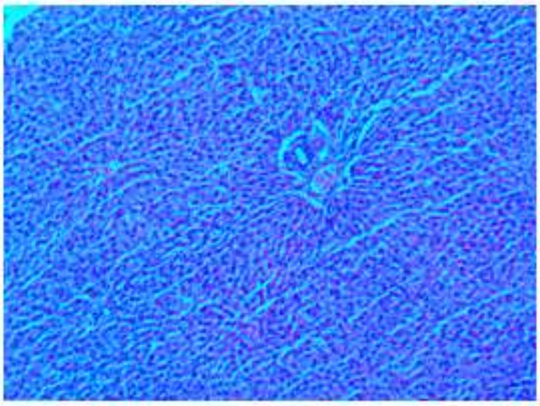

Plate 5: Photomicrograph of the Control Rats (x 400)

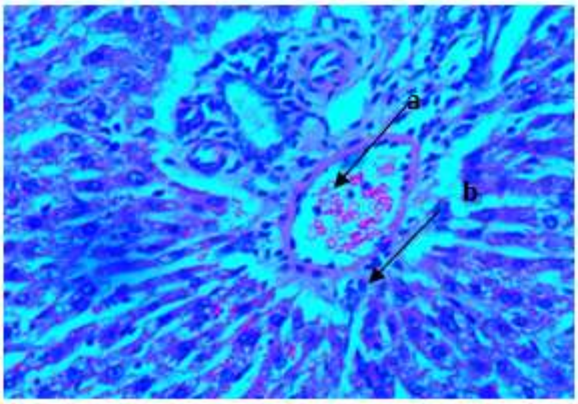

Plate 6: Photomicrography of the liver tissues of the rats treated with 25 % of the effluent (x 400)

a = Congestion in central vein and b = Karyolysis

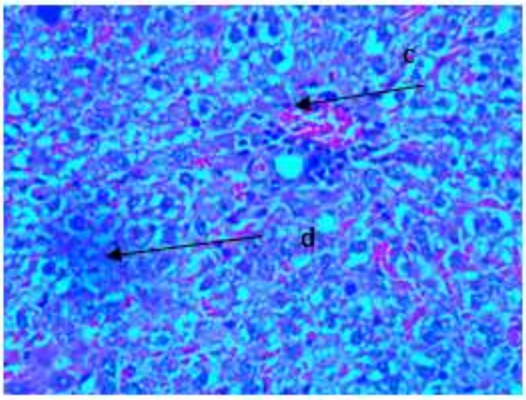

Plate 7: Photomicrograph of the liver tissues of the rats treated with 50 % of the effluent (x 400)

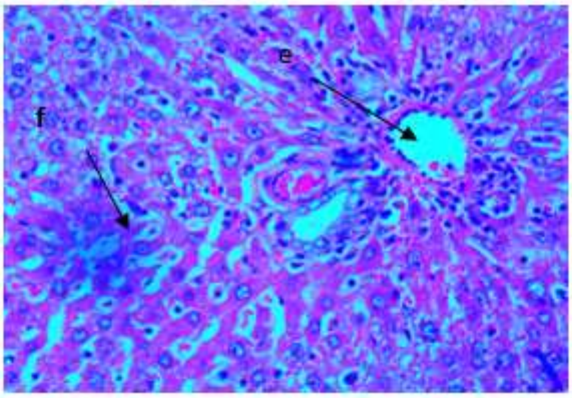

Plate 8: Photomicrograph of the liver tissues of the rats treated with 100 % of the effluent (x 400)

c = Karyolysis, d = Congestion within the vein and cellular infiltration, e = Partial hepatic necrosis, and f = Lymphatic infiltration

4. Conclusions

The Labana Rice Mill Limited wastewater contained non-permissible levels of Ca, Ni, Pb, DO, BOD, COD, EC, TDS, turbidity, and nitrate as well as bacteria and yeast. As such, the wastewater is toxic and can pose a significant health risk as evident in the abnormal blood parameters, liver enzymes and histological sections of the treated rats. The microbial populations and heavy metals in the wastewater may contaminate food plants and public water supply, leading to the outbreak of waterborne diseases. While we recommend a confirmatory study, agencies responsible for pollution management in the city should keep a close monitor of the rice mill. Similar studies should be done on other rice mills in the country.

References

ATSDR (Agency for Toxic Substances and Diseases Registry) (2017) Lead Toxicity. What Are Possible Health Effects from Lead Exposure? Available at https://www.atsdr.cdc.gov/csem/csem.asp?csem=34&po=10

Akpor, O.B. and Muchie, M. (2011) Environmental and public health implications of wastewater quality. African Journal of Biotechnology, 10(13): 2379-2387.

Alsaid, M., Abuseliana, A.F., Daud, H.H., Mustapha, N.M., Bejo, K.S., Abdelhadi, Y.M. and Hamdan, R.H. (2015) Haematological, biochemical and clinical signs changes following experimental infection of Streptococcus agalactiae in red hybrid tilapia (*Oreochromis sp*.). Basic Research Journal of Agricultural Science and Review, 4(9): 289-295.

Ali, H., Khan, E. and Ilahi, I. (2019) Environmental Chemistry and Ecotoxicology of Hazardous Heavy Metals: Environmental Persistence, Toxicity, and Bioaccumulation. Journal of Chemistry, 2019: 1-14

APHA (American Public Health Association) (2012) Standard Methods for the Examination of Water and Wastewater, 22nd edition, American Public Health Association, Washington, DC.

Behl, M. (2016) Rice mills at Gondia causing pollution. The Time of India City. Available at https://timesofindia.indiatimes.com/city/nagpur/Rice-mills-at-Gondiacausingpollution/articleshow/5380168 8.cms

Bergmeyer, H.U. and Bernt, E. (1974) In: Methods of Enzymatic Analysis; Bergmeyer, HU 2nd ed.; 2:574-579. Academic Press, New York.

Bull, B.S. and Hay, L.K. (2001) Is the Park Cell Volume (PCV) Reliable? Laboratory Haematology, 7**:** 191-196.

FAO (Food and Agricultural Organization) (2001) Rice in the World. Report of the Fifth External Programme and Management Review of the International Plant Genetic Resources Institute (IPGRI); FAO: Rome, Italy. Available at http://www.fao.org/wairdocs/tac/x5801e/x5801e08.htm.

FAO (Food and Agricultural Organization) (2004) The state of food insecurity in the world. Rome, FAO. Available at http://www.fao.org/3/y5650e/y5650e00.htm.

Frimpong, S. (2013) Urbanization and Pattern of Urban Food Consumption in Ashanti Region, Ghana, implications for Food Security. Journal of Economics and Sustainable Development, 4(9): 104-112

Heather, R. and Tim, J. (2016) Blood Differential

Test: what is a blood differential test? Available at https://www.healthline.com/health/blood-differential#1

Hesser, E.F. (1960) Method for routing fish hematology. Progressive fish culturist, 22: 164-171

Hoff, J. (2000) Method of blood collection in mouse: technique. Journal of laboratory animals, 29(10): 47-53.

IRRI (International Rice Research Institute) (2013) World Rice Statistics 2013. Los Baños, the Philippines. Available at http://irri.org/index.php?option=com_k2&view=item&id=9081&Itemid= 100481&lang=en.

Jayalakshmi, V. and Lakshmiwas, N. (2014) Assessment of Microbiological Parameters of Water and Waste Waters in and Around Vijayawada. IOSR Journal of Environmental Science, Toxicology and Food Technology, 8(2): 53-57.

Javed, M., Usmani, N., Ahmad, I. and Ahmad, M. (2015) Studies on the oxidative stress and gill histopathology in Channa punctatus of the canal receiving heavy metalloaded effluent of Kasimpur Thermal Power Plant. Environment Monitor and Assessment, 187(1): 4179-4185.

Layne, E. (1957) Spectrophotometric and Turbidimetric Methods for Measuring Proteins. Methods in Enzymology, 10*:*447-455.

Lili, Q., Feng, Z., Hongyan, L., Caidong, Z., Xiaoxing, C. and Dongjie, Z. (2019) Determination of Geographical Origin of Wuchang Rice with the Geographical Indicator by Multielement Analysis. Journal of Food Quality, 2019:1-7.

Mahananda, M.R., Sahu, P.N. and Seth, P. (2015) Effect of rice mill effluent on growth and biochemical parameters of Lycopersicon esculentum in pot culture, International Journal of Pure and Applied Bioscience, 3(6): 192-203.

Millaku, L., Imeri, R. and Trebicka, A. (2015) The impact of lead and nickel in some hematological parameters in house sparrow (Passer domesticus). Advances in Applied Science Research, 6(1):34-37.

MPCA (Minnesota Pollution Control Agency) (2008) Nutrients: Phosphorus, Nitrogen Sources, Impact on Water Quality - A General Overview. Available at https://www.pca.state.mn.us/sites/default /files/wq-iw3-22.pdf

Muthayya, S., Sugimoto, J.D., Montgomery, S. and Maberly, G.F. (2014) An overview of global rice production, supply, trade, and consumption. Annal of Newyork Academy of Sciences, 1324 (1): 7-14.

Moosavi, M.J. and Shamushaki, V.J. (2015) Effect of Sub-Acute Exposure to Nickel on Hematological and Biochemical Indices in Gold Fish (Carassius auratus*)*. Journal of Clinical Toxicology, 5(1): 228-233.

NBEP (Narragansett Bay Estuary Program) (2017) Technical Report State of Narragansett Bay and Its Watershed, pp 222-234. Providence, RI.

Pirsaheb, M., Sharafi, K., Ahmadi, E. and Moradi, M. (2017) Prevalence of the waterborne diseases (diarrhea, dysentery, typhoid, and hepatitis A) in West of Iran during 5 years (2006–2010). Annal of Tropical Medicine and Public Health, 10:1524-1532.

Pittol, M., Scully, E., Miller, D., Durso, L., Mariana, L., Fiuza, M. and Valiati, V.H. (2017) Bacterial Community of the Rice Floodwater Using Cultivation-Independent Approaches. International Journal of Microbiology, 2018:1-13.

Paul, J., Abhijith, D., Arjun, R.V., Jilu, J. and Shamila, L. (2015) Environmental Impact of Rice Mills on Groundwater and Surface Water. International Journal of Civil and Structural Engineering Research, 3(1):11-15.

Padhan, A. and Sahu, K.S. (2011) Effect of Rice Mill Wastewater on Population, Biomass, Rate of Reproduction and Secondary Production of *Drawida willsi* (Oligochaeta) in Rice Field Agroecosystem. International Journal of Research and Reviews in Applied Sciences, 6(2): 138-146.

Roy, P. and Shiina, T. (2010) Global Environment, Biofuel: Sustainable Food Production and Distribution. In Global Environmental Policies: Impact, Management and Effects. Nova Science pp. 29-58. New York.

Srinivasan, S. and Shende, K.M. (2015) A Study on the Benefits of Convenience Foods to Working Women. Atithya, 1(1): 57-63.

Taylor, D.J., Green, N.P.U. and Stout, G.W. (2003) Microscope Techniques. Biological Science. 3rd edn., pp 163-164.Cambridge University Press, UK.

Velma, V. and Tchounwou, P.B. (2010). Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, carassius auratus. Mutation Research, 698(1–2):43–51.

Whitby, L.E. and Britton, C.J. (1935) Disorders of

the Blood. Pp113, Churchill, London.
Zaman, M.A., Farouk, S.M. and Islam, A.K. (2006) Work Environment and Environmental Pollutions in Rice Mills of Bangladesh. Journal of Agricultural Machinery and Bioresource Engineering, 4(1-2): 67-73.