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# Effect of diethyl phthalate and lead leached from PVC water pipes in densely populated neighborhood of Lagos on Wistar rats (*Rattus norvegicus*)

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## Abstract

**Background:** This study investigates the possibility of PVC pipes leaching into municipal water and the effects of the amount leachates such as diethyl phthalates and lead on liver and kidney of Wistar rats.
**Methods:** Water samples were collected from two separate PVC pipes distributing water aged 10 and 2 years respectively from two households in Akoka, Yaba area of Lagos State. Sixteen Wistar rats each (sex ratio 1:1) were orally administered 0.079mg/L of lead, 0.57µl/L of diethyl phthalate and a mixture of lead and diethyl phthalate using the same dosage in water. For control, Sixteen Wistar rats with the same sex ratio were orally administered deionized water. Using the Dunnett's multiple comparisons test, control population were compared with treated population for the third and sixth week, the results showed a significant difference between the treatments and control model animal ($P < 0.05$).
**Results:** Water analysis showed higher concentration of lead (0.079mg/L) and diethyl phthalate (0.57µl/L) in the 10-year-old pipe than the 2-year-old pipe with 0.069mg/L and 0.034µl/L of lead and diethyl phthalate, respectively. The level of phthalate was 17 times higher in old pipes than the new ones. Blood biochemistry and hematology analyzed. Histopathological studies done on the liver and kidney of the rats. The levels of aspartate transferase (AST) (129±2mg/dL), protein (90.67±1.53mg/dL), total bilirubin (44.33±1.15mg/dL), and globulin (54.33±1.15mg/dL) in the serum were highest in rats which suggest hyper-toxicity and possible organ failure.
**Conclusion:** Liver and kidney problem in Nigeria might connected with drinking water contaminated with PVC pipes leachates.

## 1. Introduction

The water resource of Nigeria annually is about 267 billion cubic meters of surface water and about 52 billion cubic meters of groundwater, but the average national water supply coverage is only about 57% **(Ince *et al.* 2010)**. Access to safe drinking water, sanitation and hygiene (WASH) is key to the Sustainable Development Goals (SDGs) of the United Nations **(Hutton and Chase 2016)**. Safe drinking water has been defined as water free from disease causing organisms, toxic chemicals, color, smell, and unpleasant taste **(Bartram *et al.* 2018)**. There is, however, a strong link between safe water and the quality of distribution systems. Distribution system infrastructure and appurtenances, including piping, linings, fixtures, and solders can react with the water they supply as well as the external environment **(Talsness *et al.* 2009)**. These interactions can result in degradation of the distributed water. Permeation of plastic pipes and leaching from linings and metal appurtenances are known pathways for water quality gradation. Permeation of piping materials and non-metallic joints can be defined as the passage of contaminants external to the pipe, through porous, non-metallic materials, into the drinking water. The problem of permeation is generally limited to plastic and non-metallic materials **(Lei *et al.* 2018)**. The mechanism of leaching involves the dissolution of metals, solids, and chemicals into drinking water can result in elevated levels of metals, organic contaminants, or asbestos in water consumed from the tap which causes adverse effects when such contaminated water is consumed **(Tabelin *et al.* 2018)**.
Polyvinyl chloride (PVC) plastics are widely used in drinking water distribution systems. PVC is often preferred over metal due to several inherent advantages: PVC piping is lightweight and open flame is not required for joining, the flexibility of plastic can simplify installation and reduces breaks due to freezing. PVC is typically lower in cost and resists the corrosion and scaling that affect metals in some applications **(Lasheen *et al.* 2008)**. Despite the obvious advantages of PVC pipe, there are concerns about chemical contaminants migrating from the plastic to the water it carries **(Skjevrak *et al.* 2003, Kowalska *et al.* 2011)**. Polyvinyl chloride (PVC) is a chlorinated thermoplastic polymer, which can be adapted for various uses by the addition of fillers, stabilizers, lubricants, plasticizers, pigments flame retardants. PVC can be converted either into rigid product or into flexible articles when compounded with plasticizers **(Rahman and Brazel 2004)**. Between 1980 to 2015, the PVC consumption significantly rose from 0.4 metric ton (0.4 kg/capita) in 1980 to 14.5 metric tonnes (10.7 kg/capita) in 2015 and by the end of 2050, China alone will account for 508 metric tons of PVC wastes **(Liu *et al.* 2020)**. In

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recent years, the use of PVC pipes has been increasingly considered for replacing the old steel copper pipes in public drinking water distribution systems. Its widespread use is due to its low cost, ease of installation, excellent structural strength, its light weight and resistance to corrosion **(Arthur *et al.* 2020)**. PVC leachates including phthalate esters are a growing source of concern in municipal water distribution. Phthalates are a family of chemicals that are produced in millions of tons annually worldwide and are a principal component of many diverse products that consumers are exposed to. They include products made of flexible polyvinyl chloride plastic (PVC), cosmetics and other personal care goods, pesticides, building materials, lubricants, adhesives, and film, among other items **(Davies *et al.* 2010)**. Phthalates are released into the environment by manufacturers and escape from consumer products in which they are used. Worldwide ecosystem contamination and direct contact with phthalate-containing products result in virtually ubiquitous human exposures **(Heudorf *et al.* 2007)**.

Excretion data from animal experiments have shown that diethyl phthalate is absorbed following oral and dermal exposure. Studies on related phthalic acid esters would indicate that the compound may also be absorbed by inhalation **(Li *et al.* 2020)**. Rats excreted the majority of an oral dose in the urine, indicating extensive absorption **(Pereira and Rao 2007, Kumar *et al.* 2014)**. One of the earliest studies of the contaminant observed that twenty-four hours after dermal application of $^{14}$C-diethyl phthalate, approximately 9 % of the radioactivity was recovered in the urine of rabbits, 24 % in the urine and 1% in the faeces of rats. A cumulative total of 50% of the dose was excreted by rats after 7 days. **Scott et al. (1987)** measured the *in vitro* absorption of diethyl phthalate through the epidermis of human and rat skin. The steady-state absorption rate was 1.27 and 41.37 µg/cm2/hour for human and rat skin, respectively, indicating that human skin is less permeable to diethyl phthalate than rat skin. No data was found concerning the tissue distribution of diethyl phthalate following oral or inhalation exposure.

The acute symptoms observed in rats, rabbits, dogs, and chicks after oral or intravenous administration were increased respiration rate, disequilibrium, convulsion, and lethargy respiratory arrest **(Elsisi *et al.* 1989)**. Other research shows adverse effect on kidney, liver, and brain. Biological toxicity and adverse health effect, which include, but not limited to, premature breast development, premature births and dermatitis have also been report in humans **(Pak *et al.* 2011, Wittassek *et al.* 2011, Kumar 2018)**.

A few studies in Nigeria have shown the various effects of lead and phthalates in water bodies on organisms such as mice, rats, and fishes **(Adeyemi *et al.,* 2010; Chinwe *et al.,* 2010: Unyimadu *et al.,* 2008)** but there are no published works investigating the possibility of PVC pipe leaching into the water it distributes and the possible effect on humans **(Adeyemi and Osubor 2010, Chinwe *et al.* 2010, Unyimadu *et al.* 2010)**. This has necessitated the need to investigate the effect of PVC pipes in the quality of water and the possible effect on Wistar rats, which may serve as a hint on associated health risk of these chemical on man.

Therefore, this study examines the possibility of chemical leaching that might be caused by long-term use of PVC pipes in water distribution, determine the level of as well as the effect of age of PVC pipes on lead and phthalates leaching into the municipal water distributed by PVC pipes and examine the effect of diethyl phthalates and lead on liver and kidney of Wistar rats.

## 2. Material and methods

### Study Area and Sample Collection

The samples were collected from 2 and 10-year-old laid polyvinyl chloride pipe installations located in Akoka, Yaba Local Council Development Area in Lagos State, Nigeria (6.498 °N 3.38285 °E) using simple random sampling technique. Water samples were collected randomly from the municipal drinking water (flushed pipes) supply of two selected households in Akoka, Lagos. One set of the samples collected was in an area where PVC pipes have been installed for about two years while the other was obtained from PVC pipes which have been laid for more than ten years. Information was obtained from the department of production, Lagos State Water Corporation (LSWC), Shomolu, Lagos. The samples were collected in 1 L amber glass bottle with metal screw cap. Each bottle was well labeled for each of the selected sites. The water samples were analyzed for lead and phthalates analysis using atomic absorption spectrophotometer (AAS) (Agilent, USA) and gas chromatography coupled with mass spectrometer (GC-MS) (Shimadzu, Japan) respectively.

### Test Animals (*Rattus norvegicus*)

Sixteen Wistar rats (*Rattus norvegicus*) used in this research were purchased from the same stock at the department of Veterinary Anatomy, University of Ibadan Breeding House. The rats were transported to the laboratory in Lagos and acclimatized for four days to allow for physiological adjustment to laboratory conditions. Animals were feed with standard feed described by **Vento et al. (2008)**.


**Figure 1** Map of Lagos showing sampling location (Lagos Mainland LGA) of water samples indicated by an arrow of within the Lagos Metropolis (*n* = 6).

### Laboratory Analyses and bioassays

Water samples were processed and digested using standard protocol (Federation 1999) while the bioassays were conducted in the laboratory using lead salt and phthalate against the model animal Wistar rat (*Rattus norvegicus*).

### Extraction of Phthalates in Water Samples using Gas Chromatography-Mass Spectrometer (GC-MS)

Phthalate in water was determined following standard GC-MS procedure described **(Dargahi *et al.* 2017, Kiani *et al.* 2018)**.

### Preparation of Concentration of Lead and Diethyl Phthalate

The treatment for the experimental water supplied to the Wistar rats was prepared as follows: lead plus water only, diethyl phthalate plus water only, and a mixture of diethyl phthalate and lead concentrations in water to simulate water obtained from PVC pipe installation system. For lead, 0.079mg/L which was the highest concentration obtained in the pipes was used as the sub-lethal concentration for the bioassay. Lead acetate was weighed out using a digital weighing balance (A&D Orion series, Model HR-60) into 1L of deionized water to get a concentration of 0.079mg/L. For diethyl phthalate concentration (DEP), 0.6µL/L of diethyl phthalate ((Sigma-Aldrich, St. Louis, USA) which was the highest level measured in water obtained from municipal supply pipes was prepared by administering 6µL of 99.99 % pure diethyl phthalate (Sigma-Aldrich, St. Louis, USA) with the aid of micropipette into 1 L of deionized water (stock solution). Further dilution of the phthalate solvent was made by taken 10mL from the stock solution into another bottle containing 1L of deionized water to obtain a concentration of 0.6µl/L. And for the mixture of lead and diethyl phthalate concentrations (DEP + Pb), 0.079mg of lead acetate was weighed using a digital weighing balance into 1 L of deionized water to get a concentration of 0.079mg/L and 10mL from the stock solution of diethyl phthalate solvent (6 µl/L) prepared earlier was added to it.

**Bioassay**

A total of 16 four months old Wistar rats, 8 males and 8 females of average weight 130 g were used for this experiment. The Wistar rats were housed in four transparent plastic containers measuring 70 cm × 40 cm × 50 cm which were kept in a well-ventilated laboratory in Zoological Garden maintained by the Department of Zoology, University of Lagos. Each of the transparent plastic containers was partitioned with a wooden plank with each of the partition housing two Wistar rats of the same sex. Each group was treated as follows: group A – diethyl phthalate only, group B – lead only, group C – lead and diethyl phthalate and group D was for control (i.e., rats supplied with deionized water only). The rats were fed with commercial feed (pellets) obtained from the Department of Biochemistry, Nigerian Institute of Medical Researcher (NIMR), Yaba, Lagos. 75mL of experimental water containing either lead, diethyl phthalate, or the mixture of both toxicants was daily supplied as drinking water to the rats in each partition of the cage. The rats were supplied feed every two days over a six-week period. At the end of the third week two randomly selected rats – a male and female – were sacrificed from each of the group of the exposed rats and as well as control, while the others were left for six weeks after which they were sacrificed.

**Collection of Blood Sample**

Collection of blood samples were in accordance with the methods described by the approved protocol of Institutional Animal Care and Use Committee (IACUC) for cardiac puncture **(Suckow and Lamberti 2017)**. To minimize discomfort to the rats and alterations in blood parameters each rat was deeply anesthetized using chloroform. The rat was then laid on its back as the syringe was pushed vertically through the sternum. As the blood appeared in the syringe, it was held still, and the plunger was gently pulled back to obtain the maximum amount of blood available. Blood was stored in serum separating tube and EDTA (ethylenediaminetetraacetic acid) as appropriate. These were then taken to Department of Haematology, University of Lagos Teaching Hospital (LUTH) for full blood count and blood chemistry assay.

**Dissection of Animals**

Dissection of albino rat was performed with medical dissecting sets in strict adherence to pathological techniques and Organization for Co-operation and Economic Development (OCED) guidelines on dissection. The livers and kidneys were quickly collected in universal bottles containing Bouin's fluid (Sigma-Aldrich, St. Louis, USA) and stored in an ice-packed flask to avoid tissue degradation prior to transportation for analysis.

**Assessment of Blood Chemistry**

The blood chemistry was analysed for the following parameters: alkaline phosphatase (ALP), albumin (ALB), globulin, total protein (TP), alanine amino transferase (ALT), aspartate aminotransferase (AST) and cholesterol using the standard protocols (Lee *et al.* 2011). For the alkaline phosphatase, the method of **Rivadeneyra-Domínguez et al. (2018)** was employed in the determination of alkaline phosphatase. In this method, the amount of phosphate ester that is split within a given period is taken as a measure of the phosphatase enzymes. Total protein concentration was determined using the method described by **Moradi et al. (2019)**.

**Histological analysis of Liver and Kidney**

Histological study on tissues obtained from experimental rats was carried out. The tissues were subsequently read at the Morbid Anatomy laboratory LUTH for abnormalities **(Somasundaram *et al.* 2017, Baralić *et al.* 2020)**.

**Statistical Analysis**

All experiments were performed three times in duplicate (*n=6*). Analysis of variance (ANOVA) was done using GraphPad Prism (version 8.0.2) using Dunnette multiple comparison hypothesis testing to determine significant differences between the between control and exposed Wistar rats ($P \leq 0.05$).

**3. Results**

Using the Dunnett's multiple comparisons test to compare the control Wistar rats with the treatment for male and female rats for the third and sixth week, the results showed a significant difference between the treatments and control model animal ($P < 0.05$). It was also clearly shown that regardless of the period (third or sixth week) the measurement was taken and the sex of the Wistar rats, there is a varying degrees of interaction effects on the blood chemistry which are all extremely significant. For the male rats in the third week, there was an overall significant difference of $F$ (24,64) = 505.59, $P$ = 0.0001 with interactions between blood chemistry and treatments accounting for 17.81 of the total variance. While for the male (sixth week), the significant difference of $F$ (24,64) = 260.76, $P$ = 0.0001 with interactions between blood chemistry and treatments accounting for 11.32 of the variance. Almost the same results were observed in the female Wistar rats which have of $F$ (24,64) = 288.45, $P$ = 0.0001 and $F$ (24,64) = 411.38, $P$ = 0.0001 for the third and sixth week, respectively. If there are no interactions between the blood chemistry and treatments, there is a less than 0.01 % probability of the observation occurring at random.
The result of the chemical analysis of the municipal water samples revealed that some of the impregnated additives were present in the water samples. The level of lead and phthalates in water were 0.079mg/L and 0.57µl/L respectively for the 10-year-old pipe while the levels were 0.069mg/L and 0.034µl/L respectively in the 2-year-old pipe. The level of phthalate was 17 times higher in old pipes than the new ones while there was no significant different in the level of lead in the new and old pipe.
It was observed that the results for blood biochemistry in the albino rats both in the exposed rats and the controls. Generally, serum concentrations of aspartate transferase (AST), alkaline phosphatase (ALP), alanine amino transferase, protein, total bilirubin, direct bilirubin, and globulin were found to be

significantly higher in rats exposed to lead, diethyl phthalate and mixture when compared to control (*P*<0.05). Mean serum level of AST was highest in rat exposed to lead (129±2mg/dL) followed by rats exposed to diethyl phthalate (90.33±1.53mg/dL), and mixture (88 ±1.73mg/dL) while the controls had lowest level of 28.33±1.15 mg /dL.

Serum level of Protein was highest in rat exposed to lead (90.67±1.53mg/dL) followed by rats exposed to diethyl phthalates (80±1.0mg/dL) and the mixture (72.3±1.53mg/dL) while the controls had lowest level of 54±1.0mg/dL. Serum level of alanine amino transferase was highest for rats exposed to lead (36.67±1.53mg/dL) followed by diethyl phthalates (25±2mg/dL) and the mixture (24.67±0.58mg/dL) while the controls had the lowest level of 15.33±1.53mg/dL. Serum level of globulin was highest for rat exposed to lead (54.33±1.15mg/dL), followed by the mixture (47.67±1.53mg/dL) and diethyl phthalates (40.67±2.08mg/dL) and while the control had lowest level of 36.67±0.57mg/dL. Serum level of albumin was highest for rat exposed to diethyl phthalate (41.67±1.15mg/dL), followed by the lead (37.33±1.53mg/dL) and mixture (40.67±2.08mg/dL) and while the control had lowest level of 37±1.0mg/dL. Serum level of alkaline phosphatase was highest for rat exposed to diethyl phthalate (26.33±1.53mg/dL), followed by the lead (25.33±1.53mg/dL) and mixture (22.33±1.53mg/dL) and while the control had lowest level of 9.67±1.52mg/dL.

Serum level of total bilirubin was highest for rats exposed to lead (44.33±1.15mg/dL) followed by the mixture (38.0±0mg/dL) and diethyl phthalates (350±0mg/dL) and while the controls had the lowest level of 8±0 mg/dL, respectively. Serum level of direct bilirubin was highest for rats the mixture of diethyl phthalate and lead (11.33±1.53mg/dL) followed by lead (8.33±0.57mg/dL) and diethyl phthalates (5.67±0.57mg/dL) while the control had the lowest of level of 1.1±0.1 mg/dL.

Generalized blood haemolysis was observed in rats exposed to experimental water contaminated with either lead, diethyl phthalate or the mixture. Generally, Red blood cell (RBC) counts, and Platelets counts in the albino rats in all treatment dropped significantly in the sixth of exposure when compared to the third week. Red blood cell counts were lowest in rats exposed to lead (3.320±2.376), followed by diethyl phthalates (6.445±2.044) and the mixture (6.445±2.044) while the control had highest of 8.9±0. Blood Platelet count was lowest in rats exposed to lead (206.0±97.58), followed by diethyl phthalates (540.5±7.778) and the mixture (540.5±7.778) while the control was highest for 984±0.

The result for histological examination for both liver and kidney of the rats are shown in Figure 3 (A-D). The histological examination of both liver and kidney were generally normal with no sign lesion, blood vessels are not congested except for one (plate 3) of the kidneys of male rat exposed to the mixture of concentration of diethyl phthalate and lead was observed to have interstitial inflammation.

**4. Discussion**

The findings in this study show clearly that components of PVC municipal water supply pipes have the capacity to leach into the water they convey. The result of analysis of water samples indicated that diethyl phthalates (plasticizer) and lead (stabilizer) which are components of the pipes were found in detectable levels. Lead leachate concentration observed for both old and new pipes installation (0.079mg/L and 0.069mg/L respectively) were found to be higher than WHO and FEPA limits (0.015mg/L), with lead leachate in old and new PVC pipes being five times higher than the WHO and FEPA limits **(Nkwocha et al. 2017, Ezeji et al. 2019)**. From this result, it is evident that the age of pipe has a role to play in the concentration of stabilizers and plasticizers in the water, the older the pipe the higher the level of the additives that may be leached into water. This case appears to be aggravated with diethyl phthalates leachates in which water from old PVC pipes (0.57µl/L) were found to be 17 times higher than the phthalate leachates in relatively new PVC pipes (0.034µl/L).

The danger of using PVC for water distribution has been reported by **Whelton and Nguyen (2013)** who established that vinyl chloride monomers (VC) and organotins are leached off from PVC pipe used for water distribution system in several countries. Vinyl chloride is a known human carcinogen and central nervous system depressant **(Brandt-Rauf et al. 2012)**. It is known that organotins are known to produce depression with loss of memory and aggressive behavior in humans. It also produces oedema, encephalopathy, and immune system depression in human. Some organotins are unusually toxic to the nervous system **(Budny 2015, Bolognesi et al. 2017)**. These facts make the use of PVC pipe in drinking water distribution of serious public health concern.

The increasing liver and kidney disease in Nigerians is a cause for concern although these diseases have their roots partly from nutrition and partly from inheritance **(Kris-Etherton et al. 2002)**. Serum bilirubin, globulin, alanine amino transferase (ALT), aspartate transferase (AST) and albumin concentrations are some biochemical indices for monitoring liver function. Abnormal levels of these proteins have been reported to be associated with haemolysis or increased breakdown of RBC and/ or liver damage **(Islam et al. 2004)** which appears to be the case in this study.

In this study, rats fed with drinking water contaminated with phthalate and lead after 6 weeks of exposure showed elevated serum concentrations of both total and direct bilirubin suggesting that the liver is not able to excrete bilirubin which is an evidence of liver dysfunction. Bilirubin is a breakdown product of heme, a part of haemoglobin in red blood cells. The liver is responsible for clearing the blood of bilirubin. It does this when bilirubin is taken up into hepatocytes, conjugated (modified to make it water soluble) and secreted into the bile, which is excreted into the intestine. Increased total bilirubin causes jaundice and can signal several problems **(Sunmonu and Oloyede 2009)**.

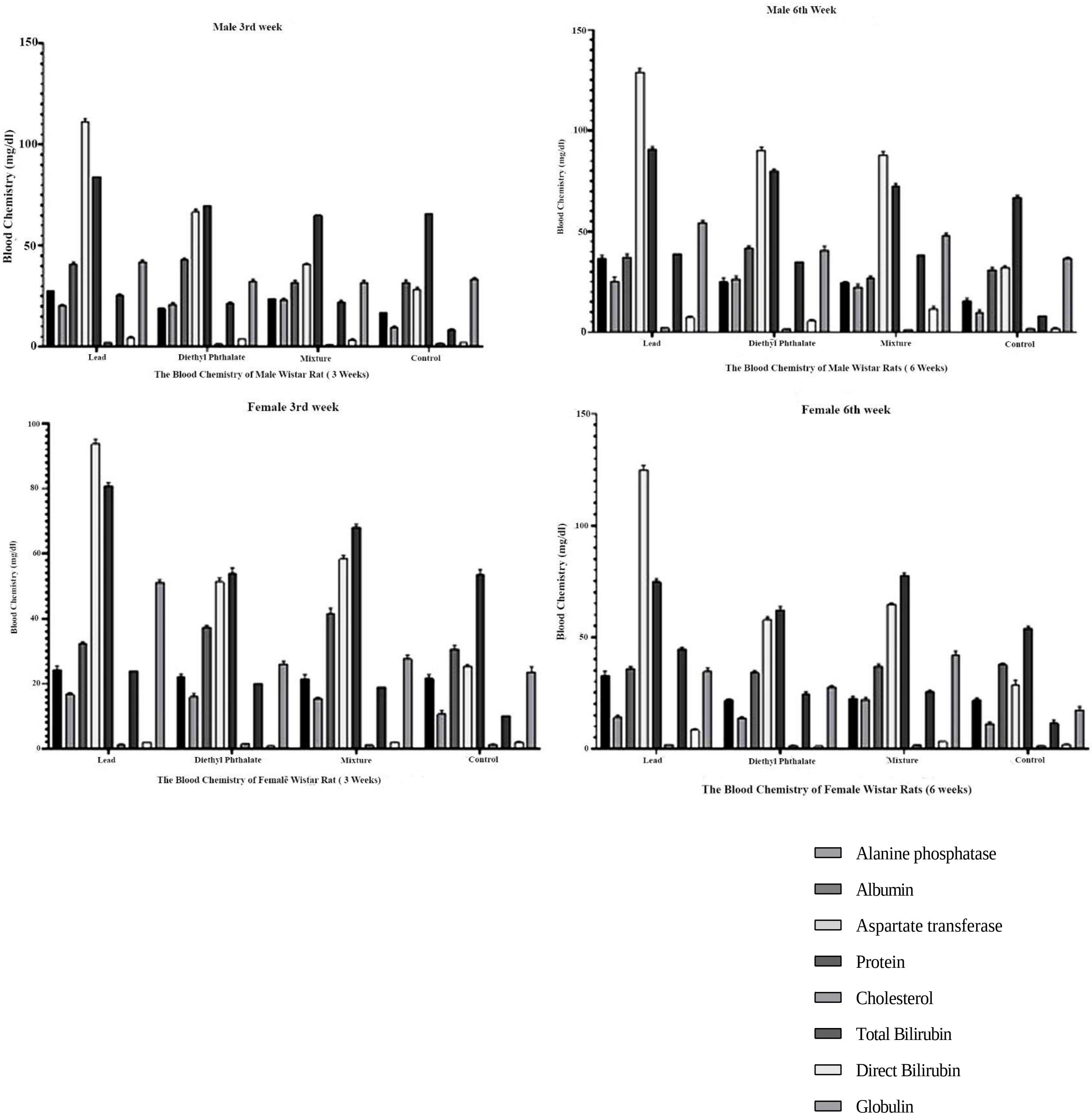


**Figure 2** Histological section of kidney of the control Wistar rat. Glomerulus with blood capillaries, normal convoluted tubules. A histological section of kidney of male rat after 6-week exposure to mixture of diethyl phthalate and lead. The section shows interstitial inflammation indicating accumulation of fluids (Oedema).

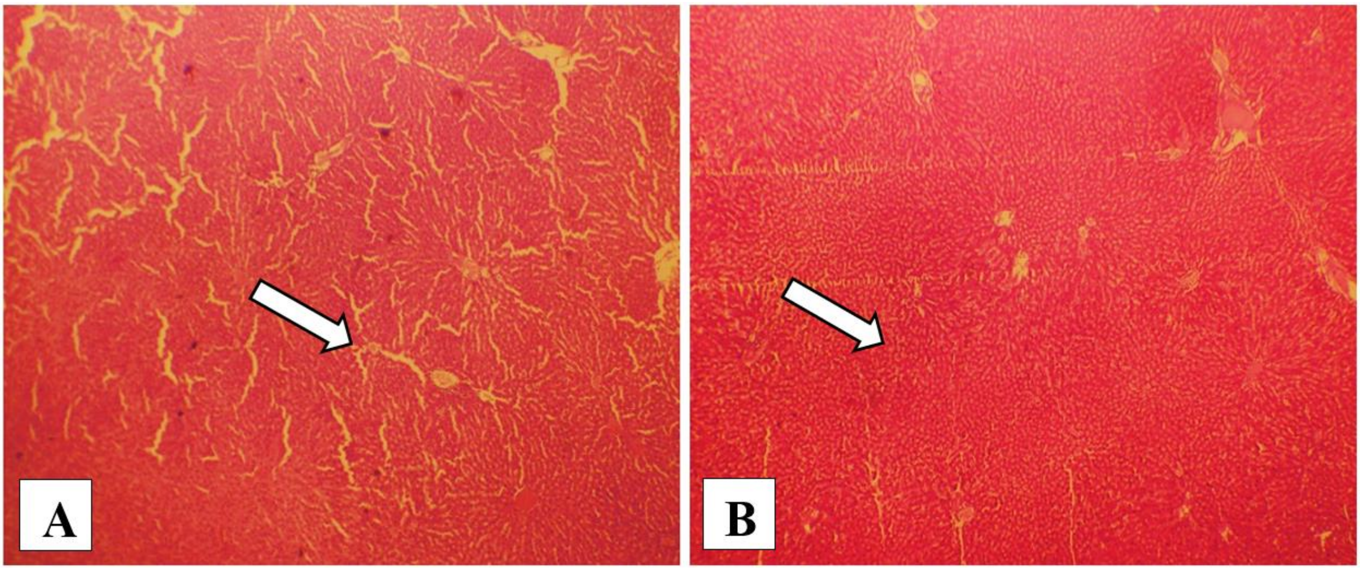


**Figure 3** Histological section of the liver of control Wistar rat. Figure 1A shows the normal hepatocytes without lesions and congested blood vessels (×100). 1B shows a histological section of the liver after exposures to lead for 6 weeks. The section is innervated by blood vessels with some showing blood clotting and mild inflammations.

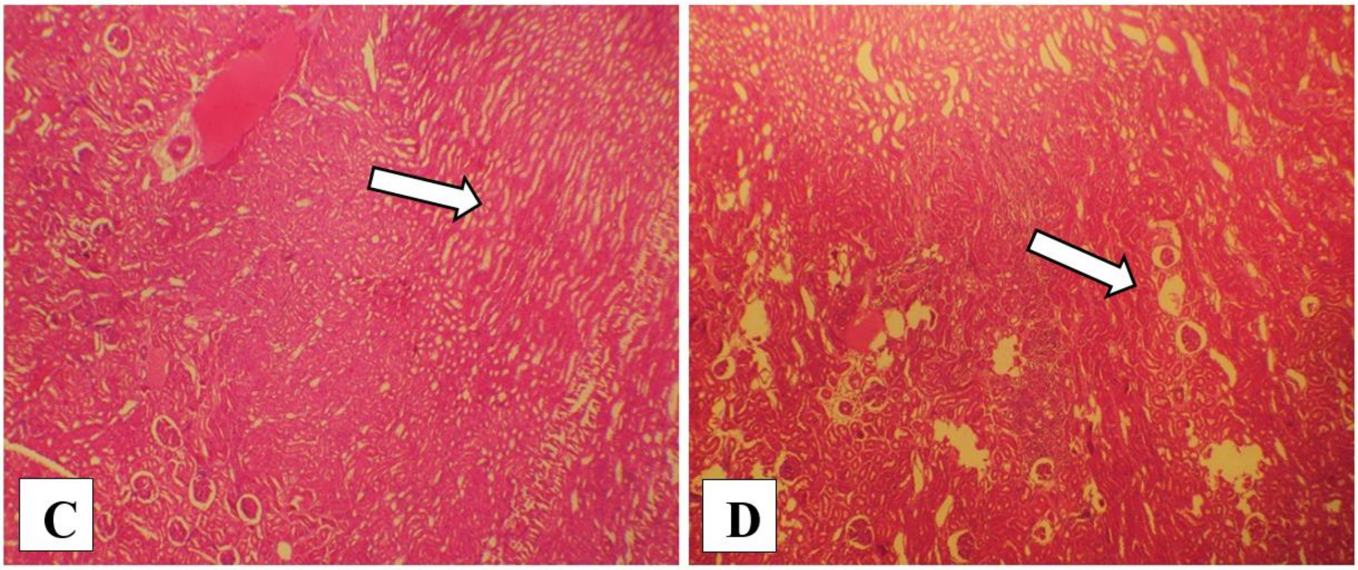


**Figure 3** Histological section of kidney of the control Wistar rat. Glomerulus with blood capillaries, normal convoluted tubules. A histological section of kidney of male rat after 6-week exposure to mixture of diethyl phthalate and lead. The section shows interstitial inflammation indicating accumulation of fluids (Oedema).

Decrease in the level of serum concentration of albumin and a corresponding increase in the level of globulin as was the case in this study might be an indication that the liver function may be impaired although histopathological study did not indicate any liver impairment. Both globulin and albumin are produced by the liver and if the liver is damaged, it can no longer produce these essential proteins. The results in this study show that serum proteins are consistently high, which implies that the liver might have been damaged probably due to the intake of water that has been contaminated with lead and diethyl phthalate.

A general significant increase in AST and ALP was observed in serum of rats administered with contaminated water relative to the control ($P < 0.05$). The increase in the ALP and AST in the serum as observed in this study suggests that drinking water contaminated with diethyl phthalates and lead possibly led to inhibition of ALP and AST activity in the liver. Aspartate is found in many body tissues including the heart, muscle, kidney, brain, and lung. A significant rise in serum concentration of AST may be indicative of kidney dysfunction. However, another evidence in this study points to possible kidney dysfunction in exposed animals is the observed anaemia as shown by low RBC and platelet counts observed in exposed rats when compared to control ($P$ <0.05). Anaemia is common in people with kidney disease. Healthy kidneys produce a hormone called erythropoietin, or EPO, which stimulates the bone marrow to produce the proper number of red blood cells needed to carry oxygen to vital organs. Diseased kidneys, however, often do not make enough EPO. As a result, the bone marrow makes fewer red blood cells **(Daugirdas *et al.* 2012)**. Further, conclusive kidney function tests are needed to confirm this submission, however.

Only 1% of the histological examination was positive for interstitial inflammation of the kidney while the rest were normal. Probably, due to the short duration of this experiment; a longer period of exposure of these Wistar rats to the toxicants is likely to present with liver and kidney degeneration that may be significant. The findings in this study give us a clue to the potential adverse effect of drinking contaminated water with levels of lead and diethyl phthalate for a long period of time on human health. Further research is however needed to investigate the contribution of the long-term effect of exposure of these levels of lead and diethyl phthalate in the Nigerian population.

## 5. Conclusion

This research has been able to establish the fact that PVC pipes additives such as lead, and phthalates leached into water they distribute which may cause physiological changes that are indicative of potential organ dysfunction such as the liver and kidney. The public should be concerned about the safety in the use of PVC pipes for distribution drinking water. This, therefore, inform us that drinking water contaminated with lead far beyond WHO and FEPA limit is highly unsafe for the population. Thus, there is urgent need for an improved technology in PVC pipe production in such a way that will be safe when used in water distribution systems or a total replacement with safer substitutes to either minimize or completely eradicate the exposure of adult and children to the adverse effect of lead and phthalate.

## Declarations

### Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests

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### Authors' contributions

**OA:** Writing - original draft, resources, investigation, conceptualization, methodology

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