Original Article

Toxicological Impacts of Pharmaceutical Industrial Effluents on Hematology, Biochemical and Histological Indices of Rattus Novergicus

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Received: 05 August 2024	Revised: 08 September 2024	Accepted: 22 September 2024	Published: 05 October 2024

Abstract - The most harmful and potentially lethal wastes are heavy metals. In this investigation, the hematology, biochemical and histopathological effects on Rattus novergicus of the compositions of effluent from the pharmaceutical industry were evaluated. The effluent was taken from a pharmaceutical industry at Ilorin, Kwara State. Thirty-six (36) laboratory rats were obtained, and six groups were made up of each of the six rats. The first group was served as the control group. Distilled water was given to them, while groups 2 to 5 had 20, 40, 60, 80 and 100 % v/v of the pharmaceutical effluent as the exclusive water source, respectively. By the fourth week, the rats were sacrificed for hematology and biochemical analysis, and GraphPad Prism version 5.0 was used, while the liver and the kidney were subjected to histopathological examinations, which showed mild portal congestion and mild to moderate periporal cellular infiltration in the liver and focal extension area of tubular degeneration, necrosis around the renal capsule, and moderate interstitial congestion of the kidney due to the concentrations of the effluents. These results demonstrate that the rats were fairly impacted, which may confirm the effect on aquatic and terrestrial life forms that depend on the river for home and agricultural purposes.

Keywords - Biochemical, Effluents, Hematology, Histopathology, Rattus novergicus.

1. Introduction

Industrial effluents are considered to be the most dangerous waste since they contain poisonous chemicals like heavy metals (Dhevagi and Oblisami, 2002; Azim et al., 2017). These wastewater or effluents are mostly discharged into lakes, rivers, and streams, which serve as the primary supply of water for residential and agricultural use (Manunatha, 2008; Rakesh et al., 2021). Toxic materials settle on the bed when they get into bodies of water. Aquatic ecosystems are impacted by the pollution that emerges from this, which causes materials to disintegrate, float, or get absorbed in water (Osaigbovo and Orhue, 2006; Razzak et al., 2022). The effluent may impact the quality of the soil if this water is used for long-term irrigation. In addition to affecting the groundwater deposits, pollutants can flow downward (Kolpin et al., 2002; Anser et al., 2020). Industrialization in recent decades has generally resulted in the release of solid, liquid, and gaseous emissions into natural systems, leading to environmental damage (Adebisi et al., 2007; Yan et al., 2018). The development, utilization and elimination of diverse chemicals that facilitate progress in industry, farming, healthcare, and daily domestic amenities have led to growing worries in recent decades over possible negative impacts on human and ecological health (Saeed et al., 2011; Vanerkar et al., 2013). Waste produced during the drug-making process by the pharmaceutical industry is known as pharmaceutical effluent. Increased amounts of organic compounds, total solids, cadmium, mercury, and isomers of 1,2-dichloroethane, hexa-chlorocylohexane, among other solvents, have been found in certain pharmaceutical effluents (Savita and Deepa et al., 2013). Depending on the product made, the ingredients utilized, and the specifics of the procedure, wastewater from the pharmaceutical industry might pollute water. Industrial wastes that cause environmental degradation have been mainly accountable for some terrible environmental effects and human disasters within the past 40 years (Srinagar, 2000; Yan et al., 2018). Due to the presence of harmful compounds for human health, the waste products released by these companies constitute a health hazard to people and other living things in the environment (Singare and Dhabarde, 2014; Marzieh et al., 2020) when it is not properly discharged into

the environment. There is a need to evaluate the effects of these metals and to evaluate the level of impact at which the effluents may affect the blood parameters and organs of living organisms.

2. Materials and Methods

2.1. Collection and Analysis of Effluent

Wastewater samples were obtained from the pharmaceutical industry and sent directly to the laboratory for examination of the physical and chemical characteristics, as reported by Kesalkar et al., 2012, Fekede et al., 2020 Dalal et al., 2013, Bhartia et al., 2018 of the samples such as temperature, total hardness, pH, conductivity, dissolved oxygen, and biochemical oxygen demand. Following filtration using a Whatman filter and acidification with concentrated HNO₃ to reduce the pH, fifty milliliters (50 ml) of each wastewater sample were collected. After that, 40 milliliters of the sample were mixed with 5 milliliters of concentrated HNO₃, and it was digested for 30 minutes in an enclosed area. Thereafter, it was lessened in strength to 100 ml with distilled water (Ahaneku and Animasahun, 2013; Ogundiran and Fawole, 2014)) and the metal concentrations of the digested samples (Islam et al., 2016) were ascertained using an Atomic Absorbance Spectrophotometer (Perkin Elmer 3110)

2.2. Procurement and Treatment of Rats

A total of thirty-six male adult rats with good health (Rattus novergicus) of (120±20g) were procured from Adeleke Farms, Ogbomosho, Oyo State. Before the experiment started, the rats had a two-week acclimatization period. Throughout the study, the rats were fed with farmers' mash, ad libitum supplies of food and water were made available. The rats were housed in six plastic cages with adequate ventilation before being moved to the Animal House for two weeks of acclimation. There were six groupings formed from the animals, each receiving dilutions of different effluent concentrations as follows: By measuring 0.2 liters of wastewater sample and adding it to 0.8 liters of potable water, 20% of the wastewater was obtained. For the remaining 40%, 0.4 liters of wastewater were measured and added to 0.6 liters of potable water. Measurements of 0.6 liters of wastewater and 0.4 liters of potable water were used to get the 60%, and 0.8 liters of wastewater and 0.2 liters of potable water were used to get the 80% and 100%, respectively. Twice a day, for thirty days, at 12-hour intervals, 0.5 milliliters of the diluted water were gavaged into each rat. The rats in the control group were placed unconscious after the experiment in a chamber that was soaked with chloroform. Next, a five-milliliter blood sample was obtained by means of a cardiac puncture in each individual. To guarantee homogeneity and prevent blood clots, each blood sample was well mixed with lithium heparin anticoagulant before being spun for 10 minutes at a speed of 2500 revolutions per minute (rpm) utilizing a Gulfex Medfield Equipment and Scientific Limited macrocentrifuge (type 800D). The resultant plasma was then put to use to measure biochemical parameters (Owoade et al., 2019). After that, the *Rattus novergicus* rats were euthanized, and the liver and kidney were taken, respectively, from the experimental and control groups. Following their fixation in 10% formal saline, the organs were ready for histomorphological analysis (Asyura et al., 2016; Fikre et al., 2020)

2.2.1. Statistical Analysis

The data was subjected to Graphpad prism version 5.0 to analyze the hematology and biochemical analysis, and SPSS 22.0, the Statistical Packaging for Social Sciences, was utilized for data analysis for physicochemical and heavy metal determination following a Duncan multiple range test and one-way Analysis of Variance (ANOVA) was done. A significance level of p < 0.05 was established.

3. Results and Discussion

3.1. Physicochemical Parameters

The physicochemical results, as shown in Table 1, indicate that all the parameters were above the permissible standard of WHO except the pH, Electrical Conductivity and Alkalinity, which were below the standard limits indicating that the effluent is being polluted in agreement with the submission of Ashok et al., 2006 that the variation recorded in the physicochemical parameters is attributed to the product made, the ingredients utilized, and the specifics of the procedure of the effluent being released during manufacturing at the time of sample taken.

3.2. Heavy Metal Determinations

According to the report obtained by James et al. (2013), the heavy metals concentration value in the effluent was higher and exceeded the allowable limit set by WHO (2011). This could potentially have detrimental effects on our environment, and humans may not be exempt from them.

3.3. Haematological Analysis

The average PCV values of Rattus novergicus are displayed in Figure 1, which also depicts the influence of pharmaceutical effluent on rats' PCV. The findings for each treated group PCVs were discovered to be similar to the control group. However, the 80% treated group was significantly increased compared with 20%, 40% and 60% treated rats. The effect of pharmaceutical effluent on hemoglobin, RBC, MCV, MCH, lymphocyte, monocytes and platelets with no significant difference was observed between treated groups and control. Hence, no effect on the concentrations, as shown in Figures 2,3,4,5,6,7 and 8. Depicts the effect of pharmaceutical effluent on the WBC concentration of rats with no significant difference between the 20% treated group and control while 40%, 60%, 80% and 100% were all substantially (P<0.05) less than the control and 20% treated group in a way independent of concentration as shown in Figure 9. Hematological measures and complete blood counts can be employed as useful tools to evaluate the harm caused by various chemicals and as stress, indicators to ascertain an organism's physiological status (Flaiban et al., 2008). In this study, the blood parameters investigated for the hematological test for all the treated groups were PCV, WBC, Hb, and RBC. Neutrophil concentration showed a significant decrease in various treatment groups in contrast to the control.

3.4. Biochemical Analysis

Figure 10 shows no significant difference when comparing the treatment groups with the control, and the treated groups were not significantly different from one another. Hence, there was no effect on serum albumin, albumin, globulin and ALP. Figure 11 shows a substantial (p< 0.05) non-concentration dependent rise in every treatment group in contrast to the control group, while an increment was observed in 20%, 40%, 80%, and 100% in ALT. Figure 12 shows a non-concentration dependent significant increment in all the treatment groups with respect to the control group. However, there was not a statistically significant variation between the treated groups (P<0.05) non-concentration dependent increase in all the treated groups when compared with control in AST. There was a slight increase noted in all the treated groups when compared with the control as shown with no significant difference between the treated groups observed in bicarbonate ion. There was a noticeable decline in 20% and 60% when compared with the control, while 40%,80%, and 100% were not notably distinct from the control, and the effect was not concentration dependent, as shown in Figure 13 in chloride ion. A noticeable decrease was observed in all the treated groups when compared with the control, as shown in Figure 14, while there were no appreciable differences between the treatment groups hence, the effect was not concentration dependent in sodium ion, Conjugated bilirubin, Total bilirubin, Unconjugated bilirubin and Creatinine has when drawing comparisons to the treated groups and the control group, there was no discernible difference and were not significantly different from one another as shown in Figures 15 to 31. The treated groups of ALT only caused a significant increase in ALT activity, while

in AST, all the treated groups significantly increased the serum activity of the enzyme. This also showed that effluent from the pharmaceutical industry has embedded substances that can adversely affect liver cells. This result is consistent with the submission of Singh and Pandev (2021), who reported a significant elevation in AST and ALT activities in stinging catfish from fertilizer industrial effluents. The treated groups were significantly increased in Urea in contrast to the control. Heavy chemicals and various nephrotoxic compounds present in the effluent might have elicited various biochemical pathways, resulting in toxic effects on nephrons, hence elevating the concentration of serum urea. This is also consistent with the preceding report of Alimba et al.,2019, who reported pathological lesions on the gills, liver and kidney of pharmaceutical effluent treated fish of Clarius gariepinus. A significant increase in bicarbonate concentration and conjugated bilirubin was discovered in all treated groups when compared with control, having various chemical substances that stimulate the production of bicarbonates from stomach and pancreas, hence regulate the concentration of hydrogen ion and thereby prevent metabolic acidosis with a significant decrease in chloride ion concentration and no effect on serum albumin concentration and total bilirubin.

3.5. Histopathology Analysis

Histopathological alterations seen in the rats' kidneys and liver after various dosages of the concentrations in the form of periportal cellular infiltration and congestion of the renal cortical interstitium are in agreement with the observations of Bhushan et al. (2013) in rat liver biochemical and histopathological alterations caused by Cypermethrin and beta-cyfluthrin, George et al.,(2014) in renal failure caused by the increased level of creatinine and urea levels as markers and Emmanuel et al., 2020 in biochemical and histomorphological changes in liver and kidney of *Rattus novergicus* administered with tetracycline as shown in Figures 33 and 34 respectively.

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				WHO, NESREA, 2011				
Temp (°c)	25.67	26.00	25.78	25				
pH	6.10	6.23	6.0	6.5				
EC (sm ⁻¹)	2.07	2.14	2.18	1000				
DO (mg/l)	39.17	44.39	32.50	5-9.5				
Hardness (mg/l)	236.33	216.02	280.40	100				
Alkalinity (mg/l)	410.67	428.31	384.48	500				
Turbidity	17.51	16.52	14.36	<5				

 Table 1. Physicochemical parameters of the effluent from the Pharmaceutical industry

 Table 2. Heavy metal determination of the effluent from the Pharmaceutical industry.

				WHO,2011
Fe (mg/kg)	10.764	7367	9.145	0.30
Cu (mg/kg)	0.062	0.024	0.198	0.01
Zn (mg/kg)	1.172	0.659	0.081	0.03
Cd (mg/kg)	0.022	0.031	0.035	0.10
Cr (mg/kg)	0.278	0.129	0.215	0.003
Pb (mg/kg)	0.000	0.01	0.000	
Ni (mg/kg)	0.113	0.153	0.171	0.02



Effect of different percentage of pharmaceutical effluent on PVC of rats Fig. 1 Effect of pharmaceutical effluents on PCV

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with alphabet '**a**' are not noticeably different from the control. Bars with alphabet '**b**' are noticeably different from 80% of pharmaceutical wastewater



Effect of different percentage of pharmaceutical effluent on PVC of rats

Fig. 2 Effect of pharmaceutical wastewater on Hemoglobin

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. No significant difference between the treated groups



Effect of different percentage of pharmaceutical wastewater on RBC conc. of rats Fig. 3 Effect of pharmaceutical wastewater on RBC concentration

A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control.No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on MCV conc. of rats Fig. 4 Effect of pharmaceutical wastewater on MCV concentration of the rats

A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. No significant difference between the treated groups.





A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on MCHC conc. of rats

Fig. 6 Effect of pharmaceutical wastewater on MCHC of rats

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. The bar with the alphabet 'b' is noticeably different from control.

Bar with the alphabet 'c' is noticeably different from 60% pharmaceutical wastewater. Bar with the alphabet 'd' are noticeably different from 100% pharmaceutical wastewater. Bars with the alphabet 'e' are noticeably different from 80% of pharmaceutical wastewater.



Effect of different percentage of pharmaceutical wastewater on WBC conc. of rats Fig. 7 Effect of pharmaceutical wastewater on WBC concentration

A significance threshold of (P<0.05) was applied to each group of five rats. Bar with alphabet 'a' not noticeably different from control. Bars with the alphabet 'b' are noticeably different from control.

Bars with the alphabet 'c' are noticeably different from 20% pharmaceutical wastewater. Bars with the alphabet 'd' are noticeably different from 100% pharmaceutical wastewater. Bars with the alphabet 'e' are noticeably different from 100% pharmaceutical wastewater.



Effect of different percentage of pharmaceutical wastewater on Neutrophil conc. of rats Fig. 8 Effect of pharmaceutical wastewater on Neutrophil concentration

A significance threshold of (P<0.05) was applied to each group of five rats.Bar with alphabet 'a' are not noticeably different from control. Bars with the alphabet 'b' are noticeably different from control. Bars with alphabet 'c' are noticeably different from 20% of pharmaceutical wastewater Bars with the alphabet 'd' are noticeably different from 40% of pharmaceutical wastewater. Bars with alphabet 'e' are noticeably different from 80% of pharmaceutical wastewater.



Effect of different percentage of pharmaceutical wastewater on Lymphocyte conc. of rats Fig. 9 Effect of pharmaceutical wastewater on lymphocyte concentration

A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. No significant difference between the treated groups.



Fig. 10 Effect of pharmaceutical wastewater on monocyte concentration

A significance threshold of (P<0.05) was applied to each group of five rats.Bars with the alphabet '**a**' are not noticeably different from control.



Effect of different percentage of pharmaceutical wastewater on Platelet conc. of rats Fig. 11 Effect of pharmaceutical wastewater on platelet concentration

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. No significant difference between the treated groups



Effect of different percentage of pharmaceutical wastewater on serum total protein

Fig. 12 Effect of pharmaceutical wastewater on total protein

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on serum Albumin Fig. 13 Effect of pharmaceutical wastewater on serum Albumin

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. No significant difference between the treated groups.

A significance threshold of (P<0.05) was applied to each group of five rats.



Effect of different percentage of pharmaceutical wastewater on serum Globulin

Fig. 14 Effect of pharmaceutical wastewater on serum globulin

Bars with the alphabet 'a' are not noticeably different from control.

Bar with alphabet 'b' noticeably different from control.

Bars with alphabet 'c' are noticeably different from 20% of pharmaceutical wastewater.

Bar with alphabet 'd' noticeably different from 40% pharmaceutical wastewater.



Effect of different percentage pharmaceutical wastewater on serum ALP activity

Fig. 15 Effect of pharmaceutical wastewater on serum ALP

A significance threshold of (P<0.05) was applied to each group of five rats.

Bars with the alphabet 'a' are not noticeably different from control. Bar with alphabet 'b' noticeably different from control.



Effect of different percentage of pharmaceutical wastewater on serum ALT activity

Fig. 16 Effect of pharmaceutical wastewater on serum ALT activity

A significance threshold of (P < 0.05) was applied to each group of five rats.

Bars with the alphabet 'a' are not noticeably different from control.

Bar with alphabet 'b' noticeably different from control.



Effect of different percentage of pharmaceutical wastewater on serum AST activity Fig. 17 Effect of pharmaceutical wastewater on serum AST activity

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. Bar with alphabet 'b' noticeably different from control.



Effect of different percentage of pharmaceutical wastewater on serum Bicarbonate conc.

Fig. 18 Effect of pharmaceutical wastewater on serum bicarbonate

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on serum Chloride conc.

Fig. 19 Effect of pharmaceutical wastewater on Chloride ion.

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on serum sodium conc. Fig. 20 Effect of pharmaceutical wastewater on sodium concentration.

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on serum Total Bilirubin. Fig. 21 Effect of pharmaceutical wastewater on total bilirubin.

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on serum creatinine. Fig. 22 Effect of pharmaceutical wastewater on serum creatinine

Bars with the alphabet 'a' are not noticeably different from control.

A significance threshold of (P < 0.05) was applied to each group of five rats.

Bars with the alphabet 'b' are noticeably different from control.

Bars with alphabet 'c' are noticeably different from 20% of pharmaceutical wastewater.

Bars with alphabet 'd' are noticeably different from 80% of pharmaceutical wastewater.



Fig. 23 Effect of pharmaceutical wastewater on serum conj. Billrubin

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. Bar with the alphabet '**b**' are noticeably different from control.



Effect of different percentage of MS water on serum unconj. Bilirubin. Fig. 24 Effect of pharmaceutical wastewater on unconjugated bilirubin

A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on Liver SOD activity. Fig. 25 Effect of pharmaceutical wastewater on SOD activity A significance threshold of (P < 0.05) was applied to each group of five rats.Bar with alphabet '**a**' not noticeably different from control.Bars with the alphabet '**b**' are noticeably different from control.



Effect of different percentage of pharmaceutical wastewater Liver Total protein conc.

Fig. 26 Effect of pharmaceutical wastewater on liver total protein

A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control.



Effect of different percentage of pharmaceutical wastewater Liver Total protein conc.

Fig. 27 Effect of pharmaceutical wastewater on MDA concentration

A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. The bar with the alphabet '**b**' is noticeably different from control.



Effect of different percentage of pharmaceutical wastewater on Liver GSH conc. Fig. 28 Effect of pharmaceutical wastewater on GSH concentration A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet 'a' are not noticeably different from control. The bar with alphabet 'b' is noticeably different from control. Bars with alphabet 'c' are noticeably different from 20% of pharmaceutical wastewater.



Effect of different percentage of pharmaceutical wastewater on kidney SOD activity. Fig. 29 Effect of pharmaceutical wastewater on SOD activity

A significance threshold of (P<0.05) was applied to each group of five rats. Bar with alphabet 'a' not noticeably different from control. Bars with the alphabet 'b' are noticeably different from control. Bars with alphabet 'c' are noticeably different from 100% pharmaceutical wastewater



Effect of different percentage of pharmaceutical wastewater on Kidney Total protein conc. Fig. 30 Effect of pharmaceutical wastewater on total protein

A significance threshold of (P<0.05) was applied to each group of five rats.

Bars with the alphabet 'b' are noticeably different from control. No significant difference between the treated groups.



Effect of different percentage of pharmaceutical wastewater on Kidney MDA conc. Fig. 31 Effect of pharmaceutical wastewater on MDA concentration

A significance threshold of (P < 0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control. Bars with the alphabet '**b**' are noticeably different from control. Bars with alphabet '**c**' are noticeably different from 80% of pharmaceutical wastewater.





A significance threshold of (P<0.05) was applied to each group of five rats. Bars with the alphabet '**a**' are not noticeably different from control.

Organ	rgan Concentration Histopathological charges			
Liver	20	Mild portal congestion	+	
	40	Mild portal congestion	+	
	60	Mild to moderate periportal cellular Infiltration	+	
	80	Mild to moderate periportal cellular infiltration	+	
	100	Mild to moderate periportal cellular infiltration	+	

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1 able 4.	Aberrations were	observed in the	kidneys of rat	s given var	ving concentrations	s of treatment from	pnarmaceutica	wastewater
					/ 8		1	

Organ	Concentration	Histopathological charges	Significance
Liver	20	Focally extension area of tubular degeneration.	-
	40	Necrosis around the renal capsule	+
	60	Moderate interstitial congestion	+
	80	Moderate interstitial congestion	+
	100	Moderate interstitial congestion	+







Control







Fig. 34 Aberration observed in the kidney of rats given varying concentrations of treatment from pharmaceutical wastewater

4. Conclusion

Before being released into the environment, wastewater from the pharmaceutical industry needs to be carefully monitored and treated. This is necessary for avoiding contaminating rivers and streams, which can build up in the kidneys and liver of organisms that depend on water for longterm domestic use and avoid consumption of aquatic organisms like fish from streams and rivers where the industrial effluents are being discharged because of deposit of heavy metals like lead and various injurious substances in the tissue of the animals.

Funding Statement

The corresponding author and the co-authors fully funded the research on a reasonable amount.

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