

## Reviewer' query and suggestions

**Dear Authors,**

This is a virtuous piece of work however, I would like to remarks on this manuscript entitled as *“Bioaccumulation of Heavy Metals in Organs of Clarias gariepinus Juveniles Exposed to Sub-Lethal Concentrations of Waste Tire Residues”*

Authors highlighted on the subject of Waste Burnt Tire Residues pollution and bioaccumulation of heavy metals in the organs of *Clarias gariepinus*.

### Specific comments:

1. Title of the manuscript was vague and it should be revised with relevance of the work.
2. Introduction seems to be very short and needs to update with latest references
3. Introduction didn't have enough content to reflect the importance of the current work. The overall introduction needs to revise according to objective of the work
4. Conclusion” didn't reflect the importance of the research. Authors needs to mention the novel findings of the work
5. **RECOMMENDATION-** There is no need to include under a separate title of recommendation of further work rather encompassed in the last paragraph of discussion section.
6. English language and scientific nomenclature should be in italics so needs to be improve in the manuscript.
7. Check references for some errors.

\*This manuscript is written in underprivileged script all over the text I recommend to revised it with help of English scholarly with line wise.

**Some Suggestions for different sections are as followings:-**

### **Title**

✓ Author may replace with the more relevant title as:

**“Analyses of bioaccumulation of heavy metals on the Organs of *Clarias gariepinus* following exposure of Sub-Lethal Concentrations of waste tire residues”**

### **Abstract Section**

Abstract should be concise and highlights aim, methods, results and the main outcomes.

Abstract may be restructure as followings:-

This study evaluated certain heavy metals concentrations of waste burnt tire residues (WBTRs) and bioaccumulation of these heavy metals in the certain organ systems (viz-gills, liver, kidney and muscles) of *Clarias gariepinus* following exposure to sublethal concentrations (SLCs) of water-soluble fractions (WSFs) of WBTRs. *C. gariepinus* (average weight of  $47.95 \pm 0.34$ g and length of  $15.54 \pm 0.36$ cm) were exposed to SLCs of different concentrations (0.00, 0.23, 0.47, 0.94, 1.87, and 3.74 ppm) of WSFs of WBTRs for a period of fifty-eight days. Heavy metal concentrations in WBTRs and in the organs of the experimental fish were measured using a handheld X-Ray Fluorescence Analyzer (NitonXL3T). Results showed that strontium, lead, zinc, cobalt, bismuth, rubidium, gold, tungsten, iron, thorium, arsenic, copper, and niobium were detected in WBTRs although the maximum level of zinc was perceived. However, no significant difference was observed as compared to the control group regarding heavy metal accumulation in muscles,  $53.10 \pm 12.78$ ; liver,  $56.30 \pm 76.96$ ; kidney,  $164.54 \pm 12.78$ ; and gills,  $241.36 \pm 146.87$  of fish, ( $P > 0.05$ ). The high heavy metal concentrations present in WBTRs are really concerned as having the capacity to pollute surrounding environments with the aquatic ecosystem through sewage flow and rain water effluents. Upon regular consumption of fishes from aquatic body with WBTRs also caused the pathogenic impact on the other fauna including human beings.

**Key words-** placed alphabetically

### **Introduction Section**

Background of introduction should be pivoted around toxicity and bioavailability of such heavy metals that found in the waste burnt tire residues at global and the country where investigation was being carried out.

\*\*Author must need to incorporate the (references of) fact of elevated heavy metals chromium/ cobalt, bismuth, rubidium, gold, tungsten, iron, thorium, copper, niobium and arsenic exposure that pose or/ and exhibit a significant negative impact on aquatic as well as human life. However, Authors had cited the very few research papers that showed the frequency of metal pollution in fish particularly in areas where used tires are burned or disposed of as fuel.

## **Materials and Methods Section**

How was the LC<sub>50</sub>/sub lethal dose of such WBTRs / heavy metals determined? Again how was the selected concentration of WBTRs/heavy metals in the exposed water determined? (reference?). These are not clear and must be explained by the authors

**why did not Author perform One-way ANOVA followed by Dunnett's *t*-test to detect the level of significance at 5% of error probability (p<0.05) between the exposed and untreated fish groups**

**\*\*Author also need to be performed one way ANOVA /posthoc test for Comparative analysis of Heavy Metals Loads in different tissue /organ system (Viz-Gills, Liver, Kidney and Muscles) of *Clarias gariepinus***

## **Result Section**

Author need to tabularize the references of permissible level set by different agencies (viz-USEPA/WHO/Canada Health Agencies) of observed heavy metals from WBTRs

**\*\*\*Author did not show any mortality of fish during entire experimentation following exposure of sub lethal WBTRs**

In Table 3: Authors showed Characteristics (only three heavy metals load ())of the eligible studies.

Author showed only three heavy metals load (Sr, Fe and Nb) in liver tissue of *Clarias gariepinus* following 58 days of experimentation while Chromium, Arsenic and Mercury etc. highly accumulated heavy metals are well documented.

**\*\*As heavy metal species concentrations in addition to other continuous variables should be figured using the binary logistic regression possible associations between heavy metals exposure and health status/ fish wellbeing.**

## **Discussion section**

**This section should be revised with new and latest references**

**\*\*Authors observed and showed significantly increasing trends of heavy metal in specific organs. Author need to specific reason and citation of such significant pattern of bioaccumulation and altered pathway of metabolism and vital function of that organ( context of different period of exposure)**

## **Conclusion Section**

**Conclusion section may be written as:**

**“The investigation of the heavy metal loading in WBTRs from Ring Road, Jos, Plateau State, and their bioaccumulation in *Clarias gariepinus* showed concerning pollution levels. Although, no significant mortality of the fish occurred till the experiment (after 58 days of exposure). Excessive deposition of heavy metals from WBTRs (Zinc, lead, cobalt, copper, and strontium) in the different tissues of *C. gariepinus***

made them unsuitable to serve as food. As a result, there may be health risks to aquatic life and organisms up the food chain. Fishes living in natural waters even having low concentration of heavy metals for prolonged periods might accumulate substantial amount of the toxic metal and make themselves unsuitable for human consumption.

### **Recommendation section**

\*\*RECOMMENDATION- There is no need to include under a separate title of recommendation of further work rather encompassed in the last paragraph of discussion section.

### **Reference Section**

All references should be in proper format as long as by journal guidelines (Year of publication in journal should be in similar pattern)

Following references are required to be revised-

10. Jacqueline Q M C, Christian R, Fengxiang X. H, Paul B. T. (2014). Rapid screening of heavy metals and trace elements in environmental samples using portable X-ray fluorescence spectrometer, A comparative study, Water Air Soil Pollution. 2014; 225(2169):1-10.

24 Latifa M, Zahoorb M, Muhammada A, Nazb S, Kamranc AW, Ullahd R, Shahe AB, Almeerf R, Sayedg A. (2022). Bioaccumulation of lead in different organs of *Ctenopharyngodon idella* (grass fish) and *Tor Putitora* (Mahseer) fish. Brazilian Journal of Biology. 2022; 84: 1-7.

25 Selvanathan, J., Chitrarasu, P., Manickam, N., & Babuthangadurai, T. (2017). Accumulation of heavy metal (Mercury) in the different tissues of walking catfish (*Clarias batrachus*). *Journal of Terrestrial and Marine Research*, 1(1) 2017: 12-15.

26. Vardi V, Chenji, V. (2020). Bioaccumulation of heavy metal lead (Pb) in different tissues of brackish water fish *Mugil cephalus* (Linnaeus, 1758). *Journal of Applied Biology and Biotechnology*. 2020; 8(02): 1-5.

27. Sehar A, Shafaqat A, Uzma SA, Mujahid F, Saima A B, Fakhir H, Rehan A. (2014). Effect of different heavy metal pollution on Fish. *Research Journal of Chemical and Environmental Sciences*. 2014; 2(1): 74-79.

28. Mastan S A, (2014). Heavy metals concentration in various tissues of two freshwater fishes, *Labeo rohita* and *Channa striatus*. *African Journal of Environmental Science and Technology*, 2014; 8(2);166-170.

29 Siraj M, Khisroon M, Khan A. (2016). Bioaccumulation of heavy metals in different organs of Wallago Attu from river Kabul Khyber Pakhtunkhwa, Pakistan. *Biological Trace Element Research*, 2016; 172(1): 242-250.