

To Study The Effect Of Dried Banana Peel Biosorbent On Heavy Metal-Contaminated Water And Its Impact On Fish

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Abstract:

There Has Been An Increase In Water Pollution By The Toxins Released From Industries And Pesticides From The Agricultural Fields , This Poses A Significant Threat To Aquatic Organisms. The Heavy Metals Like Copper And Mercury, As Well As Pesticides, Were Found To Damage Both Marine And Freshwater Life By Affecting The Vital Organs Of Fish Like Gills, Liver, Brain And Heart. The Accumulation Of Heavy Metals And Pesticides In Fish Tissues And Organs Increases The Need For Understanding The Impact Of Heavy Metals On Aquatic Organisms. As Fish Are A Vital Source Of Protein And Vitamins For Humans, They Are Consumed By Other Living Organisms, Including Humans, Which Leads To Transmission Of Heavy Metals Through The Food Chain. Ingestion Of These Chemicals Can Cause Various Health-Related Disorders Like Abdominal Pain, Headache, Nausea, Dizziness, Vomiting, Diarrhea, Tachycardia, Respiratory Difficulty, Hemolytic Anemia, Damage To The Liver, Damage To The Genetic Material (Dna, Rna) And Gastrointestinal Bleeding.

Various Technologies Have Been Developed To Remove Metal Toxicants From Polluted Waters Such As Silica, Alumina, Resins, And Activated Carbon. While These Methods Are Successful, They Are Expensive And Require Advanced Facilities. The Developing Countries May Not Have The Necessary Resources For Treating The Contamination. As An Alternative, Natural Waste Products Like Dried Banana Peel Have Shown Promise In Removing Mercury, Copper, Lead, And Pesticides From Contaminated Water Bodies. Banana Peels Contain Sulfur, Nitrogen, And Carboxylic Acid, Which Are Responsible For Their Ability To Bind With Toxic Elements And Remove Them From The Contaminated Water.

The Present Work Deals With The Study Of Natural Products, Which Are Usually Considered Waste, Such As Banana Peels, Which Have Been Used As Good Adsorbents Of Heavy Metals And Pesticides. Dried Banana Peel Successfully Removes Toxic Elements Without Any Harmful Effect On The Organs Of *L. Rohita*. The Present Study Was Carried Out In A Laboratory On *Labeo Rohita* (Rahu), As It Is One Of The Most Economically Vital Freshwater Fish That Is Extensively Cultured In India And Other Countries. The Study Investigates The Efficacy Of Dried Banana Peel As A Biosorbent For Mercury Elimination From Contaminated Water. The Histological Study Reveals The High Adsorption Capacity Of Dried Banana Peel Powder For Mercury, Resulting In Minimum Damage To The Most Vital Organ Of Fish I.E Gills. The Result Shows A Degenerative Effect On The Gills Of *L. Rohita* Kept In Water Containing Mercury, And There Remained A Negligible Degenerative Effect On The Gills Of *L. Rohita* Kept In Water Containing Both Mercury And Dried Banana Peel Powder.

Keywords: banana peel, mercury, *Labeo rohita*, biosorbent, contaminated water.

INTRODUCTION:

Aquatic ecosystems play a significant role in the environment, and their contamination with heavy metals leads to a major global problem. The group of communities in cities and intensive activity in industry and agriculture have inevitably elevated the levels of heavy metals in water bodies; the higher amount of heavy metal cause toxicity, resulting in variations in the genetic makeup of living organisms (jorao et al., 2002; vardhan et al., 2019; kumar et al., 2019). Heavy metals accumulated in soil and water are of particular importance due to their impact upon human health through possible contamination of food (filippini et al., 2020; abu reza md towfiqul islam et al., 2020). With the increasing demand for water for agricultural, industrial use, domestic use, and recreational purposes, remediation and reuse of contaminated waters is considered as prime attention globally (opeolu and fatoki, 2012). As mercury is highly toxic, it causes oxidative stress, neurological damage, effect on growth, and decreased immunity in fish and other aquatic fauna (zheng et al., 2019). Mercury removal methods often cause inefficiency and high costs and involve the risk of secondary pollution. The biosorbents are often low cost and effective, as they are derived from agricultural waste, such as dried banana peel and biodegradable solution rich in pectin, cellulose, and metal-binding functional groups (farias et al., 2023). Elevated pollution in water bodies leads to severe effects on the aquatic fauna by bringing changes into their morphology and physiology (mazon et al.,

1999; zhou et al., 2008). Chemical and physical parameters are used as biomarkers to monitor water pollution and to determine the adverse effects of heavy metals on aquatic fauna (van der oost et al., 2003; au, 2004).

The non-essential heavy metals in biological processes show that they usually get detoxified or stored in the body, the essential metal can also get stored exceeding the permissible limits in the various organs of the body like gills, liver, kidney, muscle, intestine, skin, and bones. When the detoxification mechanism fails, accumulation of heavy metal in different organs of fish in varying degrees causes pathological changes in these tissues, mainly affecting critical organs such as gills, liver, kidney, etc. (benjamin and kutty, 2019; guardiola et al., 2013; kawade, 2020; naz et al., 2021; rajeshkumar et al., 2017).

Heavy metals are a major hazard among all environmental pollutants due to their toxicity and bioaccumulative nature; thus, it is of great importance to evaluate the degree of potential impact of heavy metals on the environment (elnaggar et al., 2009, samanidou et al., 1991). Fish are the primary aquatic organisms that collect considerable amounts of heavy metals exceeding their concentrations in the aquatic ecosystem. The relationship between metal accumulation and the feeding behavior of a species shows the significant role of bioavailability of different metals in the aquatic environment, which get transferred to the food web (monperrus et al., 2005; ambedkar & muniyan, 2011).

Fish are considered as good bioindicators of contaminated heavy metal water bodies, and even other aquatic fauna are proven as great bioindicators (livingstone, 2003; hemmadi, 2017). To illustrate the mechanism of action adopted by several stress agents, histological changes are shown to evaluate the health of fish, which serve as the bioindicators; hence, the histopathological studies are conducted to establish causal relationships between contaminant exposure and various biological responses. (nascimento et al., 2012). This helps in identifying the effects of irritants, mainly chronic ones, on various tissues and organs (johnson et al., 1993; velmurugan et al., 2009). Fish: gills are the first organ to which any pollutant comes in contact. Gills are efficient biomonitoring tools as they are very sensitive to changes in the composition of the environment's toxicants (bose et al., 2013). Fish gills are highly effective biomonitoring organs due to their large surface area, which remains in direct contact with environmental irritants (bernet et al., 1999; sweidan et al., 2015). Gills play a very crucial role in facilitating respiratory gas exchange and additionally play a role in osmoregulation and maintaining acid-base balance (fernandes & mazon, 2003; fernandes et al., 2007). Alterations in the gill epithelium occur due to exposure to a different contaminant and the exposure time to the pollutants (franchini et al., 1994; evans et al., 2005; gomes et al., 2012). Moreover, such modification in the histology of gills and other organs indicates early indication of fish health (sorour, 2001). So, the present study was carried out to observe the changes that occur in the gills of fish when exposed to the heavy metal mercury and when exposed to mercury along with dried banana peel powder.

MATERIAL AND METHODS:

The fingerlings measuring 10-18 cm of *L. rohita* were collected from nearby fish farm (Chhatrapati Sambhajanagar). They were acclimatized for one week in the laboratory condition with dechlorinated water with pH 7. The fish fed twice a day with fish food.

Preparation of solution:

1) Mercury: 0.010 g 2) Distilled water: 100 ml
0.010 g of mercury dissolved in 100ml of distill water.

Preparation for adsorbent:

Banana peels collected and washed several times with distilled water then were dried in sun light for 4-5 days and kept in oven for 30 minute and made fine powder. The banana peel adsorbent samples were stored in an air tight container.

Control:

The fish kept in different aquarium as control group to examine the difference between control and exposed fish.

Experiment:

Equal sized 05 fingerlings each were placed in seven different aquariums and labelled as control Group I and exposed Group II, III, and IV. Group II, III, and IV exposed with mercury (Hg) of 40µ/L, 80 µl/L and 120 µl/L concentration at different hours 24hrs, 48hrs and 96hrs. Group V, VI, VII were exposed with heavy metal mercury with same as above concentration of 40µl/L, 80µl/L and 120µl/L along with

dried banana peel powder with weigh 30 gm, exposed for 24hrs, 48hrs and 96hrs. The effect of Hg was observed on the histological slide of gill.

The dead fish were taken out and the LC50 values were determined with the formula $LC50 = LC100 - \frac{\sum \text{conc. diff.} \times \text{mean \% mortality}}{\% \text{ control}}$. Fish from each control group and exposed groups are collected and offered as sacrifices after 24hrs, 48hrs and 96hrs.

Histological slides:

After the exposure of 24hrs, 48hrs and 96hrs fish and control fish were sacrificed to collect tissues for histological studies. The selected tissue gills kept in Bouin's fluid for 24 hours for fixation. Then tissues were washed in running tap water overnight to remove the fixative. After the removal of the fixative tissues were dehydrated.

For dehydration, tissues were passed through the different alcohol grades. In 30%, 50%, 70%, 90% and absolute alcohol, each for 2 hours finally two washes of xylene were given to the tissues for 1 hour each. In hot impregnation, tissues were transferred from xylene to molten wax (58-60°C) in three washes each for half an hour.

The tissues were then embedded in paraffin wax. Sectioned with 4-micron rotary microtome. The sections were stained with Hematoxylin and eosin and mounted in DPX. The slides were examined under a light microscope and photographed for histopathological effects.

RESULTS:

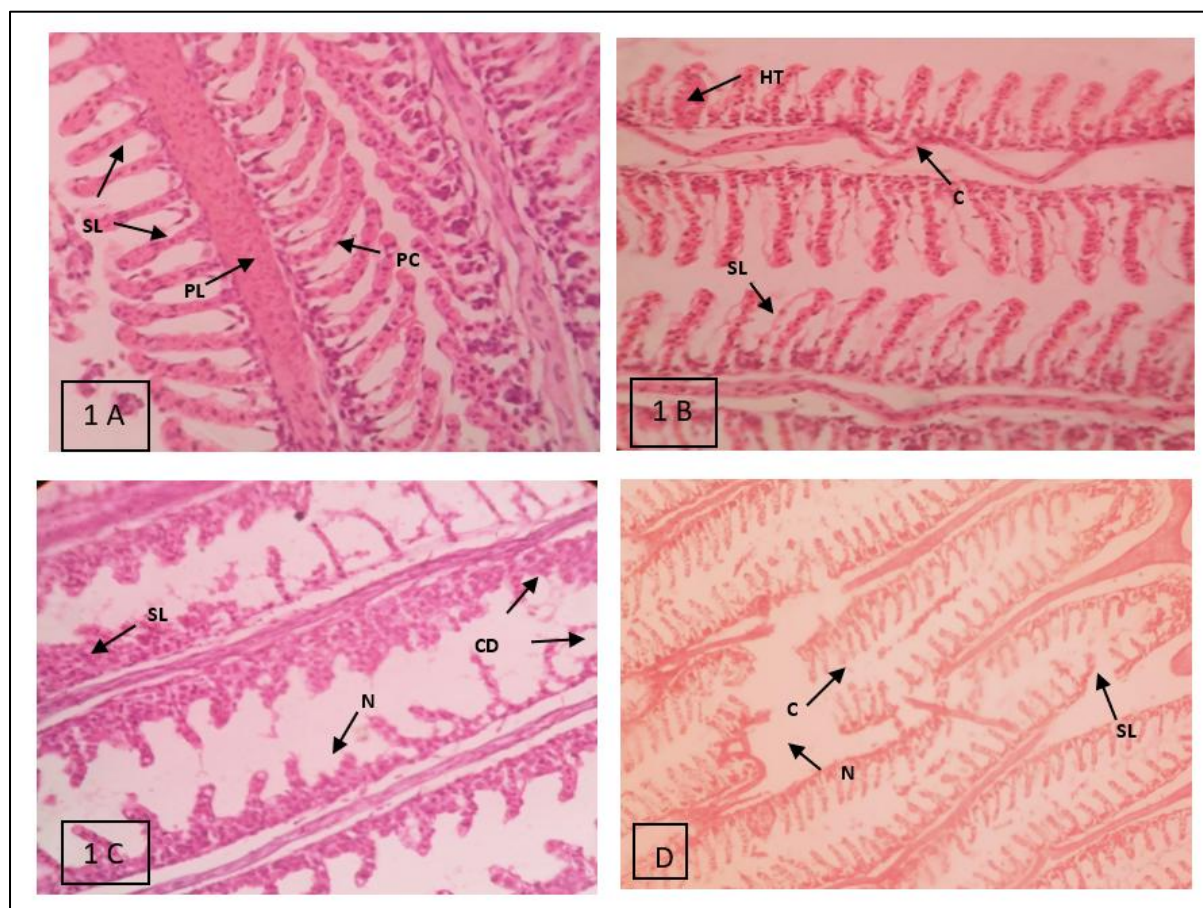


Figure 1:

Labeo rohita, normal structure of gill consists of, Primary Lamellae (PL), Secondary Lamellae (SL), Epithelial Cells (EC), Erythrocytes (E), Cartilages (C), Necrosis (N). The **Image A** control group I gill section of fish displays healthy gills, its primary and secondary lamellae are clearly differentiated with evenly spaced secondary lamellae. Epithelial layer is intact. **Image B** exposed to 24 hours to mercury show accumulation of blood cells, (SL) Secondary Lamellae shows thickening of lamella (edema) detachment of gill epithelium, (C) Cartilage seen as intact, indicating primarily damage to the lamellae and epithelium. **Image C** exposed to 48 hours shows disorganization of the secondary lamellae and capillary dilation, Necrosis. **Image D** exposed to 96 hours to mercury- (PL) primary Lamellae, (SL) secondary lamellae, rupture of epithelial layer

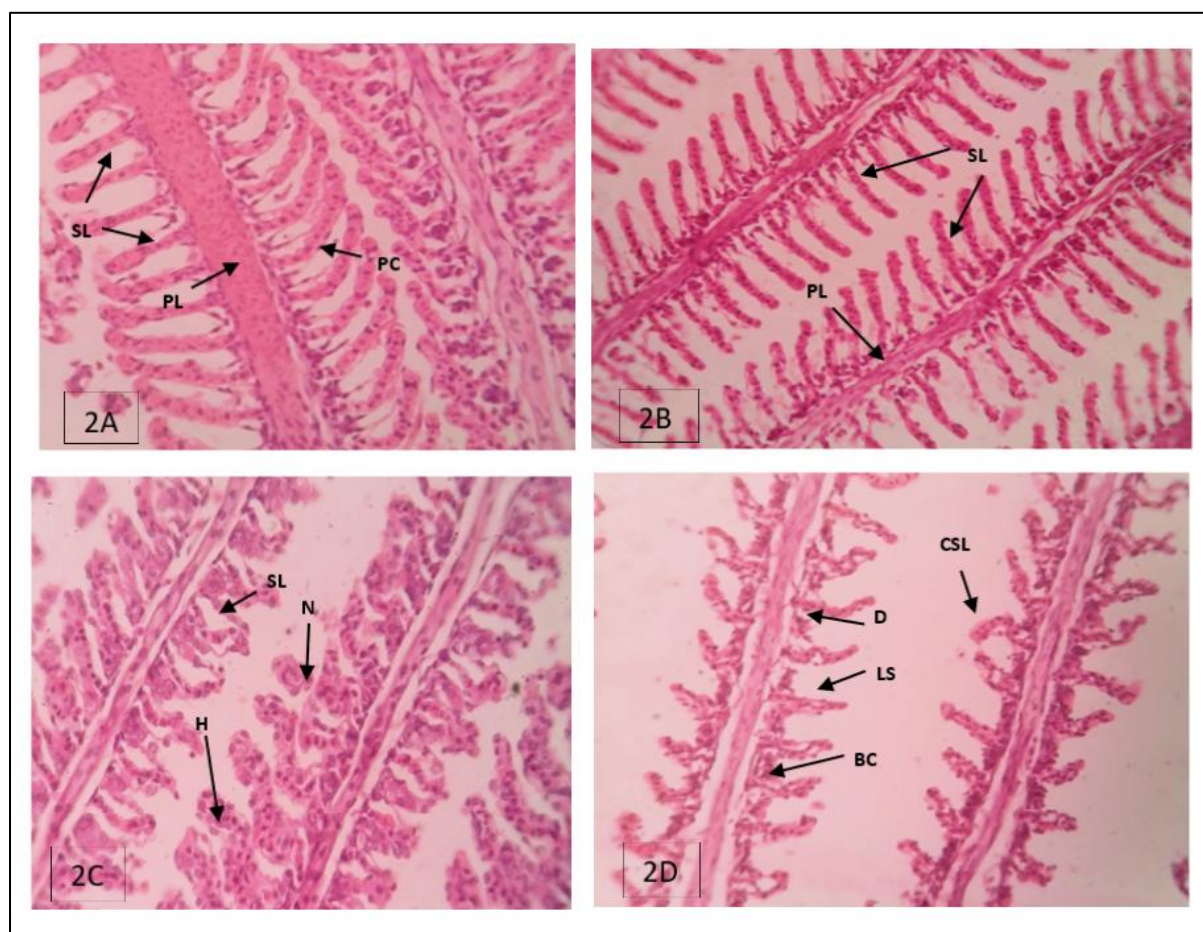


Fig 2: Description of histological changes in gills of *Labeo rohita* exposed to mercury with dried powder of banana peels, **Image 2 A** is control group I. **Image 2 B** with secondary lamellae (SL), primary lamellae (PL) with no damage. **Image 2 C** secondary lamellae (SL), Necrosis (N), hemorrhage (H). **Image 2 D** shortening of lamellae (LS), blood congestion (BC) and curling of secondary lamellae (CSL).

In figure 1 A and Figure 2 A, the H&E stained image of control group I observed that fish gill filament were seen and secondary lamellae were lined on both sides of gill filaments, it appeared distinct and well separated and no signs of fusion was visible. Gill lamellae was covered with epithelial cells parallel to each other, epithelium consist of different cells types mucous (goblet), pavement (epithelial) and showing no hyperplasia (cell proliferation). There was no aneurysmal swelling, hemorrhage and no deformation of cartilage was observed (fig.1A and 2A). The gill structure of *Labeo rohita* exposed to 24 hrs. mercury (fig.1B), gill cartilage shrinks, increased volume of tissue causes the hypertrophy (HT) in secondary lamellae and sublethal edema (SE) the space within the gill tissue formed due to fluid accumulation or detachment of gill epithelium observed in secondary lamellae. The cartilage appears intact, indicating damage to lamellae and epithelium (Fig. 1B). At 48 hrs. of mercury exposure, (Fig. 1C), showed the moderate hyperplasia (HP) of interlamellar epithelium, severe blood congestion of secondary lamellae and hypertrophy in pavement cell or respiratory wall, edema (EM), the presence of edema along the detachment of the lamellar epithelium, chloride cell hypertrophy (CCH), desquamation (D), lamellar shortening (LS), degeneration of epithelium was observed (fig.1C). At 96 hrs. of mercury, showed the severe damage to cartilage matrix, degeneration of secondary lamellae and primary lamellae, proliferation and gill lesion, distortion of lamellae, hypertrophy, hyperplasia, edema, separation of the secondary lamellae, curling of the secondary lamellae, hemorrhage between gill filaments were observed (fig.1 D). The H&E-stained image of control group I (Fig. 2 A) shows healthy image of gill filaments, after 24 hours exposure to mercury with banana peel powder Group II, shows no significant changes in gill structure (fig.2 B). At 48 exposures of mercury with banana peel powder, thickening of lamellar occurs because of proliferation (P) of cell rich in mitochondria which results in partial or complete fusion of lamellae, hypertrophy, lamellar disorganization, no massive necrosis was observed, the organization of lamellae was

uneven, hinting as the partial protective effect by dried banana peel powder under mercury exposure (fig. 2 C). At 96 hours of mercury and banana peel exposure, irregular lamellar space, shortening of lamellae (LS), blood congestion (BC) and curling of secondary lamellae (CSL) was found (fig.2 D) the image shows the moderate epithelial damage, thickening and fusion of secondary lamellae due to mercury toxicity with protective effect of dried banana peel powder.

DISCUSSION:

The histological changes observed in the gills due to heavy metals indicate damage to the gills which is a vital organ for the fish, it is noteworthy to know the extent to which the changes occurring in the organs due to environmental pollution, (Agbugui and Marian, 2022). Heavy metal toxins lead to many pathological changes in the organs of fish as observed in *Labeo rohita* exposed to mercury chloride and effect of phenyl mercury acetate on *Chana Punctatus* (Karuppasany, 2000). It was observed that the *O. hepsetus* and *H.auroguttatus* had gills prone to changes, making them suitable for the histological biomarkers to determine the water quality in entrophized tropical rivers (Nascimento et al., 2012), similar study was carried out by Tirkey et al., (2024) on *L. rohita* when exposed to malathion showed abnormal alignment in gill's primary and secondary lamellae without interspace when compared with the control group whereas thymoquinone showed normal alignment of both primary and secondary lamella as the group lamellae were equally interspaced in comparison with the control group. The group with malathion along with thymoquinone showed the restoration of primary and secondary filament with the repair of damages. In gills, the number of minor, moderate and severe changes were identified. Minor damage included interstitial edema, leukocyte infiltration, hypertrophy, hyperplasia, lamellar fusion, vasodilatation, and blood congestion. This minor damage can be repaired naturally by organs as the environmental conditions improve. Even moderate damage is repairable but the severe damage cannot naturally be reversible to normal functioning. Necrosis is the most severe histopathological damage observed in gills exposed to CuSO_4 and ZnCl_2 and such damage cannot be repaired naturally (Aliza et al., 2024). The finding of the present study, where *L. rohita* was exposed to mercury, caused severe damage to gills and when exposed mercury along with dried banana peels powder minimum changes was observed in the histological image of gills, these findings correspond closely with those reported in a previous study (Tirkey et al., 2024) of SEM analysis which observed that mercury and malathion cause damage to the primary and secondary lamella of the gill arches, curling of the pillar cells. Heavy metal exposure is often associated with histopathological damage in fish organs such as the gills, liver, kidney, muscle, small intestine and other organs. Gill filaments specifically result in cell proliferation, lamellar cell hyperplasia, lamellar fusion, loss of secondary lamellae and inflammatory cells (Yaqoop and paul, 2003). In present study *L. rohita* exposed to mercury for 24 hours show gill cartilage shrinkage, edema and fluid accumulation or detachment of gill epithelium observed in secondary lamellae (Elshaer et al., 2015), epithelial lifting which also represents one of the earliest histological damage when exposed to toxic agents such as oils, detergents, ammonia and heavy metals like mercury (Poleksic and Mitrovic-Tutundzic, 1994).

It was observed in the present study that *L. rohita* when exposed to mercury for different hours showed gill cartilage shrink, increased volume of tissue cause the hypertrophy in secondary lamellae and sublethal edema in secondary lamellae, degeneration of epithelial lining was observed in 24 hours. The severe damage to gill occurred in 96 hours exposure to mercury which showed degeneration of primary and secondary lamellae, hypertrophy, hyperplasia, edema, hemorrhage, necrotic changes were observed. There was no aneurysmal swelling, hemorrhage and no deformation of cartilage in the control group but it was observed in the gills exposed to mercury with elevated concentration, where lamellar aneurysms were also observed in the gills of fish exposed to cadmium, an aneurysm is characterized by leakage within the lamellae and rupture of the pillar cell system, followed by dilation of blood vessels (Martinez et al., 2004). The laboratory study indicates the effect of heavy metals on accumulation in the environment from industrial and domestic waste as it causes great damage to the aquatic environment. Banana peel is a low cost biosorbent that reduces the concentrations of various toxic metals like mercury, cadmium and lead (Mihai et al., 2021)

CONCLUSION: The dried banana peel is a low cost biosorbent which effectively reduces the concentration of heavy metals dispelled in water bodies, which harm the aquatic organisms. The

laboratory study, effect of Dried Banana Peel Biosorbent on Heavy Metal-Contaminated Water and Its Impact on Fish observed that the histological section of fish gills which was exposed to different concentration of mercury revealed the classic signs of epithelial thickening, lamellar fusion, tissue damage, necrosis and other severe damage to the functioning of gills. The dried banana peel introduced as a biosorbent to the mercury exposed water reveals a noticeable gill histopathology, the gill lamellae retained greater definition and organization of gill filament. The result confirmed that dried banana peel biosorbent ameliorates mercury induced toxicity and it is an effective natural sustainable, efficient and low-cost remediation for aquatic environments.

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