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BIO ACCUMULATION AND BIOCHEMICAL CONSTITUENTS CHANGES IN GILL TISSUES OF A FRESHWATER FISH *LABEO ROHITA* EXPOSED TO METAL AND METAL OXIDE NANOPARTICLES IN PERSONAL CARE PRODUCTS

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Abstract: Personal care products (PCPs) are among the most commonly detected compounds in surface water. PCPs have used large amount of metal and metal oxide nanoparticles (NPs). In the present study an attempt was made to study the bioaccumulation and biochemical constituents changes in gill tissues of a freshwater fish *labeo rohita* exposed to metal and metal oxide nanoparticles in domestic wastewater collected from student hostel. The observed results suggested that the structural conformation of proteins in fish gill tissues was significantly influenced by wastewater exposure when compared to control and the uptake of various NPs in gill tissue was very high.

Keywords: Bio accumulation, Nanoparticles**,** Toxicity, Gill tissues, Metal, Metal oxide, Personal care products, *Labeo rohita*

Introduction

PCPs are used extensively by people to maintain their appearance and looks and also to be attractive and gaze younger in all the ages. For this purpose world's people consume enormous quantities of skin care products, soaps, sunscreen lotions, hair styling products, hair removers, shampoos, conditioners, moisturizers, anti-agers, nail and cuticle care products, oral care including toothpastes, and whiteners (Daughton and Ternes, 1999). Metal and metal oxide NPs are among the most commonly used engineered NPs in cosmetics. Noble metals like gold, platinum, and silver are used as well as oxides of titanium, zinc, cerium, and iron. Some of these engineered metal-based NPs (e.g. aluminum and iron oxide) are used as concealers in foundations. Others (e.g. silver) are used for their antibacterial properties in beauty soaps as well as their claimed ability to remedy pimples and acne. Together with platinum NPs, which

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are claimed to have an adsorptive function, silver NPs are also used in creams to enhance the appearance of facial skin. Gold NPs are also commonly used in cosmetic products such as facial gold masks. (Mihranyan *et al.,* 2011).

PCPs may affect health either directly by absorption through the skin and into the bloodstream, when they might affect internal organs such as kidneys and liver, or indirectly, when they are washed off the body and enter the aquatic environment through drainage systems or during immersion in swimming pools or natural water bodies. (Wood, 2010). Several studies about the above said ingredients of PCPs have been published but nowadays metal and metal oxide nanoparticles are added in personal care products and there is no much work done in this concern.

Keeping all this in mind to understand the effects of metal and metal oxide nanoparticles present in the PCPs, this study was carried out and freshwater fish *Labeo rohita* was used as a model organism. Considering the effects of personal care products in an ecosystem and their hazardous effects on the aquatic organisms particularly fish species. In the present study, an attempt was made to assess the changes in the biochemical contents in general, and the protein structural changes in the gill tissues of *Labeo rohita* due to the various NPs present in the PCPs, by FT-IR spectroscopy with TEM analysis.

Materials and Methods

Collection of wastewater sample

The domestic wastewater was collected from Bharathiar University Kannamma Ladies hostel, there are around 450 students staying from various department. A 10000 L capacity water container was placed in order to collect the wastewater sample from morning 6 AM to next day morning, 6 AM. The wastewater from different application like bathing, brushing and face washing, was discharged in to the water container. From this wastewater the appropriate sample was taken for the experiment.

Animal maintenance

The freshwater fingerlings, *Labeo rohita* (length 7.2 ± 0.4 cm and weight 8.5 ± 1 g) were procured from Tamil Nadu Fisheries Development Corporation Limited, Aliyar, Tamil Nadu, India. The collected fish were safely brought to the laboratory and acclimatized for one month in a large cement tank (1000L capacity). During the acclimatization period, the fish were fed *ad libitum* with rice bran and groundnut oil cake. Food was provided once in a day. Fish showing any abnormal behavior was removed as soon as possible. In the present study tap water free from chlorine was used which had the following physicochemical

characteristics (APHA, 2005); temperature 26±1ºC, pH 7.2 ±1, salinity 0.30±0.1 ppt, dissolved oxygen 7.0 ±0.02 mg/L, total hardness 17±0.5 mg/L, alkalinity34.0±5 mg/L, calcium 4±0.51 mg/L and magnesium 2±0.2 mg/L.

Experimental study

The acclimated test fish were divided into three groups, each containing 15 fish. Group I was used as control, group II and III are exposed to treatment with the time duration was 96h and 30days (short time and long time studies respectively). After the exposure period, the fish were sacrificed, and gill tissues were removed and stored at -20ºC until sample preparation for further studies. No mortality was observed in any group during the 30-days exposure period.

Sample preparation

The gill tissues were dried in a lyophilizer (VIRTIS 6KBEL85) for 12 h to remove water content in the samples. The dried samples were then ground in an agate mortar and pestle in order to obtain gill powder. The gill powder was mixed with completely dried potassium bromide at a ratio of 1:100, and then the mixture was subjected to a pressure of 5 tons for 5 min in an evacuated dye to produce a clear transparent KBr disc of 13-mm diameter and 1-mm thickness for use in FT-IR spectrometer.

Spectroscopic measurement

The measurement of FT-IR spectroscopy was performed on a Nicolet-Avatar 300FT-IRspectrometer equipped with a DTGS detector, using KBr pellets. To prevent absorption from ambient (mainly water and CO_2), the system was purged using dry nitrogen. For each spectrum, 128 scans were co-added at a spectral resolution of 4 cm^{-1}. The spectra covered the wave number ranging from 4000 to 400 cm-¹.The frequencies for all sharp bands were accurate to 0.01 cm-¹. Absorption intensity of the peaks was calculated with base-line method

Results and Discussion

96 hours study

Fig. 1 & 2 have shown the representative FT-IR spectra of the control, and exposed samples in the 4000–400 cm^{-1}region. In these studies, fig.2 exposed samples an increase in carbonyl groups centered around 1740 $cm^{-}$and a degradation of acyl chains are observed when lipids become oxidized, since oxidation to lipids causes the formation of carbonyl groups and a breakdown of long chains into smaller fragments. In addition, the amide I peak at 1654 cm^{-1} broadens when proteins become oxidized

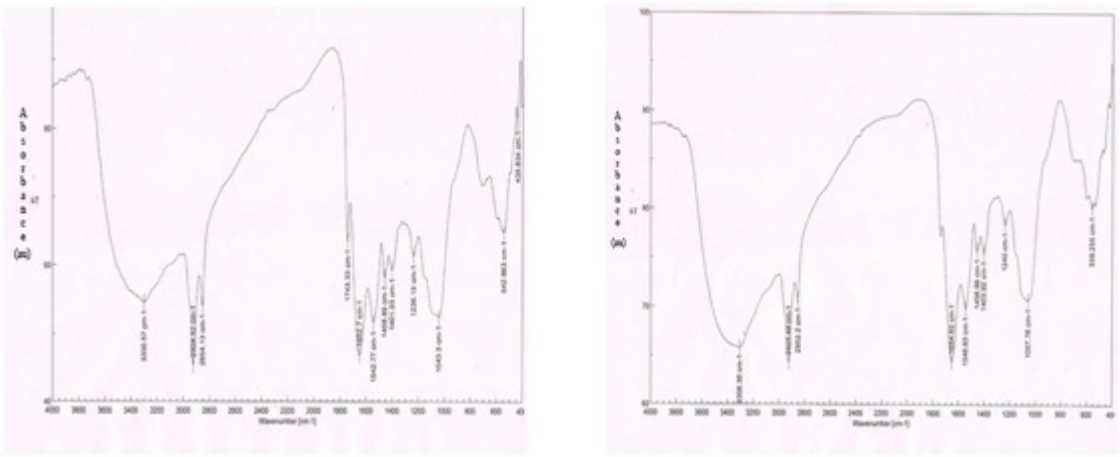

Fig 1&2 are the representative of FT- IR Spectra of the control and the 96 h exposed gill tissues of *Labeo rohita* in the 4000 – 400 (cm^{-1}) regions, respectively

30 days study

Fig. 3 & 4 have shown the representative FT-IR spectra of the control, and exposed samples. These spectra are very similar at the wavelength, however, the slight differences between the controls and exposed are remarkable. For example, the similar intensities at amide I and amide II of proteins but lower at 2924 cm^{-1} (CH_2 asymmetric stretching of lipids), 2924 cm^{-1} (CH_2 symmetric stretching of lipids) and 1743 cm^{-1} (C-O stretching of phospholipids) when compared with the control.

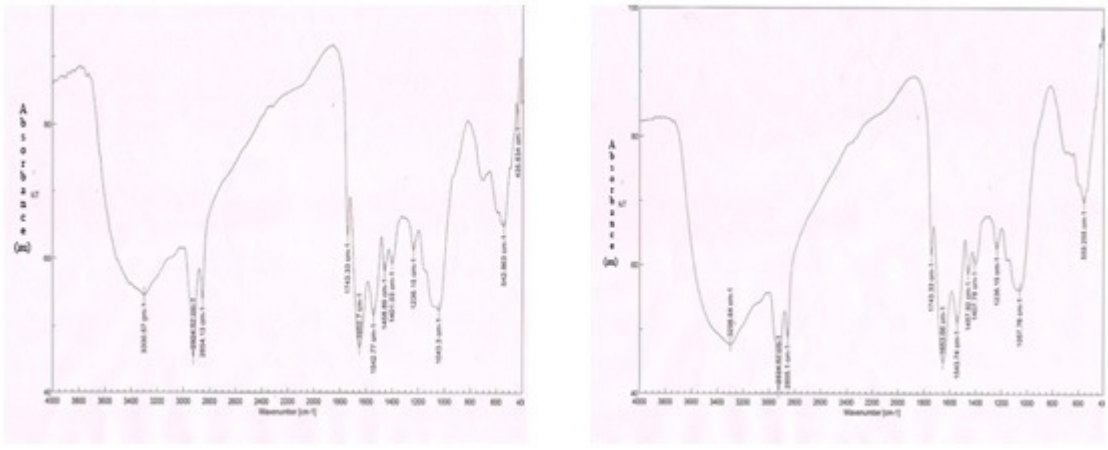

Fig 3&4 are the representative of FT- IR Spectra of the control and the 30 days exposed gill tissues of *Labeo rohita* in the 4000 – 400 (cm^{-1}) regions respectively.

This suggests that there is an increase or decrease in the percentage of certain types of bimolecular relative to the total infrared-active constituents in the gill tissues. Detailed spectral analyses were performed in three distinct frequency ranges: Between 3600 and 2800 cm^{-1}, the absorption is dominated by the stretching vibrations of the CH_2 and CH_3 groups

contained mainly in the lipid acyl chains and N–H stretching vibrations of proteins. Between 1800 and 1300 cm^{-1}, the absorptions are primarily due to proteins, with some absorbance from the lipids.

Uptake of nanoparticles into the organs

Gills cells

The uptake mechanism of nanoparticles was confirmed by the TEM images taken for both short and long-term exposures, the control fish possessed normal cytological structures of gill tissue including epithelial cell and chloride cell with intact cell membranes, nuclei and organelles. The 96 h exposure caused some cytological changes including irregular cell outlines, abnormal pyknotic nuclei, shrinkage or loss of cell cytoplasm (Fig. 5, b). However, more remarkable pathologies with exposure to 30 day such as atrophic and damaged cell membrane, swollen and distorted organelles and a trend of total disruption of gill cells could be observed in this study (Fig. 5, c). Additionally, some epithelioid granulomas were found to appear on the epithelial cell exposed to 30 day. And, some black blocks accumulated on the mucus of chloride cell. The EDX spectrum analysis data (Fig. 5, aa, bb and cc) clearly demonstrated the weight percentage of various elements present in the gill tissues of fish for 96 h and 30 days exposure period. In 96 h period we observed Ag- 14.97, Al- 57.23, Cu- 6.04, Zn- 3.25 and Ti- 0.54%, in this 96 h exposure Ag was high in the gill tissue. During long-term exposure (30 day) Al- 19.65, Cu- 28.70, Zn- 10.27 and Ti- 3.80% were observed, but Ag was not detected. In control group there were no NPs or element observed.

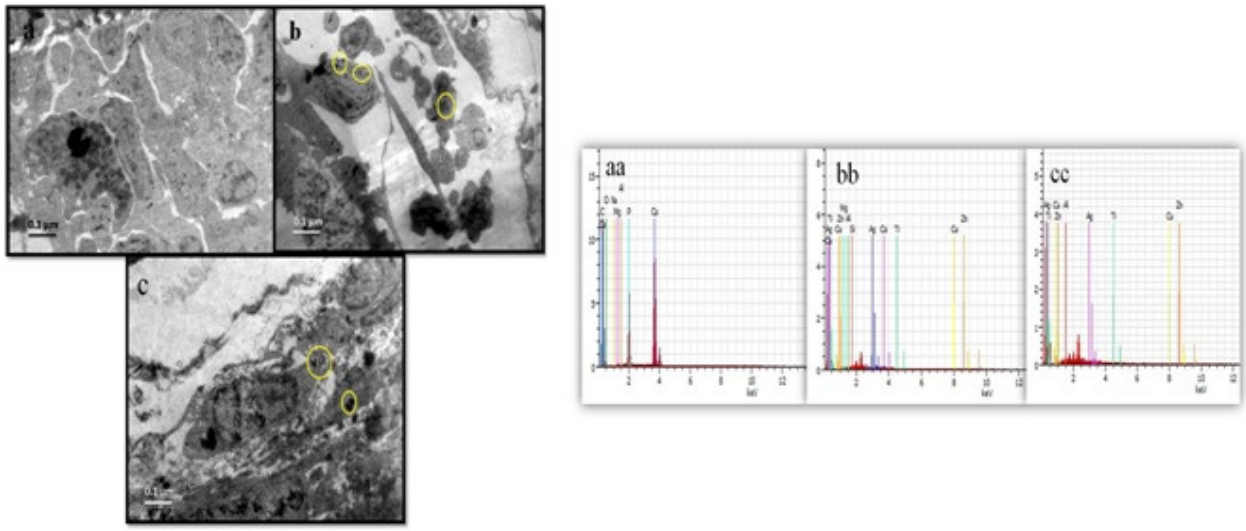

Fig. 5. TEM image of gill tissues of a freshwater fish *Labeo rohita* exposed to domestic wastewater. (a) control, (b) exposed to 96 h, (c) exposed to 30 days. The aa, bb and cc are corresponding EDX spectrum analysis

Discussion

There are also obvious changes in FT-IR peak positions and intensities due to the PCPs exposure in the 1800 to 1300 cm^{-1} region (Fig. 4), which are related to the amide I and amide II of proteins, and C–C stretching of phospholipids. The sharp band at 1743 cm^{-1} in the control gill tissues is assigned to the C=O stretching mode of carboxylic acid in hydrogen bonding (Lin-Vien *et al.,* 1991). The band in the spectral range 1700 to 1600 cm^{-1}, called the amide I band, is due to stretching vibrations of C=O groups of peptide chains. The position of this absorption is sensitive to protein conformation. The amide II bands observed at 1542 cm^{-1} arise from the C–N stretching coupled with N–H bending vibrations of proteins. The weaker amino acid side chain from peptides and proteins at 1458 and 1401 $cm^{-1}$are associated with the asymmetric and symmetric CH_2 bending vibrations, respectively. The peak intensity variations of these bands indicate the conformations of the side chains from peptides and proteins due to PCPs exposure. The band at 1236 cm^{-1} is due to the asymmetric phosphate PO_2^- stretching modes (Parker, 1971). The vibrations from PO_2^- groups originate mainly in the phosphodiester groups of nucleic acids. The amide III band could theoretically contribute to the intensity of the PO_2^- band at 1236 cm^{-1}. However, this contribution does not happen here for the following reason. The frequency of the amide III band depends on the secondary structure of proteins, which, in turn, is determined by the position of the amide I band. In the present work, the amide I band is observed at 1652 cm^{-1}, indicating that the proteins are predominantly a-helical. The amide III band of proteins with a-helical structure will be located at 1260– 1290 $cm^{-1}$and, therefore, it does not contribute to the intensity of the PO_2^- band at 1236 cm^{-1}(Wong *et al.,* 1991).

Comparisons of the present FT–IR spectrum analysis and the previously published FT–IR spectra results (Gomes *et al*., 2007) showed a strong similarity of the main absorption bands in various regions. From the above evidence, it could be concluded that exposed gill tissues shows significant alteration on the major biochemical constituents.

Conclusion

The FT-IR spectra of the domestic wastewater exposed gill tissues show significant alteration on the major biochemical constituents, demonstrating the ability of fish model for toxicity study and in estimating the possible biotoxicity. The observed results suggest that the structural conformation of proteins in fish gill tissues is significantly influenced by wastewater exposure when compared to control. The amide I bands observed at 1654 cm^{-1}in the gill tissues suggest that the protein is dominated by a-helical structure is due to the

wastewater exposure. These changes in the secondary structure might be an indication of some important structural alterations in the existing proteins and/or the expression of new types of proteins. The uptake of various NPs in these tissues is very high. TEM/EDX studies shows that there is a significant damage and accumulation in these tissues.

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