



(RESEARCH ARTICLE)



## Mixture toxicity of mercury and cadmium on the mineral content of the Indian major carp, *Labeo rohita*

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### Abstract

Industrial activities, domestic wastes and agriculture release increased quantities of heavy metals into the environment. As they cannot be degraded, they accumulate in organisms and cause toxic effects. When present in different combinations, their effects can be additive, synergistic, or antagonistic. Hence the present work has been carried out to study the mixture toxicity of mercury and cadmium on the mineral content of the fish, *Labeo rohita* after ten days of exposure to sublethal concentrations. Except potassium content, the two selected metals were antagonistic in their effects.

**Keywords:** Mixture toxicity; Metals; Minerals; *Labeo rohita*; Mercury; Cadmium

### 1. Introduction

Water is an integral constituent of all living things and it is the universal biological solvent. The rapid industrialization is one of the major causes of water pollution. The discharge of untreated and partially treated waste water from various industries like chemical, pesticides, fertilizers, pulp and paper and sugar has polluted the aquatic bodies such as rivers, ponds and ditches [1]. The safe disposal of industrial waste water is one of the major ecological challenges. Therefore, environmental degradation has now become a global problem and maintaining ecosystem health is a serious issue being confronted by the environmentalists. Due to lack of effluent treatment facilities and proper disposal system of waste water, water bodies are getting polluted day by day causing adverse effect on soil, water bodies, agriculture, flora and fauna, because of the presence of toxic and persistent chemicals [2]. The industrial effluents contain large number of pollutants in higher concentration. These effluents are commonly used for irrigation in adjoining areas of the industries in most of the cities in India because of the availability of polluted water at free of cost [3].

The contamination of aquatic environment with heavy metals is of serious concern as they cannot be removed or degraded. They pose a severe threat to aquatic organisms, which form important members of food chain of man [4]. In the recent years, the levels of the heavy metals in the environment increased naturally [5]. In the developing countries environmental monitoring strategies sufficient to identify potentially detrimental concentration of heavy metals in the environment are often poorly developed [6]. Recently, fresh water pollution due to heavy metals has become a hazard due to discharge of industrial effluents. This wide spread problem ultimately affects aquatic fauna [7]. The human activity has continuously disturbed the natural environment particularly the aquatic ecosystem. The use of organochlorine insecticides and heavy metals in industry has led to wide spread environmental contamination [8].

Heavy metal contamination can start from both natural and anthropogenic sources. Mining and smelting operations and agriculture have polluted large areas of world like Japan, Indonesia and China mostly by metals such as Cd, Cu and

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Zn [9], Cu, Cd and Pb in North Greece [10], and in Albania [11] and Cu, Pb, Cr, Ni, Zn and Cd in Australia [12]. Generally the industrial wastes contain high quantities of dissolved and suspended solids, organic and inorganic chemicals, oils, grease, besides toxic metals which cause deleterious effects on fresh water fish when discharged into water bodies [13, 14].

Heavy metals have been recognized as strong biological poisons because of their nature, toxicity, and tendency to persist, accumulate in organisms and undergo food chain amplification [15]. Heavy metals are inorganic elements and they cannot be broken down. Hence they persist in the environment [16]. Therefore, aquatic animals are often exposed to elevated levels of heavy metals [17]. This leads to biomagnification of heavy metals in ecosystem and a major threat to human life [18]. If the present situation of water pollution continues in future, the survival of fish population and other aquatic animals will be extremely difficult. The fish forms the basic protein supplies to mankind but its body has dangerous levels of heavy metals. According to Dhanapakiam [19] the tannery effluent leads to change in the architecture of gills. Metals transferred through aquatic food chain and webs to fish, humans and other animals are of more environmental concern and human health [20]. In this context, the present study was undertaken to investigate the sub lethal effects of mercury, cadmium and mixture toxicity (Hg and Cd) on Na, K, Ca, P and Mg concentration in *Labeo rohita*.

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## 2. Material and methods

For the present study, the fingerlings of *L. rohita* were purchased from local aquafarm in Madurai, Tamil Nadu, India. The fish were acclimatized for more than ten days in large aquaculture tanks (75 L). The fishes were fed with commercially available feed daily. The excreta and excess food were siphoned out to avoid contamination and ammonia stress. Once in a day water was changed. From the laboratory acclimatized fishes, fishes with  $5 \pm 0.5$  cm length and  $5 \pm 1$  g were selected and they were again acclimatized for one or two days in experimental tanks prior to commencement of the experiment. The capacity of experimental tank was twenty liters. The tank was closed by net to prevent the jumping of fish.

1.353 g of  $\text{HgCl}_2$  was dissolved in one liter double distilled water to get 1000 ppm of mercury stock solution whereas 2.031 g of  $\text{CdCl}_2 \cdot 2 \frac{1}{2} \text{H}_2\text{O}$  was dissolved in one litre of double distilled water to get 1000 ppm of cadmium stock solution. The acclimatized fishes were introduced into five experimental tanks. Among these five tanks, four tanks served as experimental tanks and the remaining one as control. The ground water was used in the present study. Each tank was filled with five liters of ground water with five fishes.

Based on the acute toxicity test results, the sublethal concentrations were fixed and prepared ( $1/10^{\text{th}}$  96 hr  $\text{LC}_{50}$  value of individual metals and metal mixtures) from the respective stock solutions [0.064 ppm of Hg, 8.09 ppm of Cd, (0.064 ppm of Hg)+ 5 ppm of Cd and (8.09 ppm of Cd) + 0.025 ppm of Hg]. The control tank had only ground water with fish.

The experimental fishes were exposed to the sublethal concentrations of Hg, Cd and metal mixtures [(Hg)+Cd and (Cd)+Hg] for ten days. The water was renewed daily and the concentrations of metal/metal mixtures in the experimental tanks were maintained. The fish were fed with commercial fish feed. For the estimation of minerals present in the fish body, the following routine procedure was followed.

### 2.1. Triple acid digestion

The dried and powdered fish samples were weighed (0.5g) and taken in clean boiling test tubes. The sample was then treated with triple acid mixture, nitric acid ( $\text{HNO}_3$ ), sulphuric acid ( $\text{H}_2\text{SO}_4$ ) and perchloric acid ( $\text{HClO}_4$ ) in the ratio of 9:2:1. To complete the digestion process, the digestion was done three times and the samples were evaporated to dryness. The sample residues were dissolved in 1 % nitric acid, cooled and made upto 50 ml in volumetric flask with the help of double distilled water. Following the dilution, the samples were centrifuged at 2000 rpm for about 30 minutes and the supernatant liquid was decanted into polypropylene tubes that were then capped and stored pending analysis. It was then neutralized using ammonium hydroxide with the help of phenolphthalein as an indicator. The above procedure was carried out for preparing a blank.

### 2.2. Sodium and potassium

The concerned element concentration of the present study (sodium and potassium) was found out using flame photometer (ELICO make). The triple acid digested samples were taken for analysis. For different elements specific filters were used. A monochromator which allowed to pass the wavelength of light specific to that of particular element was used.

### 2.3. Calcium

Calcium was estimated by complexometric titration method. Exactly 10ml of the sample was taken in a clean Erlenmeyer flask. About 0.4 ml of 1 N sodium hydroxide solution and 20-30 g of murexide indicator were added. The solution was titrated against 0.01 N EDTA solutions. The change of pink colour to purple marked the end point. Calcium content was calculated employing the following formula.

$$\text{Calcium } (\mu\text{g/g}) = \frac{\text{Volume of EDTA} \times 400.8 \times 1000}{\text{Volume of sample}}$$

### 2.4. Magnesium

To find out the magnesium content of the sample, the total hardness of the sample was first estimated by complexometric titration using EDTA. A 50 ml sample was taken in Erlenmeyer flask. About 0.4 ml of buffer solution (pH 10) and approximately 10 mg of Eriochrome black T indicator were added. The sample was titrated against 0.01 N EDTA solution taken in the burette. The end point was the change from wine red to blue colour.

$$\text{Total hardness } (\mu\text{g/g}) = \frac{\text{Volume of EDTA} \times 1000 \times 1000}{\text{Volume of sample}}$$

Based on the EDTA consumption in the total hardness and calcium determinations, the magnesium content was calculated using the following relationship.

$$\text{Magnesium } (\mu\text{g/g}) = \frac{(Y - X) \times 400.8 \times 1000}{\text{Volume of sample} \times 1.645}$$

Where, Y = volume of EDTA used in the total hardness determination; X = volume of EDTA used in the calcium determination.

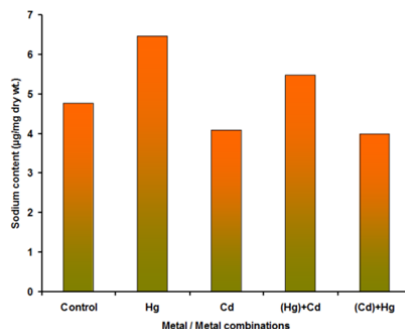
### 2.5. Phosphorus

10 ml of sample was taken and to this 0.4ml of ammonium molybdate and 3 drops of stannous chloride were added. The colour of the solution changed to blue. Then the reading was measured at 690 nm in a spectrophotometer. The concentration of phosphorus was calculated from a standard curve.

## 3. Results and discussion

The effect of mercury, cadmium and their mixtures on the mineral content of *L. rohita* was analyzed.

### 3.1. Effect on sodium content

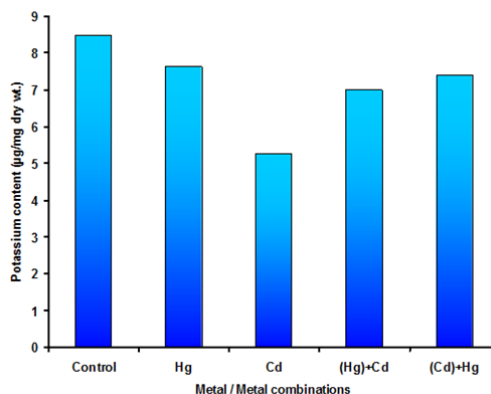


**Figure 1** Effect of Mercury, cadmium and their combinations on the sodium content of *L. rohita*

The sodium contents of *L. rohita*, exposed to metals/metal mixtures and control fish are given in Fig.1. The control fish exhibited 4.77 µg / mg dry wt. of sodium in their body. The mercury exposed fish had 6.46 µg of sodium / mg dry

wt. Here mercury caused an increase in the sodium level in fish body. The cadmium exposed fish had  $4.09 \mu\text{g} / \text{mg}$  dry weight of sodium in their body. Cadmium caused reduction in the sodium content in fish body. Metal mixture [(Hg) + Cd] exposed fish had  $5.48 \mu\text{g}/\text{mg}$  dry weight of sodium in their body. In this case, mercury and cadmium interacted against each other. So sodium level was present in the fish body in between the mercury and cadmium exposed fish. In another metal mixture [(Cd) + Hg] exposed fish, the sodium level was  $3.99 \mu\text{g} / \text{mg}$  dry weight. Both cadmium and mercury were antagonistic in their action.

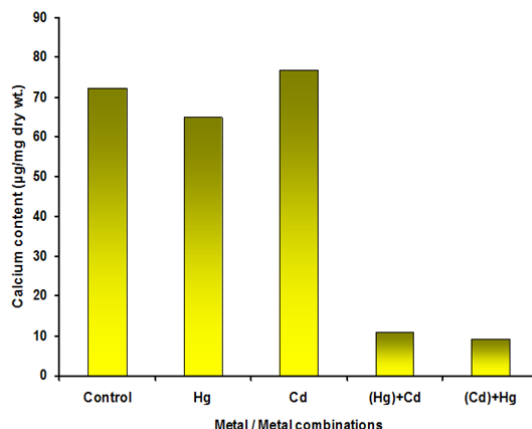
### 3.2. Effect on potassium content



**Figure 2** Effect of Mercury, cadmium and their combinations on the potassium content of *L. rohita*

The potassium content of *L. rohita*, exposed to metals/metal mixtures and control fish is given in Fig.2. The control fish had  $8.48 \mu\text{g} / \text{mg}$  of dry weight of potassium in their body. The mercury exposed fish had  $7.636 \mu\text{g} / \text{mg}$  dry weight of potassium. Mercury decreased the potassium level in fish body while cadmium exposed fish had  $5.26 \mu\text{g}/\text{mg}$  dry weight of potassium. Metal mixture [(Hg) + Cd] exposed fish had  $7.0 \mu\text{g}$  of potassium / mg of dry weight of fish. Hence both metals decreased the potassium content compared to control fish. In another metal mixture [(Cd) + Hg] exposed fish, potassium was  $7.39 \mu\text{g} / \text{mg}$  dry weight of fish. Here also both cadmium and mercury decreased the potassium content. The potassium content in fish body decreased with mercury, cadmium and their combinations.

### 3.3. Effect on calcium content

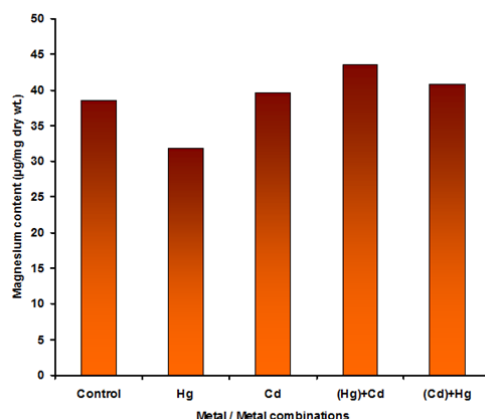


**Figure 3** Effect of Mercury, cadmium and their combinations on the calcium content of *L. rohita*

The calcium content of *L. rohita* exposed to metals/metal mixtures and control fish is given in Fig.3. The control fish exhibited  $72.34 \mu\text{g} / \text{mg}$  dry weight of calcium in their body. The mercury exposed fish had  $65.04 \mu\text{g} / \text{mg}$  dry weight of calcium and mercury slightly decreased the body calcium level. The cadmium exposed fish showed  $76.86 \mu\text{g} / \text{mg}$  dry weight of calcium in their body and cadmium slightly increased the calcium level in fish body. In metal mixture [(Hg) + Cd] exposure, fish exhibited  $10.94 \mu\text{g} / \text{mg}$  dry weight of calcium in their body. In another metal mixture

[(Cd)+Hg], the exposed fish had 9.3  $\mu\text{g} / \text{mg}$  dry weight of calcium in their body. Both the metal mixtures drastically reduced the calcium level.

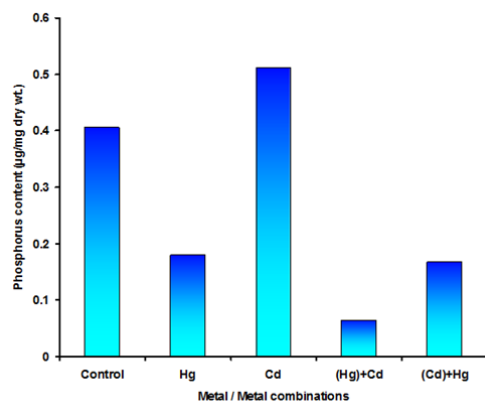
### 3.4. Effect on magnesium content



**Figure 4** Effect of Mercury, cadmium and their combinations on the magnesium content of *L. rohita*

The magnesium content of *L. rohita* exposed to metals/metal mixtures and control fish is given in Fig.4. The control fish exhibited 38.59  $\mu\text{g}/\text{mg}$  dry weight of magnesium in their body. The mercury exposed fish showed 31.81  $\mu\text{g}/\text{mg}$  dry weight of magnesium in their body. Mercury decreased the magnesium content in fish tissues. The cadmium exposed fish exhibited 39.63  $\mu\text{g}/\text{mg}$  dry weight of magnesium in their body and cadmium slightly increased the magnesium level. In metal mixture [(Hg) + Cd] exposure, the fish had 43.57  $\mu\text{g}/\text{mg}$  dry weight of magnesium in their body. In the case of another metal mixture [(Cd) + Hg], the exposed fish showed 40.83  $\mu\text{g}/\text{mg}$  dry weight of magnesium in their body. The metal mixtures, [(Hg) + Cd] and [(Cd) + Hg] slightly increased the magnesium level than that of the control fish.

### 3.5. Effect on phosphorus content



**Figure 5** Effect of Mercury, cadmium and their combinations on the phosphorus content of *L. rohita*

The phosphorus content of *L. rohita* exposed to metals / metal mixtures and control fish is shown in Fig.5. The control fish had 0.406  $\mu\text{g}/\text{mg}$  dry weight of phosphorus in their body. The mercury exposed fish exhibited 0.180  $\mu\text{g}/\text{mg}$  dry weight of phosphorus in their body. Mercury caused a drastic reduction in the body phosphorus content. The cadmium exposed fish showed 0.511  $\mu\text{g}/\text{mg}$  dry weight of phosphorus in their body. Cadmium induced an increase in the phosphorus content in fish body. In metal mixture [(Hg) + Cd] exposure, the fish exhibited 0.064  $\mu\text{g}/\text{mg}$  dry weight of phosphorus in their body. In another metal mixture [(Cd) + Hg] exposure, the fish showed 0.167  $\mu\text{g}/\text{mg}$  dry weight of phosphorus in their body. Mercury, individually and in combination with cadmium drastically reduced the body phosphorus content while cadmium caused an increase in phosphorus content.

#### 4. Discussion

The micronutrients interact with metals at several points like absorption and excretion, metal transport in the body, binding to target proteins, metabolism and sequestration of metals, and in secondary mechanisms of toxicity like oxidative stress [21]. Sodium present in extra cellular fluid is involved in maintaining osmotic pressure of the cell and acid-base equilibrium. It also plays a role in water metabolism and muscle irritability. Sodium is essential for the maintenance of membrane potential, blood volume and blood pressure. In the present investigation, the level of sodium increased with mercury alone and in combination with cadmium. These findings were supported by Subashini and coworkers [22]. According to them, the level of plasma sodium increased in *Cyprinus carpio* exposed to chromium sulphate. But sodium level decreased with cadmium alone and in combination with mercury in the present study. It is contrary to the findings of Shukla [23]. According to him, liver and muscle sodium level increased in *Channa punctatus* exposed to cadmium. The metal mixture (mercury and cadmium) decreased the percentage of alteration of sodium in *Labeo rohita*. Here the metals in mixture interact with one another. So the toxic effect is minimized than that of individual metals. The decrease of sodium was observed in *Channa punctatus* exposed to zinc and selenium than that of individual metals. The decrease of sodium level in gill was noticed when *Onchorhynchus mykiss* was exposed to lithium [24].

Potassium is essential for the maintenance of membrane potential and it is a co-factor for enzymes. It regulates intracellular osmotic pressure. It is also required for glycogen and protein synthesis and the metabolic break down of glucose. The toxic effects are often due to physical changes in the tissue at the cellular or ultracellular levels and can only be speculated, unless they are visualized. The potassium level was increased in blood when *Channa punctatus* was exposed to cadmium. In contrast, in the present investigation the potassium content decreased with the exposure of mercury, cadmium and their mixtures [25].

More amount of calcium is present in the skeletal system of animals and very less amount of calcium is present in blood and muscle. For normal physiological function, narrow concentration of calcium in extra cellular fluid is essential. It is an activator for several enzymes like acid phosphatase, cholinesterase, ATPase and dehydrogenases. Calcium stimulates the muscle contraction and regulates the transmission of nerve impulses from one cell to another through its control over acetylcholine production. The calcium homeostasis was disrupted when rats were subjected to nephrotoxic doses of platinum compounds [26]. Similar trends were noticed in the present investigation also i.e., the calcium content either increased with cadmium or decreased with mercury and their mixtures. This study was also supported by Thatheyus [27]. He reported decline of calcium content in the vertebrae of the scale carp, *Cyprinus carpio communis* exposed to nickel and chromium. The nonphysiological cations  $Pb^{2+}$  and  $Hg^{2+}$  as well as their organic derivatives are environmental neurotoxicants, which are highly potent  $Ca^{2+}$  channel blockers [28].

Magnesium is present in the skeleton, muscle and extra cellular fluid. It is involved in energy production, synthesis of essential molecules like DNA, RNA and protein. It plays a structural role (cell membrane and chromosome) in the transport of ions across membrane and it is an activator of several key enzymes like kinases, mutases, muscle ATPase, cholinesterase, alkaline phosphatase, enolase, isocitric dehydrogenase, arginase and deoxyribonuclease. It plays an important role in carbohydrate, protein and lipid metabolism. The mercury, cadmium and their mixtures slightly influenced the magnesium content in the present study. In contrast, nickel and chromium reduced the magnesium level in the vertebrae of the scale carp [27].

The ethological response of the fish *Channa striatus* treated with industrial waste water was found to depend on its concentrations and duration of exposure time [1]. Phosphorus is an essential mineral which is required by every cell in the animal body for normal function. Phosphorus is present in the body as phosphates ( $PO_4$ ). It is an essential component of phospholipids, nucleic acids, phospho proteins, high energy phosphate esters (ATP), hexose phosphates and creatinine phosphate. The inorganic phosphate serves as an important buffer to regulate the normal acid-base balance of animal body fluid. Phosphorus is also present in some amino acids. The decrease in protein along with an increase in levels of free amino acids and decrease in the tissue levels of RNA might indicate an increased catabolism of protein and decreased synthesis, when fresh water mussel *Lamellidens marginalis* was exposed to chromium [4]. Phosphorus content decreased in mercury and its mixture (mercury and cadmium) treated fish, but cadmium alone increased the phosphorus content. Both nickel and chromium caused the depletion of phosphorus in the vertebrae of *Cyprinus carpio communis*. The decline in the content of phosphorus was directly proportional to nickel and chromium concentration [27].

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## 5. Conclusion

The present study indicates that both metals altered the mineral metabolism of fish. Consequently, in terms of ecological significance, fish are irreplaceable bio-indicators of the degree of damage to the water environment. Moreover, it is also important to monitor the contaminated fish with heavy metals, because frequent consumption of the contaminated fish presents a variety of serious health risks.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors namely Ramesh D, Surendran Appasamy and Joseph Thatheyus Antony state that the article has not been published in another publication and is not being submitted simultaneously to another journal.

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