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# *In vitro* acute toxicity and bioaccumulation of manganese in common carp fish (*Cyprinus carpio*)

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## Abstract

The present research and experimental study were conducted out in order to know bioaccumulation of manganese in the gills, intestine and muscles and its acute toxicity to the Common carp (*Cyprinus carpio*) fingerlings by using manganese sulphate solution. Atomic Absorption Spectrophotometer (Model: Analyst 700, Parkin Elmer, USA, Serial No: 700S5040102) was used for determination of concentration of Manganese (Mn) in the Skin, Intestine and Gills tissue. During experiment common carp fingerlings were exposed to a specific concentration of manganese sulphate solution. In the experiment manganese sulphate solution of 5.6 ppm were applied to 24, 48, 72 and 96 hours exposures of fingerlings to the specific solution. Highest concentration of manganese was in found in gills that is21.6, 26.4, 14.4, 10.32 in gills, 5.49, 3.33, 5.85, 6.96 in intestine and 3.99, 2.22, 2.37, 2.55µg/g in muscles respectively after 24, 48, 72 and 96 hours. Gills, intestine, muscles were not looking much affected and were easily extracted for sampling, but the last one fish were having more affected viscera as compared to the previous fishes which indicated that the fingerling exposed to 96 hours of exposure got much affected by manganese sulphate solution. With increasing of exposure time the morphometric and behavioral changes and abnormalities became more prominent. Out of total 10 common carp fingerlings no mortality occurs all were remained alive. The above observations proved that manganese is toxic to common carp fingerlings and other fishes as well as animals when it increases from its normal and optimal limit.

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**Introduction**

Common carps normally spawn in spring water as a response to rise in temperature and rainfall besides these the common carp also spawn multiple times in a season (Narayanan and Vinodhini, 2008). Pollutants are those substance found in the environment which causes different hazardous effects to living forms, these reduces the quality of life and certainly cause death of the living organisms when found in a higher amount than that of normal tolerable limit (Duruibe *et al,* 2007).

Heavy metals occur as natural constituents of the earth crust, and are persistent environmental contaminants since they cannot be degraded or destroyed. To a small extent, they enter the body system through food, air, and water and bio-accumulate over a period of time (Lenntech *et al*, 2004).

Heavy metals effects fishes when its concentration arises from its normal and required ranges, these metals are essential for fish body but in an optimum levels, whenever fishes got into the environment having maximum amount of these metals, can severely affects the fish health as these metals starts accumulating different tissues of different organs in fish body and causes physical as well internal damage to the fish body (Azmat *et al.,* 2006).

These heavy metals commonly and generally accumulates in the Skin, Gills, intestine, Liver and Kidney and causes various defects to the fish growth and in certain severe cases may leads to death of the fish (Adeyeye *et al.*, 1993).

Aquatic organisms have maximum chances to be affected heavy metals because the aquatic environment is more susceptible for heavy metals pollution and as the aquatic organisms specially fish live there for long time which provide prolong contact between the heavy metals and aquatic organisms (Huton and Lenntech, 2013). Heavy metals effects fishes when its concentration arises from its normal and required ranges, these metals are essential for fish body but in an optimum levels, whenever fishes got into the environment having maximum amount of these metals, can severely affects the fish health as these metals starts accumulating different tissues of different organs in fish body and causes physical as well internal damage to the fish body (Azmat *et al,* 2006). The gills of fishes when heavily polluted by heavy metals causes lamellar edema and separations which is dangerous to fish health in which appearance of hyaline casts occurs in the kidney and chances for renal necrosis also enhances when fish kidney faces heavily polluted environment including heavy metals (Bolis *et al,* 1984).

Bioaccumulation is generally known as the intake of substance by a living organism from their surrounding environment as well as from their diet which stored in the body but this is generally an unnecessary and unwanted phenomenon and commonly occurs due to man-made hazardous chemicals which are thrown to different water bodies, these chemicals build up a certain harmful levels inside the body of aquatic organisms, this mainly happens when certain heavy metals like, Cu, Zn, Hg, Cd, and Manganese accumulates in different tissues of the living organisms specially in fishes, when they accumulates above their normal limits may cause deaths of fishes which further effects the food chain so far, The word bioaccumulation is used to describe the buildup of chemicals in fish (Yousafzai and Shakoori, 2006).

Bioaccumulation of heavy metals is a spontaneous process which is somehow useful as it maintain the required normal level of essential metals in the body of aquatic organisms mostly the fish. When the level of surrounding environment metals exceeds and become hyper saturated then maximum amount of heavy metals accumulates in different organs of the living organisms (Nriagu, 1989).

Manganese is an essential element and the body of living organisms requires it for normal body growth and functions, it's basically a mineral found also in different food substances like nuts, legumes, seeds, tea and grains as well as in leafy vegetables (Lönnerdal *et al*, 1983).

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Currently no in vivo work is done on accumulation of heavy metal (manganese) in *Cyprinus carpio*. Present study was performed by keeping the *Cyprinus carpio* in aquarium and manganese sulphate solution was applied to determine the accumulation of manganese in different organs of *Cyprinus carpio* and to observe the physiological, behavioral and morphological changes and the study will also assist to provide new data on the mentioned topic.

**Materials and methods**

*Fish collection and acclimatization*

Common carp *(Cyprinus carpio)* fingerlings with average body weight of 6-10g were obtained from the Sher Abad Carp Hatchery situated near Charsadda, 10 fingerlings were kindly provided by the hatchery authorities. The fingerlings were put in a shopping bag having water and filled with oxygen gas, the shopping bag were covered and packed tightly by a cotton wire so that to prevent the out flow of water and oxygen. The fingerlings were brought to home and transferred to the aquarium already being set it up for them, the fingerlings were acclimatized for about 2 weeks maintaining all the parameters at optimal and normal ranges, the temperature was kept at room temperature and oxygen was provided to the fingerlings through an electronic air pump. Fingerlings were feed twice a day by giving 2% food by body weight. The water was changed on a daily basis to prevent suffocation from excreta. After 2 weeks of acclimatization the fingerlings did not show any mortality and all were fresh and healthy which shows the fingerlings were free from any kind of disease or death precursors, they were performing an active movements in the aquarium, the fingerlings were now ready for the experiment.

*Experimental design*

For sub-lethal tests (10% of 96-h $LC_{50}$ value). The fingerlings were randomly distributed in two different aquariums with a density of 5 fishes per aquarium having 27 liters of water in each, one was labeled as control group and other was treated group. They were then exposed to medium with concentration of 5.6 mg/L for Manganese (Mn) to the treated group and 0.0 mg/L for control group after 24 hours one fish from treated and one from control group was collected and sacrificed according to the ethical codes, and were transferred to the laboratory for further dissection and removal of different organs where accumulation is to be observed. The same procedure was performed for 4 days (96 hrs.) continuously; the water was changed on a daily basis to keep metal concentration near the nominal ones.

*Preparation of stock and test solution of manganese*

The test chemical used for the experiment was Manganese sulphate ($MnSO_4$). A stock solution 1000 mg/L (1000 ppm) of reagent $MnSO_4$ was prepared by adding 1g of Manganese to 1 liter of distal water and stored in a clean glass bottle. Four different sub-lethal concentrations (5.6 mg/L) of $MnSO_4$ were used based on the four days $LC_{50}$ values. These concentrations were obtained by the dilution of stock solution daily with the help of calculations to maintain the required concentration. A control solution was prepared without manganese.

*Water chemistry*

In the present work, de-chlorinated water (tap water) was used. The water was taken from the aquarium in a small flask and was tested accordingly. The physio-chemical characteristics of tap water are shown in Table 1.

*Sampling*

The fingerlings were dissected and different body organs were isolated, about 0.5 g of tissue were cut off from Gills, Skin, and Intestines, these were then put in different labeled test flasks and made three different samples, 10 ml of nitric acid (Merck, Darmstadt, Germany) were added to the test flasks the tissues were then digested in nitric acid for 24 hours.

The test flasks were then placed in a 100 ºC Hot plate (Gallenkamp: England, CAT No: SS260, APP No: 4-SS260, 6.5 Amp, 220/240 Volt) to be digested completely; the samples were then cooled at room temperature and were added 30 ml of distilled water to each sample, the samples were then filtered by whatman filter paper (Whatman 42). The filtrate was now ready to be analyzed for heavy metal.

Atomic Absorption Spectrophotometer (Model: Analyst 700, Parkin Elmer, USA, Serial No: 700S5040102) was used for determination of concentration of a heavy metal Manganese (Mn) in the Skin, Intestine and Gills tissue samples of each fish. Each sample was analyzed in a triplicate.

**Results**

The presence of manganese sulfate in control as well as treated population were observed. The highest concentration of manganese sulfate was found in gills at 48 hours. After that the concentration decreased with lowest concentration at 96 hours. Second highest concentration was observed in intestine with highest absorbance at 96 hours.

**Table 1.** Physio-chemical characteristics of tap water used in research work.

| Parameter | Value | Status |
|---|---|---|
| Temperature | 23±1ºC | Safe |
| Dissolved oxygen | 6.0±1.5 | Normal |
| Nitrate (NO3) | 0 ppm | Safe |
| Nitrite (NO2) | 0.5 ppm | Caution |
| Total Hardness | 98 ppm | Slightly hard |
| Total chlorine | 0.4 ppm | Safe |
| Total alkalinity | 345 ppm | Highly alkaline |
| PH | 8.3 | Alkaline |

In muscles the bioaccumulation of Manganese sulfate was found to be at lowest concentration with highest absorbance at 24 hours.

All the values observed after 24, 48, 72 and 96 hours post treatment was found to be higher than the control group. The results were shown in table 2.

**Table 2.** The uptake and accumulation values of Manganese sulfate (ug/g) in various tissues treated population and control group of *Cyprinus carpio,* exposed to concentration of 5.6 ppm Manganese sulphate solution at 24, 48, 72 and 96 hours.

| Organs | Control population | | | Bioaccumulation of Manganese after 24 hours. (µg/g) | | | Bioaccumulation of Manganese after 48 hours. (µg/g) | | | Bioaccumulation of Manganese after 72 hours. (µg/g) | | | Bioaccumulation of Manganese after 96 hrs (µg/g) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Standard Deviation | %RSD | Mean | Standard Deviation | %RSD | Mean | Standard Deviation | %RSD | Mean | Standard Deviation | %RSD | Mean | Standard Deviation | %RSD |
| Gills | 3.06 | 3.06±0.0069 | 6.74 | 21.6 | 21.6±0.0107 | 1.48 | 26.4 | 26.4±0.0013 | 1.46 | 14.4 | 14.4±0.0035 | 0.73 | 10.32 | 10.32±0.0045 | 1.31 |
| Intestine | 2.61 | 2.61±0.0017 | 10.13 | 5.49 | 5.49±0.0041 | 2.25 | 3.33 | 3.33±0.0021 | 1.93 | 5.85 | 5.85±0.0043 | 2.18 | 6.96 | 6.96±0.0039 | 1.68 |
| Muscles | 2.58 | 2.58±0.0087 | 1.98 | 3.99 | 3.99±0.0035 | 2.66 | 2.22 | 2.22±0.0037 | 5.04 | 2.37 | 2.37±0.0020 | 2.48 | 2.55 | 2.55±0.0007 | 0.85 |

*Behavioral and morphological changes*

The fingerlings showed different certain behavioral changes to the manganese concentration used. The rate and condition as well as duration of these changes increased on daily basis, during all of the treatment the concentration of manganese was the same.

At first day of the experiment when concentration of manganese was added to the aquarium the fingerlings did not show any abrupt behavioral changes but as the time passes and they swam in water which was contaminated by manganese sulphate solution, the fingerlings became a bit dizzy and their movements got slow as compared to the movement of the control population.

There were no behavioral and morphological changes observed in the control population during all the sub-lethal tests. Fish behaviors and swimming patterns were normal in the control population of fingerlings. The behavioral changes increased day by day in the treated population, the swimming patterns changed from normal to slow, the feeding behavior was not much affected but not that much active as compare to the control fingerlings. The least behavioral changes were observed in the very first fingerling which was exposed to 24 hours of manganese concentration while the most evident behavioral changes were observed in the last fingerling which was exposed to 96 hours of manganese concentration. There were no specific morphological changes observed in the fingerlings of *Cyprinus carpio* during the 96 hrs. Exposure of manganese concentration, just copious mucus secretion on gill surfaces of the fingerlings was evident in all sub-lethal tests.

**Discussion**

Manganese found in environment in a high and wide range in different forms which directly and indirectly influence the aquatic environment specially the fish and its behavior adversely when the concentration exceed than the normal, manganese is an essential chemical element and the body of living organism need it in a proper and normal dosage (Lonnerdal *et al.,* 1983).

All aquatic organisms particularly fishes are directly and indirectly affected by the physical characteristics of aquatic environment, especially the physiochemical parameters of the water (Exley *et al.,* 1991). The physio-chemical characteristics of metal mixture which were used as a exposure media during certain growth trails significantly affect the condition factor, food intake, growth and the feed conversion capability of fish *viz. Catla catla, labeo rohita* etc (Alam *et al.,* 1995).

Acute metal toxicity of water-borne and dietary metals to the fish is influenced by various abiotic environmental factors such as oxygen, hardness, pH and temperature (Nath *et al.,* 1987).

Short and long term toxicity of metals can affect the other essential element of the body such as; $Na^{+}$, $Mg^{+2}$, $K^{+}$ and $Ca^{+2}$ of fishes (Hansen *et al.,* 2000).

We studied that the water chemistry such as temperature, pH, alkalinity, dissolved oxygen and hardness etc. has extreme effect on the uptake and the bioaccumulation of metals which ultimately resulted in variable tolerance in the tissues of *Cyprinus carpio.*

There was no mortality cases in *Cyprinus carpio* fingerlings exposed to sub-lethal metal concentrations during 96 hours exposure period. This shows that the selected concentrations were truly sub-lethal for *Cyprinus carpio* fingerlings under the stated laboratory conditions. Sub-lethal metal challenges resulted significant accumulations of metals in fish gills in the experimental group compared to the control. It should be stressed that even 10% of $LC_{50}$ dose (as maximum acceptable concentration) can accumulate in fish tissue in high water hardness and even during short term exposure (four days). In addition, increase in the mucus secretion was similar to the results obtained by (Alkahem *et al.,* 1995) in catfish during cadmium exposure that could affect metal uptake.

AF (Accumulation Factor) is a useful factor to compare the metal body burden of an organism with the degree of exposure dose (Kim *et al.,* 2004). It is documented that gills are the primary target organ for manganese uptake in fishes (Dussault *et al.,* 2001).

Laboratory experimental work shows that the fish takes most of the heavy metals from water by gills and that's the reason gills shows higher concentration of heavy metals as that of the rest of the body organs, while some research work also shows that fish accumulates metals from food shows higher metals level in the digestive track as compare to that of gills (Ney and Hassel, 1983; Clements, 1991).

According to the present research work the heavy metal accumulates in different organs of *Cyprinus carpio* was in order of Gills>Intestines>Muscles, the same results were also obtained by (Gbem *et al.* 2009).

The gill surface are in direct contact with water born metals and as gills contain thin epithelial membranes having phospholipids surrounded by a slimy mucuos layer, all of them make it more easy for metals accumulation (Bolis *et al.,* 1984).

Conversely in the present study muscles were third in the order of metal bioaccumulation after gills and intestine. But according to (Santry *et al.*, 1997) accumulation of heavy metals in fish may also be attributed to the variability in size and age of individual, feeding habits and seasonal changes in living condition (Debs *et al.,* 1997).

The overall studies and research works shows that manganese is an essential metal required by all living organisms in its respective levels same in the case of fish but when its level increases from normal then it starts accumulating in different organs and tissues of them, there is no much more research works has been done on manganese accumulation in fish but some other related research works also explain the same about the metal accumulation.

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