Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6[1] December 2016: 48-55

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Acute Toxicity and Bioaccumulation Of Chromium In Gills, Skin And Intestine Of Gold Fish *(Carassius auratus)*

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ABSTRACT

The present research and experimental study were conducted out in order to know the rate of bioaccumulation of Chromium (Cr (III) in the gills, intestine, and skin and its acute toxicity to goldfish (Carassius auratus) fingerlings by using Chromium Chloride salt solution. During this experiment goldfish fingerlings were exposed for 96 hours to Chromium chloride salt by preparing a stock solution from 4.6 gram of greenish chromium chloride salt. The dose of chemicals provides to the fingerlings are increasing day by day, i.e. 4ppm, 6ppm, 8ppm and 12ppm chemical are added to the test group aquarium at 24, 48,72, and 96 hours of experiment respectively. The increase in the concentration of chromium chloride salt solution brought about various morphological and behavioral changes, but no mortality occurs in goldfish fingerlings. Behavioral changes included irregular swimming, loss of schooling behavior in fingerlings, imbalance and abrupt movements, gradual deserve in food intake. Irregular swimming indicated that due to the toxicity Chromium chloride solution brought about abnormal changes in goldfish fingerlings. Imbalance and fast movements were due to in order to escape from a toxic and stressful environment. Increased secretion of mucus was due to the response of the fish immune system to the irritating effect of toxicants. When the fish regulatory system responds to the toxicants, it regulates the level of chromium in the fish body.The result shows that rate of accumulation of chromium in Gills > Intestine > Skin of gold fish. The behavioral change occurs in the fish is that all the fingerlings of goldfish come to the corner of the aquarium and their appetite also decrease due to chemical effect.Out of total 10 fingerlings none of the fish died during the experiment. No mortality occurs because they were subjected to the sub lethal concentration during 96 hours exposure respectively. The above observation conclude that the chromium has toxic effects on goldfish fingerlings, and also on other fish and animals.

Key Words. *Acute toxicity,gills,skin,intestine,goldfish.*

Received 21.10.2016 Revised 09.11.2016 Accepted 09.12. 2016

INTRODUCTION

The term fish are most strictly used to describe any animal with a backbone that has gills through life and has limbs in the shape of fins [1]. A typical fish is a cold blooded animal that lives in water, breathes with the help of gills usually has scales and fins for swimming. They live under the water and are dependent on water for the dissolved oxygen. It also includes the living hag fish, lampreys and cartilaginous and bony fish, as well as various extinct related groups. On the basis of temperature maintenance Fishes are divided into two types, the Cold blooded Fish and The Warm blooded Fish.The Fish meat is considered an important source of protein for good human health. In 2009 survey, the fish meat accounted for 16.6 %of the world population's intake of animal's protein and 6.5 % of all protein consumed.Worldwide, the fish provide round about 3.0 billion peoples with almost 20 % of their intact of animal protein, and the 4.3 billion people with about 15 % of such protein. Capture fisheries and aquaculture provide the world about 148 million tons of fish meat in 2010 and 154 million tons in 2011. Fats in fish are useful for

patients of cardiovascular disease the reason behind this is that the Fats in fish is different from other food, because it has omega-3 polyunsaturated fatty acids [2].

It is a source of two different kinds of omega-3 polyunsaturated fatty acids, which are i.e. the eicosapentenoic acid (EPA) and the docosahexenoic acid (DHA) [3]. These two Omega-3 (n-3) fatty acids are very much useful and important for the normal growth; it reduces the cholesterol levels in the human body and also controls high blood pressure and also the incidence of heart disease, stroke, and premature delivery. Omega-3 fatty acids are the main component of fish which lower the risk of age related muscular degeneration and vision impairment, also decreases the risk of bowel cancer, and helpful to reduce skeletal muscle against insulin. AHA (American Heart Association) recommended fish use for people at least two times per week to ensure the daily required intake of the important omega-3 fatty acids [4].

Fish accumulate toxic chemicals directly from water such as heavy metals, and contaminant residues ultimately reach concentration hundreds or thousands of times above those measured in the water, sediments and food [5]. The Heavy metals are normal constituents of aquatic environment that occur as a result of pollution which is due to the discharge of untreated wastes of many different types of industries into rivers.The bioaccumulation of these metals in organs of aquatic organism like fish has been identified as an indirect measure of the abundance and great availability of metals in the aquatic environment [6].

The contamination of fresh water with wide range of pollutant is due to increase human activity especially with rapid development of agriculture and industries has resulted in considerable increase in level of pollutant such as the heavy metals which is the main anthropogenic pollution causing serious a and long lasting damage to all live organisms especially Fishes.Pollution is considered as one of the most serious threat that has brought drastic change and ill-effects to the growing population, human societies in the whole world and as well as our planet earth. Since from past two decades, Aquatic pollution is a major environmental problem [7]. Water is the real source of life, due to continuous growth of the population rapid increase in urbanization and industrialization is the cause of increase disposal of pollutant such as heavy metals ,radioactive nuclides and many other types of organic and inorganic substances into the aquatic system and have measurably influenced the quality of water of rivers lakes and ponds all over the world, industrial wastes is the major source of heavy metals contamination of the aquatic environment [8].

The heavy metals produce toxic effects on organisms by generating reactive oxygen species (ROS), which cause oxidative stress. Mostly the heavy metals when produce ions become toxic in nature and become a threat to the living organisms like fish and human health, and also damage the environment [9].

MATERIAL AND METHODS

Thirty (30) Goldfish (*Carassius auratus*) fingerlings with average body weight of 10-15g and body length 4-6 inches were bought from the Sheikh Abad Carp hatchery situated near Charsadda. Then the fishes were brought safely to the lab and put in aquariums which were already being set up for them. The fingerlings were acclimatized for about 2 weeks maintaining all the parameters at optimal and normal ranges.Then two aquaria were placed side by side on a large table one aquarium was labeled as “Control Group” for the control fishes and the other was labeled as “Treated Group” for fishes treated with heavy metal Chromium Chloride. The duration of the experiment was 4 days (96 Hours). 10 fishes were placed in “Control Group” labeled aquarium and 10 were placed in “Treated Group” labeled aquarium. The amount of water in each aquarium was 52 liters.

The treated group was exposed to Chromium Chloride (4ppm, 6ppm, 8ppm and 14 ppm) on 1^{st}, 2^{nd} 3^{rd} and 4^{th} days respectively. The control group was run parallel to the experimental group. The fish of both the groups were feed twice a day with 3% of their body weight during experimental period with artificial meal. The fish which was taken from treated and control group aquarium was taken to the laboratory for dissection. In the dissection the different organs like skin, intestine and gills were separated from both the treated and normal fish in order to detect the amount of chromium in both fishes. A 0.5g portion of all organs were separated then washed with distal water. Then kept in oven for drying at 80-90 C.

Acid digestion

For heavy metal analysis a 0.5g grinded tissues of all organs were transfer to 100ml flasks for digestion. For complete digestion 10ml of nitric acid will be put in each flask. The flasks were kept air tight for one night. On the next day the flasks were placed in a digital oven and provide the temperature of 150-160^{o}C for minimum 1 hours and when a transparent and clear solution were form than turn off the oven and wait for 10 minutes so that the flasks become cool.

Than 30ml of distilled water was poured in each flask. After diluting the solution, each sample was filtered through whatman paper. All the Samples are appropriately stored in washed and labeled bottles. Now all the samples were ready to find out the absorption of heavy metals in it. Atomic Absorption spectrophotometer was used to find out the heavy metal (Chromium) in the different organs of fish i.e.

Gills, Intestine and Skin in the CRL (central research laboratory) laboratory Peshawar University. Data obtain was analyze and the results were articulated as mean ±S.E.

RESULTS

During the experiment accumulation of chromium^{+3} different organs of the fish, its toxicity on fish and comparison of mortality rate of both control and treated or experimental groups were studied. The accumulation of Cr^{+3} in different organs of goldfish are discussed as under.The accumulation of chromium occur in the gills of the treated group fishes are 5.43µg/g or 0.181mg/l, 5.04µg/g or 0.168mg/l, 4.59µg/g or 0.153mg/l, 4.11µg/g or 0.137mg/l, when tested at 1st, 2nd, 3rd, and 4th days respectively. Due to essentiality of chromium a small was also present in the gills of control fish such as 2.55µg/g or 0.085mg/l at the 4th days of experiment.

The intestine from both treated and control groups fish were taken and tested to determine the accumulations of chromium in it. So the accumulation of chromium in the intestine of the treated group fishes observed were as follow: 3.9µg/g or 0.13mg/l, 3.7µg/g or 0.123mg/l, 3.63µg/g or 0.12mg/l, and 3.5µg/g or 0.11mg/l at 1st, 2nd, 3rd, and 4th day respectively. But small amount of chromium was also found in the intestine of fish from control group at about 2.22µg/g or 0.074mg/l throughout the 4 days when tested because chromium is essential for the normal metabolism of fish.

Accumulation of Chromium in the skin of the fish from treated and control group are as follow: 3.21µg/g or 0.107mg/l, 3.18µg/g or 0.106mg/l, 3.03µg/g or 0.101mg/l, and 1.98µg/g or 0.066mg/l at 1st, 2nd, 3rd, and 4th day respectively. Very little amount of chromium is also accumulated in the skin of the control group fishes at 1.32µg/g or 0.044mg/l throughout the 4 days when tested.Overall accumulation of chromium in the Gills, Intestine and Skin of the treated and control groups with the mean difference statistically significant at $P<0.05$ level with are given tables and graphs.

Control Group:

Table 1: Shows the result of test data of control group Gold fish (*Carassius auratus*)

Tissue	Mean(µg/g)	Mean ±SD	RSD
Gills	**2.55**	**2.55±0.0018**	**2.20**
Intestine	**2.22**	**2.22±0.0017**	**2.00**
Skin	**1.32**	**1.32±0,0070**	**15.81**

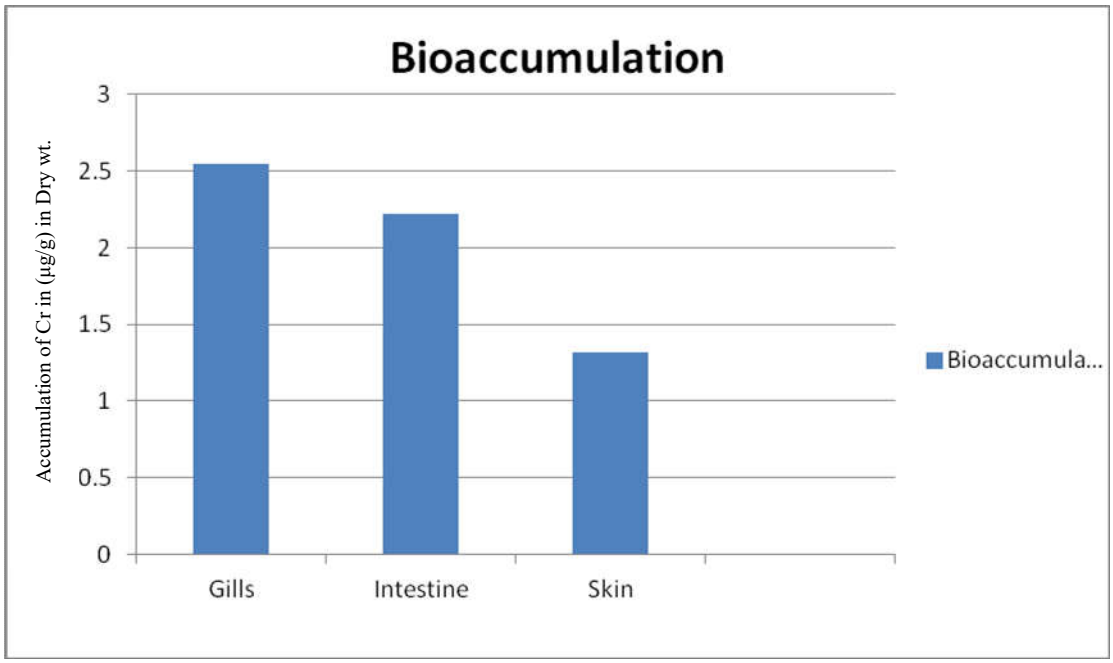

Fig.1: Shows Chromium uptake and bioaccumulation (µg/g) in various organs of Gold fish (*Carassius auratus*).

Treated Group:

Table 2: Shows the results of accumulation of Chromium in various Tissues of Gold fish (*Carassius auratus*) during the 24 hours of exposure to the concentration of 4 ppm.

Tissue	Mean(µg/g)	Mean ±SD	RSD
Gills	**5.43**	**5.43±0.0060**	**3.35**
Intestine	**3.9**	**3.9±0.0028**	**2.13**
Skin	**3.21**	**3.21±0.0028**	**2.61**

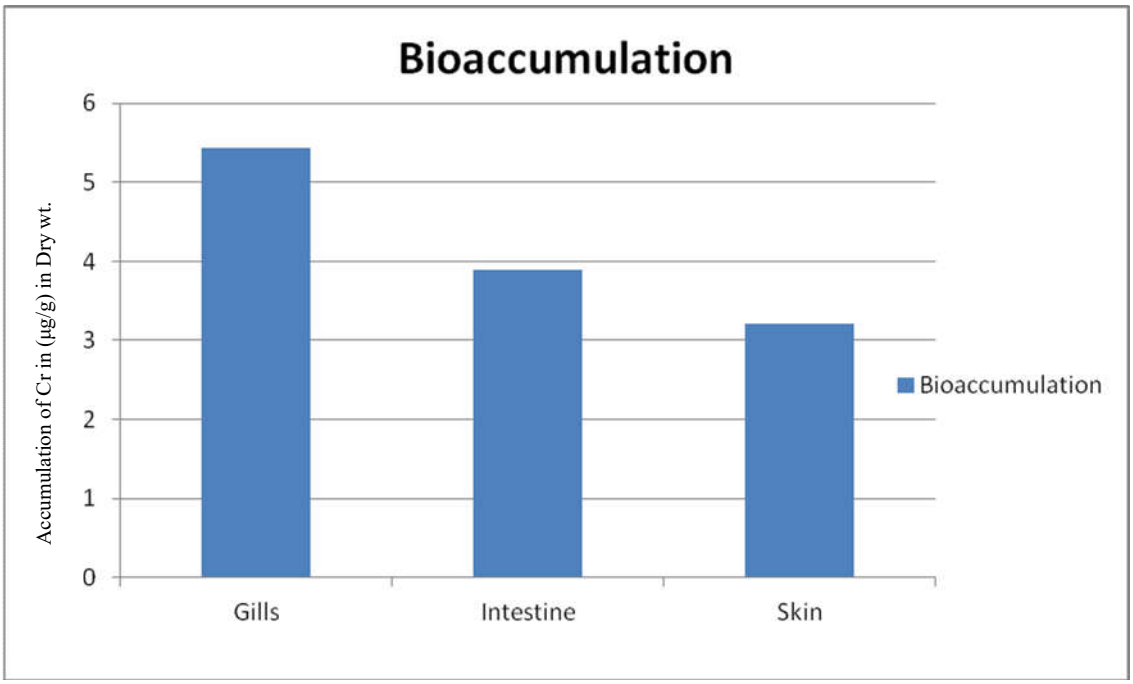

Fig.2: Shows chromium uptake and bioaccumulation (µg/g) in various tissues of Gold fish (*Carassius auratus*) exposed to concentration of 4.6 ppm of Chromium chloride solution in 24 hours.

Table 3: Shows the results of accumulation of Chromium in various tissues of Gold fish (*Carassius auratus*) during the 48 hours exposure to the concentration of 6 ppm Chromium chloride

Tissue	Mean(µg/g)	Mean ±SD	RSD
Gills	**5.04**	**5.04±0.0048**	**2.88**
Intestine	**3.72**	**3.72±0.0028**	**2.04**
Skin	**3.18**	**3.18±0.0085**	**7.96**

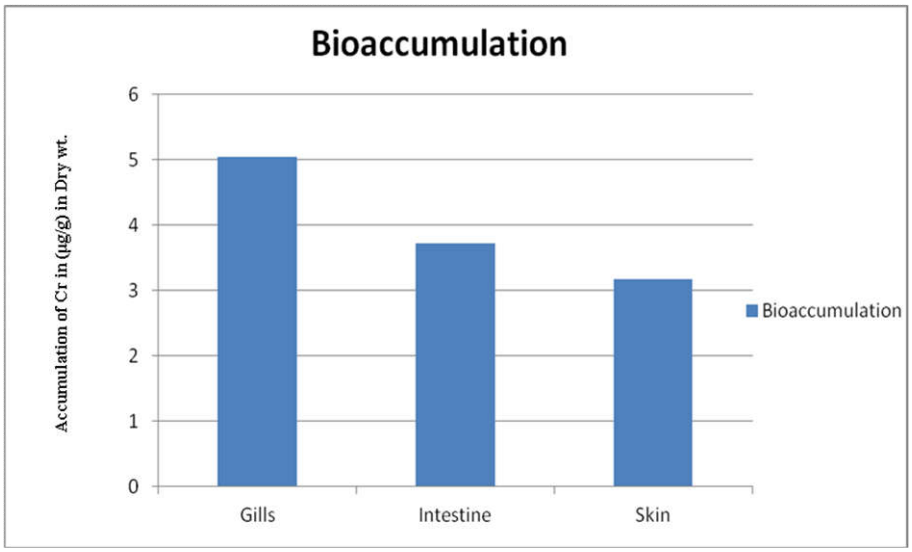

Fig.3: Shows chromium uptake and bioaccumulation (µg/g) in various tissues of Gold Fish (*Carassius auratus*) exposed to the concentration of 6 ppm of Chromium chloride in 48 hours.

Table 4: Shows the results of accumulation of Chromium in various tissues of Gold fish (*Carassius auratus*) during the 72 hours of exposure to the concentration of 8 ppm chromium chloride.

Tissue	Mean(µg/g)	Mean ±SD	RSD
Gills	**4.59**	**4.59±0.0012**	**0.78**
Intestine	**3.63**	**3.63±0.0015**	**1.28**
Skin	**3.03**	**3.03±0.0028**	**2.95**

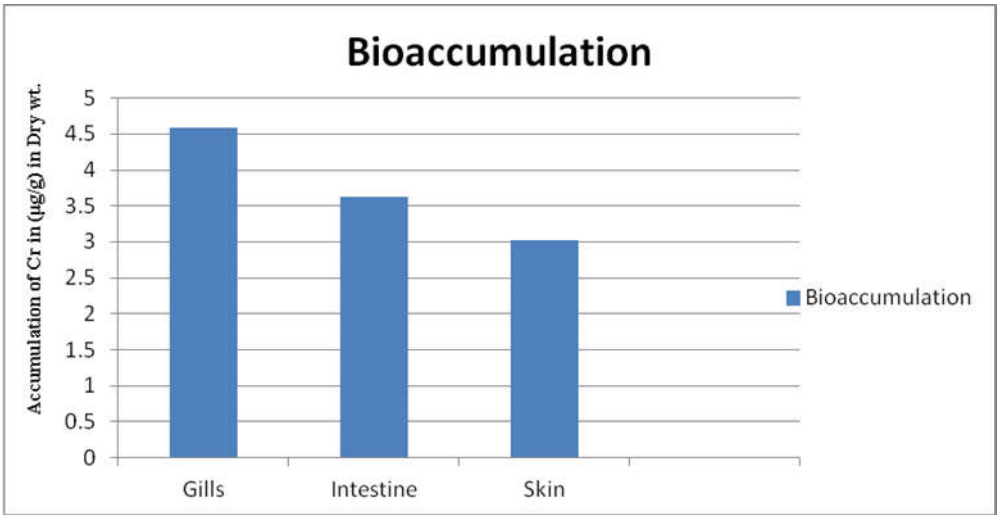

Fig.4: Shows chromium uptake and bioaccumulation (µg/g) in various tissues of Gold Fish (*Carassius auratus*) exposed to the concentration of 8 ppm of Chromium chloride in 72 hours.

Table 5: Shows the results of accumulation of Chromium in various tissues of Goldfish (*Carassius auratus*) during the 96 hours of exposure to the concentration of 12 ppm chromium chloride.

Tissue	Mean(µg/g)	Mean ±SD	RSD
Gills	**4.11**	**4.11±0.0062**	**4.51**
Intestine	**3.51**	**3.51±0.0059**	**5.08**
Skin	**1.98**	**1.98±0.0073**	**11.09**

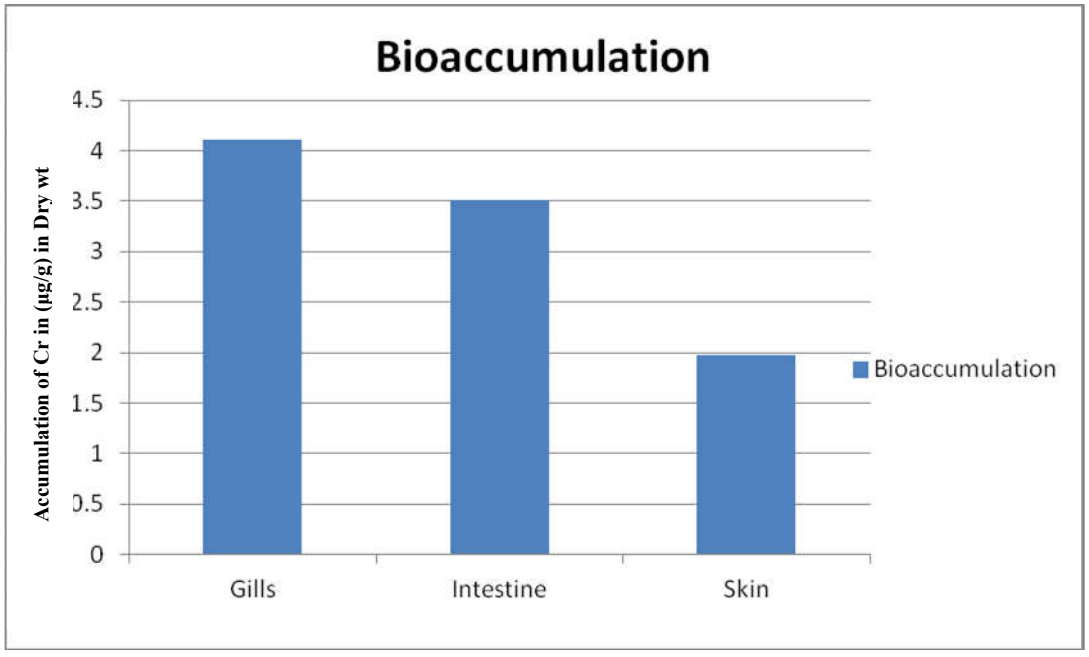

Fig.5: Shows chromium uptake and bioaccumulation (µg/g) in various tissues of Gold Fish (*Carassius auratus*) exposed to the concentration of 12 ppm of Chromium chloride in 96 hours.

DISCUSSION

Metal concentration in aquatic organisms appears to be of several magnitudes higher than concentration present in the ecosystem [10] and this is attributed to bioaccumulation, whereby metal ions are taken up from the environment by the organism and accumulated in various organs and tissues. Metals also become increasingly concentrated at higher tropical levels, possibly due to food-chain magnification [11].

Accumulation of heavy metals by fish is influenced by various factors like; feeding behavior, ambient temperature, water hardness, pH, salinity, age, sex and mutual interactions, etc. In the present study, the mortality increased with an increase in the concentration of the toxicant and also the duration of exposure. This is in agreement with earlier studies explaining the relationship between exposure duration, tissue residues, growth and mortality in goldfish (*Carassius auratus*) when exposed to Chromium.

All of the above studies showed that the chromium is the essential element when used in traces; Chromium is an essential component for the biological and physiological functions such as the maintenance of normal glucose tolerance [12]. It is an essential metal in a limited amount, but exceeding the normal or permissible limits it turns into destructive toxicant. When in greater amount it accumulate in different organs like liver, muscle, gills, and greatly affects the mortality rate of the fish. Accumulation patterns of chromium in different organs of the fish in the experimental group were Gills>Intestine>Skin.The chronic exposure of fish to the Chromium cause a variety of physiological and behavioral changes including loss of appetite, reduced growth ionic loss increased fish mortality [13]. Gills are the primary site of osmoregulation in the aquatic vertebrates. In aquatic organisms like fish gills surface are the first target of the heavy metals. These are the main target organ, which gets affected very easily when the organisms like fish if exposed to dissolved heavy metals [14]. Chromium was accumulated in greater amount in gills from the water to which the fish were exposed during the experiment. The accumulation of Chromium in gills affects the fish badly and even can cause mortality, as due to the Chromium accumulation in gills the respiration of fish were disturbed and there the breathing occur in fish due to which oxygen concentration becomes low. Due to decrease the oxygen concentration the fish behavior change and they all come to one corner of the aquarium. It is believed that dissolved oxygen content 5-7ppm is sufficient for the most of aquarium occupants, while the sign of stress will show if the content drops below 4 ppm and fatalities can be expected at the 2 ppm. So the less amount of dissolved oxygen concentration can result in the mobilization of trace metals. The gill is an important site for the entry of heavy metal that provokes lesions and gill damage [15]. So during the experiment this point comes in front that mostly gill is affected during Chromium toxification.The accumulation of heavy metals in water, followed the order Zn >Ni >Cr >Pb >Cu >Cd >Hg. The highest amount of Zn and the lowest amount of Hg was also reported by [16]. Some non essential heavy metals like lead, cadmium, etc, are toxic when present in small amount and have very low lethal concentration. They are considered as non essential elements. While other heavy metals like nickel, copper, cobalt, iron etc, are toxic when present in large amount. Because of this Chromium in small amount were present in the body of fish as it is essential for many functions in the body of fish like in metabolism. So a little amount of copper was found in the control groups which indicate its essentiality for the fish.The sensitivity and accumulation of heavy metals in different organs of fish and the heavy metals accumulation in the gills and intestine was Pb >Cd >Ni >Cr and Pb >Cd > Ni >Cr. Similarly, in case of skin and flesh tissues, the order was Pb >Cd >Cr >Ni and Pb >Cr >Cd >Ni. In all heavy metals, the accumulation of lead and cadmium proportion was significantly increased in the tissues of *Cyprinus carpio* (Common Carp) [17].

The result of the experiment showed that accumulation of Chromium in the different organs of *Carassius auratus* are in the order of gills > intestine >Skin. So the gills was the most sensitive for the Chromium and intestine comes second and in the skin were the least.The concentration was sub lethal, but the fish showed various morphometric and behavioral changes and abnormalities were observed in the experimental aquarium fingerlings of goldfish. During the experiment highest accumulation of chromium was observed in gills and lowest in the skin.In the treated group the fish shows abnormal swimming behavior, jerking movement, increased breathing rate and increased mucus secretion was showed by fishes when exposed to sub lethal concentration. At higher concentration these changes become more clear and prominent than lower concentration. In the experiment the fishes didn't die as the concentration of chromium was sub lethal and the chromium used was Cr (III) which are not as toxic as Cr (VI). All fishes survive but shows irregular movements as swim bladder is present in the fishes, therefore the fishes are unstable. The loss of balance is due to the toxicant effect of Chromium on the lateral line system. The fast movement of the fishes is due to the toxicant medium in the fishes were kept and the fishes want to escape from the medium as fishes jumped several times from the water of aquarium. The fish during exposure to chromium eat less food which is provided twice a day. Increased

secretion of mucus is the response of body immune system of fish to the irritating effect of the toxicants, Mucus coating the body in order to protect the body from the stressful and unfavorable environment.
As the experiment was on sub lethal concentration of chromium in goldfish fingerlings, all the fishes survive during the 96 hour experiment and none of the fish died.The gill filament and its constituents were also damage gradually which reached to the total collapsed level at the highest concentration of (40mg/L). At the higher concentration the gill rakers start hypertrophy and that was followed by the hypertrophy in gill filaments. During this time the disintegration if the chloride cells and the pillar cells were noted follow by their hypertrophy.
According to the different research works every researcher has its own values of accumulation and that's because of the different research protocols and different water quality variables which also affect the rate of accumulation in different tissue, organs of the fish and also depends on the type of heavy metal and the type of specie of fishes.

CONCLUSION

The experimental work proved that heavy metals has been accumulated in different organs of the fish body and its accumulation pattern has different in different organs like the more accumulation occur in the gills because the gills are in direct contact with the heavy metal chromium dissolved in water. The result from the experiment revealed that the gills of the fish are the primary target organ in the fish. The result shows intestine as the second organ that has accumulated more chromium metal during the experiment. The high accumulation of chromium in the intestine is due to the food they eat and they intake chemical along with food. The skin accumulated lower amount of chromium as compare with gills and intestine. The high amount of accumulation in the gills can damage the structure of the gill filament thus affect the respiration process of the fish and this damage can even cause death of fish, while accumulation in the intestine can also damage the digestive system of the fish. Heavy metal accumulation in the skin and flesh of the fish can affect humans as humans consume it as a primary source of protein.
In the experiment trivalent chromium chloride was used which are less toxic than the hexavalent chromium chloride. In the conclusion chromium is essential metal, but it becomes toxic when its concentration increases from the required or normal level, it can cause death of the fishes. *Carassius auratus* were exposed to the sub lethal concentration of trivalent chromium for 4 days in our experiment we find out that more accumulation occurred in the gills as compared to the intestine and skin because gills are directly exposed to the chemical. In intestine the rate of accumulation is greater than the skin because it is the main part of the digestive system. The less chromium is accumulated in chromium because of the mucus secretion of skin. The accumulation rate of chromium in different organ is Gills > Intestine > Skin. In 96-hrs its accumulated remarkable in different organs of fish while the fish in natural aquatic system are continuously kept on accumulating these pollutants in large amount the accumulation in them will be danger and alarming for the fish population and as well as for the humans, because humans are the biggest consumer of fish.

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CITATION OF THIS ARTICLE

M Fawad, A M Yousafzai, A Haseeb, H Ur Rehman, F Rasheed, Azam jan Afridi, N Akhtar, K Saeed, M G Ul Islam, S H Din,T Maqbool, M Ateeq. Acute Toxicity and Bioaccumulation of Chromium In Gills, Skin And Intestine Of Gold Fish (Carassius auratus). Bull. Env. Pharmacol. Life Sci., Vol 6 [1] December 2016:48-55