



## Dynamics of Hexavalent Chromium in Four Types of Aquaculture Ponds and Its Effects on the Morphology and Behavior of Cultured *Clarias gariepinus* (Burchell 1822)

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Hexavalent chromium is a bio accumulative toxic metal in water and fish. It enters aquaculture ponds mainly through anthropogenic sources. Hexavalent chromium concentrations and its effects on the morphology and behavior of *Clarias gariepinus* were investigated from four aquaculture ponds for 12 weeks. Chromium was measured using diphenyl carbohydrazide method; alkalinity and hardness were measured using colometric method and analyzed with Bench Photometer. Temperature and pH were measured using pH/EC/TDS/Temp combined tester. Temporal and spatial replications of samples were done with triplicates morphological and behavioural effects of the metal on fish were observed visually. Chromium ranged from no detection to 0.05 mg/L, alkalinity 105 to 245 mg/L, hardness 80 to 165 mg/L, pH 6.35 to 8.03 and temperature 29.1 to 35.9°C. Trend in the chromium concentrations in the ponds is natural > earthen > concrete > collapsible. There was a significant difference ( $P < 0.05$ ) in chromium, alkalinity, water hardness, pH and temperature among the four ponds. Significant positive correlation also existed between alkalinity, water hardness, pH, with chromium. Morphological and behavioural changes observed in the fish include irregular swimming, frequent coming to the surface, dark body colouration, mucous secretion on the body, erosion of gill epithelium, fin disintegration, abdominal distension and lethargy. High chromium concentration in natural pond was due to anthropogenic run-off of materials in to the pond. Acidic pH, low alkalinity, low water hardness also contributed to the high chromium concentration. Morphological and behavioural changes observed were attributed to the high concentrations, toxicity and bio accumulative effect of the metal. Toxicity of chromium to fish in aquaculture could threaten food security. Watershed best management practices and remediation could be adopted to reduce the effects of toxicity of chromium on pond water quality, fish flesh quality and fish welfare.

**Key words:** Chromium, Toxicity, Ponds, Aquaculture, Anthropogenic, Bioaccumulation

### INTRODUCTION

Hexavalent chromium otherwise known as chromium VI or Cr VI is an important heavy metal pollutant in aquatic medium due to its solubility, mobility and long residence time in water. Its toxicity in water and bioaccumulative property in organisms especially fish species is due to its high oxidizing potentials, high solubility, low adsorption

rate in sediments and ease of permeation of biological membranes. According to Doudoroff and Katz, chromium behaves toxicologically in a manner quite different from most heavy metals (1). It possess carcinogenic, mutagenic and tetragenic properties (2) which could manifest in organisms after a long time of exposure and bio accumulation in organs and tissues of organisms.

Chromium toxicity in water depends on multiple factors including temperature, concentration of chromium, oxidation state of chromium, pH, alkalinity, hardness and salinity of the water, while toxicity to fish depends on the species, age, developmental stage of the fish, exposure time and exposure concentration (3). Several anomalies seen in fish at physiological, histological, biochemical, enzymatic and genetic levels have been linked to the toxic effects of hexavalent chromium (3).

Chromium enters aquaculture facilities such as ponds through

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natural and anthropogenic sources with anthropogenic sources accounting for about ninety percent of total input. The anthropogenic sources of chromium in aquaculture include metals and plastic pipes and plates, pigments and paints, fertilizers, municipal waste and sewage sludge incineration (4). Ambient sources of chromium into aquaculture ponds may include vegetative debris, road dust containing chromium on account of wear of tires and brake linings (5).

Various laboratory studies have been carried out on the acute and chronic toxicity of hexavalent chromium in various species of fish. These studies include the works of Vincent *et al.* (6) on *Catla catla*, Mishra and Mohanty (7) on *Channa punctatus*, Yilmaz *et al.* (8) on *Oreochromis aureus*, Vera-Candioti *et al.* (9) on *Cnesterodon decemmaculatus*, Johnson and Radhakrishnan (10) on *Clarias batrachus*, and Nisha *et al.* (11) on *Danio rerio* among several others. There have been very few studies on the field assessment of chromium concentrations either in the wild or aquaculture ponds as well as the toxicological effects of the metal on the fish population in these habitats.

The objective of this study is to assess the dynamics of hexavalent chromium concentration in four aquaculture ponds and the effects of the metal on the cultured fish species in the ponds over time.

## MATERIALS AND METHODS

Four different aquaculture ponds of the same size and dimensions of 4.5 m × 4.5 m × 1 m, (20 m<sup>2</sup> × 1 m) volumetric capacity of 20 m<sup>3</sup> (20000 litres), containing a population of 2000 *Clarias gariepinus* and total biomass of 1000000 ± 50 g were used for the measurement of chromium and to test for the effect of elevated chromium concentrations on the morphology and behavior of the fish population. The pond types included collapsible pond, concrete pond, earthen pond and a natural pond. Well water was the source of water to the collapsible pond, concrete tank, and earthen pond while the natural pond has its water source from the rains and run-off from the watershed. The

water is constantly renewed through a flow-through system weekly to maintain the volumetric capacity of 20 m<sup>3</sup> (20000 litres) in the ponds.

Water for analyses was collected from the ponds with 50 mL plastic water bottle in the afternoons at 1 pm weekly for 12 weeks between February and April 2015. Chromium concentration was measured using the diphenyl carbohydrazide method, while alkalinity and hardness were measured using colometric method. The three parameters were analyzed with the aid of Hanna Multiparameter Bench Photometer for Laboratories Model HI 83200-02 (Hanna instruments, RI, USA). Temperature and pH of the ponds were also measured in situ at the same time for the same period using Hanna Portable pH/EC/TDS/Temp combined waterproof tester Model HI 98129 (Hanna instruments, RI, USA). Temporal and spatial replications of samples were done. Triplicates water samples were obtained and measured for chromium, alkalinity and hardness. Similarly, pH and temperature measurements were also done temporally and spatially and replicated in triplicates in situ.

Although, chromium concentration in the fish tissue was not analysed, the morphological and behavioural changes in fish were monitored daily to examine the potential effects of high chromium levels in the ponds and subsequent toxicity to the fish.

**Statistical analysis.** Stata ver. 13 was used to analyze the results (StataCorp LLC, TX, USA). Weekly means of each parameter were compared using ANOVA at  $P < 0.05$  to test for the significant differences in each parameter among the four ponds, while correlations between the parameters were also determined.

## RESULTS

The mean results of chromium, alkalinity, hardness, pH concentrations and temperature fluctuations in the four ponds are presented in Tables 1, 2, 3, 4 and 5 respectively. Chromium concentrations ranged from no detection in col-

**Table 1.** Mean chromium VI concentrations in the four ponds

Week	Collapsible pond mg/L	Concrete pond mg/L	Earthen pond mg/L	Natural pond mg/L
1	0.01 ± 0.1	0.03 ± 0.1	0.03 ± 0.1	0.05 ± 0.1
2	0.01 ± 0.1	0.03 ± 0.1	0.03 ± 0.1	0.05 ± 0.1
3	0.01 ± 0.1	0.02 ± 0.1	0.03 ± 0.1	0.05 ± 0.1
4	0.01 ± 0.1	0.02 ± 0.1	0.03 ± 0.1	0.05 ± 0.1
5	0.01 ± 0.1	0.02 ± 0.1	0.03 ± 0.1	0.05 ± 0.1
6	0.01 ± 0.1	0.01 ± 0.1	0.03 ± 0.1	0.04 ± 0.1
7	0.00 ± 0.0	0.01 ± 0.1	0.02 ± 0.1	0.04 ± 0.1
8	0.00 ± 0.0	0.01 ± 0.1	0.02 ± 0.1	0.04 ± 0.1
9	0.00 ± 0.0	0.01 ± 0.1	0.02 ± 0.1	0.04 ± 0.1
10	0.00 ± 0.0	0.00 ± 0.0	0.02 ± 0.1	0.03 ± 0.1
11	0.00 ± 0.0	0.00 ± 0.0	0.01 ± 0.1	0.03 ± 0.1
12	0.00 ± 0.0	0.00 ± 0.0	0.01 ± 0.1	0.03 ± 0.1

**Table 2.** Mean alkalinity concentrations in the four ponds

Week	Collapsible pond mg/L	Concrete pond mg/L	Earthen pond mg/L	Natural pond mg/L
1	225 ± 0.2	200 ± 0.2	130 ± 0.2	105 ± 0.2
2	225 ± 0.2	205 ± 0.2	130 ± 0.2	105 ± 0.2
3	235 ± 0.2	205 ± 0.2	140 ± 0.2	120 ± 0.2
4	240 ± 0.2	205 ± 0.2	150 ± 0.2	120 ± 0.2
5	240 ± 0.2	205 ± 0.2	150 ± 0.2	125 ± 0.2
6	240 ± 0.2	205 ± 0.2	160 ± 0.2	135 ± 0.2
7	240 ± 0.2	210 ± 0.2	160 ± 0.2	140 ± 0.2
8	240 ± 0.2	210 ± 0.2	200 ± 0.2	140 ± 0.2
9	245 ± 0.2	210 ± 0.2	200 ± 0.2	140 ± 0.2
10	245 ± 0.2	220 ± 0.2	205 ± 0.2	150 ± 0.2
11	245 ± 0.2	220 ± 0.2	205 ± 0.2	150 ± 0.2
12	245 ± 0.2	220 ± 0.2	210 ± 0.2	160 ± 0.2

**Table 3.** Mean water hardness concentrations in the four ponds

Week	Collapsible pond mg/L	Concrete pond mg/L	Earthen pond mg/L	Natural pond mg/L
1	145 ± 0.2	135 ± 0.2	120 ± 0.2	80 ± 0.2
2	145 ± 0.2	135 ± 0.2	120 ± 0.2	80 ± 0.2
3	155 ± 0.2	140 ± 0.2	125 ± 0.2	80 ± 0.2
4	160 ± 0.2	140 ± 0.2	125 ± 0.2	80 ± 0.2
5	160 ± 0.2	140 ± 0.2	130 ± 0.2	80 ± 0.2
6	160 ± 0.2	145 ± 0.2	130 ± 0.2	85 ± 0.2
7	160 ± 0.2	145 ± 0.2	130 ± 0.2	85 ± 0.2
8	160 ± 0.2	145 ± 0.2	130 ± 0.2	85 ± 0.2
9	165 ± 0.2	150 ± 0.2	140 ± 0.2	85 ± 0.2
10	165 ± 0.2	150 ± 0.2	140 ± 0.2	90 ± 0.2
11	165 ± 0.2	150 ± 0.2	145 ± 0.2	90 ± 0.2
12	165 ± 0.2	150 ± 0.2	145 ± 0.2	90 ± 0.2

**Table 4.** Mean pH measurements in the four ponds

Week	Collapsible pond	Concrete pond	Earthen pond	Natural pond
1	8.01 ± 0.2	7.88 ± 0.2	6.75 ± 0.2	6.35 ± 0.2
2	8.01 ± 0.2	7.88 ± 0.2	6.82 ± 0.2	6.45 ± 0.2
3	8.01 ± 0.2	7.90 ± 0.2	6.85 ± 0.2	6.50 ± 0.2
4	8.02 ± 0.2	7.90 ± 0.2	6.90 ± 0.2	6.55 ± 0.2
5	8.02 ± 0.2	7.90 ± 0.2	6.90 ± 0.2	6.60 ± 0.2
6	8.02 ± 0.2	8.00 ± 0.2	7.10 ± 0.2	6.92 ± 0.2
7	8.02 ± 0.2	8.01 ± 0.2	7.15 ± 0.2	7.15 ± 0.2
8	8.02 ± 0.2	8.01 ± 0.2	7.60 ± 0.2	7.15 ± 0.2
9	8.02 ± 0.2	8.01 ± 0.2	7.60 ± 0.2	7.20 ± 0.2
10	8.03 ± 0.2	8.01 ± 0.2	7.85 ± 0.2	7.40 ± 0.2
11	8.03 ± 0.2	8.02 ± 0.2	7.85 ± 0.2	7.50 ± 0.2
12	8.03 ± 0.2	8.02 ± 0.2	7.88 ± 0.2	7.50 ± 0.2

lapsible pond to 0.05 mg/L recorded in natural pond. Alkalinity ranged from 105 to 245 mg/L with the highest obtained in collapsible pond and lowest recorded in natural pond. Natural pond recorded the lowest mean water hardness of 80 mg/L and the highest mean of 165 mg/L was found in collapsible pond. Lowest mean pH of 6.35 was obtained in natural pond and highest pH of 8.03 was recorded in collapsible pond. Temperature fluctuations ranged between 29.1 to 35.9°C among the four ponds.

Weeks 1 and 12 recorded the highest and lowest Cr VI concentrations respectively in all ponds, while lowest and highest alkalinity, water hardness, pH and temperature were obtained in weeks 1 and 12 respectively. The trend in the Cr VI concentrations in the ponds was natural pond > earthen pond > concrete pond > collapsible pond.

There was a significant difference ( $P < 0.05$ ) in the concentrations of chromium, alkalinity, water hardness, pH and temperature among the four ponds. There was also a signifi-

**Table 5.** Mean temperature measurements in the four ponds

Week	Collapsible pond °C	Concrete pond °C	Earthen pond °C	Natural pond °C
1	31.9 ± 0.1	30.9 ± 0.1	29.4 ± 0.1	29.1 ± 0.1
2	32.5 ± 0.1	31.1 ± 0.1	30.1 ± 0.1	29.8 ± 0.1
3	32.7 ± 0.1	31.6 ± 0.1	30.5 ± 0.1	30.1 ± 0.1
4	32.8 ± 0.1	31.9 ± 0.1	31.1 ± 0.1	30.7 ± 0.1
5	33.1 ± 0.1	32.5 ± 0.1	31.7 ± 0.1	31.3 ± 0.1
6	33.4 ± 0.1	33.1 ± 0.1	32.3 ± 0.1	31.8 ± 0.1
7	33.8 ± 0.1	33.7 ± 0.1	32.7 ± 0.1	32.2 ± 0.1
8	34.3 ± 0.1	34.0 ± 0.1	33.6 ± 0.1	33.0 ± 0.1
9	34.6 ± 0.1	34.1 ± 0.1	33.8 ± 0.1	33.3 ± 0.1
10	35.0 ± 0.1	34.6 ± 0.1	34.0 ± 0.1	33.9 ± 0.1
11	35.7 ± 0.1	34.8 ± 0.1	34.2 ± 0.1	34.1 ± 0.1
12	35.9 ± 0.1	35.0 ± 0.1	34.8 ± 0.1	34.5 ± 0.1

**Table 6.** Summary of statistical analyses of significance difference between pond types

Pond types	Chromium	Alkalinity	Hardness	pH	Temperature
Collapsible	0.005 ± 0.0 <sup>a</sup>	238.75 ± 0.2 <sup>a</sup>	158.75 ± 0.1 <sup>a</sup>	8.02 ± 0.0 <sup>a</sup>	33.8 ± 0.1 <sup>a</sup>
Concrete	0.013 ± 0.0 <sup>b</sup>	191.25 ± 0.2 <sup>b</sup>	143.75 ± 0.1 <sup>b</sup>	7.96 ± 0.0 <sup>b</sup>	31.1 ± 0.1 <sup>b</sup>
Earthen	0.023 ± 0.1 <sup>c</sup>	170.00 ± 0.1 <sup>c</sup>	131.67 ± 0.1 <sup>c</sup>	7.27 ± 0.1 <sup>c</sup>	32.4 ± 0.1 <sup>c</sup>
Natural	0.042 ± 0.1 <sup>d</sup>	132.5 ± 0.1 <sup>d</sup>	84.17 ± 0.1 <sup>d</sup>	6.94 ± 0.1 <sup>d</sup>	31.9 ± 0.1 <sup>d</sup>

Overall means values with STD having different alphabet shows significant difference at  $P < 0.05$ .

**Table 7.** Summary of statistical analyses of correlation of chromium with other parameters

Pond types	Alkalinity	Hardness	pH	Temperature
Collapsible	0.14 <sup>+</sup>	0.10 <sup>+</sup>	0.88 <sup>+</sup>	-0.90
Concrete	0.16 <sup>+</sup>	0.12 <sup>+</sup>	0.92 <sup>+</sup>	-0.95
Earthen	0.19 <sup>+</sup>	0.15 <sup>+</sup>	0.94 <sup>+</sup>	-0.97
Natural	0.24 <sup>+</sup>	0.18 <sup>+</sup>	0.98 <sup>+</sup>	-0.98

<sup>+</sup>Significant at  $P < 0.05$ .

cant positive correlation ( $P < 0.05$ ) between the alkalinity, water hardness and pH with chromium concentrations in the ponds, while there was no correlation ( $P > 0.05$ ) between temperature and chromium concentrations in the ponds (Table 6, 7).

The temperature and pH followed seasonal pattern with lowest temperatures and pH recorded in February corresponding to the end of rainy season and highest temperatures and pH recorded in April corresponding to the dry

season. This scenario was also observed in the alkalinity, water hardness and chromium concentrations in all the ponds.

The general pattern of measured factors in the ponds showed that as pH and temperature increased, alkalinity and water hardness also increased while chromium concentration decreased.

Morphological and behavioural changes observed in the population of the cultured fish species *Clarias gariepinus* in the ponds showed that the fish in natural pond exhibited irregular swimming, accelerated operculum movement, barbells vibration, frequent coming to the surface, dark body colouration, mucous secretion on the body, reddening of urino-anal-genital pores, erosion of gill epithelium, caudal fin bending, disintegration of fin, irregular feeding and abdominal distension and lethargy (Table 8).

## DISCUSSION

The relatively high chromium concentration in natural

**Table 8.** Field observation of morphological and behavioural changes among fish in the various pond types

Observations	Collapsible	Concrete	Earthen	Natural
Behaviour		Barbells vibration	Irregular swimming, barbells vibration, frequent coming to the surface	Irregular swimming, accelerated operculum movement, barbells vibration, frequent coming to the surface, irregular feeding
Morphology		Dark body colouration, caudal fin bending, abdominal distension	Dark body colouration, mucous secretion on the body, caudal fin bending, abdominal distension	Dark body colouration, mucous secretion on the body, reddening of urino-anal-genital pores, erosion of gill epithelium, caudal fin bending, disintegration of fin, abdominal distension

pond was likely due to anthropogenic run-off of chromium bearing materials coming from the watershed into the pond and also from concentration of chromium in the rain water that fed the pond. According to (12), the average concentration of chromium in rain water is in the range of 0.2~1.0 µg/L, while that of surface water is 0.5~2.0 µg/L (13). Acidic pH, low alkalinity, low water hardness in the natural pond also contributed to the high chromium concentration recorded. This was evident in the significant positive correlations recorded between these parameters and chromium. Förstner *et al.* (14) and European Inland Fisheries Advisory Commission (15) had reported that low pH, low alkalinity and low water hardness caused higher concentration and toxicity of chromium in water and fish species with toxicity of chromium increasing by a factor of 3.4 as pH falls from alkalinity to acidity. Temperature did not have a significant effect on chromium concentration and toxicity in the pond as shown by the non-significant correlation with chromium. This scenario has also been shown by European Inland Fisheries Advisory Commission (15). The reduction in chromium concentrations temporally recorded in the ponds was likely due to increase pH, alkalinity and water hardness with time.

The low concentration of chromium in the collapsible and concrete ponds was due to the ground water used as the source of water in the two tanks. Chromium concentration have been found to be low in the range of < 1.0 µg/L to 1.0 µg/L in ground water (16). Also, correlated with the low concentration were the alkaline pH, high water alkalinity and hardness of the ponds. The water quality guideline for the protection of freshwater life was for chromium was set at 0.01 mg/L by Canadian Council of Ministers of the Environment (5). This, therefore, means that the Cr VI concentrations in natural and earthen ponds were slightly above the Canadian Council of Ministers of the Environment guidelines for the protection of freshwater life (5).

The large measurement error recorded in the pond types could have occurred due to weekly calibration of the instruments, changes in the environmental conditions in the pond types as well as the repeated measurements of the parameters in triplicates from each pond type. The readings nevertheless are very reliable as the true concentrations of chromium in the various pond types.

The effects of acute and chronic toxicity of hexavalent chromium to fish in various laboratory studies have been shown to depend on the fish species, age, developmental stage, exposure time and exposure concentrations (3). In the field, especially aquaculture ponds, the effects may not be as profound as seen in the laboratory on account of the size, volume depth and other physico-chemical and biological factors present in the pond (that typically reduce toxicity compared to more controlled laboratory exposures) as well as age, size, population and the type of fish species cultured in the pond. However, if the pond is small and the fish

species being cultured in it is small, as well at early developmental stage and sensitive to chromium, high chromium concentration in such pond will show marked acute or chronic toxicity to the fish like those observed in laboratory with morphological and behavioural changes being the first signs to be noticed among the fish population. Brooks showed that behavioural testing of fishes to measure toxicological effects is one of the best methods to measure the effects of toxicological agents (17).

The morphological and behavioural changes observed in the population of the cultured fish species *Clarias gariepinus*, especially in the natural pond of irregular swimming, accelerated operculum movement, barbells vibration, frequent coming to the surface, dark body colouration, mucous secretion on the body, reddening of urino-anal-genital pores, erosion of gill epithelium, caudal fin bending, disintegration of fin, irregular feeding and abdominal distension and lethargy could be attributed to high concentrations of Cr VI in the pond. Several workers have linked some of the observed morphological and behavioural changes recorded in the fish to acute and chronic toxicity of chromium to fish species (7,8,11,18-21).

The morphological changes were potentially due to the toxic effect of chromium VI on the body owing to the stronger oxidizing potential and biological permeation of the metal. The behavioural alterations may be due to the oxidation stress - induced neuro toxicity and overall systemic toxicity of chromium VI on the fish as reported by Yilmaz *et al.* (8). It could also be due to increased activity of cholinesterase as a result of accumulation of acetylcholine in cholinergic synapse following hyper stimulation as observed by Mathivanan (22). The bio accumulative effect of the metal on the fish species could also be responsible for the morphological and behavioural changes noticed. According to Avenant-Oldewage and Marx, Cr VI bio accumulates in the gills of *Clarias gariepinus* ten times the concentration of the metal in the surrounding water (23). The observed morphological and behavioural changes recorded in the fish species potentially due to elevated chromium concentration in the pond, could make the fish population more prone to disease and mass mortality if the concentration of the metal is not reduced and brought to minimum level at least below the protective criteria through remediation measures.

Hexavalent chromium is a toxic metal in water which may find its way into aquaculture ponds and facilities through anthropogenic and natural sources from the watershed. Its toxic effects on cultured fish species could reduce food security, protein availability, income and provision of employment to millions of people especially in developing countries who depend on aquaculture for survival. To address this situation, watershed best management practices to control anthropogenic chromium VI run-off to dug-out, earthen fish ponds as well as in cage and pen fish culture should be adopted and adapted to these aquaculture facilities.

Remediation strategies such as phytoremediation where plants that can take up Cr VI are planted in and around the ponds and the use of physical, chemical (eg. pH adjustment) and biological remediation could be employed to reduce the concentrations and effects of this toxic heavy metal on pond water quality, fish flesh quality and welfare of the fish in culture.

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