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Research Article

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# Effect of oil effluent on the cortisol level of *Cyprinus carpio*

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## Abstract

The purpose of this study was to estimate the acute toxicity of metals on *Cyprinus carpio* and to evaluate the lethal levels of Oil Effluent. The 120 hrs median lethal concentration of Oil Effluent were found to be 80ppt for *Cyprinus carpio*. Further experiments were proceeded with sub lethal concentration of ($1/10^{th}$ Conc. of $LC_{50}$) of Oil Effluent which were evaluated from the $LC_{50}$ value. After treatment the fishes were reared in ideal condition, then sacrificed dissected at different predetermined interval during the accumulation period, (i.e.) $1^{st}$ day to $20^{th}$ day, during the depuration period from $1^{st}$ day to $15^{th}$ day for *Cyprinus carpio* in Oil Effluent treatment for assay studies. The Cortisol studies carried out under sub lethal ($1/10^{th}$ Conc. of $LC_{50}$) in 3 different tissues of Gill, Liver and Muscle.

**Keywords:** Oil Effluent, *Cyprinus carpio*, Cortisol.

## Introduction

Water pollution is one of the most important problems of this era, affecting human and all living organisms and deteriorating natural resources (Khan, *et al.*, 2000). A large number of chemical compounds that get into aquatic ecosystems can cause hazardous effects on marine and freshwater organisms. Industrial effluents are indiscriminately discharged into aquatic without any pretreatment thus creating serious problems to the non target organisms. Discharging of effluents into freshwater systems depletes the dissolved oxygen content causing heavy mortality in fish by interfering with respiratory metabolism (Mishra, and Poddar, 2013).

Water pollution does not only greatly damage the aquatic ecosystems but even the terrestrial organisms and ecosystems are severely damaged and threatened. The natural resources of water like rivers, ponds, lakes and seas are polluted with a variety of solid and liquid wastes. Every waste is ultimately dumped or emptied in natural water bodies. Most of our rivers have become noxious sewers due to haphazard and extravagant pouring of industrial wastes into them and veritable death traps for aquatic life including fish which is highly nutritious, easily digestible and much sought after food (Garg *et al.*, 2009). As fish fauna serves as a food source, it is essential to know the impact of water pollution on these organisms. Any change in the natural conditions of aquatic medium causes several physiological adjustments in fish (Black, 1955).

Cortisol affect the carbohydrate metabolism (Andersson *et al.*, 1998) and increase the glucose 6-phosphatese activity in liver (Shahbazi and Maleknia, 1999), thus cause an elevation of glucose

concentration in blood of fish the depletion of liver glycogen (glycogenesis) and the rise in blood glucose levels were reported in carp *Cyprinus carpio* after exposure to several pollutants at sub-lethal concentration (Abdelmeguid *et al.*, 2002). Increases in plasma glucose concentration were previously described *in Salmo gairdneri* (Monteiro *et al.*, 2005) and *Heteroclarias* (Kori- Siakpere *et al.*, 2006) after exposed to copper and cadmium, respectively.

Generally, the immunosuppressive consequences of stressors are attributed to the action of circulating glucocorticodds, in particular cortisol. Both in mammals and fish, cortisol exerts powerful anti-inflammatory effects, inhibiting inflammatory mediators including cytokines (Tort 2011 and Petrovsky, 2001). In vitro studies using primary cell cultures of head kidney showed that cortisol effects on immune parameters are kidney showed that cortisol effects on immune parameters are mainly suppressive Teles *et al.*, (2009). Moreover, microarray analysis after in vivo treatments with cortisol implants of immune – related genes responsible for antigen recognition, antiviral activity, and inflammatory responses Krasnov, *et al.*, (2012); Teles, *et al.*, (2013). The present investigation was proposed to the evaluate cortisol level in *Cyprinus carpio* at different exposure period of sublethal concentration ($1/10^{th}$ Conc. of $LC_{50}$) of oil effluent. When observing the impact of toxicity on fish, it is dire necessary to know about the mechanism of detoxification also detoxification system in fishes seemed to indicate that they were in capable of detoxification. However, it was later demonstrated that some fish species could detoxify xenobiotics and that toxicity of several xenobiotics could be related to conjugative metabolic activity (Buchler, 1996; Lech, 1974).

## Materials and Methods

### Evolution of $LC_{50}$ employing acute toxicity test

The purpose of this study was to test the acute toxicity of Oil Effluent on *Cyprinus carpio* and to evaluate the lethal levels of effluent.

At first tentative experiment were conducted to fix the minimum concentration of oil effluent to obtain maximum mortality for *Cyprinus carpio* over 120 hour's duration. After confirming the minimum concentration, identical size of *Cyprinus carpio* were placed in different tubs (each group consists of 6 animals in 10 litre capacity plastic tubs) and exposed to different concentration of effluent which ranges from 10 ppt – 80 ppt at an interval of 5 ppt for *Cyprinus carpio* for a period of 120 hour. In addition to that a control was also maintained simultaneously. During the experimental period, mortality of fishes exposed to oil effluent was noted at every twenty four hours and dead fishes were removed as an when observed to avoid contamination. While approaching death, the fish become sluggish, inactive and upside down and reflecting the symptoms of death. The tests were repeated three times with the same concentration to improve the validity of the data.

The cortisol level were carried out under sub lethal ($1/10^{th}$ Conc. of $LC_{50}$) conce ntration in various tissues Gill, Liver and Muscle.

Cortisol was analysed using Cortisol EIA test kit (Monobind Inc. USA) Enzyme Immunoassay, Colorimetric Streptavidin Biotin Based Competitive Assay.

## Results

### Acute toxicity $LC_{50}$

The 120 hrs median lethal concentration of Oil effluent on *Cyprinus carpio* was 80 ppt. During the experimental period the fishes were restless aggressive and have the tendency to leap out of the tubs (struggle for existence). This may be due to the suffocation out of oxygen deficiency. Secretion of mucus in the gill chamber should the lesions in gills of fishes. The 120 hrs median lethal concentration of oil effluent were found to be 80 ppt for *Cyprinus carpio*.

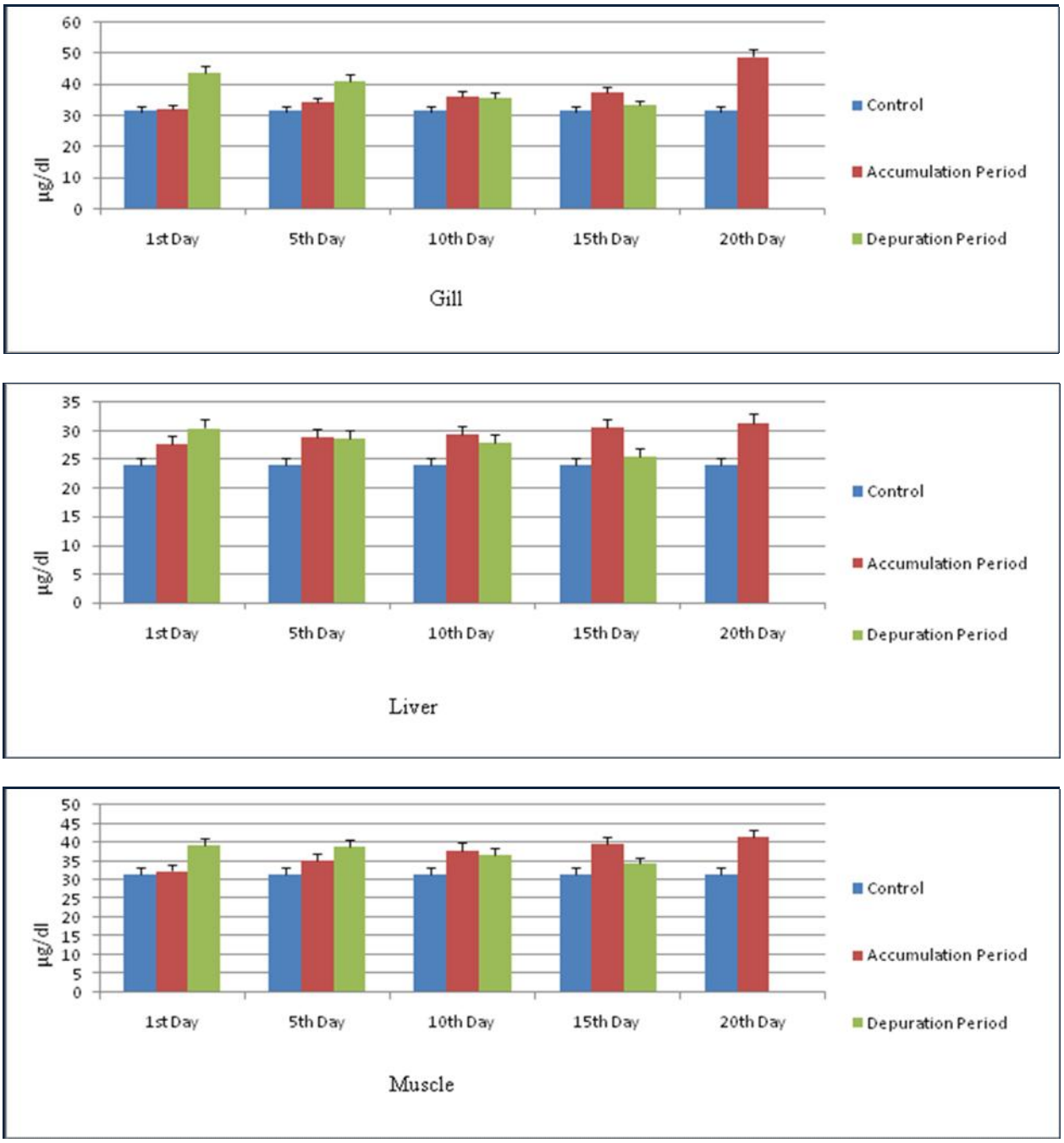
In the present experiments mortality was observed in the control tubs for the entire duration of the experiment. Oil effluent is much more toxic than the organic compounds and one readily taken up by the tissue of aquatic organisms. Many aquatic pollutants have found to be toxic to fishes.

These acute toxicity tests suggest that *Cyprinus carpio*, is sensitive to oil effluent and the caused death might be the result of difficulties in gas exchange in the gills as well as the lethality of oxidative metabolism. Hence, biotransformation and antioxidant enzymes were expected to play a major role in the removal of xenobiotic compounds.

### Cortisol

**Fig 1** represents the level of Cortisol in response of Oil Effluent in the tissue of *Cyprinus carpio*. The mean values of control were found to be 31.36±3.57 µg/dl; 24.03±3.60 µg/dl; 31.60±3.97 µg/dl in Gill, Liver and Muscle respectively.

**Fig 1** The Level of Cortisol in the tissues (Gill, Liver and Muscle) of *Cyprinus carpio* during accumulation and depuration period of sublethal ($1/10^{th}$ Conc. of $LC_{50}$) concentration of Oil Effluent




The mean value (Mean±SD) of the cortisol level for exposed and control groups of the fish are shown in **Table 1**. During accumulation period of the Cortisol level of *Cyprinus carpio* showed significant changes throughout the experiment. In Oil effluent exposed to *Cyprinus carpio* ($1/10^{th}$ Conc. of $LC_{50}$) the value of Cortisol level 32.03±1.98 µg/dl to 48.93±3.35 µg/dl for Gill, 27.70±3.35 µg/dl to 31.36±2.31 µg/dl for Liver, 32.40±3.08 µg/dl to 41.30±3.30 µg/dl for Muscle.

During the depuration period, the mean values were found to be decreased significantly from $1^{st}$ day to $15^{th}$ day. For $1/10^{th}$ concentration of $LC_{50}$, the mean values were 43.60±3.25 µg/dl to 33.26±3.61 µg/dl for Gill; 30.50±3.61 µg/dl to 25.56±2.80 µg/dl for Liver; 39.16±3.72 µg/dl to 34.26±3.55 µg/dl for Muscle. (Fig. 1)

**Table 1** The level of Cortisol content alterations in the tissues of *Cyprinus carpio* during accumulation and depuration period of sublethal ($1/10^{th}$ Conc. of $LC_{50}$) concentration of Oil Effluent.

| S.No | Days | Tissues | Control (µg/dl) | Experiments | |
|---|---|---|---|---|---|
| | | | | Accumulation period (Mean ± SD) (µg/dl) | Depuration period (Mean ± SD) (µg/dl) |
| 1 | $1^{st}$ day | Gill | 31.36±3.57 | 32.03±1.98 | 43.60±3.25 |
| | | Liver | 24.03±3.60 | 27.70±3.55 | 30.50±3.61 |
| | | Muscle | 31.60±3.97 | 32.40±3.08 | 39.16±3.72 |
| 2 | $5^{th}$ day | Gill | 31.36±3.57 | 34.16±3.80 | 41.13±3.40 |
| | | Liver | 24.03±3.60 | 28.90±3.70 | 28.60±2.72 |
| | | Muscle | 31.60±3.97 | 35.26±3.39 | 38.93 ±3.71 |
| 3 | $10^{th}$ day | Gill | 31.36±3.57 | 36.30 ±2.75 | 35.80±3.25 |
| | | Liver | 24.03±3.60 | 29.33±4.40 | 27.96±3.62 |
| | | Muscle | 31.60±3.97 | 37.93 ±2.34 | 36.73±3.81 |
| 4 | $15^{th}$ day | Gill | 31.36±3.57 | 37.36±3.55 | 33.26±3.61 |
| | | Liver | 24.03±3.60 | 30.56 ±3.72 | 25.56±2.80 |
| | | Muscle | 31.60±3.97 | 39.56±2.95 | 34.26±3.55 |
| 5 | $20^{th}$ day | Gill | 31.36±3.57 | 48.93 ±3.35 | |
| | | Liver | 24.03±3.60 | 31.36±2.31 | |
| | | Muscle | 31.60±3.97 | 41.30±3.30 | |

## Discussion

In this study the sub lethal ($1/10^{th}$ Conc. of $LC_{50}$) concentration of oil effluent exposed to *Cyprinus carpio*, the value of Cortisol level in Gill, liver and muscle it was 32.03±1.98 µg/dl; 27.70±3.35 µg/dl and 32.40±3.08 µg/dl at $1^{st}$ day and end of the experiment it was reached 48.93±3.35 µg/dl, 31.36±2.31 µg/dl and 41.30±3.30 µg/dl at $20^{th}$ days respectively. Similarly Afaghi *et al.,* (2001) stated that exposed to copper sulphate of the level of cortisol in common carp *Cyprinus carpio.* In fish copper is a classical limiting factor as it is both essential and toxic. As a micronutrient, it is necessary for haemoglobin synthesis and for being as a component of cytochrome oxidase (Benneth *et al.,*1995) copper ions are quite toxic to fish at various functional levels when environmental concentrations are increased (Vutukuru *et al.,* 2005). The toxic effect of copper is re-altered capacity for catalyzing oxidative reactions that leads to the production of reactive oxygen species. (Lopes *et al.,* 2001). This observation related with the findings of khan (1991) who observed the excessive secretion of mucus in the gills of longhorn scupin fish when exposed to Oil contaminated sediment.

Cortisol–dependent effects are mediated at the target tissue by specific receptors. Persistent stimulation of cortisol-sensitive pathways may be detrimental and consequently target tissue receptor abundance may be down-regulated (Pottinger and Mosuwe 1994a). Cortisol, known as a stress hormone, plays a major regulatory role in metabolism and is mediated by the hypothalamus pituitary-interrenal (HPI) axis (Wendelaar Bonga, 1997; Mommsen *et al.,* 1999). An elevated plasma cortisol level is a primary indicator of a stress response in fish (Barton, 2002).

Plasma cortisol concentrations of the treated fish were consistently higher than those of the control fish throughout the experiment. In general, plasma cortisol concentrations reflect the magnitude of the stress response in fish, and once adaptation to the stressors has occurred plasma cortisol concentrations return to the pre-stressed levels despite the ongoing presence of the stressors (Donaldson, 1981).

Mommsen *et al.,* (1999) reported that the toxicant-exposed fish in their study exhibited atrophied pituitary corticotropes. Thus, it appears that prolonged hyperactivity of the cortisol-producing cells associated with long-term pollutant exposure will lead to an exhaustion of the pituitary-interrenal axis in fish (Hontela, 1997; Mommsen *et al.,* 1999).

Fish exposed to heavy metals activate several compensatory mechanisms, of which some are mediated by a non-specific stress response (Wendelaar Bonga, 1997). Cortisol is a non-specific stress response that release to the blood via stimulation of the Hypothalamus-Pituitary- Interrenal (HPI) axis by heavy metal exposure (Pelgrom *et al.,* 1995; Dethloff *et al.,* 1999). Cortisol is not stored in the interregnal tissue, but is synthesized on demand (Sumpter, 1997) and so, the elevation of circulating cortisol must be a function of de novo stimulation of the HPI axis.

Cortisol affects carbohydrate metabolism and a rise in cortisol levels in frequently followed by hyperglycemia in fish (Wendelaar Bonga, 1997). Although the mechanisms involved remain unclear, the rapid rise in plasma glucose concentration following an acute stressor has been associated with the activation of the Hypothalamus-Sympathetic-Chromaffin cell (HSC) axis (McDonald and Milligan, 1997), rather than with the cortisol rise medicated by the Hypothalamus-Pituitary-Interrenal (HIP) axis (Arends *et al.,* 1999). Hyperglycemic response illustrated in this study is an indication of a disrupt carbohydrate metabolism, possibly due to enhanced the glucose 6-phosphatase activity in liver, elevated breakdown of liver glycogen (glycogenesis) (Shahbasi and Maleknia, 1999) and the synthesis of glucose from extra-hepatic tissue proteins and amino acids (Zikie *et al.,* 2001). Accordingly, the high plasma levels of cortisol and glucose observed in the present study may be indicative of the stimulation activation of HPI and HSC axes by lead and induction of different compensatory response.

In *Cyprinus carpio*, plasma cortisol and glucose levels significantly increased during the water lead exposure period. Increases in the cortisol and glucose levels were dose-dependent and previously described in *Prochilodus lineatus* (Martinez *et al.,* 2004) and *Oreochromis niloticus* (Monteiro *et al.,* 2005) in response to lead and copper, respectively. In *O.mykiss* (Dethloff *et al.,* 1999) and *Cyprinus carpio* (De Boek *et al.,* 2001), cortisol concentrations were significantly increased during the copper exposure period. Cortisol may be useful only in acute stress experiments and monitored throughout time. To be used as stress indicator, the physiological status of organisms should be standardized (Marcer martinee – Porchar *et al.,* 2009).

Cortisol and glucose as stress indicators the researcher needs to be careful to identify possible situations or factors that may influence the stress response of the fish as long as possible and to be certain they are not part of the experiment. On the other hand, the use of these two indicators as pollution or toxic stress indicators is not adequate, rather behavior and oxidative stress tests are recommended.

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