Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

Behavioral Intoxication of *Channa marulius* and *Wallago attu* during Acute Exposure of Cadmium and Copper

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ARTICLE INFO
Received: Aug 23, 2013
Accepted: Jan 30, 2014
Online: Feb 21, 2014

Keywords
Behaviour
Wallago attu
Cadmium
Channa marulius
Copper
Toxicity

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ABSTRACT

Aquatic contamination by heavy metals is a worldwide environmental problem. This contamination is due to different anthropogenic activities of human. Different metals have the tendency to induce harmful effect on living organisms. In order to evaluate the acute response of cadmium and copper alterations in the behaviour of freshwater fish, *Channa* (*C.*) *marulius and Wallago* (*W.*) *attu,* the static bioassay tests were carried out. The LC_{50} values of cadmium and copper were determined as 75.70±0.91; 32.96±0.36 and 1.07±0.01; 0.60±0.02 mg L^{-1} for *C. marulius and W. attu*, respectively. During the acute exposure of both metals, the fish showed some abnormal activities like erratic swimming, equilibrium loss and enhanced surfacing behaviour. The metals concentrations showed direct relationship with fish hyperactivity and convulsions rate. The cadmium and copper exposed test mediums showed directly maximum relationship with somersaulting activity (R^2=0.838; 0.861) for *C. marulius* while the same for *W. attu* was maximum with hyperactivity (R^2=0.797; 0.882). These indications were more pronounced for both fish species in the copper than cadmium exposed test mediums. The fish kept in the medium without metal exposure showed normal behaviour and activity.

INTRODUCTION

Due to industrialization and urbanization the number of factories and inhabitants has increased rapidly. The contamination of freshwaters with a wide range of pollutants has become a matter of serious concern over the last few decades (Canli et al., 1998; Dirilgen, 2001; Vutukuru, 2005). The natural aquatic systems extensively are polluted with heavy metals released from household, industrial and other man-made activities (Velez and Montaro, 1998). Heavy metal contaminations have destructive effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Ashraf, 2005; Vosyliene and Jankaite, 2006). The equilibrium of the aquatic environment and organisms is disturbed by these actions (Farombi et al., 2007). In order to manage aquatic ecosystem it is important to know the biological status of the system, especially when evaluating the impact of a chemical that create a stressor on the aquatic biota. Bioavailability is a dynamic process with two different phases: a physico-chemically driven desorption and a physiologically driven uptake (Embrahimpour and Mushrifah, 2008). Atmospheric deposition, erosion from the geological matrix, or from anthropogenic sources, such as manufacturing discharges, and mining wastes can introduce heavy metals into the aquatic ecosystem (Alam et al., 2002). Pollution of freshwater reservoirs (water, sediment and fish) by cadmium and other heavy metals have been paid attention for considerable time (Srivastav and Srivastav, 1998; USATDR, 1999; Murugan et al., 2008; Tripathi and Dubey, 2008; Ebrhimi and Taherianfared, 2009).

Some heavy metals like copper is important in small quantity for biological processes and occur naturally in many riverine systems; however, when it is discharged in large quantity from sewage or agricultural runoffs, it can be extremely harmful. Copper in the ionic forms Cu_2^{+}, $Cu_2OH_2^{\ 2+}$ and $CuOH^{+}$ is toxic to fish (Moore, 1991). Copper, an essential metal for organisms, may become extremely toxic for aquatic animals as its concentration in water increases. Among animal species, fishes are the inhabitants that cannot escape from the harmful effects of these pollutants (Olaifa et al., 2004). Fish may absorb metal directly from contaminated water or indirectly from feeding (Suedel et al., 1997; Javed, 2005). Fish may act as sentinel organism for indicating the potential for exposure of human population to pollutants in water reservoir and recognized as major vectors for contaminant transfer to humans (Tripathi and Dubey, 2008; Murugan et al., 2008; Kasherwani et al., 2009). Fishes are relatively sensitive to changes in their surrounding environment. Fish health may therefore reflect and give a good indication of the status of specific aquatic ecosystem (Gupta, 2009; Mokhtar et al., 2009). The toxic pollutant may affect water quality, feeding, swimming, delayed hatching and maturation period of fish (Atif et al., 2005; Laovitthayanggoon, 2006 and Kumar, 2007; Srivastava and Srivastava, 1998). Behaviour is obviously a very important individual's level response that is the result of molecular, physiological and ecological processes (Scott and Sloman, 2004; Weis, 2005). Very few studies, up to date, have been performed on the effect of metals exposure on fish behavior and behavioural abnormalities are still poorly understood.

Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems and has been widely used to identify suitable organisms as a bio indicator and to derive water quality standards for chemicals. The purpose of toxicity test is to assess various abnormalities caused due to administration of a chemical or heavy metal to fish on occasion or other and to determine the order of lethality of the chemical (Shuhaimi- Othman, 2010). Acute toxicity caused by different toxicant on freshwater fish can evaluate by quantitative parameters like survival and mortality of test animals and sensitivity of different fish species against metal's toxicity (Kausar and Javed, 2012; Azmat et al., 2012; Ebrahimpoure et al., 2010).

In Pakistan riverine, freshwater ecosystem are enriched with highly diversified and representative of all warm water fish species. A lot of work has been done on major and Chinese carps. The fish species viz. *Channa* (*C.*) *marulius* and *Wallago* (*W.*) *attu* were important because of their abundance, common distribution and market value. *C. marulius* locally called as Sol and *W. attu* locally named as Mulee are fresh water predatory fish species present in Azad Jamu Kashmir, Balochistan, Khyber Pakhtunkhwa, Punjab and Sindh Provinces. The distributional status of both fish species is indigenous and according to IUCN status these are near to be threatened but its commercial value is very high (Rafique and Khan, 2012). The main objective of this study was to determine the lethal concentration (LC_{50}) of cadmium and copper compounds at 96 hours and the behavioural alterations due to action of cadmium and copper compounds on *C. marulius* and *W. attu*.

MATERIALS AND METHODS

The fish, *C. marulius* and *W. attu* fingerlings were collected from their natural breeding grounds and transported to the wet laboratory and placed in cemented tanks having 1000 liter water capacity. The fingerlings were fed with diet, containing 40 % crude protein. The acclimatization period in the laboratory was last for 15 days having photoperiod of 12h Light: 12h Dark regime.

Test chemicals

The pure chloride compounds of cadmium and copper of analytical grade (Merk) were used as metal toxicant for acute toxicity tests and behavioral study.

Test procedure

The 96-hr LC_{50} and lethal concentrations (Finney, 1971) were determined in static bioassay system. Twenty fish of each species, separately, were placed in aquarium of 100-liter water capacity. Metal's toxicity concentration for each fish species were started from zero and increased as 0.05 and 5 mg L^{-1} (as total concentration) for low and high metals concentrations, respectively. Acclimated fish were not fed for one day before the start of experiments until the end of the 96-h experimental period. In each aquarium, the concentration of metal was increased gradually in order to avoid the fish from stress. Continuous air was supplied to all the test and control media with an air pump through capillary network. Stock solutions were prepared for required metals dilutions in double deionized water. Three replicates were used for each toxicity test. Fish mortality and behavior were regularly monitored for each test dose during 96-hr exposure period. Dead fish was immediately removed from the aquarium to prevent contamination of the test solution.

LC_{50} Assessment

To estimate 96-hr LC_{50} and lethal values of cadmium and copper (LC_{50}: the concentration of the toxicant that caused 50% mortality in fish and lethal: 100% mortality after a specific exposure time), the total mortality of *C. marulius* and *W. attu* in each concentration of the toxicant was recorded, separately and calculated by the Probit Assay Method (Finney, 1971).

Behavioural studies

During the acute exposure period, the behavioural changes and morphological abnormalities of the healthy/control fish and the fish exposed to various concentrations of cadmium and copper were regularly monitored and evaluated for behavioural changes. A regression analysis was performed to find-out relationships among various parameters under study (Steel et al., 1997).

RESULTS

LC_{50} Assessment

Figure 1 represents the data of cadmium and copper concentrations and the mortality rate of *C. marulius* and *W. attu* used during the acute toxicity of metals. During

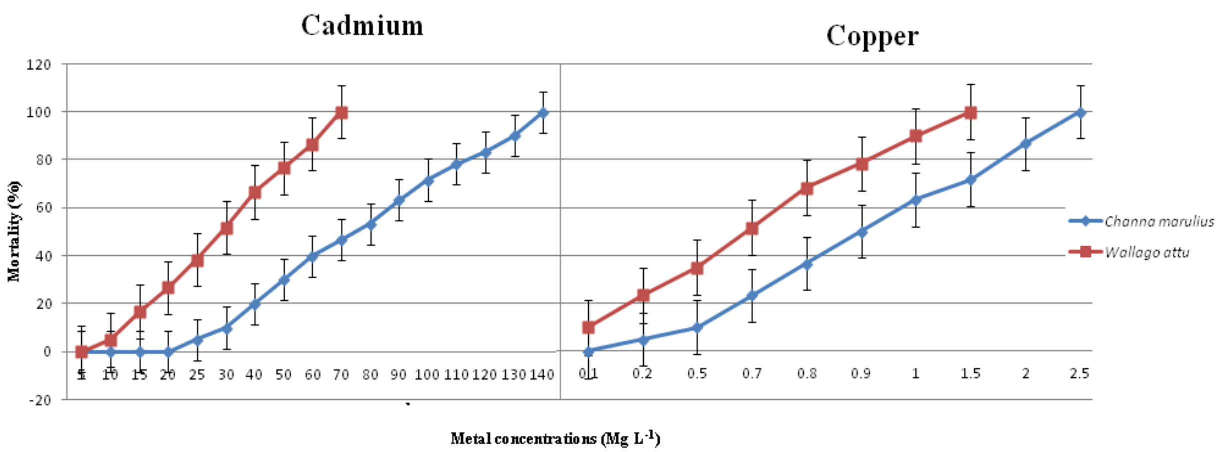

Fig. 1: The percentage mortality of *Channa marulius* and *Wallago attu* at different cadmium and copper concentrations during 96-hr acute toxicity tests.

the experiment it was observed that mortality depends on metals concentration. Mortality was 100% for *C. marulius* and *W. attu* at 140 and 70 mgL^{-1} for cadmium while it was 2.5 and 1.5 mg L^{-1} for copper, respectively. The values of 96-hr LC_{50} and lethal concentrations of cadmium and copper with 95% confidence interval for both fish species are shown in Table 1. Among the selected toxicants, copper is more toxic than cadmium. The *W. attu* was significantly more sensitive to copper having lower mean LC_{50} value (0.60+0.02 mgL^{-1}) than *C. marulius* (1.07+0.01 mgL^{-1}). Both fish species were showed least sensitively towards cadmium exposure. The 96-hr LC_{50} values of fish vary from species to species and from metal to metal. The different LC_{50} values also depended on the different methods used to determine it. During this experiment Finny's Method was used as recommended by EPA.

Behavioural studies

The behaviour and condition of both fish species in the control and exposed media were noted for 96-hr. Throughout the 4 day test period (96-hr), fish behaviour was monitored and recorded. Behavioural changes in the test organisms are the most sensitive indication of potential toxicant. The fish showed a marked change in their behaviour when exposed to various concentration of the toxicant as shown in figures 2 and 3. The intensity of toxicity of copper chloride concentrations was most obvious than cadmium chloride in the first hour of exposure. The introduction of chemical in the test media showed that fish try to jump out of aquarium to avoid the chemical followed by increased swimming, surfacing and hyper activity.

The data in figure 2 represents the relationship between fish behaviour and cadmium concentrations used during this experiment. The exposed cadmium concentrations showed strong and direct relationship with somer-saulting activity followed by convulsions, equilibrium status, hyperactivity, fin movement and swimming rate for *C. marulius* with R^2 value of 0.838; 0.796; 0.784; 0.749; 0.701 and 0.644, respectively. The fish, *W. attu* showed this relationship in the following order hyperactivity> somersaulting activity> Equilibrium status> swimming rate> fin movement> convulsions. The relationship computed between copper concentrations and fish behaviour was presented in figure 3. The computed R^2 values showed that the relationship trend between fish (*C. marulius*) behaviour and metal (Copper) concentrations was somersaulting activity> Equilibrium status> hyperactivity> swimming rate> convulsions> fin movement. The same for *W. attu* remained as hyperactivity (0.882)> Equilibrium status (0.840)> somersaulting activity (0.827)> fin movement (0.771)> swimming rate (0.696)> convulsions (0.493). The *C. marulius* in both Cadmium and Copper exposed test mediums showed maximum relationship with somersaulting activity while the same for *W. attu* was maximum for hyperactivity. The high values of R^2 (Co-efficient of determination) computed for each regression equation reveals high reliability of these regression models. No behavioural changes were observed in the control group of fish that showed normal behaviour throughout the experiment. The reaction and survival of aquatic animal not only depended on the biological state of animals and physiochemical characteristics of water but also on the kind, toxicity, type of exposure to the toxicants.

DISCUSSION

It was observed during this experiment that fish mortality is concentration dependent, because the fish mortality increased as metals concentrations increased in the test mediums. The comparison made between two metals, showed that copper was more toxic than

Table 1: The 96-hr LC_{50} and lethal (mg L^{-1}) values of cadmium and copper for fish with 95% confidence interval

Species	96-hr LC_{50} with 95% C.I		96-hr lethal with 95% C.I	
	Cadmium	Copper	Cadmium	Copper
Channa marulius	75.70±0.91a	1.07±0.01 a	166.03±2.53 a	2.44±0.06 a
	(68.56-82.65)	(0.94-1.22)	(149.13-191.56)	(2.07-2.90)
Wallago attu	32.96±0.36 b	0.60±0.02 b	77.54±2.06 b	1.50±0.04 b
	(28.73-43.89)	(0.50-0.69)	(67.45-94.25)	(1.29-1.87)

cadmium. The *W. attu* was significantly more sensitive to copper having lower mean LC_{50} value of 0.60+0.02 mgL^{-1} than *C. marulius* that showed LC_{50} value of 1.07+0.01 mgL^{-1}. Both fish species were showed least sensitively towards cadmium exposure. The 96-hr LC_{50} values of fish varied from species to species and from metal to metal. The different LC_{50} values also depended on the different methods used to determine it. Tiwari et al. (2011) reported LC_{50} values with 95% confidence interval of cadmium for freshwater teleost, *C. punctata* (Bloch) at 24, 48, 72 and 96-hr exposure durations as 26.88 (21.69-71.68), 18.76 (17.13-20.81), 16.70 (14.77-17.96) and 14.95 (13.13-15.88) mgL^{-1}, respectively. The 96-hr LC_{50} values for *Oreochromis niloticus* and *Clarias gariepinus* were determined as 58.837 and 70.135 mgL^{-1}, respectively that showed different sensitivity patterns (Ezeonyejiaku et al., 2011). Javed and Abdullah (2006) and Azmat et al. (2012) reported *Catla catla* as most sensitive specie against Nickel and Aluminum as compared to *Labeo rohita* and *Cirrhina mrigala*. LC_{50} obtained in the present study compared with corresponding values that have been published in the literature for other species of fish, showed different LC_{50} of copper sulphate in different species. The 96-hr LC_{50} values of copper sulphate on rainbow trout (*Oncorhynchus Mykiss*) were reported to be 0.094 mg L^{-1} by Gundogdu, 2008; while Shuhaimi-Othman, 2010 reported the 96-hr LC_{50} value of copper sulphate on two freshwater fishes, *Rasbora sumatrana* (Cyprinidae) and *Poecilia reticulata* (guppy). For *R. sumatrana*, LC_{50} for 96-hr were 5.6 µg L^{-1} and for *P. reticulata* were 37.9 µg L^{-1}. Gomes et al. (2009) reported that with juvenile Brazilian indigenous fishes, *curimata Prochilodus vimboides* and *piaucu Leporinus macrocephalus*, 96-hr LC_{50} of copper were 0.047 and 0.090 mg L^{-1}, respectively.

Behavioural changes in the test organisms were the most sensitive indication of potential toxicant. Behavioural changes were observed in both fish species and exposed metals concentrations. During present investigations fish showed a marked change in their behaviour when exposed to various concentrations of the metals. The exposed cadmium concentrations showed strong relationship with somersaulting activity followed by convulsions> equilibrium status> hyperactivity> fin movement> swimming rate for *C. marulius* with R^2 value of 0.838; 0.796; 0.784; 0.749; 0.701 and 0.644, respectively. The same for *W. attu* remained as hyperactivity> somersaulting activity> Equilibrium status> swimming rate> fin movement> convulsions. The *C. marulius* in both Cadmium and Copper exposed test mediums showed maximum relationship with somersaulting activity, while the same for *W. attu* was maximum for hyperactivity. These indications were more pronounced in Copper exposed fish. No behavioural changes were observed in the control group of fish that showed normal behaviour throughout the experiment. These findings are in confirmatory with the results of kaushal and Mishra (2011; 2013). They investigated the acute toxicity of cadmium compounds and their toxicological effects on LC_{50} and behaviour of fish, *C. punctatus* for 96 hr. The fish showed markable changes in behaviour i.e. increased swimming, restlessness, surfacing and hyperactivity, when exposed to various concentrations of chemicals. Nawani et al. (2013) determined the LC_{50} value and behavioral responses of commercial formulation of chlorpyrifos (Termifos) on the freshwater fish *Clarias gariepinus*. Fish exposed to various concentrations of the pesticide showed uncoordinated behavior such as erratic and jerky swimming, attempt to jump out of water, frequent surfacing and gulping of air, decrease opercula movement and secretion of mucus on the body and gills followed by exhaustion and death. These findings are also in confirmatory with the results of Biuki et al. (2010). Exposure of high concentration of cadmium chloride to fish (*Chanos chanos*) showed behavioural changes such as swimming disorder, loss of balance, somersaulting activity and fin movements. Our results are also in line with the findings of Hesni et al. (2010). They observed the lead nitrate effect on behavioural changes of milkfish (*Chanos chanos*). The behavioural changes observed in fish were, hyperactivity, loss of balance, vertical and downward swimming patterns, convulsions, attaching to the surface and increased mucus secretion. The result showed that acute lead toxicity severely affects the mortality and normal behaviour which may be deleterious for milk fish. The abnormal behavior like hyperactivity was dose and duration dependent (Tiwari et al., 2011). Change in the behavioural patterns of individuals is the main sensitive indicator of stress induced by chemicals (Remyla et al., 2008). The behavioural changes of copper sulphate exposed tilapia (*Oreochromis niloticus*) and catfish (*Clarias gariepinus*) were studied by Ezeonyejiaku et

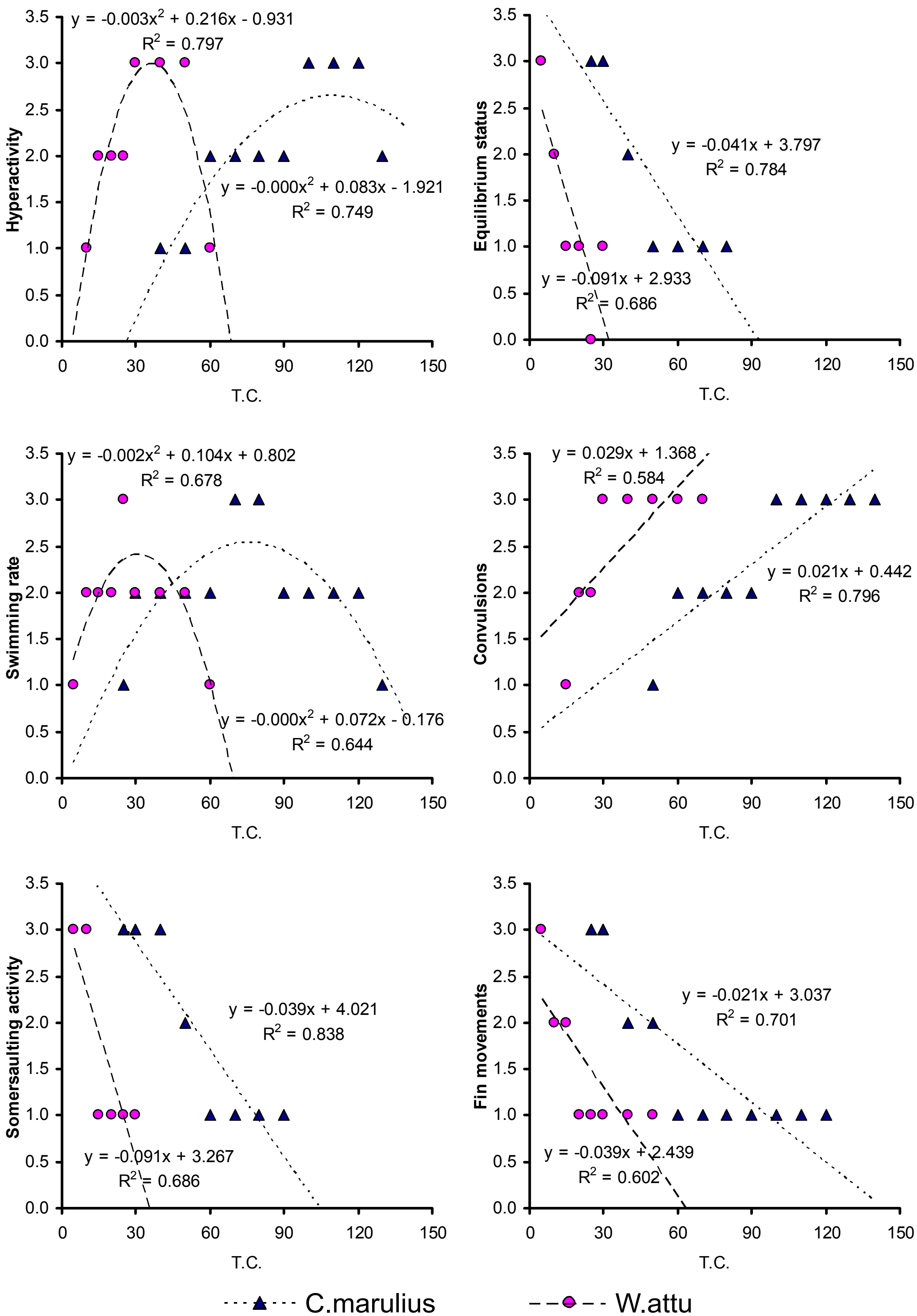

Fig. 2: The computed relationships between fish behaviuor and cadmium concentrations of the test mediums.
y = Dependent variable; x = Independent variable; R^2 = Coefficient of determination; T.C. = Toxicant concentration

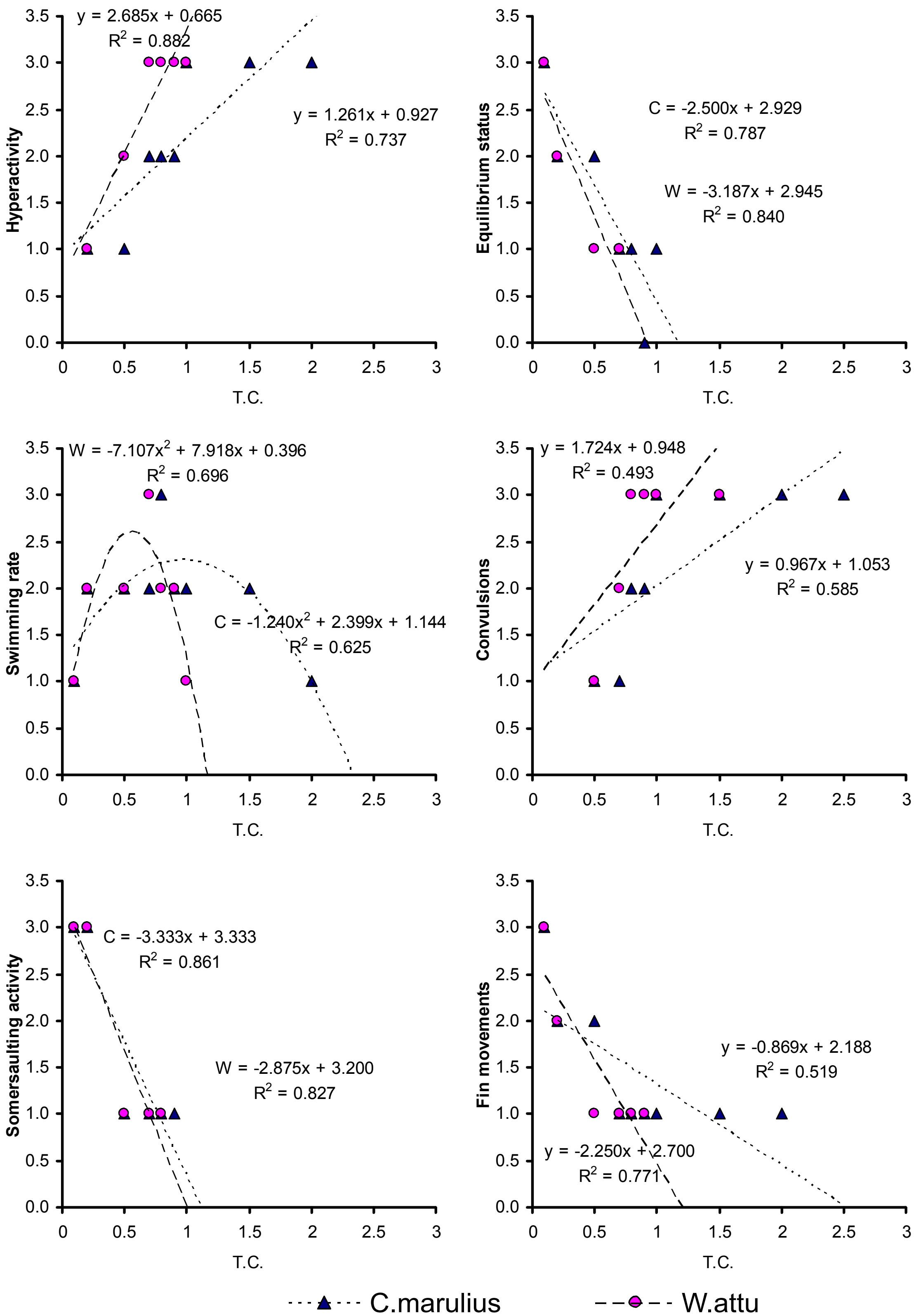

Fig. 3: The computed relationships between fish behavior and copper concentrations of the test mediums.
y = Dependent variable; x = Independent variable; R^2 = Coefficient of determination; T.C. = Toxicant concentration

al. (2011). There was avoidance of the copper sulphate contaminated water through unsteady swimming pattern with jerky movements. In conclusion, our results pointed out that the cat fish may be used as a bio-indicator for acute exposure to cadmium and copper but more bahavioural studies in ecotoxicolgy are now needed. The results of this study, acute toxicity and behaviour responses in this state of affairs, justify the need for regulatory monitoring of pollutants discharged into the aquatic environments.

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