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Cellular energy budget in tropical freshwater fish following exposure to sublethal concentrations of cadmium

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Under stressful conditions (toxicity), organisms often try to detoxify by mobilizing available energy sources with costs to various metabolic functions, such as growth or reproduction. Cellular energy allocation (CEA) is a methodology used to evaluate the energetic status and which relates with organisms' overall condition and response to toxic stress. It consists of the integration of the energy reserves available (Ea) and energy consumption (Ec). The effects of different sublethal concentrations (0.828, 0.0828, and 0.00828 mg/l) of cadmium (Cd) was evaluated on the total energy budget of African catfish (*Clarias gariepinus*) juveniles over 56-day period of exposure. A total of 180 *C. gariepinus* were exposed under the static renewal assay, and parameters measured were the total energy reserves available (protein, carbohydrate and lipid budgets) and the energy consumption (based on electron transport system activity assay) being further integrated to obtain the CEA. The Bradford method, Phenol-sulphuric acid method and Bligh and Dyer method were used to evaluate the protein, carbohydrate, and lipid contents, respectively of the test animals. Significant changes ($p < 0.05$) in energy reserves and energy consumption were observed upon Cd exposure. Among the three energy reserves obtained, carbohydrate offered the least energy fraction (0.23%), and followed by protein (20.27%). The highest energy fraction was offered by lipid (79.50%). The effect of cadmium brought about a tremendous decrease in Carbohydrate (from 0.04 kJ/g in day 7 to 0.01 kJ/g in day 28). Lipid always stepped up (from 9.84 kJ/g on day 7 to 34.48 kJ/g on day 28) to compliment energy loss whenever carbohydrate was exhausted. Protein was least affected (from 1.78 kJ/g on day 7 to 1.23 kJ/g on day 28) with mild reduction in its reserve. Increased energy consumption was recorded amongst the exposed groups, with the highest concentration (0.828 mg/l) offering the most Ec of 58.54 kJ/g on day 56. Significant reduction in CEA occurred across the exposed groups as Ea and Ec dwindled. These results have shown the adverse effects of Cd on the energy status of *C. gariepinus* and the sensitive effectiveness of CEA technique in assessing the toxic effects of metallic pollutants on freshwater animals.

Key words: *Clarias gariepinus*, cellular energy allocation, energy available, electron transport system, biomarker, energy metabolism.

INTRODUCTION

Metal contamination in aquatic ecosystem is considered to cause deteriorating effects for both the inhabiting organisms and also for terrestrial organisms including humans who depend on several of these animals as food (Perera et al., 2015). Most of the discharges of industrial, agricultural, domestic, and urban wastes eventually end up in the aquatic environment (Hourkpatin et al., 2012). These discharges introduce and increase the levels of heavy and trace metals in soil and water bodies and these have negative effects on aquatic organisms (Azmat et al., 2016).

A variety of heavy metals contaminants including (cadmium, copper, mercury and zinc) are reported to be ubiquitously present in rivers, reservoirs, they are not biodegraded, they bioaccumulate in fish, oyster, mussels, sediments and other components of aquatic ecosystems (Bhattacharya et al., 2009). Contamination of aquatic ecosystems with cadmium and various physiological adverse effects caused by cadmium have raised genuine concern (Perera et al., 2015). Cadmium is xenobiotic which is widely used in electric, electronic, automotive, metal plating, battery, dye, plastic and synthetic fiber industries and in nuclear reactor control systems (Okocha and Adedeji, 2011). It is known to have toxic effects on animals even at very low concentrations (Kaoud and Eldahshan, 2010). Cadmium pollutants in freshwater habitat get into the body systems of the inhabiting organisms, bioaccumulates and cause undesirable consequences (Perera et al., 2015). Extensive studies have been conducted on Cadmium toxicity in freshwater. Anaemia, leukocytosis, neutrophilia, and eosinophilia have been reported (Karuppasarmy et al., 2005).

The stress caused by toxicants to an organism can lead to the depletion of energy reserves especially under long time of exposure (Ferreira et al., 2014). Different factors affect the total amount of energy available, reserved and consumed by an organism. The available energy is required for the maintenance of the basic physiological processes of the organism such as growth, replacement of worn-out tissues, reproduction and basal metabolism (Gourley, 2009). Thus, additional energy is required for dealing with stress. If this is not provided via the diet, growth, reproduction and overall body function can be compromised (De Coen and Janssen, 2003b).

Cellular energy allocation (CEA) as a biomarker, has been developed based on the "metabolic cost" hypothesis, which suggests that toxic stress induces metabolic changes which might lead to a depletion of energy reserves resulting in adverse effects on growth

and reproduction (Gomes et al., 2015). CEA is based on a biochemical comparison of the organism's energy consumption and energy reserves available for metabolism (Novais et al., 2013). The method can be used as a marker of the available energy content of an organism and also as a rapid and instantaneous method for measuring the energy content of an organism (Verslycke et al., 2004; Smolder et al., 2004).

In this study, we examined the effect of Cadmium on the energy reservoir (Energy available, E_a), the mitochondrial energy consumption (E_c) rate. The application of Cellular Energy Allocation (CEA) methodology for the assessment of toxic stress on *Clarias gariepinus* was also tested/optimized.

MATERIALS AND METHODS

Test species and maintenance

The research was carried out at the animal house, Department of Zoology, Nnamdi Azikiwe University Awka, Anambra State, Nigeria. The experimental fish were *C. gariepinus* (African catfish) Juveniles. A total of 200 juveniles with an initial average weight and length of 42.5 g and 14.2 cm respectively were procured.

On settling the animals at the house, they were allowed to acclimatize for two weeks in ordinary tap water (pH 6.4, conductivity 160.3 μ S/cm, dissolved oxygen 5.6 mg/l) in four separate 60 L aquaria (35 fish per aquarium) before the commencement of the toxicity test. The water temperature was 25°C with a 12:12-h light: dark photoperiod, though light conditions were also influenced by fluctuations in day/night durations. Fish were fed to satiation with commercial fish feed twice daily (7:30 am and 16:00 pm) at a ratio of 5% of the body weight throughout the period of the experiment.

Chemicals

Cadmium chloride salt used for the study was bought from industrial chemical market, Bridge head Onitsha, Anambra State. Reagents such as Vanillin, Anthron, Triton X-100, nicotinamide adenine dinucleotide phosphate (NADPH), p-iodo-nitro-tetrazolium (INT), glycogen, Tris-HCl, glucose, and $MgSO_4$ (Sigma grade) were also obtained. Standard stock solution of cadmium was prepared in the laboratory using the same water source from which the experimental fish were exposed.

Experimental design

A total of 180 juvenile fishes were selected from the initial stock. The fishes were weighted after acclimatization period and divided into 4 groups (three treatments and one control). Each group including the control contained 10 fish in three replicates.

The animals were grouped based on the different concentrations of the test substance, cadmium. To avoid mortality during the experiment the concentrations of the test cadmium used were 0.828, 0.0828, and 0.00828 mg/l. These represent 1/10, 1/100,

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and 1/1000, respectively of 8.280 mg/l (the reported 96 h LC₅₀ of Cadmium for *C. gariepinus* juvenile) (Ezeonyejiaku, 2013).

After acclimatization, the fish were randomly transferred from their acclimatization tanks to experimental plastic tanks of 40-L capacity, measuring 30 cm in width × 45 cm in length and 28 cm in height. Before stocking the fish, their baseline weight was taken and recorded and homogenous sizes were randomly distributed in twelve experimental tanks of 40-L water capacity, with each tank containing ten juveniles of *C. gariepinus*. Each of the groups was placed in triplicate (including control) according to the different concentration of the test substance.

Appropriate concentrations of cadmium for each group was measured from the prepared standard stock solution and gently introduced into each tank accordingly. The control group was never exposed to any concentration of cadmium throughout the experiment.

The water was changed every three days to ensure constant and fresh concentration of the test substance, to maintain adequate dissolved oxygen content and also to remove waste and unfed food. At each change of water, they were re-contaminated with appropriate doses of cadmium according to the groupings.

Every seven days, one fish was randomly removed from each experimental tank for dissection. The fishes were dissected to extract the liver which is the organ to be used for the Cellular Energy Allocation (CEA) biochemical analysis. The CEA was determined in the liver of the fish, since a large proportion of substrate transformation or changes in nutrient supply occur in this organ (Whitlow et al., 2008). Also the fish liver is considered as a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment (Dural et al., 2007).

At the laboratory, homogenate samples of the liver were prepared and used for the CEA biochemical tests. The liver samples were cut into small pieces and then homogenized.

Biochemistry and cellular energy allocation measurements

Protein assay

Protein content was determined following the Bradford method (Bradford, 1976). Total protein contents were determined from 200 µL of homogenate. Proteins were precipitated by adding 15% of trichloroacetic acid (TCA) and incubated at 4°C for 10 min. The samples were centrifuged (3000×g, 10 min, 4°C) and the pellets were washed with 5% TCA and both supernatant fractions were combined and used for the total carbohydrate analysis. The remaining pellets were resuspended in NaOH, incubated at 60°C for 30 min and neutralized with HCl. The total protein content was then measured using Bradford's reagent. The absorbance was measured at 590 nm using bovine serum albumin as standard.

Carbohydrate assay

Carbohydrates content were analyzed using both supernatant fractions after centrifuging and washing the samples. In using the microplating method, the extract of samples, phenol 5% and concentrated H₂SO₄ (95-97%) were added at a proportion 1:2:4 to the microplate. After 30 min incubation at room temperature, the absorbance was measured at 492 nm using glucose as standard.

Lipid assay

Lipids were extracted according to the method used by Bligh and

Dyer (1959). The extract was obtained from 500 µL of homogenate sample, after which 500 µL chloroform (spectrophotometric grade) and 500 µL methanol (spectrophotometric grade) were added. After centrifugation (10,000×g, 5 min, 4°C), the top phase was separated and 500 µL of H₂SO₄ was added to 100 µL of lipid extract, and then charred for 15 min at 200°C. The burned residue was then diluted in 1 ml of distilled water and total lipid content was determined by measuring the absorbance at 370 nm using Tripalmitin as standard.

Energy consumed (Ec)-electron transport system (ETS) activity assay

The energy consumed at mitochondrial level was measured according to King and Packard (1975) as described by De Coen et al. (1995) and De Coen and Janssen (1997, 2003) as electron transport system activity (ETS) based on the spectrophotometric determination of INT (*p*-Iodonitro tetrazolium Violet; Sigma) reduction.

The homogenates of the samples were centrifuged at 4°C, 3000×g for 10 min. After centrifugation, 50 µL of extract was added into the microplate well together with 150 µL of buffered substrate solution (BSS; 0.13 M Tris-HCl and 0.3% (w/v) Triton X-100, pH 8.5) and 50 µL NADPH solution (1.7 mM NADH and 250 µL NADPH). The reaction was induced by adding 100 µL INT (*p*-Iodo Nitro Tetrazolium 8 mM). Absorbencies were measured kinetically every minute using a spectrophotometer at 20°C for 10 min. The wavelength filter used was 490 nm.

The aforementioned assays were carried out in triplicate and for all assays appropriate blanks were run. The total energy stored in the protein, carbohydrate, and lipid fractions was summed as a measure of the total energy available (Ea), for the organism. These values were obtained by multiplying the nutrient contents by their respective energetic equivalents using enthalpy combustion (17.5 kJ/g carbohydrates, 24 kJ/g protein and 39.5 kJ/g lipids) (Gnaiger, 1983)

CEA was calculated after determination of Ea and Ec according to the following formula:

$$Ea = \text{glucose} + \text{lipid} + \text{glycogen} + \text{protein (kJ/g)}$$

$$Ec = \text{ETS activity (kJ/g)}$$

$$CEA = Ea/Ec.$$

Statistical analysis

Data collected from CEA analysis were subjected to Analysis of variance (ANOVA), while LSD Multiple Comparison Test was used to separate the sample means. All statistical analyses were performed using SPSS for Windows version 20 (SPSS, Inc.).

RESULTS

Protein

In the first treatment (0.828 mg/l), a marked increase and reduction in the protein content of the organisms occurred intermittently (Table 1) between the days of the experiment. From day 7 to day 14, protein increased from 1.613 to 1.933 kJ/g and then decreased to 1.564 and 0.327kJ/g on days 21 and 28, respectively. Day 35 however recorded a resurgence of protein (1.691 kJ/g)

Table 1. Protein, carbohydrate and lipid contents of *C. gariepinus* exposed to different sublethal concentrations of cadmium for 56 days.

Day	Treatments (mg/l)	Protein±SD (KJ/g)	CHO±SD (KJ/g)	Lipid±SD (KJ/g)
7	0.828	1.613±0.26 ^a	0.036±0.00 ^a	12.508±3.29 ^a
	0.0828	1.999±0.03 ^{ab}	0.036±0.00 ^a	18.565±0.38 ^{ab}
	0.00828	1.477±0.03 ^a	0.036±0.00 ^a	22.778±4.09 ^b
	Control	2.021±0.04 ^{ab}	0.036±0.00 ^a	24.358±10.01 ^b
14	0.828	1.933±0.56 ^a	0.027±0.00 ^a	6.816±2.29 ^a
	0.0828	1.629±0.14 ^a	0.031±0.00 ^a	4.655±0.95 ^{ac}
	0.00828	2.239±0.95 ^{ac}	0.032±0.00 ^a	12.237±7.60 ^c
	Control	2.494±0.41 ^{ac}	0.036±0.00 ^a	15.645±3.09 ^c
21	0.828	1.564±0.89 ^a	0.020±0.00 ^a	9.348±3.42 ^{ac}
	0.0828	1.599±1.14 ^a	0.021±0.00 ^a	12.377±4.21 ^a
	0.00828	2.059±0.61 ^{ab}	0.021±0.00 ^a	12.113±8.19 ^a
	Control	2.508±0.40 ^b	0.041±0.01 ^a	21.988±4.67 ^{ab}
28	0.828	0.327±0.21 ^a	0.000±0.00 ^a	28.308±0.81 ^a
	0.0828	0.738±0.48 ^a	0.000±0.00 ^a	27.913±0.81 ^a
	0.00828	1.388±0.46 ^b	0.000±0.00 ^a	26.728±1.34 ^a
	Control	2.480±0.86 ^c	0.049±0.00 ^b	46.083±13.74 ^b
35	0.828	1.691±0.10 ^a	0.000±0.00 ^a	0.295±0.07 ^{ab}
	0.0828	2.153±0.13 ^{ab}	0.000±0.00 ^a	0.316±0.05 ^a
	0.00828	2.494±0.13 ^b	0.001±0.00 ^a	0.407±0.11 ^a
	Control	2.997±0.11 ^b	0.051±0.00 ^b	0.740±0.36 ^{ac}
42	0.828	1.656±0.26 ^{ab}	0.018±0.00 ^a	15.723±5.50 ^{ab}
	0.0828	2.013±0.07 ^a	0.018±0.00 ^a	26.876±0.39 ^a
	0.00828	2.129±0.19 ^a	0.030±0.00 ^a	27.108±0.29 ^a
	Control	2.672±0.04 ^{ac}	0.061±0.00 ^b	29.354±3.74 ^{ac}
49	0.828	2.387±0.10 ^a	0.059±0.00 ^a	4.879±2.31 ^{ab}
	0.0828	2.322±0.53 ^a	0.068±0.00 ^a	11.153±3.95 ^a
	0.00828	2.469±0.37 ^a	0.072±0.00 ^a	14.328±0.72 ^a
	Control	3.094±0.04 ^a	0.073±0.00 ^a	21.067±2.12 ^{ac}
56	0.828	2.779±0.26 ^a	0.071±0.00 ^a	9.759±3.29 ^a
	0.0828	2.900±0.03 ^a	0.072±0.00 ^a	16.497±0.38 ^a
	0.00828	2.943±0.03 ^a	0.072±0.00 ^a	15.955±4.09 ^a
	Control	3.188±0.04 ^a	0.071±0.00 ^a	40.817±10.01 ^b

Columns sharing similar superscripts are not significantly different at $P>0.05$. CHO: Carbohydrate.

and that continued all through the remaining days of the study. Day 28 recorded the lowest protein content (0.327 kJ/g), while the highest protein content was recorded on day 56 (2.779 kJ/g).

In the second treatment (0.0828 mg/l), protein decrease consistently (but slightly) from day 7 (1.999

kJ/g) through day 28 (0.738 kJ/g) (Table 1). A recovery in protein was recorded on day 35 (2.153 kJ/g), and this went on all through the period of the experiment. Day 28 recorded the lowest protein content (0.738 kJ/g), while the highest protein content was recorded on Day 56 (2.900 kJ/g).

In the third treatment (0.00828 mg/l), the protein content increased markedly throughout the period of the experiment, save for days 21 and 28 which recorded slight decrease in protein from 2.059 to 1.388 kj/g (Table 1). Day 28 recorded the lowest protein content (1.388 kj/g), while the highest protein content was recorded on day 56 (2.943 kj/g).

In the control group, there was consistent and significant ($p < 0.05$) increase in protein from 2.021 kj/g on day 7 to 3.188 kj/g on day 56. Statistically, there was a significant difference in protein content between the treatments ($p < 0.05$) and also between the days ($p < 0.05$) of the study.

Carbohydrate

Generally, carbohydrate offered the least fraction of the energy content of the test organisms (Table 1). In the first treatment (0.828 mg/l), carbohydrate decreased from 0.036 kj/g on day 7 to 0.027 and 0.020 kj/g on days 14 and 21, respectively (Table 1). A marked total depletion of carbohydrate (0.000 kj/g) was recorded on days 28 and 35. From day 42 however, carbohydrate recovered to 0.018 kj/g and increased to 0.71 kj/g on day 56 which was the highest carbohydrate content.

In the second treatment (0.0828 mg/l), carbohydrate decreased from 0.036 kj/g on day 7 to 0.031 and 0.021 kj/g on days 14 and 21, respectively (Table 1). A marked total depletion of carbohydrate (0.000 kj/g) was recorded on days 28 and 35. Day 42 however recorded a recovery of carbohydrate to 0.018 kj/g which increased to 0.72 kj/g on day 56 being the highest carbohydrate content.

In the third treatment (0.00828 kj/g), carbohydrate decreased from 0.036 kj/g in day 7 to 0.000 kj/g on day 28. A consistent increase in carbohydrate was recorded from day 35 (0.001 kj/g) up to day 56 (0.072 kj/g) (Table 1).

The control group recorded consistence and continuous increase in carbohydrate content all through the period of the experiment. Days 7 and 14 recorded the least carbohydrate content of 0.036 kj/g, while day 49 recorded the highest carbohydrate content of 0.073 kj/g (Table 1). Statistical analysis showed a significant difference between the days ($p < 0.05$) in the carbohydrate content of the organism, whereas an insignificant difference ($p > 0.05$) was found between the treatments.

Lipid

In the first treatment (0.828 mg/l), lipid decreased and increased intermittently throughout the period of the experiment (Table 1). There was a decrease in lipid from 12.508 kj/g on day 7 to 6.816 kj/g on day 14, then followed by an increase to 9.348 and 28.308 kj/g on days

21 and 28, respectively. Day 35 recorded the least amount of lipid of 0.295 kj/g, which then increased to 15.723 kj/g on day 42 which being the highest lipid content (Table 1).

In the second treatment (0.0828 mg/l), there was also an intermittent increase and decrease in lipid content (Table 1). Lipid decreased from 18.565 kj/g on day 7 to 4.655 kj/g on day 14, then followed by an increase to 12.377 and 27.913 kj/g on days 21 and 28, respectively. Day 35 recorded the least amount of lipid of 0.316 kj/g, while day 28 gave the highest lipid of 27.913 kj/g (Table 1).

In the third treatment (0.00828 mg/l), a similar trend of intermittent increase and decrease in lipid content occurred. The highest lipid content was recorded on day 42 (27.108 kj/g) while day 35 gave the least lipid content of 0.407 kj/g (Table 1).

The control group recorded the highest lipid content of 40.817 kj/g on day 56, while day 35 has the least lipid of 0.740 kj/g, like in the other groups, lipid decrease and increase as the days went by. Statistical analysis showed a significant difference between the days ($p < 0.05$) in the lipid content as well as between the treatments.

Energy available (Ea)

The energy available as obtained from the study is the sum of the aforementioned three energy parameters (Protein, Carbohydrate, and Lipid) (Table 2). Consequently, every variation in any and/or all of the three energy parameters will have a corresponding effect in the value of the energy available (Ea). From Table 2, the Ea was alternatively decreasing and increasing throughout the study in all experimental groups including the control. In the first treatment (0.828 mg/l), Ea decreased from 14.157 kj/g on day 7 to 8.775 kj/g on day 14. An increase in Ea was recorded on days 21 (10.933 kj/g) and 28 (28.636 kj/g), followed by a drastic decrease to 1.986 kj/g on day 35 which was the lowest Ea (Table 2). Day 42 recorded the highest Ea of 17.396 kj/g (Table 2).

In the second treatment (0.0828 mg/l), Ea decreased from 20.60 kj/g on day 7 to 6.315 kj/g on day 14 and then increased to 13.996 and 28.651 kj/g on days 21 and 28, respectively. Day 35 recorded the least Ea of 2.469 kj/g, while the highest Ea (28.907 kj/g) was recorded on day 42 (Table 2).

In the third treatment (0.00828 mg/l), Ea decreased from 24.291 kj/g on day 7 to 14.193 kj/g on day 21 and then increased to 28.117 kj/g on day 28. Day 35 recorded the least Ea of 2.903 kj/g, while the highest Ea (29.267 kj/g) was recorded on day 42 (Table 2).

The control group, Ea decreased from 26.416 kj/g on day 7 to 18.176 kj/g on day 14. However, Ea increased to 24.176 and 48.613 kj/g on days 21 and 28, respectively.

Table 2. Ea, Ec and CEA of *C. gariepinus* exposed to different sublethal concentrations of cadmium for 56 days.

Day	Treatments (mg/l)	Ea±SD (KJ/g)	Ec±SD (KJ/g)	CEA±SD
7	0.828	14.157±3.18 ^{ac}	4.527±31.44 ^a	3.127±0.25 ^a
	0.0828	20.60±0.41 ^a	4.962±10.51 ^a	4.152±0.12 ^{ab}
	0.00828	24.29±4.071 ^a	2.389±4.87 ^a	10.169±2.83 ^b
	Control	26.416±9.98 ^b	2.811±5.77 ^a	9.397±0.43 ^b
14	0.828	8.775±2.30 ^a	4.578±4.00 ^{ac}	1.917±2.89 ^a
	0.0828	6.315±0.84 ^{ac}	4.292±4.36 ^a	1.471±15.94 ^a
	0.00828	14.508±6.87 ^a	1.209±0.2 ^{ab}	11.999±3.95 ^b
	Control	18.176±2.86 ^{ab}	2.352±1.20 ^a	7.728±5.54 ^b
21	0.828	10.933±2.86 ^a	4.689±5.29 ^{ac}	2.332±8.98 ^a
	0.0828	13.996±3.37 ^a	3.428±2.14 ^a	4.083±10.55 ^a
	0.00828	14.193±8.73 ^a	5.235±0.81 ^{ac}	5.371±1.27 ^a
	Control	24.537±4.59 ^a	1.158±0.49 ^{ab}	21.197±18.56 ^b
28	0.828	28.636±0.75 ^a	9.474±6.43 ^a	3.023±34.73 ^a
	0.0828	28.651±1.28 ^a	9.234±1.28 ^a	3.103±23.67 ^a
	0.00828	28.117±1.32 ^a	5.235±1.32 ^a	5.371±23.85 ^{ab}
	Control	48.613±12.88 ^a	4.792±12.88 ^a	10.144±7.56 ^b
35	0.828	1.986±0.11 ^a	5.795±1.02 ^a	0.343±0.08 ^a
	0.0828	2.469±0.18 ^a	2.853±0.42 ^a	0.865±0.17 ^a
	0.00828	29.267±0.39 ^a	11.944±5.79 ^{ac}	2.450±1.75 ^{ab}
	Control	2.086±3.76 ^b	22.413±5.22 ^a	1.432±0.32 ^a
42	0.828	17.396±5.2 ^a	348.218±51.97 ^{ab}	0.361±0.74 ^{ac}
	0.0828	28.907±0.46 ^a	21.234±1.95 ^a	1.361±0.12 ^a
	0.00828	29.267±0.39 ^a	11.944±5.79 ^{ac}	2.450±1.75 ^{ab}
	Control	2.086±3.76 ^b	22.413±5.22 ^a	1.432±0.32 ^a
49	0.828	7.326±2.28 ^{ab}	52.421±5.69 ^a	0.140±0.03 ^a
	0.0828	13.543±4.01 ^a	46.301±52.05 ^a	0.293±0.76 ^a
	0.00828	16.871±0.04 ^a	9.511±4.03 ^c	1.774±1.54 ^b
	Control	24.233±2.14 ^{ac}	22.856±2.90 ^a	1.060±0.21 ^{ab}
56	0.828	12.609±3.18 ^{ac}	58.540±31.44 ^a	0.215±0.25 ^a
	0.0828	19.469±0.41 ^a	47.554±10.51 ^a	0.409±0.12 ^a
	0.00828	18.969±4.09 ^a	9.953±4.87 ^b	1.906±2.83 ^b
	Control	44.075±9.98 ^{ab}	21.012±5.77 ^a	2.098±0.43 ^b

Columns sharing similar superscripts are not significantly different at P>0.05. Ea: Energy available, Ec: energy consumed, CEA: cellular energy allocation.

Thus day 28 recorded the highest Ea, while day 35 gave the least Ea of 3.788 kJ/g. From day 42 Ea increased to 32.086 kJ/g and then to 44.075 kJ/g on day 56 (Table 2). Statistical results showed a significant difference in energy available (Ea) between days ($p<0.05$) and treatments ($p<0.05$).

Energy consumption (Ec)

Cellular respiration was measured in *C. gariepinus* following sublethal cadmium exposure for 56 days through the *in vitro* electron transport system (ETS) activity. The energy consumption (Ec) was the calculated

value of the energy consumed by the test organisms at the cellular level, which mirror the overall energy consumption (E_c) of the whole organism. The results of energy consumption over periods of exposure are shown in Table 2.

In the first treatment (0.828 mg/l), E_c increased consistently from day 7 (4.527 kJ/g) up to day 28 (9.474 kJ/g), before taking a dive on day 35 to 5.795 kJ/g. The E_c recovered on day 42 to 48.218 kJ/g and continued on the increased to 58.540 kJ/g on day 56 (Table 2).

In the second treatment (0.0828 kJ/g), there was a decrease in E_c from 4.962 kJ/g on day 7 to 3.438 kJ/g on day 21. The E_c rose to 9.234 kJ/g on day 29 and then dropped to 2.853 kJ/g on day 35 which was the least E_c recorded for the treatment. Day 56 recorded the highest E_c of 47.554 kJ/g (Table 2).

In the third treatment (0.00828 mg/l), there was a decrease in E_c between Day 7 (2.389 kJ/g) and day 14 (1.209 kJ/g). The E_c spiked to 5.235 kJ/g on day 28, dropped to 2.293 kJ/g on day 35 which was the least E_c of the treatment before rising to 11.944 kJ/g on day 42 which has the highest E_c (Table 2).

In the control group, E_c decreased from 2.811 kJ/g on day 7 to 1.158 kJ/g on day 21. After day 35 with E_c of 2.455 kJ/g, the E_c increased to 22.856 kJ/g on day 42 was the highest E_c (Table 2). There was significant difference ($p < 0.05$) in the energy consumption (E_c) between the days, but not between the treatments ($p > 0.05$).

Cellular energy allocation (CEA)

Cellular energy allocation (CEA) was calculated as the ratio of the available energy (E_a) to the energy consumption (E_c) (Table 2). Results showed that in general, there was a decrease in the net energy budget with increasing exposure time (Table 2).

In the first treatment (0.828 mg/l), CEA decreased from 3.127 on day 7 to 1.917 on day 14, then rose to 2.332 and 3.032 on days 21 and 28, respectively. Starting from day 35 with CEA of 0.343, CEA decreased to 0.140 on day 49 which was the least CEA of the treatment (Table 2).

In the second treatment (0.0828 mg/l), CEA decreased from 4.152 on day 7 to 1.471 on day 14, it rose to 4.083 on day 21 and then decreased to 0.293 on day 49 which was the least CEA of the treatment (Table 2).

In the third treatment (0.00828 mg/l), CEA decreased from 10.169 kJ/g on day 7 to 1.266 on day 35 which was the least CEA. Day 14 recorded the highest CEA of 11.999 (Table 2).

In the control group, CEA decreased from 9.397 on day 7 to 7.728 on day 14, and afterwards rose to 21.197 on day 21. From day 28 onward, there was continuous decrease in CEA up to day 49 (1.060 kJ/g) which recorded the least CEA (Table 2). Statistically, it showed

significant difference between the days ($p < 0.05$), as well as between the treatments ($p < 0.05$) in the CEA of the test organisms.

DISCUSSION

The results of this study clearly demonstrate that exposure of *C. gariepinus* to sublethal cadmium concentrations interfered with energy metabolism by disrupting energy-producing processes of the catabolism thus causing a decrease in the net energy budget of the test organisms. The amounts of carbohydrate, protein, and lipid recorded over the 56 day exposure period were significantly lower in the cadmium exposed groups compared to the control.

From the individual energy reserve budgets, it is possible to say that carbohydrates were mostly used as the first energy source in all cadmium exposed groups. Depletion of carbohydrates by toxicants has been documented for several species exposed to various toxicants, including *Enchytraeus albidus* when exposed to cadmium (Novais et al., 2013). Soengas et al. (1996) also reported decrease in stored carbohydrate in Atlantic salmon (*Salmo salar* L.) exposed to cadmium. This depletion of carbohydrate came as no surprise since it is the most readily available source of energy (Smolders et al., 2003; Novais and Amorim, 2013).

Together with carbohydrate, lipids constitute the more readily available energy reserve and this was shown in the works of De Coen and Janssen (2003b), Novais and Amorim (2013) and Gomes et al. (2015). In this study, lipid mobilization occurred with a simultaneous increase in energy consumption (E_c) between day 21 and 28 (Table 1). This trend also was observed when carbohydrate depletion was severe. We can say that this mirrors the biological knowledge that lipid reserves serves as a buffer/fall back to cushion and supply energy in stress conditions when carbohydrate supply seriously dwindles (Gomes et al., 2003). Amongst the three energy reserves studied, protein was the least affected following exposure to cadmium (Cd). Despite being less immediate, protein reserves are also known to be an important source of energy (Smolders et al., 2003; Gomes et al., 2015). In this study, exposure of *C. gariepinus* to cadmium (Cd) for 56 days caused a dose-related increase in protein budget. Protein reserves were however observed to increase occasionally (from day 7 to 21, and again from day 35 all through day 56) (Table 1). This pattern of protein increase has been observed in other studies by De Coen and Janssen (1997, 2003a). Smolders et al. (2003) and Gomes et al. (2015) suggested that this occasional increase in protein could be the result of an induction in protein synthesis for detoxification or other defense mechanisms, while the other energy sources (carbohydrate and lipid) are

available.

Although the feeding rate was not measured, the organisms had food *ad libitum* during the entire experiment. As the carbohydrate and lipid were depleting, protein was called up (so to say) to supply the needed nutrient/energy used in repair and replacement of worn out tissue and growth. In addition, the energy consumption rate of the animals significantly increased. Hence effects of cadmium on energy status of the tested animals were not likely related to changes in feeding rate.

Cadmium (Cd) was found to alter the energy consumption rate of the exposed organisms. There was a significant increase in the energy consumption in higher concentration groups compared to the control (Table 2). This trend continued and lasted all through the experiment. Energy consumption (Ec) increased as the exposure time increased. *C. gariepinus* exposed to highest concentration of cadmium (Cd) had Ec rates that were more than twice the rate of the control groups. Novias et al. (2013) also reported increased rate of oxygen consumption in *E. albidus* when exposed to Cd and Zn. Reason for this could be the high energetic cost in the organism to deal with the chemical stress in the process of detoxification, defense, repair and replacement of damaged cells (Gomes et al., 2015).

The effect of Cd in the CEA of *C. gariepinus* was significant at both treatment level and the exposure time. There was reduction in cellular energy allocation (CEA) of exposed groups as well as the control as the study went on. This reduction in cellular energy allocation (CEA) can be explained partly as a result of increased energy consumption and also reduction in the energy reserves. Curiously in certain days 21 and 28, there was an increase in the cellular energy allocation (CEA) in all the treatments including control (Table 2), this can be attributed to the increase in Ea without a corresponding increase in energy consumption (Ec) at the time. De Coen and Janssen (1997) reported similar trend of reduction and seldom increase in cellular energy allocation (CEA) in their study with *Daphnia magna*.

In this study, we found that effect of cadmium on the cellular energy allocation (CEA) of *C. gariepinus* progressed as the experiment went on, suggesting that not only do cadmium toxicity on energy budget dose-dependent, but also depends on the length of exposure. This was evident from the result which showed that the second part of the study recorded the least CEA in the test organisms. Thus in relation to the population level (growth and reproduction), effects of cadmium (Cd) pollution at higher levels of biological organization could be seen in the cellular energy allocation (CEA) when investigated over a considerable length of time in sublethal exposures.

The biomarker cellular energy allocation (CEA) technique was successfully optimized and applied to *C. gariepinus* exposed to cadmium (Cd). The technique

clearly revealed the energy budget of *C. gariepinus* and the distinction between the metal concentration and the exposure time. It was shown that *C. gariepinus* responded differently to cadmium at different sublethal concentrations and at over a prolonged time interval. The technique offered a higher sensitive approach to energy endpoints at lower level of biological organization (e.g. biochemical and physiological).

Conclusion

Exposure to cadmium (Cd) caused significant effects on the physiology of *C. gariepinus* which were detected through the cellular energy allocation (CEA) assay. The study showed that the CEA assay can be used to detect sublethal interactions of environmental pollution with the energetic processes of an organism. The changes at the cellular level occurred at environmentally relevant concentrations of the toxicant. The cellular energy allocation (CEA) results indicate that the toxicity of cadmium (Cd) alters the energy budget of *C. gariepinus* even at sublethal concentrations. The energy available (Ea) and energy consumption (Ec) results pointed to that fact. Changes in the cellular energy allocation (CEA) at both treatment levels and the exposure time resulted more or less from the increase in energy consumption as well as depletion of energy reserves.

Thus from this study, we comment on the cellular energy allocation (CEA) as a more sensitive biomarker in assessing the toxic effects of toxins since it measures effects at a lower level of biological organization (cellular). The present study however, has shown the use of cellular energy allocation (CEA) as an alternative or complementary approach to measure physiological observations in organisms.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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