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Comparative Studies of the Mineral Composition of Two Age-Groups of *Heterobranchus bidorsalis* Exposed to Graded Concentrations of Bonny-light Crude Oil

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ABSTRACT: The macro-and trace-element component of the mineral composition of *Heterobranchus bidorsalis* juveniles (JVs)(14.06 ± 0.38g) and adults (Ads) (138.24 ± 0.16g) were studied on exposure to graded concentrations (1.00 – 8.00 ml L^{-1}) of Bonny-light crude oil (BLCO). The experiment was monitored for 4 days (toxicity) and 42 days (recovery) periods. Insignificant decreases ($P > 0.05$) in the values of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), phosphorus (P), iron (Fe), vanadium (Va), lead (Pb) and manganese (Mn) in the *H. bidorsalis* JVs and Ads corresponded with the increasing concentrations of BLCO in water. The values of Na, K, Mg, Ca, P, Zn Fe, Va Pb and Mn recorded in the JVs were generally lower than those recorded in the ADs and the control fish. Although the removal of the oil pollutant elicited increases in the values of the macro elements (Na, K, Mg Ca and Pb) in the magnitudes of 15% at day 14, 20% at day 28 and 20% at day 42, no definite pattern of increases was established for the trace elements (Zn, Fe, Va Pb and Mn). The apparent depletion in the values of the macro elements with the increasing concentrations of BLCO implies that the crude oil compounds might have impacted negatively on the deposition of these elements in the fish tissues. In addition, the higher values of the trace elements in the ADs than in the JVs implies that irrespective of the BLCO concentration, the Ads incorporated more trace elements in their body tissues than the JVs although the differences in values were insignificant.

Keywords: *Heterobranchus bidorsalis*, Macro element, Trace element, Bonny-light crude oil, Toxicity, Recovery.

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Introduction

Increased industrialization associated with the oil boom of the 1970's in Nigeria has led to increased urbanization and pollution stress on the environment both from industrial and domestic sources (Oguzie, 1976). This author posited that metal inputs from rural areas include metals contained in pesticides, while atmospheric sources include the burning of fossil oils, incineration of wastes and industries. The need to make an assessment of the level of heavy metal contamination in the African aquatic environment has been stated by Calamari and Naeve (1994). Consequently, several pollution monitoring programmes, including the Mediterranean Pollution Monitoring Programme (MEDPOL) covering North, West and Central African Marine Pollution and Research Programme (WACAF 3) and the Eastern African Marine Pollution and Research Programme (EAF/6) were established. It was opined that for effective water pollution control and management, there is need for a clear understanding of the principles of metal contamination.

Fufeyin (1994) investigated the heavy metal contamination of some dominant fish species in Ikpoba River, Benin City, Nigeria and observed that substances discharged into the river were fatal to fish. Fishes are noted to absorb minerals from food the ambient water environment (Lagler *et al.,* 077) hence, it is expected that sea fishes should contain more minerals in their body tissues than freshwater fish. The higher mineral content (calcium) in female osteichthyes than in males especially during the breeding season has been suggested to be due to increase in protein bound calcium during the breeding period (Urist and Schyeide, 1961).

Varying levels of petroleum hydrocarbons have been recorded in the body organs of fishes, frog and snails exposed to oil spills (Akingbade, 1991). Freshwater fishes have been used as 96 – hour bioassay test organisms for the determination of crude oil toxicity (Kopperdaul, 1976). Owing to this reliance, freshwater quality near oil spills is used for estimating the survival and growth of sensitive stages of aquatic organisms. These are no reports in the literature on the effect of crude oil concentrations on the mineral constituents of different age-groups of *Heterobranchus bidorsalis*. Various methods of collecting and integrating data from many specific tests to arrive at a general assessment of the risk posed by chemical pollutants to the aquatic environment have been developed (Cairns and Dickson, 1978; Calaman *et al.,* 1979; Oronsaye and Obano, 1998). These protocols for hazard evaluation provide working models for the extrapolation of single species data to ecosystem predictions.

Many aquatic organisms and fishes have been affected by the incessant oil spillages in the Nigerian coastal environment, that indepth studies of their biochemical and physiological status become imminent. Since various age-groups of fishes are prone to contamination by oil spills, whether from accidents or from the activities of the petroleum industries, this study therefore investigated the mineral composition of two age-groups (juveniles and adults) of *H. bidorsalis* exposed to different concentrations of Bonny-light crude oil. The essence was to ascertain the status of some mineral constituents of the fishes consequent upon the infiltration of crude oil compounds into their body tissues.

Materials and Methods

Six hundred (600) fish specimens of two age-groups of *Heterobranchus bidorsalis* (Geofferey St. Hilaire, 1809) comprising 300 adults (138.24 ± 0.16g) and 300 juveniles (14.06 ± 0.36g) were randomly stocked in 30 aerator-fitted glass aquaria (55 x 30 x 30cm^3) at 20 fish per aquarium. The experiment was designed to have two sets of aquaria in a 4 x 3 arrangement (Completely Randomized Design) to constitute 24 aquaria inundated with 25cm^3 of dechlorinated tap water and contaminated with 5 ml each of Bonny-light crude oil (BLCO) at 1.00, 2.00, 4.00 and 8.00 ml L^{-1} concentrations. Six (6) aquaria were not contaminated with BLCO and were left as the controls. Mosquito-mesh nets were used to cover the aquaria to prevent fish escape.

Two experimental periods were adopted for the study. The toxicity period lasted for 4 days (96h) while the recovery period lasted for 42 days and was monitored at fortnightly (14 days) intervals. Fishes were also monitored daily for mortality and survival records and the data obtained summed up at days 4, 14, 28, and 42. At the end of the toxicity period, the surviving fish and glass aquaria were washed and replenished with dechlorinated tap water. A 38% crude protein diet (Table 1) was fed to fish at 3% body weight per day (b.w.d^{-1}) during the toxicity period (4 days) and at 5% b.w.d^{-1} during the recovery period (42 days). Fish were weighed fortnightly during the recovery period with the aid of a top-loading electronic Mettler balance (Model 600PT) and the diet adjusted in accordance

with the body weight of fish. The filtration systems of the aquaria helped in the elimination of faeces and other residues.

The mineral compositions of the fish were determined at days 4, 14, 28 and 42 of the study period using the method described by Windham, (1996); while that of the diet was determined at the beginning of the experiment. The flame photometric method was used to determine the values of sodium (Na) and potassium (K), while etheylenediamine-tetra-acetic acid (EDTA) titrations were used for those of calcium (Ca) and magnesium (Mg). Complexometric titration method was used for zinc (Zn). For all other minerals tested, the spectrophotometric method of assessment was used (Windham, 1996) and these were all compared with calibrated series. All the data obtained were analyzed using descriptive statistics and analysis of variance (ANOVA) to indicate statistical significance ($P < 0.05$) (Steel and Torrie, 1990). The Duncan's (1955) Multiple Range Test method was employed to partition the differences.

Table 1. Gross and Proximate Compositions of the Experimental Diet Fed to Two Age Groups (Juveniles and Adults) of *Heterobranchus bidorsalis* Stocked in Crude Oil Polluted water

Feed ingredient	**%Composition**
Yellow maize	9.29
Soyabean meal	54.84
Fish meal	16.65
Blood meal	10.97
Palm oil	5.00
Salt	0.25
Vitamin mix[1]	0.60
Mineral mix[2]	2.40
Total	100.00
Nutrients	
Crude protein	37.58
Ether extract	5.18
Ash	10.48
Dry matter	11.80
Nitrogen-free-extract	36.46
Total	100.00

[1]Vitamin mix provided the following constituents diluted in cellulose (mg/kg of diet): thiamine, 10; riboflavin, 20; pyridoxine; 10; folacin, 5; pantothenic acid, 40; chloline chloride, 3,000; niacin, 150; menadione_Na-bisulphate, 80; inositol, 400; biotin 2; vitamin C, 200; alphatocopherol, 200, cholecalcipherol, 1,000,000 1U/g.

[2]Contained as g.kg of premix: $FeSO_4$. $7H_2O$;5; $MgSO_4$. 7HO, 132; K_2SO_4, 329.90; KI, 0.15; NaCl, 45; Na_2SO_4, 88; $AlCl_3$, 0.15; $CoCl_2$. $6H_2O$, 0.50; $CuSO_4$. $5H_2O$; 0.50; $NaSeO_3$, 0.11; $MnSO_4.H_2O$, 0.70; and cellulose, 380.97.

Results

The macro-element components of the mineral composition of the juvenile (JV) and the adult (AD) *Heterobranchus bidorsalis* exposed to 1.00-8.00 ml L^{-1} concentrations of Bonny-light crude oil (BLCO) are shown in Table 2. The values of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca) and phosphorus (P) recorded in the JVs and ADs decreased significantly ($P > 0.05$) with the increasing concentrations of exposure of the fish to the oil pollutant (Table 2). This situation was observed during the 4 days (toxicity) and 42 days (recovery) periods. Nonetheless, the values of these elements in the control fish were higher than those in the JVs and the ADs (Table 2). Comparatively, the values of each macro-element in the ADs were higher than those in the JVs during both experimental periods; although these variations were insignificant ($P > 0.05$) (Table2). Increases in the values of Na, K, Mg, Ca and P in the fishes irrespective of the BLCO concentration were recorded between day 14 and day 42 of the recovery period. These increases were computed to be in the magnitudes of 15% at day 14, 20% at day 28 and 20% at day 42.

Table 3 shows the trace element components of the mineral composition of the test fish. The values of iron (Fe), vanadium (Va), lead (Pb) and manganese (Mn) in both the JVs and ADs also decreased insignificantly ($P > 0.05$) as the BLCO concentrations increased from 1.00 to 8.00 ml L^{-1}. The values of zinc (Zn), however, decreased significantly ($P < 0.01$) and tremendously in the fishes as the BLCO concentration increased (Table 3). Comparatively, the values of each trace element (Zn, Fe, Va, Pb or Mn) recorded in the adult fishes during the toxicity and recovery periods of this study were higher than those in the juveniles. Unlike the macro-elements, no trend was established in the pattern of variation of the trace elements in the fishes as they recuperated from their esposures to the oil pollutants.

Discussion

Calamari and Naeve (1994) expressed the need for researchers to make an assessment of the level of heavy metal contamination of the African aquatic environment. These workers noted that for effective water pollution control and management, there is need for a clear understanding of the principles of metal contamination. In order to achieve this objective, some pollution monitoring programmes were established to cover North, West, Central and East Africa.

The monovalent cations: sodium (Na^+) and potassium (K^+) are primarily involved in ion transport and exchange in fish. An absolute requirement of Na has only been demonstrated in few plants. Wetzel (1975) stated that Na requirements are particularly high in some species of blue green algae and argued that K and other elements cannot be substituted for Na. The concentration of divalent metal ions: magnesium (Mg^{2+}), iron (Fe^{2+}) and zinc (Zn^{2+}) measures the total hardness of water bodies. Both the total hardness and alkalinity of water are measured in $mgCaCO_3$/litre, since calcium carbonate usually dominates (Fufeyin, 1994).

Decreases in the macro-element components of *H. bidorsalis* juveniles and adults, fed with a 38% CP diet in a crude-oil polluted water in this study, were BLCO concentration dependent (Table 2). The deposition of these elements namely: Na, K, Mg, Ca and P in the fishes were, however, relatively better expressed in the ADs than in the JVs. This implies that the adult *H. bidorsalis* were more disposed to absorb these minerals from food and the oil-polluted water environment than the juveniles. Since fishes are noted to absorb minerals from food and the ambient water environment (Lagler *et al.*, 1977), the adult *H. bidorsalis* were more amenable to incorporate these minerals in their body tissues than the juveniles. This state of affairs was evident during the 4 days (toxicity) and the 42 days (recovery) periods. Increased concentrations of exposure of the fish to BLCO (1.00 – 8.00 ml L^{-1}) apparently depleted the values of the macro-elements in both the JVs and the ADs. This implies that increased crude oil concentrations in water might have impacted negatively on the deposition of these macro-elements in the fish tissues. The important role played by Ca, P and Mg in bone formation in animals (in order to keep shape) is well known. Hence, the JVs subjected to crude oil pollution in this study had a higher propensity to retarded formation of their bone/skeletal structure than the ADs. Welcome (1979) reported that nitrate ions as well as calcium, magnesium, phosphorus and chloride ions/elements could enhance the growth, survival and reproduction of fish in its water medium. Hence, the utility of dietary and aqueous minerals by fishes in this study in order to ensure growth and survival was better expressed in the ADs than in the JVs. The improvement in the values of the macro-elements during the recovery period (Table 2) implies that the removal of the crude oil stress improved the quantity of these minerals deposited in the fish. This improvement was also better expressed in the ADs than in the JVs.

Table 2. Macro-elements of the Mineral Composition of Juvenile* and Adult *Heterobranchus bidorsalis* Exposed to Graded Concentrations of Bonny-light Crude Oil for 4 Days (Toxicity) and 42 Days (Recovery) Periods**

Study period	Duration (days)	Nutrient	Control 0.00 ml L^{-1}		BLCO[1] Concentrations (ml L^{-1})							
					1.00		2.00		4.00		8.00	
			JV[2]	AD[3]	JV	AD	JV	AD	JV	AD	JV	AD
Toxicity period	4	Na[4]	0.06 ± 0.02[a]	0.07 ± 0.03[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.01 ± 0.00[ab]	0.01 ± 0.00[ab]
		K[5]	0.09 ± 0.02[a]	0.11 ± 0.04[a]	0.09 ± 0.03[a]	0.11 ± 0.03[a]	0.08 ± 0.02[a]	0.09 ± 0.03[a]	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.03 ± 0.01[b]	0.04 ± 0.02[b]
		Mg[6]	0.05 ± 0.01[a]	0.07 ± 0.03[a]	0.05 ± 0.02[a]	0.06 ± 0.02[a]	0.03 ± 0.01[a]	0.04 ± 0.02[a]	0.02 ± 0.01[ab]	0.02 ± 0.01[ab]	0.01 ± 0.00[ab]	0.01 ± 0.00[ab]
		Ca[7]	0.18 ± 0. 02[a]	0.21 ± 0.05[a]	0.17 ± 0.03[a]	0.20 ± 0.04[a]	0.16 ± 0.02[a]	0.19 ± 0.04[a]	0.15 ± 0.03[ab]	0.18 ± 0.03[a]	0.12 ± 0.02[b]	0.14 ± 0.03[b]
		P[8]	0.24 ± 0.03[a]	0.29 ± 0.04[a]	0.23 ± 0.03[a]	0.27 ± 0.04[a]	0.22 ± 0.03[ab]	0.26 ± 0.05[a]	0.20 ± 0.01[b]	0.23 ± 0.04[a]	0.16 ± 0.03[c]	0.19 ± 0.03[c]
Recovery period	14	Na	0.07 ± 0.02[a]	0.08 ± 0.03[a]	0.04 ± 0.02[a]	0.05 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.02 ± 0.01[ab]	0.03 ± 0.02[a]	0.01 ± 0.00[ab]	0.02 ± 0.01[ab]
		K	0.11 ± 0.02[a]	0.03 ± 0.03[a]	0.11 ± 0.03[a]	0.13 ± 0.02[a]	0.09 ± 0.03[a]	0.11 ± 0.03[a]	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.04 ± 0.01[b]	0.05 ± 0.01[b]
		Mg	0.07 ± 0.02[a]	0.08 ± 0.02[a]	0.07 ± 0.02[a]	0.07 ± 0.02[a]	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.02 ± 0.01[ab]	0.03 ± 0.01[a]	0.01 ± 0.00[ab]	0.01 ± 0.00[ab]
		Ca	0.21 ± 0.03[a]	0.24 ±± 0.01[a]	0.20 ± 0.01[a]	0.23 ± 0.04[a]	0.19 ± 0.04[a]	0.22 ± 0.03[a]	0.18 ± 0.03[ab]	0.21 ± 0.03[a]	0.14 ± 0.03[b]	0.16 ± 0.02[b]
		P	0.29 ± 0.03[a]	0.34 ± 0.03[a]	0.26 ± 0.03[b]	0.31 ± 0.04[a]	0.26 ± 0.04[b]	0.30 ± 0.04[a]	0.23 ± 0.03[b]	0.27 ± 0.02[b]	0.19 ± 0.03[c]	0.22 ± 0.03[b]
	28	Na	0.09 ± 0.02[a]	0.10 ± 0.01[a]	0.05 ± 0.02[a]	0.06 ± 0.01[a]	0.04 ± 0.01[ab]	0.05 ± 0.02[a]	0.03 ± 0.01[ab]	0.04 ± 0.01[ab]	0.02 ± 0.01[ab]	0.03 ± 0.01[ab]
		K	0.14 ± 0.03[a]	0.16 ± 0.02[a]	0.14 ± 0.03[a]	0.16 ± 0.02[a]	0.13 ± 0.03[a]	0.15 ± 0.02[a]	0.01 ± 0.00[b]	0.01 ± 0.00[b]	0.05 ± 0.02[b]	0.06 ±0.02[b]
		Mg	0.09 ± 0.02[a]	0.10 ± 0.01[a]	0.08 ± 0.02[a]	0.09 ± 0.02[a]	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.03 ± 0.01[ab]	0.04 ± 0.01[ab]	0.01 ± 0.00[ab]	0.01 ± 0.00[ab]

Table 2 (Contd.)

Study period	Duration (days)	Nutrient	Control 0.00 ml L^{-1}		BLCO[1] Concentrations (ml L^{-1})							
					1.00		2.00		4.00		8.00	
			JV[2]	AD[3]	JV	AD	JV	AD	JV	AD	JV	AD
		Ca	0.26 ± 0.03[a]	0.30 ± 0.03a	0.24 ± 0.04[ab]	0.28 ± 0.04[a]	0.23 ± 0.04[ab]	0.27 ± 0.04[a]	0.22 ± 0.03[ab]	0.26 ± 0.04[a]	0.17 ± 0.03[c]	0.20 ± 0.04[abc]
		P	0.35 ± 0.04[a]	0.41 ± 0.03[b]	0.33 ± 0.04[a]	0.38 ± 0.04[a]	0.29 ± 0.04[ab]	0.34 ± 0.04[a]	0.28 ± 0.04[ab]	0.33 ± 0.04[a]	0.23 ± 0.03[abc]	0.27 ± 0.03[ab]
		Na	0.10 ± 0.02[a]	0.12 ± 0.02[a]	0.07 ± 0.02[a]	0.08 ± 0.02[a]	0.05 ± 0.02[ab]	0.06 ± 0.01[ab]	0.04 ± 0.01[ab]	0.05 ± 0.01[ab]	0.03 ± 0.01[b]	0.04 ± 0.02[ab]
		K	0.18 ± 0.03[a]	0.20 ± 0.02[a]	0.17 ± 0.03[a]	0.20 ± 0.03[a]	0.15 ± 0.03[a]	0.18 ± 0.03[a]	0.02 ± 0.01[b]	0.02 ± 0.01[b]	0.07 ± 0.02[b]	0.08 ± 0.02[ab]
	42	Mg	0.10 ± 0.02[a]	0.12 ± 0.03[a]	0.09 ± 0.02[a]	0.11 ± 0.02[a]	0.07 ± 0.02[a]	0.08 ± 0.02[a]	0.04 ± 0.01[ab]	0.05 ± 0.02[ab]	0.01 ± 0.00[bc]	0.02 ± 0.01[bc]
		Ca	0.31 ± 0.04	0.36 ± 0.04	0.29 ± 0.03	0.34 ± 0.04	0.28 ± 0.03	0.33 ± 0.04	0.27 ± 0.03	0.32 ± 0.03	0.20 ± 0.03	0.24 ± 0.03
		P	0.43 ± 0.04[a]	0.50 ± 0.04[b]	0.39 ± 0.04[c]	0.46 ± 0.04[a]	0.35 ± 0.03[c]	0.41 ± 0.04[a]	0.31 ± 0.03[c]	0.39 ± 0.03[cd]	0.28 ± 0.04[e]	0.33 ± 0.04[cd]

[1]Bonny-light crude oil, [2]Juvenile, [3]Adult, [4]Sodium, [5]Potasium, [6]Magnesium, [7]Calcium, [8]Phosphorus, Values in the same row followed by the same superscripts are not significantly different (P > 0.05). Values in the same row followed by different superscripts differ significantly (P < 0.05); *Seven weeks old, **Fifteen months old.

Table 3. Trace Elements of the Mineral Composition of Juvenile* and Adult *Heterobranchus bidorsalis* Exposed to Graded Concentrations of Bonny-light Crude Oil for 4 Days (Toxicity) and 42 Days (Recovery) Periods.**

Study period	Duration (days)	Nutrient	Control 0.00 ml L^{-1}		BLCO[1] Concentrations (ml L^{-1})							
					1.00		2.00		4.00		8.00	
			JV[2]	AD[3]	JV	AD	JV	AD	JV	AD	JV	AD
Toxicity period	4	Zn[4]	0.54 ± 0.03[a]	0.63 ± 0.02[b]	0.43 ± 0.03[c]	0.51 ± 0.03[a]	0.03 ± 0.01[d]	0.04 ± 0.01[d]	0.02 ± 0.01[d]	0.03 ± 0.01[d]	0.01 ± 0.00[d]	0.02 ± 0.01[d]
		Fe[5]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
		Va[6]	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]
		Pb[7]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]
		Mn[8]	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.02 ± 0.00[a]	0.03 ± 0.01[a]	0.01 ± 0. 00[a]	0.02 ± 0.01[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]	0.01 ± 0. 00[a]
Recovery period	14	Zn	0.54 ± 0.03[a]	0.64 ± 0.02[b]	0.50 ± 0.02[c]	0.59 ± 0.02[d]	0.04 ± 0.01[e]	0.05 ± 0.01[e]	0.02 ± 0.01[e]	0.03 ± 0.01[e]	0.02 ± 0.01[e]	0.03 ± 0.01[e]
		Fe	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]
		Va	0.05 ± 0.02[a]	0.06 ± 0.01[a]	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.02 ± 0.00[a]	0.03 ± 0.01[a]	0.01 ± 0.00[ab]	0.02 ± 0.01[a]	0.01 ± 0.00[ab]	0.02 ± 0.01[a]
		Pb	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]
		Mn	0.05 ± 0.01[a]	0.06 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.01 ± 0.00[a]	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]
	28	Zn	0.53 ± 0.03[a]	0.62 ± 0.03[b]	0.60 ± 0.03[b]	0.71 ± 0.04[c]	0.05 ± 0.02[d]	0.06 ± 0.02[d]	0.03 ± 0.01[d]	0.04 ± 0.01[d]	0.03 ± 0.01[d]	0.04 ± 0.01[a]
		Fe	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ±0.01[a]
		Va	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.05 ± 0.01[a]	0.06 ± 0.02[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.02 ± 0.00[a]	0.03 ± 0.01[a]	0.02 ± 0.00[a]	0.03 ± 0.01[a]

Table 3 (Contd.)

Study period	Duration (days)	Nutrient	Control 0.00 ml L^{-1}		BLCO[1] Concentrations (ml L^{-1})							
					1.00		2.00		4.00		8.00	
			JV[2]	AD[3]	JV	AD	JV	AD	JV	AD	JV	AD
		Pb	0.01 ± 0.00[a]	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.03 ± 0.01[a]
		Mn	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.02 ± 0.00[a]	0.03 ± 0.01[a]	0.01 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]
		Zn	0.54 ± 0.03[a]	0.63 ± 0.03[b]	0.72 ± 0.04[c]	0.85 ± 0.03[d]	0.06 ± 0.02[e]	0.07 ± 0.02[e]	0.04 ± 0.01[e]	0.05 ± 0.01[e]	0.04 ± 0.01[e]	0.05 ± 0.01[e]
	42	Fe	0.02 ± 0.00[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.01 ± 0.00[a]	0.02 ± 0.00[a]	0.02 ± 0.00[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]
		Va	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.06 ± 0.01[a]	0.07 ± 0.02[a]	0.04 ± 0.01[a]	0.05 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]
		Pb	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.01 ± 0.00[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]
		Mn	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.05 ± 0.02[c]	0.06 ± 0.02[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]	0.02 ± 0.01[a]	0.03 ± 0.01[a]	0.03 ± 0.01[a]	0.04 ± 0.01[a]

[1]Bonny-light crude oil, [2]Juvenile, [3]Adult, [4]Zinc, [5]Iron, [6]Vanadium, [7]Lead, [8]Manganese, Values in the same row followed by the same superscripts are not significantly different ($P > 0.05$). Values in the same row followed by different superscripts differ significantly ($P < 0.05$); *Seven weeks old, **Fifteen months old.

The trend of trace-elements deposition in the fish tissues (Table 3) closely followed the pattern shown by the deposition of the macro-elements. Decreases in the values of Zc, Fe, Va, Pb and Mn in the fish were also dependent on the increasing concentrations of BLCO in the water. The results also show that irrespective of the concentration of the BLCO in the water, the adult fishes incorporated more trace-elements in their body tissues than the juveniles; although differences in the values obtained were insignificant ($P > 0.05$) (Table 3).

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