academicJournals

Vol. 7(8),pp. 228-237, August, 2015
DOI: 10.5897/JENE2015. 0520
Article Number: 22407AA54993
ISSN2006-9847

http://www.academicjournals.org/JENE

Journal of Ecology and the Natural Environment

Full Length Research Paper

Determination of cyanide level in Igun Reservoir and in tissues of fishes and activity of cyanide-detoxifying enzymes in these tissues

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Received 10 April, 2015; Accepted 1 June, 2015

The physicochemical properties of water from Igun gold mine reservoir in Southwestern, Nigeria were assessed to estimate the impacts of human activities on the water and the level of cyanide-detoxifying enzymes in fish species were investigated. This was with a view to studying the survival of the fish species in the reservoir despite its high level of pollution. These werewith a view to studying the survival of the fish species in the reservoir despite its high level of pollution. Water parameters and level of cyanide in the reservoir were determined for two seasons using standard methods. Level of cyanide in selected fish tissues was determined using distillation and titration techniques. Enzyme activities were determined using standard methods. Results show that dissolved oxygen (3.82±0.23 mg/L), total hardness (11.83±0.31 mg/L) and pH (7.06±0.05) have higher mean values during rainy season unlike transparency (62.44±22.23 cm), total alkalinity (86.00±2.00 mg/L), temperature (27.00±1.00°C) and conductivity (170.90±0.70 mg/L) which peaked during the dry season. Analysis of cyanide in the water showed that a higher mean level of cyanide (36980±6973.57 µg/L) was recorded in the rainy season as against 26150.22±1046.59 µg/L for the dry season. Negative results recorded for cyanide level in the fish tissues indicated that fishes do not bioaccumulate the compound. In all fish tissues studied, rhodanese and 3-MST were present in different amounts. Also, inter-species differences were noticed in the levels of enzymes in the tissues. In all the fish tissues studied, the liver showed highest mean value of rhodanese and 3-MST activities while fillet showed lowest mean value. The study concludes that Igun gold mine reservoir is highly cyanide-polluted owing to indiscriminate artisanal mining activities which incessantly impact the reservoir. However, these fishes did not bioaccumulate cyanide in their tissues which make fishes obtained from the environment fit for human consumption.

Key words: Mining, cyanide, rhodanese, 3-mercaptopyruvate sulphurtransferase,Igun Reservoir.

INTRODUCTION

Nigeria has a long but discontinuous history of mining and the country was a prominent exporter of gold, tin, colombite and coal in the early 1913 (Nigeria Geological Survey Agency, 2006). The production of gold declined during the Second World War II period and never recovered as mines were abandoned by the colonial companies. The Nigerian Mining Corporation started exploration for gold in the early 1980s but failed to be

sustained due to lack of funds. The discovery of petroleum and its subsequent domination of the Nigerian economy also contributed to the lack of attention to gold exploration despite the widespread potentials (Nigeria Geological Survey Agency, 2006; Mallo, 2012). In the absence of systematic exploration and development, the Nigerian gold fields have experienced intense artisanal workings. About 0.3 to 0.4 g of cyanide per tonne of typical ore has been recommended to dissolve and extract gold (Environment Australia, 1998). However, in practice, consumption ranges from 300 g per tonne to more than 2000 g per tonne (Environment Australia, 1998).

Hydrometallurgical method based on cyanidation process has constantly been used for extraction of gold from Igun gold ore deposit in AtakunmosaWest Local Government Area of Osun State (Mesubi et al., 1999). Gold cyanidation involves the use of sodium cyanide solution in the presence of excess oxygen gas to extract gold from its ore (Jha, 1986; Young, 1993). The mining industry, and in particular the gold mining industry, has been using cyanide in its production process for many decades (Mark et al., 1999). While cyanide is commonly perceived as being a deadly substance, it is in fact a widely used chemical that is essential to the modern world. Cyanide is a common industrial chemical that is readily available at a reasonably low cost. For economic reasons, cyanide is the chemical of choice for the recovery of gold from ores (Mark et al., 1999).

The use of cyanide in the leaching of gold ore has an increased potential to impact the environment because of the greater quantity used (Da Rosa and Lyon, 1997). This has created a number of serious environmental problems affecting wildlife and water management (Eisler and Wiemeyer, 2004).Cyanide is a highly toxic compound that is readily absorbed and causes death by preventing the use of oxygen by tissues (Egekeze and Oehme, 1980). Therefore, cyanides are one of the major classes of toxic chemicals of concern for aquatic biota in certain waste-receiving waters (Towill et al., 1978).

Cyanide hazards to fish, wildlife, livestock and man are well documented. Fish and aquatic invertebrates are particularly sensitive to cyanide exposure. High fish mortality to cyanide has been documented at free cyanide concentrations > 20 µg/L and adverse effects on swimming and reproduction at > 5 µg/L (USEPA, 1989). Early research found cyanide to be acutely toxic at concentration greater than 100-300 µg/L causing death within 96 h, chronic toxicity also occurs when fish exposed to cyanide do not die within 96 h, but suffers stress which leads to their subsequent death. Environmentally, relevant exposures to cyanide ions can cause stress, increase mortality and an appreciable metabolic load on fishes and other aquatic organisms (Eisler, 1991). Massive kills of fresh water fish by accidental discharges of cyanide waste are fairly common (Holden and Marsden, 1964;Towill et al*.,* 1978; USEPA, 1980). In one case, cyanide-containing mine effluent from a Canadian tailings pond released into a nearby creek killed more than 20,000 steelhead (*Oncorrhynchus mykiss*) (Leduc et al., 1982).

Enzymes have a great potentiality to effectively transform and detoxify cyanide containing substances because they have been recognized to be able to transform cyanide at a detectable rate and are potentially suitable to restore polluted environments (Rao et al., 2010).

Two major enzymes [(rhodanese: EC. 2.8.1.1., thiosulphate: cyanide sulphurtransferase (TST) and 3-mercaptopyruvate sulphurtransferase (3-MST) EC.2.8.1.2)] represent two chief enzymes involved in cyanide detoxification (Nagahara et al., 1999; Agboola et al., 2006). The liver has always been considered to be the major source of rhodanese and is believed to be the major site of cyanide detoxification (Sorbo, 1953; Lee et al., 1995). The enzyme has also been reported to be located in the cytosol and other organelles (Nagahara et al., 1999; Agboola and Okonji, 2004). The activity of these enzymes in a particular tissue/organ reflects the ability of that tissue/organ to detoxify cyanide (Ali et al., 2001).

Therefore, the present study investigates the level of cyanide in Igun reservoir and in selected tissues of fish species from the reservoir as well as the activity of cyanide-detoxifying enzymes in these tissues.

MATERIALS AND METHODS

Winkler's I reagent (manganese (VI) sulphate), Winkler's II reagent (potassium iodide with sodium hydroxide), tetraoxosulphate(VI) acid, ethylenediaminetetraacetic acid (EDTA), eriochrome black-T indicator, sodium thiosulphate II (Pentahydrate), Bromocresol green, methyl red, mercaptoethanol, potassium cyanide, boric acid, sodium borate, formaldehyde, ferric nitrate, nitric acid, disodium hydrogen phosphate, sodium di-hydrogen orthophosphate, Tris-base, Tris-HCl, sodium chloride, sodium acetate, acetic acid and diathizone were obtained from British Drug House (BDH) Chemical Limited, Poole, England. Coomasie brilliant blue, bovine serum albumin (BSA), silver nitrate, sodium hydrogen pellet, absolute ethanol (95%), phosphoric acid, mercuric chloride, stannous chloride and N-ε-aminocaproic acid were obtained from Sigma Chemical Company, St. Louis, U.S.A. All other reagents used were of analytical grade and were obtained from either Sigma or BDH.

Fish species and water used were obtained from an abandoned gold mine reservoir located atIgun inAtakunmosa West Local Government Area of Osun State. The reservoir extends over longitude 004°30'Eto 004°45'E and from latitude 007°35'N to 0047°38'N.

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Water sampling

Water samples were collected on monthly basis between 9.00 and 10.00 am over two seasons between August 2013 and February 2014 with sampling bottles. The bottles were rinsed with distilled water. Each bottle was immersed in the reservoir to allow air out and also corked inside the reservoir when filled with water. Ten to twelve drops of 0.25 N NaOH were immediately added to the sample water in the bottles (Agrawal et al., 1991).

Fish sampling

Fish speciessamples were obtained on monthly basis between August 2013 and February 2014 covering rainy and dry seasons. Gill-net with 2.57 cm mesh size measuring 50 m long and 3 m in depth was the fishing gear used. Also, five traps made from *Eremospatha*sp.in the form of a funnel with non-return valves were baited with palm fruits and set under sedges. Live fish specimens caught were stored in ice-chest box, covered with ice and brought to the laboratory. The gills, gut, liver and fillet were taken from each fish and placed in sampling bottles, labelledand kept in freezer until the time of analysis. Fish species were identified using the keys prepared by Reed et al. (1967) and Adesulu and Sydenham (2007).

Measurement of physicochemical parameters of the water

Water temperature was determined *in situ* using mercury-in-glass thermometer. The pH of the water was measured using the pH meter, Mettler MP 200 (Okonji et al., 2013). For dissolved oxygen (DO) determination, water samples were fixed on site with 2 ml each of Winkler's I ($MnSO_4$) and Winkler's II (KI/NaOH) reagents between 9.00 and 10.00 am. Upon arrival, 2 ml of concentrated tetraoxosulphate (VI) acid was added to the fixed samples and then mixed gently. The DO was determined by titrating 50 ml of the water samples against standard sodium thiosulphate solution (0.025 N) using five drops of starch indicator until the mixture turned from blue-black to a colourless solution (Golterman et al., 1978). Total alkalinity was determined by titrating 50 ml of water samples against tetraoxosulphate (VI) acid (0.02 N) using five drops of bromocresol green-methyl red as indicator until the sample changed from blue-green to pink (Golterman et al., 1978). Water transparency of the reservoir was determined with a secchi-disc measuring 15 cm in diameter (Quayle, 1988). Conductivity meter was used to determine the electrical conductivity of the water from the reservoir (Schiefelbein et al., 1998). Water hardness was determined using hardness EDTA titration technique (Constance, 1994). All the instruments were calibrated (standardized) before use.

Determination of mean cyanide level in water

The level of cyanide in water from Igun gold mine reservoir was determined by distillation and titration according to the method of Yeoh and Oh (1979) as slightly modified by the Federal Industrial Research Institute, Oshodi, Lagos, Nigeria (Solomon, 1998). The water sample was poured into a round bottom flask of a distillation unit and a conical flask containing 50 ml of 1% alcoholic sodium hydroxide solution was placed at the collecting end of the distillation unit while the mixture in the flask was been heated. The distillate was collected into the alkaline medium until about 200 ml was obtained. The distillate was then titrated against 0.02 M $AgNO_3$ using 1 ml of freshly prepared 0.5% dithizone as indicator. The end point was indicated by a colour change from yellow to purple. Level of cyanide in the reservoir was calculated using the correlation by Yeoh and Oh (1979).

Preparation of tissue extracts

The different fish samples obtained during the sampling period were used in this preparation. Prior to extraction, fish species were dissected and various tissues of interest (gill, gut,liver and fillet) were removed. These were later kept in the refrigerator until required. Tissue extracts were prepared by homogenising 10 g (w/v) of each tissue in 3 volume of homogenization buffer (0.1 M phosphate buffer, pH 7.2). The homogenates were centrifuged for 20 min at 6708 (xg). Their supernatants were used as the source of enzyme.

Protein concentration determination

Bradford method (1976) was used to measure the protein concentrations using bovine serum albumin (BSA) as standard.

Determination of mean cyanide level in fish tissues

The method described by Solomon (1998) was adopted for this study. Fresh tissue (20 g) obtained from individual fish species was mashed in a mortar before been soaked in 20 ml distilled water, 200 ml of 0.1 M phosphate buffer (pH 6.0) and 10 ml 2% mercuric chloride solution in a conical flask. Hydrated stannous chloride (5 g) was added to the soaked mixture so as to convert the liberated cyanide into salt. The conical flask was sealed and allowed to stay for 12 h. The content of the flask was poured into the round bottom flask of a distillation unit. Distillate collected was treated as water discussed above.

Assay of enzymes

Rhodanese was assayed by the method of Agboola and Okonji (2004). 3-Mercaptopyruvate sulphurtransferase (3-MST) activity was measured according to the modified method of Taniguchi and Kimura (1974) by Agboola et al. (2006) using mercaptoethanol as substrate. Absorbance was also measured at 460nm. One rhodanese unit and one 3-MST unit were defined as the amount of enzyme which produced an optical density of 1.08 per minute at 460nm under the given condition (Sorbo, 1951).

Statistical analysis

Data were analyzed by one-way ANOVA using graphpad prism 5.0 software. Tukey's multiple comparison test was used for paired comparisons. The results were presented as means ± SEM. A p-value < 0.05 was considered statistically significant.

RESULTS

Physicochemicalparameters of water quality

The physicochemical parameters of Igun reservoir are presented in Table 1. The dissolved oxygen was fairly higher in the rainy season (3.82±0.23 mg/L) as compared to the dry season (3.32±0.02 mg/L); while total alkalinity was lower in the rainy season (78.12±1.41 mg/L) than in the dry season (86.00±2.00 mg/L). Water temperature was found to range from 22 to 27°C during the two seasons. A higher mean temperature value recorded in the dry season was 27.00±1.00°C as compared to 22.40

Table 1. Physicochemical parameters of water quality (August, 2013 – February, 2014).

Mean of water parameter	Rainy season	Dry season
Level of cyanide (µg/L)	36980.67±6973.57[a]	26150.22±1046.59[b]
Water temperature (°C)	24.40±0.81[b]	27.00±1.00[a]
Dissolved oxygen (mg/L)	3.82±0.23[a]	3.32±0.02[b]
Total alkalinity	78.12±1.41[b]	86.00±2.00[a]
Transparency (cm)	33.04±0.03[b]	62.44±22.23[a]
Hardness (mg/L)	11.83±0.31[a]	11.76±0.01[a]
Conductivity (mg/L)	153.54±6.05[b]	170.90±0.70[a]
pH	7.06±0.05[b]	7.00±0 .10[b]

Values with superscript a > b; otherwise no pronounced difference.

Table 2. Mean (±SEM) total protein in tissue homogenates of fish species.

Species of fish	Protein (mg)			
	Gill	Gut	Liver	Fillet
M. electricus	N.O	388.490	219.334	444.376
T. dageti	282.91±100.646	489.6±32.674	347.5±131.425	412.30±205.827
O. niloticus	627.180±206.117	538.968±297.104	271.303±101.996	696.39±204.264
C. guntheri	474.136±127.353	350.574±138.732	290.539±92.232	418.26±151.125
T. zillii	510.939±245.053	886.419±643.294	572.861±357.639	803.346±262.025
T. mariae	595.302±105	524.517±129.977	905.555±184.214	663.808±108.467
C. gariepinus	550.659±372.772	509.618±316.84	353.527±237.906	1176.086±132.082
S. galilaeus	376.293±43.537	506.069±87.312	179.470±28.452	215.376±148.238
C. acutirostre	612.707±465.294	444.185±253.715	274.201±182.670	706.533±233.568
P. obscura	478.488±107.613	405.272±65.228	239.716±30.996	964.783±663.418
H. odoe	8.259[e,z]	3.685[ab,y]	9.241[ae,x]	4.074[ce,y]

N.O- Not observed.

±0.80°C observed in the rainy season. Transparency was found to increase during the dry season with 62.44±22.23 cm as compared to 33.04 ± 0.03 cm recorded during the rainy season, while the electrical conductivity of the water was found to have a higher value (170.90 ± 0.70 mg/L) during the dry season than 153.54 ± 6.05 mg/L recorded during the rainy season. The mean values of pH and hardness during the seasons were 7.06±0.05 (rainy), 7.00±0.10 (dry) and 11.83±0.31 mg/L (rainy), 11.76±0.01 mg/L (dry), respectively. The mean value for the level of cyanide during the rainy season (36980.67±6973.57 µg/L) was significantly different from 26150.22±1046.5 µg/L recorded during the dry season.

Species of fish observed in Igun Reservoir

Eleven fish species were observed in the course of sampling the reservoir. They were *Malapterurus electricus*(1)*, Tilapia dageti*(2), *Oreochromis niloticus*(3)*, Chromidotilapia guntheri*(4)*, Tilapia zillii*(7)*, Tilapia mariae*(3)*, Clarias gariepinus*(2)*, Sarotherodon galilaeus*(2)*, Ctenopoma acutirostre*(2)*, Parachanna obscura*(2) and *Hepsetus odoe*(1)*.* Selected tissues of interest were found in all the species only with the exception of gill which was absent in *M. electricus*.

Total protein

The level of total protein in the tissues of the fish species are presented in Table 2. Gill of *O. niloticus;* gut of *T. zillii;* liverof *T. mariae*and fillet of *C. gariepinus*had higher protein in the tissues across the species, respectively.

Distribution patterns of mean total rhodanese and 3-mercaptopyruvate sulphurtransferase activities in tissues of fish species

The activities of the two enzymes in the selected tissues

Table 3. Mean (±SEM) total rhodanese activity in tissue homogenates of fish species.

Species of fish	Protein (RU/mg)			
	Gill	Gut	Liver	Fillet
M. electricus	N.O	$9.296^{a,x}$	$22.556^{a,y}$	$6.389^{c,x}$
T. dageti	$9.018 \pm 2.7^{a,x}$	$6.037 \pm 2.1^{a,y}$	$12.185 \pm 2.7^{a,z}$	$3.270 \pm 2.9^{c,y}$
O. niloticus	$9.241 \pm 2.7^{b,x}$	$4.136 \pm 2.6^{a,y}$	$13.380 \pm 2.7^{c,z}$	$4.302 \pm 1.5^{cd,y}$
C. guntheri	$10.931 \pm 0.6^{c,w}$	$8.245 \pm 1.2^{b,x}$	$17.074 \pm 0.9^{b,y}$	$7.778 \pm 1.8^{a,z}$
T. zillii	$8.635 \pm 1.2^{d,x}$	$6.365 \pm 0.8^{c,y}$	$16.145 \pm 1.3^{d,z}$	$4.979 \pm 0.8^{b,y}$
T. mariae	$9.302 \pm 3.8^{b,x}$	$7.629 \pm 2.0^{d,x}$	$21.994 \pm 2.2^{b,y}$	$9.080 \pm 1.8^{a,x}$
C. gariepinus	$6.472 \pm 3.0^{d,w}$	$3.657 \pm 2.3^{ab,x}$	$15.083 \pm 0.5^{a,y}$	$3.673 \pm 3.0^{cd,w}$
S. galilaeus	$4.342 \pm 0.6^{e,w}$	$2.574 \pm 0.1^{ab,x}$	$7.741 \pm 1.0^{a,y}$	$2.436 \pm 0.1^{c,x}$
C. acutirostre	$4.954 \pm 0.9^{de,w}$	$2.796 \pm 0.6^{ab,x}$	$8.250 \pm 0.7^{a,y}$	$2.425 \pm 0.4^{c,x}$
P. obscura	$5.222 \pm 0.6^{de,x}$	$3.657 \pm 0.6^{a,x}$	$8.574 \pm 1.3^{a,y}$	$3.241 \pm 0.1^{c,x}$
H. odoe	$8.259^{e,z}$	$3.685^{ab,y}$	$9.241^{ae,x}$	$4.074^{ce,y}$

$^{a–f}$Mean ± SEM in each column with no common superscript differ significantly (p <0.05); $^{w–z}$Mean ± SEM in each row with no common superscript differ significantly (p <0.05); N.O- not observed. *M. electricus* are bimodal breathers capable of using lungs for respiration in highly polluted environment when their gills degenerate (Okafor, 2004; Loong et al., 2012).

Table 4. Mean (±SEM) total 3-MST activity in tissue homogenates of fish species.

Species of fish	Protein (MU/mg)			
	Gill	Gut	Liver	Fillet
M. electricus	N.O	$6.481^{a,w}$	$11.110^{b,x}$	$2.611^{a,y}$
T. dageti	$3.556 \pm 1.1^{ad,z}$	$1.592 \pm 0.4^{ae,z}$	$6.306 \pm 1.9^{b,z}$	$5.972 \pm 4.0^{a,z}$
O. niloticus	$5.222 \pm 1.3^{a,w}$	$3.111 \pm 2.0^{a,y}$	$7.704 \pm 1.0^{a,x}$	$2.802 \pm 1.4^{a,y}$
C. guntheri	$5.417 \pm 1.1^{b,x}$	$5.560 \pm 2.6^{b,x}$	$9.153 \pm 0.9^{c,y}$	$3.033 \pm 0.7^{a,z}$
T. zillii	$6.132 \pm 1.0^{c,y}$	$4.688 \pm 1.0^{c,y}$	$9.442 \pm 0.8^{e,x}$	$3.947 \pm 1.4^{ab,w}$
T. mariae	$8.031 \pm 3.1^{b,w}$	$6.728 \pm 1.6^{b,w}$	$12.778 \pm 2.0^{c,x}$	$4.321 \pm 1.3^{a,y}$
C. gariepinus	$5.287 \pm 2.2^{a,y}$	$2.314 \pm 2.1^{ae,x}$	$10.129 \pm 1.7^{a,z}$	$3.98 \pm 2.1^{a,xy}$
S. galilaeus	$3.018 \pm 2.2^{ad,x}$	$2.518 \pm 0.4^{ae,x}$	$5.278 \pm 0.4^{b,y}$	$1.676 \pm 1.0^{a,x}$
C. acutirostre	$4.120 \pm 0.1^{ae,w}$	$2.175 \pm 0.3^{ae,y}$	$5.064 \pm 0.8^{b,w}$	$1.204 \pm 0.6^{ac,y}$
P. obscura	$4.852 \pm 0.04^{af,w}$	$2.889 \pm 0.7^{ae,w}$	$5.240 \pm 1.1^{b,w}$	$1.454 \pm 0.3^{a,w}$
H. odoe	$3.481^{ad,y}$	$2.204^{ae,y}$	$5.555^{d,y}$	$2.926^{a,y}$

$^{a–f}$Mean ± SEM in each column with no common superscript differ significantly (p <0.05); $^{w–z}$Mean ± SEM in each row with no common superscript differ significantly (p <0.05); N.O- not observed; *M. electricus* are bimodal breathers capable of using lungs for respiration in highly polluted environment when their gills degenerate (Okafor, 2004; Loonget al., 2012).

of the fish are presented in Tables 3 and 4. Livers of *O. niloticus,C. guntheri, T. zillii, T. mariae*and *H. odoe* showed significant difference in rhodanese activity as compared to that in livers of the other fish samples. Rhodanese activity in gills of *C. guntheri* was highest and was observed to be significantly different in distribution levels in gills of other fish. No significant difference in rhodanese activity was noticed in gills of *O. niloticus*and *T. mariae*. Also, rhodanese distribution in gills of *C. acutirostre* and *P. obscura*was not different from each other.

In guts, highest rhodanese activity was observed in *M. electricus*, although with no significant difference in levels observed in *T. dageti, O. niloticus*and *P. obscura.* Equally, no significant difference was noticed in rhodanese distribution in the guts of *C. gariepinus, S. galilaeus*and *C. acutirostre.* The fillet of *T. mariae* showed highest rhodanese activity with no significant difference in that of *C. guntheri.* A significant difference in rhodanese activity was observed in fillet of *T. zillii*as compared to that in *O. niloticus*and *C. gariepinus.*

The distribution of 3-MST in gills of fish samples showed that activity in *T. mariae* was highest, and was not significantly different from that observed in *C. guntheri*. There was also no difference in levels observed in gills of *T. dageti*and *S. galilaeus*. In guts and livers, highest 3-MST activity there was no significant difference in those in *M. electricus, O. niloticus, C. guntheri, T.*

Table 5. Mean (±SEM) specific rhodanese activity in tissue homogenates of fish.

Species of fish	Protein (U/mg)			
	Gill	Gut	Liver	Fillet
M. electricus	N.O	0.024	0.103	0.014
T. dageti	0.032±0.108	0.012±0.030	0.035±0.05	0.008±0.133
O. niloticus	0.015±0.109	0.077±0.054	0.049±0.035	0.006±0.019
C. guntheri	0.023±0.078	0.024±0.053	0.059±0.025	0.019±0.419
T. zillii	0.017±0.031	0.007±0.013	0.028±0.105	0.006±0.027
T. mariae	0.016±0.119	0.015±0.005	0.024±0.028	0.136±0.045
C. gariepinus	0.012±0.047	0.007±0.010	0.043±0.050	0.003±0.014
S. galilaeus	0.012±0.005	0.005±0.005	0.043±0.006	0.011±0.001
C. acutirostre	0.008±0.015	0.006±0.012	0.003±0.009	0.003±0.004
P. obscura	0.011±0.033	0.009±0.019	0.036±0.051	0.003±0.019
H. odoe	0.018	0.004	0.026	0.005

N.O- Not observed; *M. electricus* are bimodal breathers capable of using lungs for respiration in highly polluted environment when their gills degenerate (Okafor, 2004; Loong et al., 2012).

Table 6. Mean (±SEM) specific 3-MST activity in tissue homogenates of fish species.

Species of fish	Protein (U/mg)			
	Gill	Gut	Liver	Fillet
M. electricus	N.O	0.017	0.051	0.006
T. dageti	0.013±0.032	0.013±0.03	0.018±0.012	0.014±0.206
O. niloticus	0.008±0.678	0.006±0.091	0.028±0.052	0.004±0.009
C. guntheri	0.011±0.043	0.016±0.040	0.032±0.083	0.007±0.119
T. zillii	0.012±0.020	0.005±0.010	0.016±0.091	0.005±0.040
T. mariae	0.013±0.008	0.013±0.005	0.014±0.012	0.007±0.063
C. gariepinus	0.010±0.036	0.005±0.109	0.029±0.045	0.003±0.006
S. galilaeus	0.008±0.003	0.005±0.008	0.029±0.001	0.008±0.151
C. acutirostre	0.007±0.007	0.005±0.008	0.018±0.007	0 002±0.001
P. obscura	0.011±0.025	0.007±0.018	0.022±0.033	0.002±0.005
H. odoe	0.008	0.003	0.015	0.004

N.O- Not observed; *M. electricus* are bimodal breathers capable of using lungs for respiration in highly polluted environment when their gills degenerate (Okafor, 2004; Loong et al., 2012).

mariae, C. gariepinus, S. galilaeus and *P. obscura.* In all tissues tested for enzyme activities, the liver showed highest rhodanese and 3-MST activities unlike fillet that showed lowest enzymes activities when compared with gill and gut enzyme distribution levels. Furthermore, rhodanese activity was higher than 3-MST activity in all tissues of fish investigated.

Distribution patterns of mean specific rhodanese and 3-mercaptopyruvate sulphurtransferase activities in tissues of fish species

Tables 5 and 6 shows the results of specific rhodanese and 3-MST activities in the selected tissues of fish species observed. For rhodanese, highest activity was recorded in gill of *T. dageti, gut* of *O. niloticus,* liver of *M. electricus*and fillet of *T. mariae.* Activity of 3-MST was highest in gut and liver of *C. guntheri* and in gills of *T. dageti*and *T. mariae,* respectively.

DISCUSSION

Rhodanese as well as 3-MST activity are ubiquitous in nature suggesting an important physiological role (Westley, 1973; Wood, 1975). It has been suggested that the levels of these enzymes in different tissues of animals is correlated with the level of exposure to cyanide (Aminlari et al., 2000). The potential risk of cyanide toxicity to man and animals is great. It is believed that the primary function of these enzymes is cyanide detoxification

(Cerletti, 1986). Their pattern of distribution in different animals appears to be highly species and tissue specific.

In most animals studied, the liver appears to be the richest source of rhodanese (Drawbaugh and Marrs, 1987; Aminlari et al., 1994). Human activities around Igun reservoir have been discovered as those that enhance increase in environmental pollution especially the use of excessive cyanide-containing chemicals by artisanal miners around the reservoir.

In this work, the physicochemical properties and activities of cyanide-detoxifying enzymes (rhodanese and 3-MST) were investigated in water and selected tissues of fishes within the study area. Dissolved oxygen was found to be slightly lower during the dry season than the rainy season. The lower value obtained during the dry season could be due to poor ability of the water to hold oxygen at high temperature as a result of higher evaporation rate or higher organic matter decomposition during this season. Different dissolved oxygen (D.O) results in freshwater from the one observed for Igun reservoir were reported by Akinbuwa (2008) and Komolafe and Arawomo (2008) also observed variation in Osinmo and Opa reservoir. Oke (1998) had also recorded a dissolved oxygen concentration below 5 mg/ml in Owena reservoir. A number of authors have reported increase in toxicity of cyanide with reduction in dissolved oxygen below the saturation level (Doudoroff, 1976). Water with low D.O values has low nutrient levels and this implies high concentration of biochemical oxygen demand (BOD). Unpolluted natural waters are expected to have BOD values of 5 mg/L or less (Agbaire and Oyibo, 2009).

Statistical analysis of the level of cyanide in Igun reservoir revealed there was no significant difference in values recorded between the rainy and dry seasons. Varunprasath and Nicholas (2010) reported that algae and other organisms in water take up inorganic nutrients and use them in the process of building up their organic tissues.

The mean total alkalinity of 78.12 ± 1.41 mg/L recorded in rainy season is lower as compared to 86.00 ± 2.00 mg/L observed in the dry season. Atobatele and Ugwumba (2008) recorded lower values of 66.15 ± 15 and 63.68 ± 1.29 mg/L for alkalinity during rainy and dry seasons, respectively for Aiba reservoir. Significant variation in total alkalinity observed for Igun reservoir might be due to an increased mining operation involving higher usage of quicklime or nature of the bottom deposits.

The result of temperature was found to range between 22 and 27°C during the two seasons. A higher mean temperature value (27.00 ± 1.00°C) was recorded during the dry season than 24.40 ± 0.81°C observed in the rainy season. Okonji et al. (2010) recorded 24.0 ± 0.75°C and 27.0 ± 0.56°C in rainy and dry seasons, respectively, for Osinmo reservoir. The temperature of any given water determines the concentration of dissolved gases. Higher temperature recorded during the dry season might be due to the type of waste water discharged. It was also observed that the level of water in the reservoir significantly reduced during the dry season such that light rays from the sun penetrate through the water; hence the water was heated up easily to higher temperatures.

The mean value for transparency during the dry season is significantly higher than that recorded during the rainy season. The lower transparency value observed during the rainy season may be due to heavy rainfall leading to increased surface run off from upper land which carries a lot of suspended materials into the reservoir leading to high turbidity values. Generally, during rainy season, suspended particles in the water are always in motion due to high rate of water circulation whereas in the dry season, the particles tend to settle on submerged logs as there is little turbulence.

The result of hardness observed during rainy season (11.83 ± 0.31 mg/L)was higher than that in the dry season (11.76 ± 0.01 mg/L). The result may be due to increased discharge of waste water into the reservoir during the rainy season. Total hardness is due to the presence of bicarbonates, sulphates, chlorides and nitrates of calcium and magnesium. Hard water requires more soap and synthetic detergents for home laundry and washing, and contributes to furring of kettles, scaling in boilers and industrial equipments (Briggs and Ficke, 1975). The pH of 7.06 ± 0.05 and 7.00 ± 0.10 observed in rainy and dry seasons respectively were moderately acidic.

The result of conductivity (153.54 ± 6.05 mg/L) observed during rainy season was lower than 170.90 ± 0.70 mg/L recorded during the dry season. The relative lower conductivity values during the rainy season may be due to the utilization of salts, organic and inorganic substances by phytoplanktons and other aquatic organisms which eat up the dissolved solids. A number of these organisms die off during the dry season thereby allowing the concentration of dissolved solids to increase. Electrical conductivity increases with increase in total dissolved solids. Conductivity values have been reported to be indicative of pollution load of water (Deeker et al., 2010).

No gills were observed in the system of *M. electricus* examined. Okafor (2004) and Loonget al. (2012a, b) reported that the fish has small slits and it is also a bimodal breather that uses a pair of lungs for aerial respiration in highly polluted water and at higher water temperature. At peak of dry season, the fish escavates a burrow in dried land and stays breathing with the pair of lungs leading to the degeneration of the slits (Okafor et al., 2012).

The mean levels of cyanide in Igun reservoir in both rainy and dry seasons were extremely high. The level of cyanide in rainy season (36980.67 ± 6973.57 µg/L) was observed to be higher than that of the dry season (26150.22 ± 1046.59 µg/L). The long-term survival and

growth of various fresh water fish species was observed to be seriously reduced at free cyanide concentrations of about 20-50 µg/L (Kimball et al., 1978). The lower value of cyanide level recorded at Igun reservoir during the dry season may be due to sedimentation of organic and inorganic particles. Tolerance of fish to cyanide solution has been observed to decrease with rise in temperature. No pronounced relationship has been observed between acute toxicity of cyanide to fishes and alkalinity, hardness, or pH below 8.3 (Smith et al., 1978) of certain life stages and species of fishes appear to be more sensitive to cyanide than others. Embryo, sac fry and warm water species tend to be the most resistant (Oseid and Smith, 1979).

Although at varying degree, notable enzyme activities were detected in all the selected fish tissues investigated. Generally, the pattern of distribution of rhodanese in different tissues of animals is species specific. It has been reported that activity of enzymes in a particular tissue/organ may reflect the ability of that tissue/organ to detoxify cyanide (Ali et al., 2001). No cyanide was detected in the tissues of the fish samples. Negative results indicate a non-bioaccumulation of cyanide in fish tissues. These results agree with the reports (Way, 1984) that cyanide bio-accumulation and bio-magnification in food web has not been established, possibly due to rapid detoxification of sublethal doses by most species, and death at higher doses. The distribution patterns of rhodanese and 3-MST in the selected tissues of fish species in Igun reservoir may be traced to the function of these enzymes (rhodanese and 3-MST) in cyanide-detoxification.

Lower enzyme activity was observed in gills during the dry season than in the rainy season. Lowest cyanide concentration in gill occurs at elevated (summer) water temperatures; at lower temperatures, survival is greater and residues higher (Holden and Marsden, 1964). The high level of these enzymes in gills of fish had shown (Kenneth, 1991) that gill is a multifunctional organ responsible for respiration, osmoregulation and acid base balance. Therefore, the high activity of rhodanese and 3-MST in the gills of the fish species could be physiological, since the organ (gill) is involved in respiration, a function that makes the organ prone to cyanide attack. It is no surprise that activities of rhodanese and 3-MST were observed in the gut as it plays vital roles in food digestion involving secretion of pepsin and hydrochloric acid (Duke, 1986). The presence of high rhodanese and 3-MST activity in the gut of fishes probably ensures cyanide detoxification before it reaches general circulation (Aminlari and Shahbazi, 1994). The activities of rhodanese and 3-MST in the gut of *M. electricus*were found to be higher than others with no significant difference as compared to the other species which is consistent with the report of Conn (1979) that the acidic condition of the tissue might result in the spontaneous hydrolysis of cyanogenic glycoside and liberation of cyanide, which is easily absorbed.

On the distribution of rhodanese and 3-MST in the tissues, the activities of the enzymes were found to be most in the liver. This is not unexpected going by the function of liver in metabolism and in particular, detoxification. The liver has always been considered to be the major source of rhodanese and is believed to be the major site of cyanide detoxification (Marrs and Ballantyne, 1987). The occurrence of enzyme-detoxifying activities in fillet may be linked to the defensive mechanism of enzymes in the protection of organism (Smith and Urbanska, 1986). Lowest activities of cyanide-detoxifying enzymes recorded in fillets may be explained by the reports of Smith and Olson (1973) that level of cyanide distribution is increased in organs and tissues with blood, where its concentration is greater. Hence, activity of sulphurtransferases is higher in these tissues. It is apparent that although, rhodanese and 3-MST are detectable in the selected tissues studied, the amounts are low when compared with those of liver and/or gut. Reports in literatures have shown that the liver is the major source of rhodanese and is believed to be the major site of cyanide detoxification (Ali et al., 2001; Drawbaugh and Marrs, 1987). However, high levels of rhodanese activity in other tissues have also been reported and as a result there is the suggestion that rhodanese performed primarily other biological functions and that cyanide detoxification is just a secondary role.

Conclusion

Fish population and other aquatic resources are affected by changes in seasonal rhythm, water levels and water quality. These changes affect the health of aquatic ecosystems, with impacts on productivity, species diversity and species distribution (Christensen et al., 2003).

The results from this investigation on cyanide level could be used as good indices of pollution level in Igun reservoir. The study showed that fish do not bioaccumulate cyanide in their body tissues despite inhabiting a highly cyanide-polluted environment. The activities of cyanide-detoxifying enzymes:rhodanese and 3-MST in tissues of fish species from the reservoir could possibly explain the survival of inhabitant fish species in this unfriendly environment through enzyme-based mechanism.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

The authors wish to thank everyone that contributed

immensely to the success of this work. Special appreciation is due Mr. Ademakinwa Nelson.

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