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# A survey for the presence of microcystins in aquaculture ponds in Zaria, Northern-Nigeria: Possible public health implication

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**Aquaculture ponds in Zaria, Nigeria, were screened for the occurrence of the hepatotoxic microcystins using an ELISA method. Four genera of cyanobacteria (*Microcystis*, *Nostoc*, *Planktothrix* and *Anabaena*) were recorded from 11 aquaculture ponds screened. These cyanobacteria are generally known to produce microcystins and other bioactive substances. Six of the 11 aquaculture ponds had detectable concentrations of microcystins (ranging from 0.6 to 5.89 µg/L). This means that there is the possibility of bioaccumulation of microcystins in fish. The implication of this is that people that feed on contaminated fish from these ponds stand the risk of microcystins poisoning.**

**Key words:** Aquaculture, ponds, microcystins, Zaria, Nigeria.

## INTRODUCTION

Aquaculture ponds are made up of a community of photosynthesizing organisms. These organisms belong to different groups. Among these groups is the cyanobacteria group (blue green algae). The blue green algae possess the basic morphology of gram negative bacteria. The distinct differences between them and other members of the bacteria group is that they are usually larger in size and have the ability to photosynthesize. In addition, the cyanobacteria are known to have the ability to fix atmospheric nitrogen (Codd et al., 2005; Huisman et al., 2005; Hudnell, 2008). Species of blue green algae could be unicellular, colonial and filamentous in form. They are usually enclosed in mucilaginous sheaths, either individually or in colonies. Independent of the morphological form of blue green algae in an aquaculture pond, they

could become the dominant photosynthesizing group. Under the right set of conditions and concentrations of nutrients (nitrogen and phosphorus), blue green algae undergo excessive proliferation, leading to occurrence of cyanobacterial bloom (Nwaura et al., 2004). This is also called harmful algal bloom (HAB), even if the species constituting the bloom do not produce harmful bioactive substances. This is because the excessive growth of blue green algae could have other negative effects on other plants through competition for light, space, nutrients and oxygen (Mitrovic et al., 2004). All of these effects may be detrimental to other cultured organisms, which cannot withstand the associated problems of HABs. Some species of cyanobacteria need to be in higher numbers to produce significant concentrations of bioactive substances (toxins), while others do not. Between members of the same species toxin production vary in time, space, environmental conditions and strain-type. Hence, morphologically identical species could be chemically different; a chemotype producing toxins and the other not producing (Oberholster et al., 2006).

Cyanobacteria produce a wide range of bioactive substances or secondary metabolites. Toxins produced

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**Abbreviation:** BS, Biological Sciences; PN, Prof Nok; LH, Limi Hospital; RRGRA, Rock Road, Government Reserved Area.

by cyanobacteria are of several types comprising neurotoxins (aantoxins and saxitoxins), hepatotoxins, cylindrospermopsin and lipopolysaccharides (LPS) (Carmichael and Falconer, 1993; Carmichael, 1997). The hepatotoxins are cyclic peptides including microcystins and nodularin. Microcystins contain 5 invariant and 2 variant amino acids. The Adda is one of the invariant amino acids which is a unique  $\beta$ -amino. The nomenclature of microcystins is such that a two letter suffix (XY) is given to individual toxins to specify the two variant amino acids. Several reports support the occurrence of variants of the 'invariant' amino acids and the replacement of the 9-methoxy group of Adda by an acetyl moiety. Over 90 variants of microcystins have been characterized to date (Rinehart et al., 1994; Sivonen and Jones, 1999; Natural Resources and Mines, 2005; Pichardo et al., 2007). The International Agency for Research on Cancer classified microcystins as 'possible human carcinogens' (Class 2B) based on the consideration of the accumulated toxicological data. The ability of the toxins to inhibit certain protein phosphatases influenced this classification. The protein phosphatases are enzymes that are critical in cell-cycle regulation (Grosse et al., 2006; Humpage and Burch, 2007).

The effects of the toxins produced by species of cyanobacteria could pose significant threat to public health because they have the ability to bioaccumulate; thereby passing through the food chain. Fish and other animals that feed on organisms with high or significant concentrations of these toxins could be killed. This results in severe economic losses in aquaculture if they are implicated in the death of cultured animals or consumers of the cultured animals. There are reports of losses amounting to about one billion US dollars per decade (Landsberg, 2002; Hudnell, 2008). Among these toxins are the microcystins which present the most concern to public health (Chen et al., 2009a). Microcystins are known to inhibit the growth of fishes. Environmental level toxicity has been reported for several fishes such as salmon (Anderson et al., 1993), carp (Xie et al., 2004), zebrafish (Oberemm et al., 1999) and catfish (Zimba et al., 2001).

In Nigeria, there is no report on the presence of microcystins in any aquaculture facility. Most of the works from aquaculture ponds have been on the diversity and abundance of algae (Onuoha et al., 1991; Akpan and Okafor, 1997; Ekpenyong, 2000; Chindah and Pudo, 1991). To date, the studies on algal toxins have been mainly in other aquatic ecosystems and not fish farms or aquaculture ponds. The studies have been basically bioassays which ranged from fish and shell fish bioassay (Unyimadu, 2002) and mouse bioassay (Odokuma and Isirima, 2007). Even though, there is a lot of published works in other countries on the effects of microcystins (and other algal toxins) on fish, not much is known about the presence of such toxins in aquaculture facilities in Nigeria. In Nigeria, fish is a good source of protein for the

poor and the rich as well. People that cannot afford to buy meat buy fish like Tilapia. Hence, if this cheap source of protein is contaminated, the implication of this may be far reaching. The effect of this could be short term and long term poisoning from consumption of contaminated fish. In addition, there is no legislation in Nigeria to enhance proper monitoring and management of fish farms with respect to cyanotoxins. The success of any legislation on the control of the incidence of cyanotoxin poisoning will be dependent on the amount of data available. This calls for research works aimed at generating data from surveys of fish farms or aquaculture facilities in Nigeria. This will help in appraising the extent of contamination of fish farms or ponds; thereby giving room for effective monitoring and management of the facilities. This project was carried out with the aim of screening aquaculture ponds in Zaria, Nigeria for the presence of microcystins.

## MATERIALS AND METHODS

### Study area

Zaria is situated centrally in the Northern Guinea Savanna of Nigeria (11° 3' N, 7° 42' E). Climatic conditions in Zaria are tropical with well defined wet and dry seasons. The rainy season occurs from May to October while the dry season from November to April. The aquaculture ponds selected for this survey are privately owned and managed. The cultivated fish species were Tilapia and the African catfish. A few ponds were enriched with fertilizers to enhance primary productivity of algae. Fish production from most of these ponds is for commercial purposes. Some of these aquaculture ponds supply their products (harvested fish) directly to specific markets for human consumption. These ponds were mostly concrete ponds. The source of water supply to some of them was from boreholes while others had tap water as the primary source of water. The Table 1 gives the name of the ponds and their locations.

### Sampling

The period of sampling for present survey was from September 2008 to November 2008. Samples for physical, chemical and biological analysis were collected from three fixed sampling points per aquaculture pond using an integrated hose pipe sampler (2.5 cm diameter and 5 m length). The samples were collected in replicates at about 30 cm depth and 1 m away from shore (APHA, 1998). The volume of water collected for microcystins analysis was 3 liters. Samples were preserved on ice and transported to the laboratory. In the laboratory, samples for microcystins analysis were stored at -20°C.

### Cyanobacteria analysis

Analysis of cyanobacteria samples was carried out in the Hydrobiology Laboratory of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. 100 ml concentrates from initial collected water samples using the Integrated Hose pipe sampler were obtained for cyanobacteria analysis. These subsamples were fixed with 0.1 ml of Lugol's solution to precipitate and preserve algae (APHA, 1998). Laboratory analysis of cyanobacteria was done using the procedures of Prescott (1977), APHA

**Table 1.** Selected aquaculture ponds surveyed in Zaria, Nigeria.

SN	Name of Pond	Location	Grid reference
1	Engineering pond	Faculty of Engineering ABU Zaria	11°08'N, 07° 43'E
2	BS pond A	Biological Sciences Department Fountain in front of the department, ABU Zaria	11°08'N, 07° 43'E
3	BS pond B	Behind the Department of Biological Sciences, ABU Zaria	11°08'N, 07° 43'E
4	BS pond C	Behind the Department of Biological Sciences, ABU Zaria	11°08'N, 07° 43'E
5	PN pond A	Area A, ABU, Zaria	11°08'N, 7°43' E
6	PN pond B	Area A, ABU, Zaria	11°08'N, 7°43' E
7	Aliyu Fish pond	Hanwa Low-Cost	11° 07'N, 7°43'E
8	LH pond A	Limi Hospital Hanwa New Extension	11° 07'N, 7°43'E
9	LH pond B	Limi Hospital Hanwa New Extensioin	11° 07'N, 7°43'E
10	LH pond C	Limi Hospital Hanwa New Extension	11° 07'N, 7°43'E
11	RRGRA pond	G.R.A. Zaria	11°08'N, 7°50' E

BS = Biological Sciences; PN = Prof Nok; LH = Limi Hospital; RRGRA = Rock Road, Government Reserved Area.

(1998) and Bartram and Rees (2000). Cyanobacteria biomass (number of cells per ml) was determined using the Drop Count Technique (Bartram and Rees, 2000). Cells in colonies were counted were counted without separation of cells.

#### Analysis of physicochemical parameters

Physicochemical analysis and sample preservation were carried out in the Hydrobiology Laboratory of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. The Mercury thermometer was used to determine water temperature. Electrical conductivity (EC) ( $\mu\text{mhos/cm}$ ) was obtained using the E.B.A/10 Conductivity meter. Determination of pH was with the Pye Unicam pH meter model 292 at 25°C. The modified Winkler Azide method (Lind, 1974 and APHA, 1985) was used for Dissolved Oxygen (DO) and Biochemical Oxygen Demand (BOD) analysis. Total Dissolved Solids (TDS) was obtained using the procedure of Lind (1974). Nutrient concentrations (phosphates phosphorus,  $\text{PO}_4\text{-P}$  and nitrates-nitrogen,  $\text{NO}_3\text{-N}$ ) were determined spectrophotometrically using HACH DR/2000 Direct Reading Spectrophotometer. And specific nutrient concentrations were read from a calibration curve (Mackereth, 1963; Lind, 1974; APHA, 1985).

#### Immunological detection of microcystins

Enzyme Linked Immunosorbent Assay (ELISA) was used to determine the total microcystins concentrations of pond water samples. Samples were frozen and thawed three times to release the intracellular toxins (Chorus and Bartram, 1999). This permitted the analysis dissolved as well as intracellular toxins in the ponds. The ELISA analysis was carried out in the Algae Laboratory, National Research Institute for Chemical Technology, NARICT, Zaria, Nigeria. The principle of the ELISA assay used is based on the polyclonal anti body method put forward by Chu et al. (1990) and adapted by Carmichael and An, (1994). Antibody – coated tubes, standards and all reagents were supplied by Abraxis LLC (Warminster PA18974, USA). The level of sensitivity for microcystins using this kit was approximately 0.15  $\mu\text{g/L}$ . Microcystins were quantified using a Jenway spectrophotometer (Model 6400) at a wavelength of 450 nm in conjunction with a reference wavelength of 630 nm (Fischer et al., 2001; Hawkins et al., 2005).

#### Statistical analysis

Analysis of variance (ANOVA) using Microsoft Office Excel 2007 for windows was used to test for difference between means of observed parameters (Fisher, 1925). Possible relationship between analysed parameters was determined using the Pearson's correlation coefficient.

## RESULTS

The highest temperature recorded in this study was 31.80°C in LH pond A while the lowest was 24.80°C in RRGRA pond. PN pond B had the peak value of 307 $\mu\text{ohm/s}$  for EC and was closely followed by Aliyu Fish pond with 289 $\mu\text{ohm/s}$ . The lowest value for EC was 71.0 $\mu\text{ohmS}^{-1}$  and was recorded in BS pond A. TDS showed a similar trend with EC. The highest value of TDS (153.5 mg/L) was observed in PN pond B and the lowest (35.5 mg/L) in BS pond A. DO concentrations in all ponds ranged from 14.9 to 25.4 mg/L. While Biochemical Oxygen Demand (BOD) concentrations ranged from 1.00 to 11.55 mg/L. The highest BOD value was observed in BS pond B and the lowest in LH pond C. The lowest concentration of nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) was 0.07 mg/L. This was recorded in both RRGRA pond and BS pond C. On the other hand, the highest recorded value for  $\text{NO}_3\text{-N}$  was 0.14 mg/L in PN pond B. The highest concentration of  $\text{PO}_4\text{-P}$  was 0.26 mg/L (in Engineering pond), while the lowest concentration was 0.10 (in Aliyu fish pond). Except for LH pond B and BS pond A, all ponds had pH values that ranged from 6.58 - 7.88. LH pond B and BS pond A had high pH values of 9.40 and 9.75, respectively (Table 2).

Table 3 shows density and relative abundance of cyanobacteria in the fish ponds. The cyanobacteria species with the highest density was *Microcystis* spp. This observation was made in PN pond A. In addition,

**Table 2.** Physicochemical parameters of selected Aquaculture ponds in Zaria.

Parameter	Engineering pond	BS pond A	BS pond B	BS pond C	PN pond A	PN pond B	Aliyu Fish pond	LH pond A	LH pond B	LH pond C	RRGRA pond
Temp.( °C)	27.50	27.60	27.80	25.20	24.80	29.80	31.80	32.90	29.50	25.50	25.00
Elect. Cond. (µohm/s)	108.00	71.00	110.00	90.00	178.5	307.00	289.00	204.00	80.00	104.20	135.10
DO (mg/L)	19.70	16.80	25.40	15.85	17.10	16.50	20.25	20.25	16.65	14.90	16.55
Nitrate- Nitrogen (mg/L)	0.11	0.09	0.12	0.07	0.08	0.14	0.11	0.09	0.10	0.11	0.07
Phosphate-Phosphorus (mg/L)	0.26	0.24	0.21	0.15	0.16	0.25	0.10	0.22	0.25	0.16	0.16
TDS (mg/L)	54.00	35.50	55.00	45.00	89.25	153.50	144.50	102.20	40.00	52.10	67.55
pH	7.42	9.75	5.73	6.85	7.64	7.42	7.88	8.80	9.40	6.58	7.85
BOD (mg/L)	4.30	2.75	11.55	1.75	1.60	3.40	6.95	6.25	1.15	1.00	2.50

BS = Biological Sciences; PN = Prof Nok; LH = Limi Hospital; RRGRA = Rock Road, Government Reserved Area.

**Table 3.** Density of dominant cyanobacteria (No. of cells per ml x 10<sup>3</sup>) with their relative abundance (%) in the parenthesis in selected aquaculture ponds in Zaria, Nigeria.

Pond	Density and relative abundance			
	<i>Microcystis</i> spp.	<i>Nostoc</i> spp.	<i>Planktothrix</i> spp.	<i>Anabaena</i> spp.
Engineering pond	30 (75.00)	10 (25.00)	-	-
BS pond A	50 (100.00)	-	-	-
BS pond B	50 (83.33)	10 (16.60)	-	-
BS pond C	10 (50.00)	10 (50.00)	-	-
PN pond A	250 (100.00)	-	-	-
PN pond B	50 (71.43)	-	20 (28.57)	-
Aliyu Fish pond	-	20 (50.00)	-	20 (50.00)
LH pond A	30 (50.00)	-	30 (50.00)	-
LH pond B	-	-	10 (100.00)	-
LH pond C	20 (22.22)	-	50 (55.55)	20 (22.22)
RRGRA pond	30 (75.00)	-	-	10 (25.00)
Frequency of Occurrence (%) in all ponds	72.72	36.36	36.36	27.27

BS = Biological Sciences; PN = Prof Nok; LH = Limi Hospital; RRGRA = Rock Road, Government Reserved Area; figures in the parenthesis represent relative abundance (%).

*Microcystis* spp. had the highest frequency of occurrence (72.72%) in all ponds. Biomass of *Microcystis* spp. in the ponds was significantly

correlated with EC ( $r = 0.7542$ ), DO ( $r = 0.8993$ ), BOD ( $r = 0.6669$ ), PO<sub>4</sub>-P ( $r = 0.7988$ ), TDS ( $r = 0.7545$ ) and pH ( $r = 0.8444$ ) at  $P \leq 0.05$  (Table 4).

Both *Nostoc* spp. and *Planktothrix* spp. had an occurrence frequency of 36.36%. *Anabaena* spp. had the least frequency of occurrence (27.27%).

**Table 4.** Correlation coefficient between observed parameters in selected aquaculture ponds in Zaria, Nigeria.

	<i>Microcystis</i>	<i>Nostoc</i>	<i>Planktothrix</i>	<i>Anabaena</i>	Microcystins	Temp	EC	DO	BOD	NO <sub>3</sub> -N	PO <sub>4</sub> -P	TDS	pH
<i>Microcystis</i>	-	0.3613	0.5613	0.3855	0.1223	0.2427	0.7542	0.8993	0.6669	0.5545	0.7988	0.7545	0.8444
<i>Nostoc</i>	-0.3053	-	0.1816	0.3596	0.9908	0.4998	0.5190	0.1075	0.0879	0.6282	0.1692	0.5198	0.2669
<i>Planktothrix</i>	-0.1971	-0.4346	-	0.2707	0.4565	0.6186	0.7501	0.3707	0.5270	0.3642	0.8687	0.7489	0.7951
<i>Anabaena</i>	-0.2909	0.3063	0.3643	-	0.1514	0.9870	0.4858	0.6300	0.8909	0.8109	0.0171	0.4865	0.5457
Microcystins	0.4942	0.0040	-0.2511	-0.4631	-	0.6341	0.7867	0.3344	0.4697	0.1282	0.1312	0.7877	0.3411

Bolded coefficient is significant at  $P \leq 0.05$ .

The highest density of *Nostoc* spp. recorded was  $20 \times 10^3$  cells/ml while the lowest was  $10 \times 10^3$  cells/ml. The density of *Nostoc* spp. showed significant correlation with the concentrations of NO<sub>3</sub>-N ( $r = 0.6282$ ) at  $P \leq 0.05$ . *Planktothrix* spp had a density ranging from  $10 \times 10^3$  to  $50 \times 10^3$  cells/ml. In addition, the density of *Planktothrix* spp showed significant concentrations (at  $P < 0.05$ ) with temperature ( $r = 0.6186$ ), EC ( $r = 0.7501$ ), PO<sub>4</sub>-P ( $r = 0.8687$ ), TDS ( $r = 0.7489$ ) and pH ( $r = 0.7951$ ). For *Anabaena* spp., the density ranged from  $10 \times 10^3$  to  $20 \times 10^3$  cell/ml and was significantly correlated with temperature ( $r = 0.9870$ ), DO ( $r = 0.6300$ ), BOD ( $r = 0.8909$ ), and NO<sub>3</sub>-N ( $r = 0.8109$ ) (Table 4).

Microcystins were detected in fifty four percent samples of the screened aquaculture ponds and five of the six ponds had a level of microcystins above the WHO limit (1µg/L). The highest concentration of microcystins was 5.89µg/L in engineering pond. The lowest concentration detected was 0.60µg/L in LH pond C. Concentrations of microcystins in BS pond C, Aliyu pond A, LH pond A, LH pond B and RRGRA pond were below the level of detection (BLD) for the ELISA kit used for the current study (Table 5). Concentration of microcystins significantly correlated with the temperature ( $r = 0.6341$ ), EC ( $r = 0.7867$ ) and TDS ( $r = 0.7877$ ) at  $P \leq 0.05$  (Table 4).

## DISCUSSION

The whole aquaculture ponds in the present study were relatively rich in amount of dissolved oxygen. This is because even the least recorded value of 14 mg/L is high when compared to the critical level of 3 mg/L in aquatic ecosystems (Lind, 1974). The high DO values could also be attributed to the rate of photosynthesis taking place in the aquaculture ponds. The rate of photosynthesis in an aquatic system is proportional to the plant biomass in it. The more the plants photosynthesize, the more they release oxygen to the system. The observed similarity in the variations of TDS and EC is not surprising. This is because both parameters usually show a linear relationship. The differences between the conductivity of the different aquaculture ponds may also be depended on their sources of water and the nature of the dissolved substances. The variations in the activities around the catchment of the water body that serves as source of tap water for the ponds could be responsible for observed differences in TDS and EC values (Chia, 2007). Observed differences in the amount of nutrients in these ponds could be attributed to the practices of the owners of the ponds. Therefore, the nutrient content of the ponds could be a reflection of the extent of fertilization by the owners of the ponds.

Where the ponds are artificially enriched, there will be corresponding increased growth of algae. As phosphorus concentrations increase so does the cyanobacteria biomass in an aquatic ecosystem (Havens et al., 2003).

The density of these species was closely associated with physicochemical parameters of the aquaculture ponds. The water temperature of the screened ponds ranged from 24.80- 31.00°C. This temperature range is optimum for the growth of cyanobacteria in aquatic systems (Konopka and Brock, 1978; Howard and Easthope, 2002). This could have been the reason for the increased density of most species of cyanobacteria recorded in this study. Different strains of cyanobacteria have been shown to significantly alter their growth rates in response to variations in temperature, light level, and availability of nutrients (Lee et al., 2000; Oh et al., 2000; Wiedner et al., 2003). The presence of *Microcystis* spp. in these ponds may be indicative of constant nutrient enrichment, because they are known not to tolerate nutrient poor conditions in aquatic ecosystems (Finni et al., 2001).

Species of *Microcystis* (Botes et al., 1982; Watanabe and Oishi, 1985; Henriksen, 1996), *Nostoc* (Sivonen et al., 1990), *Planktothrix* (Henriksen, 1996) and *Anabaena* (Krishnamurthy et al., 1986; Vezie et al., 1998) have been repor-

**Table 5.** Concentrations of microcystins in the selected aquaculture ponds in Zaria, Nigeria.

Aquaculture pond	Wavelength		Concentration of microcystin ( $\mu\text{g/l}$ )
	450 nm	650 nm	
Engineering pond	0.666	0.263	5.89
BS pond A	1.382	0.240	2.40
BS pond B	1.050	0.332	4.48
BS pond C	2.160	0.262	BLD
PN pond A	0.920	0.211	4.50
PN pond B	0.940	0.354	4.80
Aliyu pond A	2.093	0.089	BLD
LH pond A	2.135	0.117	BLD
LH pond B	1.890	0.050	BLD
LH pond C	1.700	0.219	0.60
RRGRA pond	2.046	0.048	BLD

BS = Biological Sciences; PN = Prof Nok; LH = Limi Hospital; RRGRA = Rock Road, Government Reserved Area; BLD= Below Level of Detection.

ted to produce microcystins in aquatic systems. Although, it is impossible to state which species in the current study was responsible for the production of microcystins, 6 out of 11 ponds screened had detectable concentrations of microcystins in the water. It is probable that the *Microcystis* could be implicated for the production of the microcystins detected in this study as 2 of the 6 ponds had 100% *Microcystis* presence. This could have a serious negative implication on people that use the fish from these ponds as a source of meat. This is because there is sufficient published data that implicate microcystins of bioaccumulation in fish tissues. The bioaccumulation in fish is further supported by the evidence of histopathological effects in muscle, gill and kidney tissue of several fish species (Rodger et al., 1994; Carbis et al., 1996; Kotak et al., 1996; Fischer and Dietrich, 2000; Sipia et al., 2001; Magalhães et al., 2003; Xie et al., 2005; Ibelings et al., 2005; Gkelis et al., 2006). Zhao et al. (2006) showed that microcystin accumulation rates in muscle and liver tissues are directly proportional to ingestion rates for *Oreochromis niloticus*.

Due to technical limitations, the present study did not look at the concentration of microcystins in fish collected from the aquaculture ponds. However, there is compelling evidence available that establishes the bioaccumulation of these toxins in fish tissues. Therefore, fish from these ponds may be contaminated due to the detection of microcystins in the water. Wilson et al. (2008) are of the opinion that the consumption of fish containing cyanobacterial toxins represents a poorly studied, but potentially important mechanism for the ingestion of harmful cyanotoxins by humans. Chen et al. (2009a) recently present a milestone work in MC exposure research that microcystins were found to be transferred mainly from contaminated fisheries products to a chronically exposed human population (fishermen at

Lake Chaohu in the subtropical China) together with indication of hepatocellular damage. They identified for the first time the presence of microcystins in serum samples (average 0.39ng/ml) of humans (fishermen) under a natural exposure route. Therefore, it is quite likely that the presence of microcystins in aquaculture ponds means that the toxins contained within fish tissues may pose an alternative route of exposure to humans. Field data have been accumulating to indicate the potential chronic risk of human health by consumption of microcystins contaminated fish muscles (Magalhães et al., 2001; Xie et al., 2004; Wood et al., 2006; Wilson et al., 2008, Chen et al., 2009b).

Other probable negative effects of microcystins in aquaculture ponds are that they have the ability to reduce fish growth and productivity. Sublethal effects from exposure of fish to microcystins include liver damage (Best et al., 2001), startle response and disoriented swimming, as well as changes in ventilation rates (Li et al., 2008). The effect on livers is supported by the fact that the toxins accumulate in the liver or hepatopancreas of an exposed fish and binds to the nucleophilic site on protein phosphatases PP1 and PP2A (Robinson et al., 1991; Craig et al., 1996; Fischer and Dietrich, 2000; Fischer et al., 2001). The consequence of this is a possible result of hepatocyte degradation and fatal liver hemorrhaging (Fischer and Dietrich, 2000; Zimba et al., 2001). There is the possibility that some incidence of unexplained fish deaths in aquaculture ponds in Nigeria could be caused by microcystins or other algal toxins. In agreement with this, a number of studies in freshwater ecosystems in other countries have reported fish kills associated with microcystins producing strains of *Microcystis* (Anderson et al., 1993, Tencala and Dietrich, 1997).

In conclusion, microcystins have been detected in 6 out of 11 aquaculture ponds screened. The physicochemical

parameters of these ponds encourage the growth of four cyanobacterial species. There is the possibility of bioaccumulation of these toxins in fish tissues as supported by published literatures. People that feed on fish from these aquaculture ponds and others may be at the risk of continuous microcystins poisoning. It is therefore recommended that fish farmers should reduce the rate of artificial enrichment of their aquaculture ponds. Further studies are needed to examine the rate of bioaccumulation and contamination of fish by microcystins in Nigerian aquaculture ponds. Furthermore, it is advised that studies in the future should quantify the type and abundance of toxic intracellular cyanobacterial compounds in important commercially harvested fish species in Nigeria. These studies are required to generate data to support the formation of legislation for the management of the incidence of fish contamination from microcystins (and other algal toxins) in aquaculture facilities in Nigeria.

## REFERENCES

- Akpan FR, Okafor N (1997). On organic fertilization and plankton development in two experimental freshwater ponds of Nigeria. *J. Aquat. Trop.* 12: 147-154.
- Anderson RJ, Luu HA, Chen DZX, Holmes CFB, Kent ML, Le Blanc M, Taylor FJR, Williams DE (1993). Chemical and biological evidence links microcystins to salmon 'netpen liver disease'. *Toxicol.* 31(10): 1315-1323.
- APHA (1985). Standard methods for the examination of water and waste water, American Public Health Association, Washington D.C.
- APHA (1998). Standard methods for the examination of water and wastewater. 20<sup>th</sup> Edition. Published by American Water Works Association/Water Environmental Federation, Washington D.C.
- Bartram J, Rees G (2000). Monitoring Bathing Waters-A practical guide to the design and Implementation of Assessments and Monitoring Programmes. World Health Organisation.
- Best JH, Eddy FB, Codd GA (2001). Effects of purified microcystin-LR and cell extracts of *Microcystis* strains PCC 7813 and CYA 43 on cardiac function in brown trout (*Salmo trutta*) Alevins. *Fish Physiol. Biochem.* 24: 171-178.
- Botes DP, Kruger H, Viljoen CC (1982). Isolation and characterization of four toxins from the blue-green alga, *Microcystis aeruginosa*. *Toxicol.* 20: 945-954.
- Carbis CR, Rawlin GT, Mitchell GF, Anderson JW, McCauley I (1996). The histopathology of carp, *Cyprinus carpio* L., exposed to microcystins by gavage, immersion and intraperitoneal administration. *J. Fish Dis.* 19: 199-207.
- Carmichael WW, An J (1994). Use of a colorimetric protein phosphatase inhibition assay and enzyme linked immunosorbent assay for the study of microcystins and nodularins. *Toxicol.* 32: 1495-1507.
- Carmichael WW, Falconer IR (1993). Diseases related to freshwater blue green algal toxins, and control measures. In: Falconer, I.R. (Ed.), *Algal Toxins in Seafood and Drinking Water*. Academic Press, London, pp. 187-209.
- Carmichael WW (1997). The cyanotoxins. *Adv. Bot. Res.* 27: 211-240.
- Chen J, Xie P, Li L, Xu J (2009a). First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. *Toxicol. Sci.* 108: 81-89.
- Chen J, Zhang D, Xie P, Wang Q, Ma Z (2009b). Simultaneous determination of microcystin contaminations in various vertebrates (fish, turtle, duck and water bird) from a large eutrophic Chinese lake, Lake Taihu, with toxic *Microcystis* blooms. *Sci. Total Environ.* 407: 3317-3322.
- Chia AM (2007). Occurrence and abundance of algal species in relation to heavy metals content and physicochemical parameters of selected ponds in Zaria, Nigeria. M.Sc Thesis, Ahmadu Bello University, Zaria Nigeria.
- Chindah AC, Pudo J (1991). Physicochemistry and Phytoplankton of a Brackish water fish pond of the Bonny Estuary Nigeria. *Afr. J. Environ. Study*, 2(2): 63-67.
- Chorus I, Bartram J (1999). *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring, and Management*. E&FN Spon, London.
- Chu FS, Huang X, Wei RD (1990). Enzyme-linked immunosorbent assay for microcystins in blue-green algal blooms. *J. Assoc. Off. Anal. Chem.* 73: 451-456.
- Codd GA, Lindsay J, Young FM, Morrison LF, Metcalf JS (2005). Harmful cyanobacteria: from mass mortalities to management measures. In *Harmful cyanobacteria*. Edited by J. Huisman, H.C.P. Matthijs, and P.M. Visser. Springer, Dordrecht, Netherlands, pp. 1-24.
- Craig M, Luu HA, McCready T, Williams DE, Andersen RJ, Holmes CFB (1996). Molecular mechanisms underlying the interaction of motuporin and microcystins with type-1 and 2A protein phosphatases. *Biochem. Cell Biol.* 74: 569-578.
- Ekpenyong E (2000). Algal biomass and pigment diversity in typical tropical fish ponds. *Trop. Ecol.* 41(1): 89-94.
- Finni T, Kokonen K, Olsonen R, Wallstrom K (2001). The history of cyanobacterial blooms in the Baltic Sea. *Ambio*, 4-5: 172-178.
- Fischer WJ, Dietrich DR (2000). Pathological and biochemical characterization of microcystin-induced hepatopancreas and kidney damage in carp (*Cyprinus carpio*). *Toxicol. Appl. Pharm.* 164: 73-81.
- Fischer WJ, Garthwaite I, Miles CO, Ross KM, Aggen JB, Chamberlin AR, Towers NR, Dietrich DR (2001). Congener independent immunoassay for microcystins and nodularins. *Environ. Sci. Technol.* 35: 4849-4856.
- Fisher RA (1925). *Statistical Methods for Research Workers*. Oliver and Boyd Edinburgh, Scotland.
- Gkelis S, Lanaras T, Sivonen K (2006). The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. *Aquat. Toxicol.* 78: 32-41.
- Grosse Y, Baan R, Straif K, Secretan B, El Ghissassi F, Coglianov V (2006). Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. *Lancet Oncol.* 7(8): 628-629.
- Havens KE, James RT, East TL, Smith VH (2003). N: P ratios, light limitation, and cyanobacterial dominance in a subtropical lake impacted by non-point source nutrient pollution. *Environ. Pollut.* 122(3): 379-390.
- Hawkins PR, Novic S, Cox P, Neilan BA, Burns BP, Shaw G, Wickramasinghe W, Peerapornpisal Y, Ruangyuttikarn W, Itayama T, Saitou T, Mizuochi M, Inamori Y (2005). A review of analytical methods for assessing the public health risk from microcystin in the aquatic environment. *J. Water SRT- Aqua.* 54: 509-518.
- Henriksen P (1996). Microcystin profiles and contents in Danish populations of cyanobacteria/blue-green algae as determined by HPLC. *Phycologia* 35: 102-110.
- Howard A, Easthope MP (2002). Application of a model to predict cyanobacterial growth patterns in response to climatic change at Farmoor Reservoir, Oxfordshire, UK. 282-283: 459-469
- Hudnell HK (2008). *Cyanobacterial Harmful Algal Blooms-State of the Science and Research Needs*. New York: Springer.
- Huisman J, Matthijs HCP, Visser PM (2005). *Harmful Cyanobacteria*. Norwell, MA: Springer.
- Humpage A, Burch M (2007). Blue-green algae: Toxicity and public health Significance. *Public Health Bull.* 4(2): 25-28.
- Ibelings BW, Bruning K, de Jonge J, Wolfstein K, Pires LMD, Postma J, Burger T (2005). Distribution of microcystins in a lake foodweb: no evidence for biomagnification. *Microb. Ecol.* 49: 487-500.
- Konopka A, Brock TD (1978). Effect of Temperature on Blue-Green Algae (Cyanobacteria) in Lake Mendota. *Appl. Environ. Microbiol.* 36 (4): 572-576
- Kotak BG, Zurawell RW, Prepas EE, Holmes CF (1996). Microcystin-LR concentration in aquatic food web compartments from lakes of varying trophic status. *Can. J. Fish. Aquat. Sci.* 53: 1974-1985.
- Krishnamurthy T, Carmichael WW, Sarver EW (1986). Toxic peptides from freshwater cyanobacteria (blue-green algae). I. Isolation, purification and characterization of peptides from *Microcystis*

- aeruginosa* and *Anabaena flos-aquae*. *Toxicon* 24: 865-873.
- Landsberg JH (2002). The effects of harmful algal blooms on aquatic organisms. *Rev. Fish. Sci.* 10: 113-390.
- Lee SJ, Jang MH, Kim HS, Yoon BD, Oh HM (2000). Variation of microcystin content of *Microcystis aeruginosa* relative to medium N:P ratio and growth stage. *J. Appl. Microbiol.* 89: 323-329.
- Li D, Xie P, Zhang X (2008). Changes in plasma thyroid hormones and cortisol levels in crucian carp (*Carassius auratus*) exposed to the extracted microcystins. *Chemosphere*, 74(1): 13-18.
- Lind EM (1974). *Handbook of common methods in limnology*. C.V. Mosby publishers, St. Louis, U.S.A.
- Mackereth FJH (1963). Some methods of Water analysis for Limnologist scientist publs. *Freshwater biology* 21:71.
- Magalhães VF, Soares RM, Azevedo S (2001). Microcystin contamination in fish from the Jacarepagua Lagoon (Rio de Janeiro, Brazil): ecological implication and human health risk. *Toxicon* 39: 1077-1085.
- Magalhães VF, Marinho MM, Domingos P, Oliveira AC, Costa SM, Azevedo LO, Azevedo S (2003). Microcystins (cyanobacteria hepatotoxins) bioaccumulation in fish and crustaceans from Sepetiba Bay (Brasil, RJ). *Toxicon* 42: 289-295.
- Mitrovic SM, Pflugmacher S, James KJ, Furey A (2004). Anatoxin-a elicits an increase in peroxidase and glutathione S-transferase activity in aquatic plants. *Aquat. Toxicol.* 68(2): 185-192.
- Nwaura F, Koyo AO, Zech B (2004). Cyanobacterial blooms and the presence of cyanotoxins in small high altitude tropical headwater reservoirs in Kenya. *J. Water Health*, 2(1): 49-57.
- Natural Resources and Mines (NRM) (2005). *Monitoring Standard for Freshwater Blue-Green Algae (Cyanobacteria)*. Water Monitoring. Department of Natural Resources and Mines, Queensland Government.
- Oberemm A, Becker J, Codd GA, Steinberg C (1999). Effects of cyanobacterial toxins and aqueous crude extracts of Cyanobacteria on the development of fish and amphibians. *Environ. Toxicol.* 14: 77-88.
- Odokuma LO, Isirima JC (2007). Distribution of cyanotoxins in aquatic environments in the Niger Delta. *Afr. J. Biotechnol.* 6(20): 2375-2385
- Oh HM, Lee SJ, Jang MH, Yoon BD (2000). Microcystin production by *Microcystis aeruginosa* in a phosphorus-limited chemostat. *Appl. Environ. Microbiol.* 66: 176-179.
- Onuoha GC, Chinda A, Oladosun UA, Ayinla CA (1991). Effect of organic fertilization on pond productivity and water quality of fish ponds at Aluu, Nigeria. *Nigerian Inst. For Oceanog. And Marine Res. Lagos. Technical paper No. 74: 1-12.*
- Pichardo S, Jos A, Zurita JL, Salguero M, Cameán AM, Repetto G (2007). Acute and subacute toxic effects produced by microcystin-YR on the fish cell lines RTG-2 and PLHC-1. *Toxicology*, 21(8): 1460-1467.
- Prescott GW (1977). *The Fresh Water Algae*. WMC Brown Company Publishers, Dubuque, Iowa.
- Rinehart KL, Namikosh M, Choi BW (1994). Structure and biosynthesis of toxins from blue-green algae (cyanobacteria). *J. Appl. Phycol.* 6: 159-176.
- Robinson NA, Matson CF, Pace JG (1991). Association of microcystin-LR and its biotransformation products with a hepatic-cytosolic protein. *J. Biochem. Toxicol.* 6: 171-180.
- Rodger HD, Turnbull T, Edwards C, Codd GA (1994). Cyanobacterial (blue-green algal) bloom associated pathology in brown trout, *Salmo trutta* L., in Loch Leven, Scotland. *J. Fish Dis.* 17: 177-181.
- Sipia VO, Kankaanpaa HT, Flinkman J, Lahti K, Meriluoto JAO (2001). Time-dependent accumulation of cyanobacterial hepato-toxins in flounders (*Platichthys flesus*) and mussels (*Mytilus edulis*) from the northern Baltic Sea. *Environ. Toxicol.* 16: 330-336.
- Sivonen K, Carmichael WW, Namikoshi M, Rinehart KL, Dahlem AM, Niemelä SI (1990). Isolation and characterization of hepatotoxic microcystin homologs from the filamentous freshwater cyanobacterium *Nostoc* sp. strain 152. *Appl. Environ. Microbiol.* 56: 2650-2657.
- Sivonen K, Jones G (1999). Cyanobacteria toxins. In: Chorus, I., Bartram, J. (Eds.), *Toxic Cyanobacteria in Water: A Guide to their Public Health Consequence, Monitoring and Management*. E and FN Spon, London and New York, pp. 41-111.
- Tencala F, Dietrich D (1997). Biochemical characterization of microcystin toxicity in rainbow trout (*Oncorhynchus mykiss*). *Toxicon* 35(4): 583-595.
- Unyimadu JP (2002). Paralytic toxin profiles in shellfish. *J. Chem. Soc. Nig.* 27(1): 88-91
- Vezie C, Bertru G, Lefeuvre JC, Salkinoja-Salonen M (1998). Variance of microcystin content of cyanobacterial blooms and isolated strains in Lake Grand-Lieu (France). *Microb. Ecol.* 35: 126-135.
- Watanabe MF, Oishi S (1985). Effects of environmental factors on toxicity of a cyanobacterium (*Microcystis aeruginosa*) under culture conditions. *Appl. Environ. Microb.* 49: 1342-1344.
- Wiedner C, Visser PM, Fastner J, Metcalf JS, Codd GA, Mur LR (2003). Effects of light on the microcystin content of *Microcystis* strain PCC 7806. *Appl. Environ. Microb.* 69: 1475-1481.
- Wilson AE, Gossiaux DC, Hook TO, Berry JP, Landrum PF, Dyble J, Guildford SJ (2008). Evaluation of the human health threat associated with the hepatotoxin microcystin in the muscle and liver tissues of yellow perch (*Perca flavescens*). *Can. J. Fish. Aquat. Sci.* 65: 1487-1497
- Xie L, Xie P, Ozawa K, Honma T, Yokoyama A, Park HD (2004). Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. *Environ. Pollut.* 127: 431-439.
- Xie L, Xie P, Guo L, Li L, Miyabara Y, Park HD (2005). Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. *Environ. Toxicol.* 20: 293-300.
- Zhao M, Xie SQ, Zhu XM, Yang YX, Gan NQ, Song LR (2006). Effect of dietary cyanobacteria on growth and accumulation of microcystins in Nile tilapia (*Oreochromis niloticus*). *Aquaculture* 261: 960-966.
- Zimba PV, Khoo L, Gaunt PS, Brittain S, Carmichael WW (2001). Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from *Microcystis* toxins. *J. Fish Dis.* 24: 41-47.