Bayero Journal of Pure and Applied Sciences, 2(1):189 - 197
Received: June, 2009
Accepted: June, 2009

DETECTION OF VARIANTS OF MICROCYSTIN PRODUCED BY *MICROCYSTIS AERUGINOSA* IN SOME BURROW PITS OF KANO, NIGERIA

Indabawa, I.I.
Department of Biological Sciences, Bayero University, P.M.B 3011, Kano, Nigeria

ABSTRACT
Water samples of some burrow pits in Kano metropolis were examined for Microcystis aeruginosa and their ability to produce toxins. Identification for the organisms was achieved by the use of standard procedure and its pure culture was obtained by capillary pipette technique. The pure culture was harvested and dried at 25°C. These were kept in refrigerator before being subjected for the analysis of toxins High liquid performance chromatography was used to detect the presence of toxins. HPLC extracts on analysis, were found to contain four variants of microcystins- RR (24%), YR (26%), LR (48%), and LF (2%). Analysis of variance (ANOVA) of the result showed that the microcystin variants in all the six samples did not vary at 5%level or p<0.05 during the study period. The results of the finding show the possibility of poisoning effect on a variety of livestock, fishes, domestic animals and human beings that uses the burrow pit water for drinking.

Keywords: Microcystis aeruginosa, toxin, Burrow pits, Kano

INTRODUCTION

Cyanobacteria or blue – green algae have the potential to produce blooms, scums, mats or pea soups in natural and controlled water bodies under favourable conditions (Codd *et al.,* 2005). These blooms cause taste and odour problems, discolours water which can hamper recreation by reducing water clarity, depletion of oxygen as well as problems in the production of safe drinking water (Fogg *et al.* 1973). Furthermore, *Cyanobacteria* are well noted to be able to potentially synthesize a large number of low molecular weight, potent, bioactive compounds termed' cyanobacterial toxins 'or cyanotoxins like microcystin, nodularin, saxitoxin, anatoxin etc. Microcystin are widely distributed and are the most common of the cyanobacterial toxins found in water and was reported to have caused livestock, wildlife and pests fatalities and primary liver cancer in humans (Chorus *et al.,* 1999). An extensive study on the detection and isolation of microcystins variants from 19 strains of *Microcystis aeruginosa* isolated from ponds in Morocco was carried out by Quadra *et al* (2001).They detected and isolated three microcystins variants (MC-LR, LF and YR) from such cyan bacterium by High performance liquid chromatography with photo diodide array detection. A similar work was also carried out by Nasi *et al* (2004) from Qubera ponds located in Eastern Algeria. The result revealed that the extract of *Microcystis aeruginosa* sampled from those ponds contained four microcystins variants (MC-LR,RR,YR and LF).

A number of analytical techniques are currently available for the detection, separation and quantification of microcystins from water samples and laboratory grown toxin producing species (Rapala *et al.,* 2002). HPLC is the most widely used technique for routine analysis of microcystin because it facilitates the identification and quantification of microcystin variants due to their specific retention times (Lawton *et al.,* 1994).The study was inspired by the serious concern expressed by the members of the surrounding communities in Kano about harmful algal blooms as well as the public health implications of the formation of this bloom presently witnessed in several burrow pits around Kano metropolis.Kano burrow pits have great ecological, commercial and socioeconomic importance and values. They contained very rich components of biodiversity especially fishes, and the pond water is also being used by the surrounding community for recreation,fishing,irrigation and building etc.The incidence of algal blooms in these ponds has increased over the past few decades due to the rising nutrient levels(Fogg, *et al*.1973).This causes imbalance in the ecosystem and also environmental health problems, posing a serious threat not only to the human populace of the surrounding communities but also to the fishes and in general deterioration of water quality. The study was aimed at isolating and identifying microcystins variants from the pure culture of *Microcystis aeruginosa* isolated from Kano burrow pits by high performance liquid chromatography (HPLC). Identification and isolation of the toxins produced by *M. aeruginosa* samples from these burrow pits will provide a baseline data on different types of microcystins variants present in these burrow pits.

MATERIALS AND METHODS

Sampling sites

The sampling sites comprised of six burrow pits located within Kano metropolis located on Latitude 11° 58' 57.3' N and Longitude 8° 31' 31.1'E and they are designated I – VI.

Site I: Hauren Shanu Pond, Site II: Hauren Wanki Pond, Site III: Dan – agundi pond, Site IV: Gidan Murtala pond, Site V: Gwale pond and Site VI: Gyadi – Gyadi pond (Figure 1)

Collection of water samples for *Microcystis aeruginosa*

Water samples were collected monthly, for three years (2004 - 2006) from the six burrow pits and examined for the presence of *Microcystis aeruginosa* as described by Burns *et al,* (1974).

Identification of *Microcystis aeruginosa*

Microcystis aeruginosa was identified from the samples by the use of standard phycological keys described by Palmer (1980).

Isolation and Culturing of *M. aeruginosa*

Pure culture of *Microcystis aeruginosa* was obtained by capillary pipette isolation method described by Bold (1972) and Weidman *et al.* (1984).

Harvesting, preservation and storage of *Microcystis aeruginosa* cultures

The pure unialgal *Microcystis aeruginosa* cultures were harvested and preserved in a refrigerator 0C prior to the microcystin analysis according to the method described by Daily and Dawson (1974). The pure established culture was harvested after four weeks. 50ml of each sample was filtered through a pre-weighed glass fibre filters (GF/C 47 mm diameter Whatman) and dried. The weight of the dry cells were recorded and stored or kept in a deep freezer at 0C.

Extraction of Microcystin

A dry cell mass (50mg) of each prepared *Microcystis* sample was extracted three times with 3ml 50% methanol for 1 hour while stirring as described by Edward *et al.* (1992).

Detection and identification of the microcystin variants by High performance liquid chromatography (HPLC)

Microcystin variants in extracted samples were detected and identified by HPLC with photodiode array detection (PDA) following the method of Lawton *et al.* 1994; Rivasseau *et al.,* 1999 and Lawrence *et al.* 2001.

Statistical Analysis

Analysis of variance (ANOVA) operated using Microsoft SPSS software was used to be determined whether significant differences exist in the occurrence of microcystin variants throughout the study period.

RESULTS

The result of HPLC assay in all the six sites through out the sampling period (2004 – 2006) is presented in Figures 2 – 7. The analysis of HPLC chromatograms (Figures 5 and 6) show four fractions of microcystin from the *Microcystis aeruginosa* culture isolated from the six burrow pits. The fractions or microcystin peaks were identified by the retention time's absorption spectra of 231.0, 233.3 and 239.2 respectively. There are four variants of microcystin that were identified, these are: Microcystin- RR, MC- YR, MC- LR and MC- LF. These were confirmed by their absorption spectra profile and retention times (Figures 1 – 6).

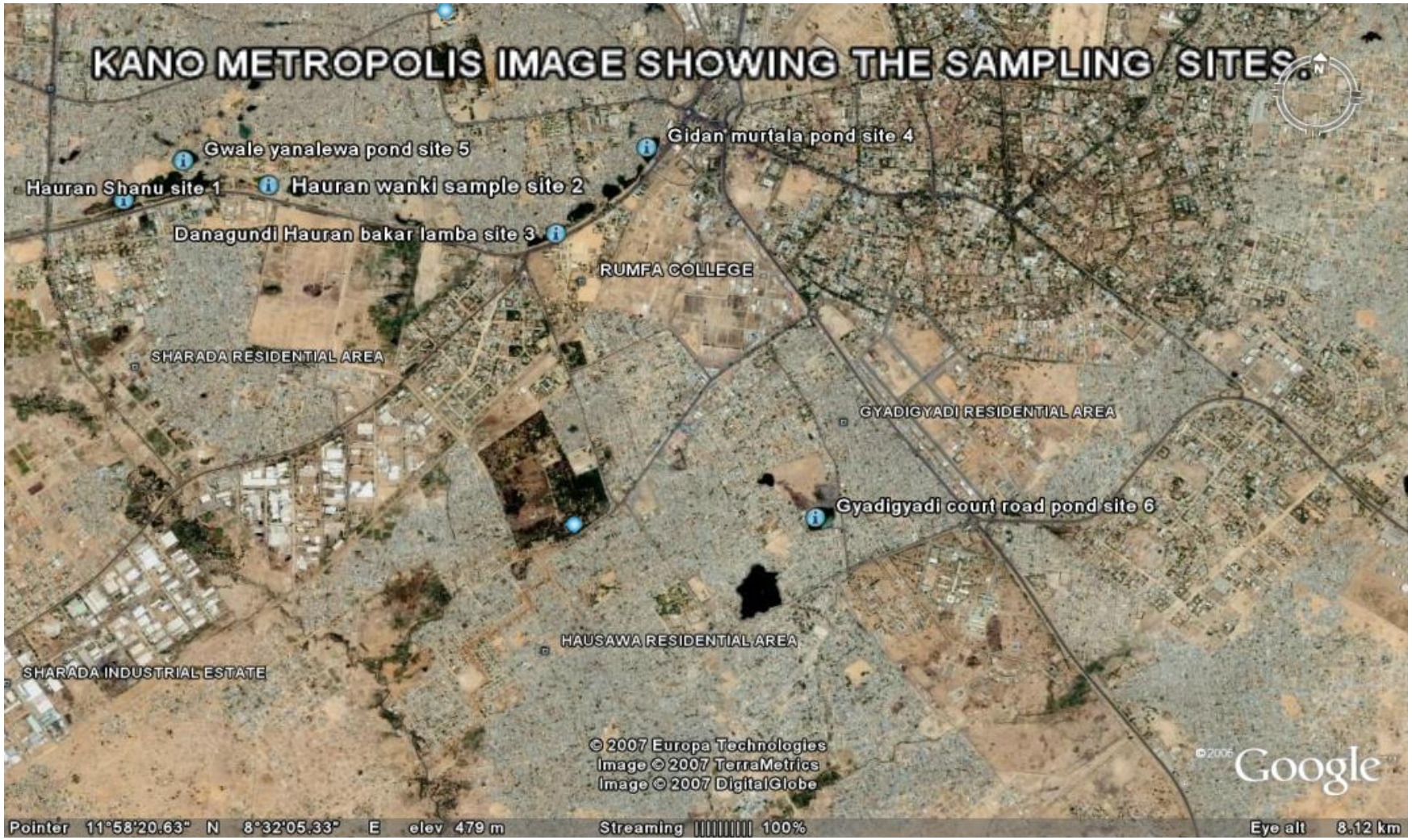

Figure 1: Map of Sampling Sites

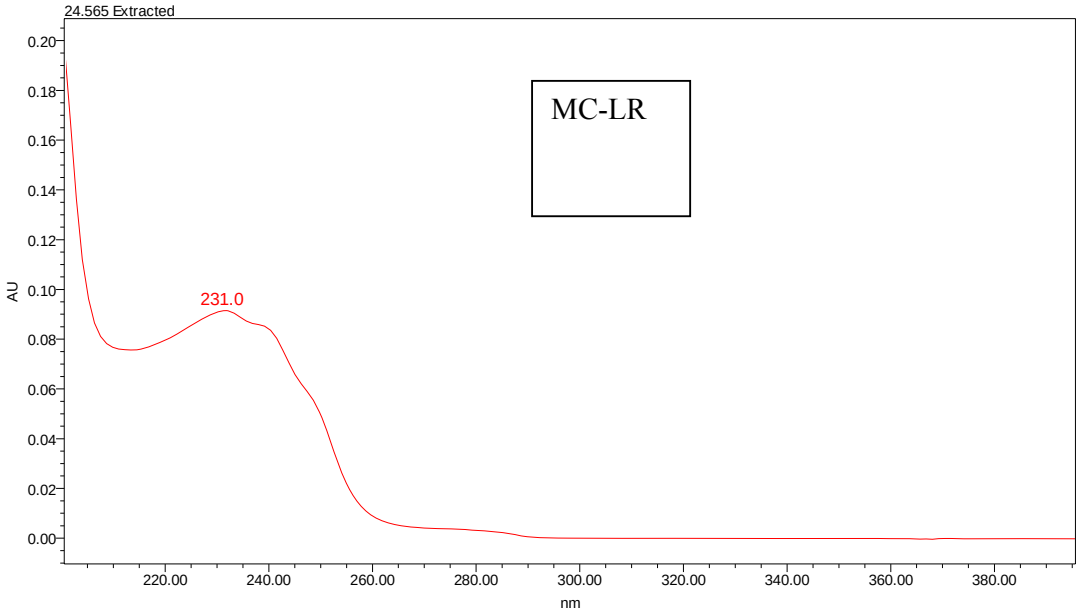

Figure 2: Absorption Spectra and retention time of *Microcystin* - LR as determined by HPLC-PDA

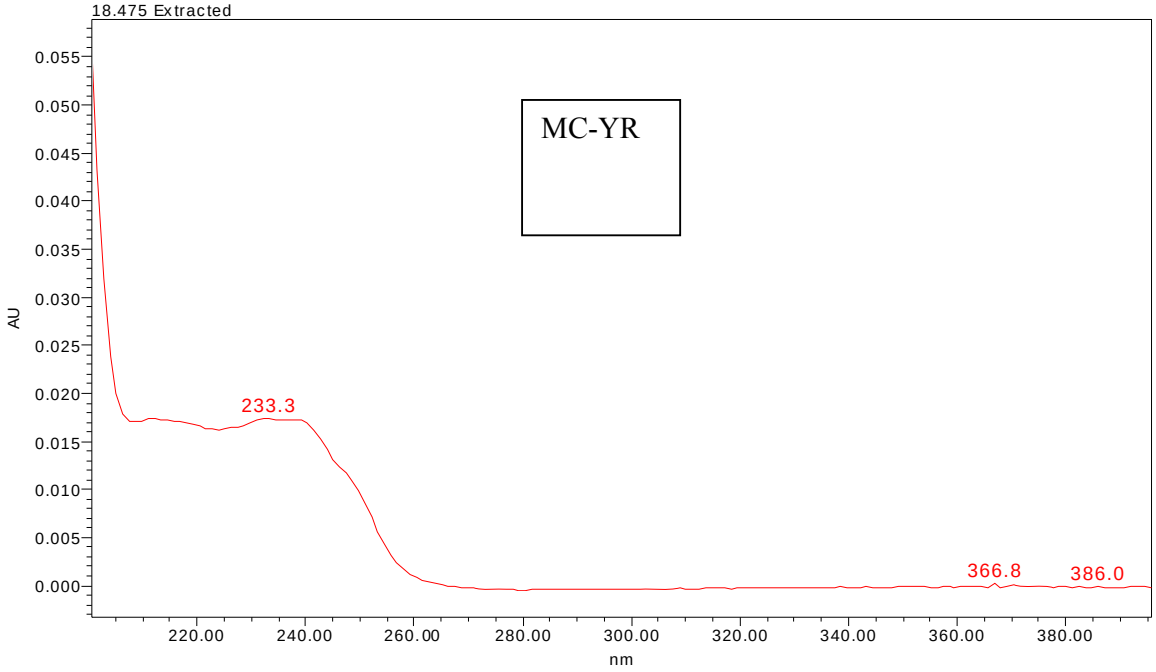

Figure 3: Absorption Spectra and retention time of *Microcystin* - YR variant as determined by HPLC-PDA

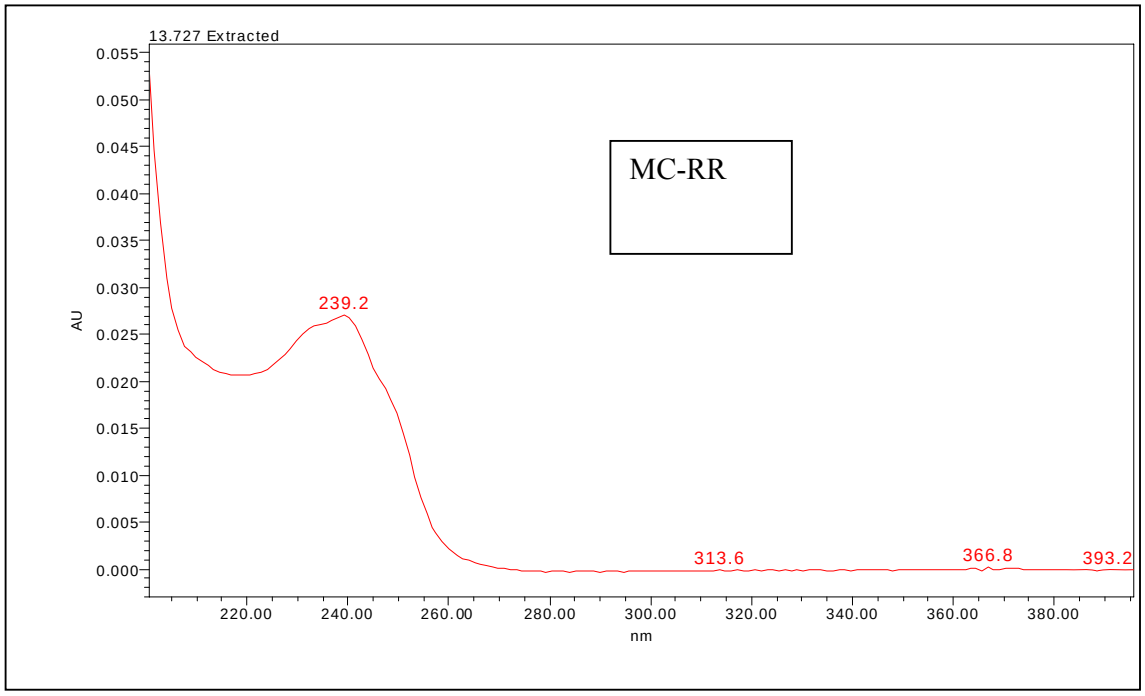

Figure 4: Absorption Spectra and retention time of *Microcystin* - RR variant as determined by HPLC-PDA

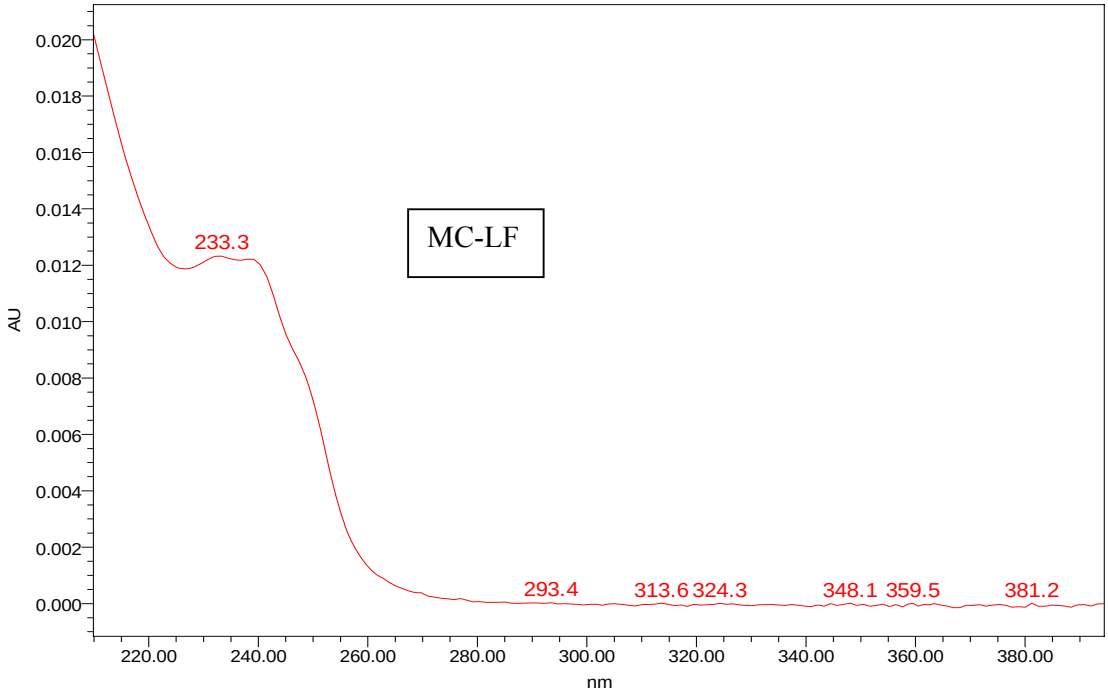

Figure 5: Absorption Spectra and retention time of *Microcystin* - LF variant as determined by HPLC-PDA

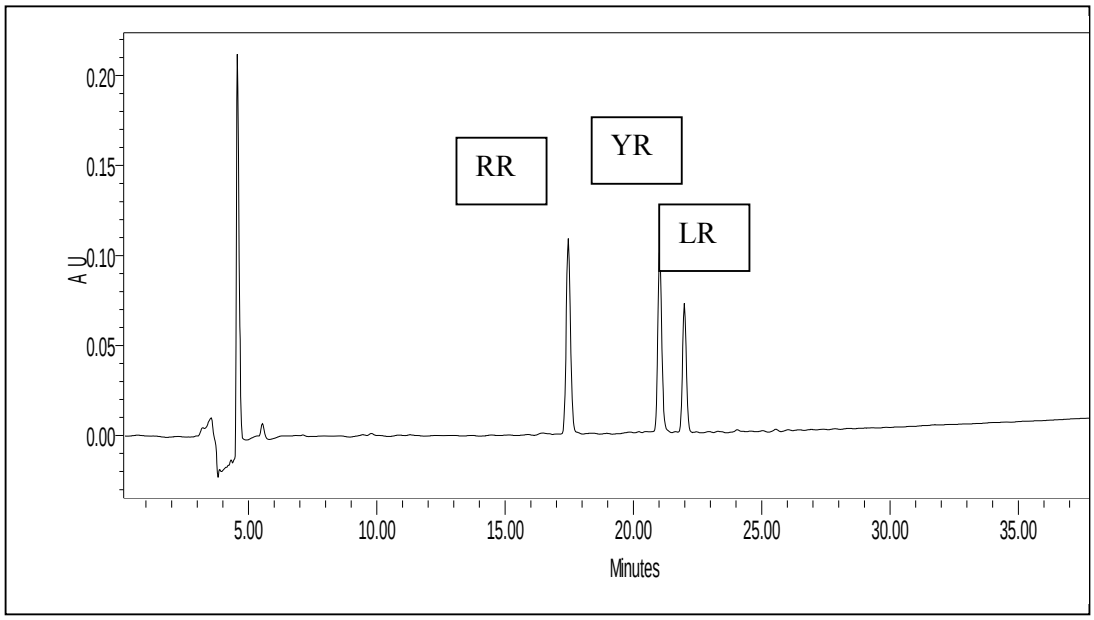

Figure 6: HPLC chromatograms of the toxin variants (RR, YR, LR) detected and identified in *M aeruginosa* isolates from six burrow pits in Kano metropolis

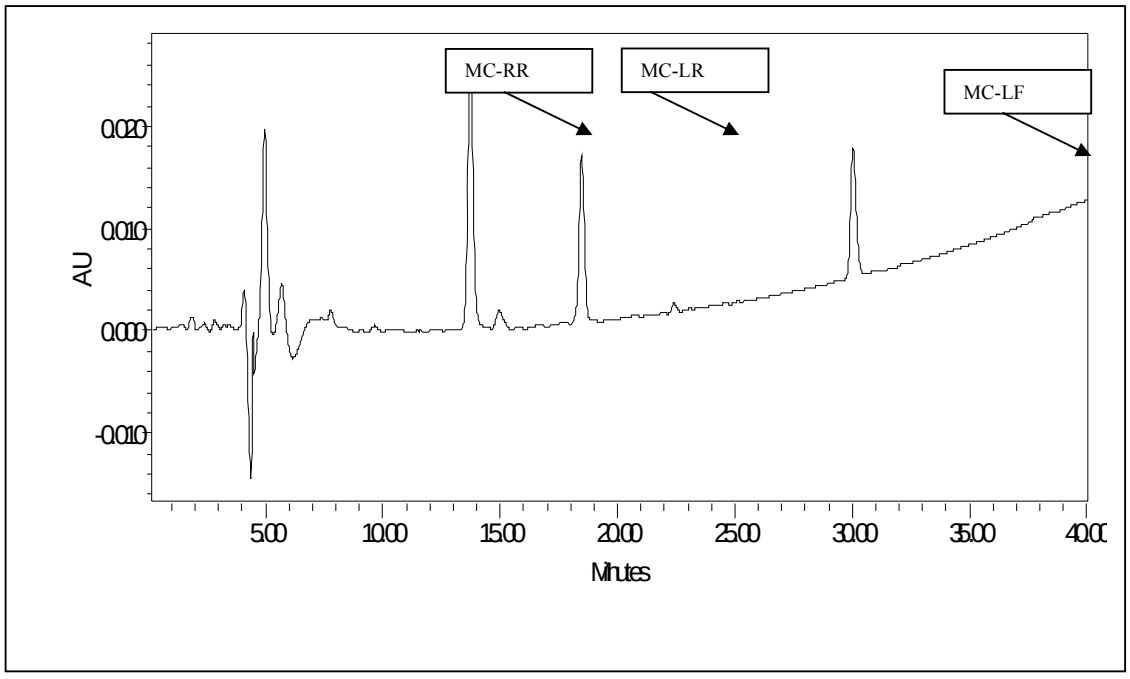

Figure 7: HPLC chromatograms of the toxin variants (MC, RR, LR and LF) detected and identified in *M. aeruginosa* isolates from six burrow pits in Kano metropolis

Table 1 shows the retention times of the four detected variants. It was observed that the retention times of these four variants were between 14 and 30 mins. Microcystin RR has the lowest retention time of 14min and was eluted first been less hydrophobic followed by Microcystin YR (18min), Microcystin LR (25min) and Microcystin LF (30min).

The result also showed that the microcystin variants in all the six samples varied during the study period (Table 2). Microcystin LR was detected and isolated in all samples through out the study period, so also microcystin RR apart from samples IV and V; while other variants were found to change (Table 2). In sample I Table 2, only three variants each (MC- RR, LR and LF) and (MC – LR, LR and YR) were isolated in 2004 and 2006 respectively while MC- YR and LF were not detected during these periods and all the four variants (MC RR, LR, YR and LF) were detected and isolated in 2005. Sample from site II also showed a marked variation of microcystin variants throughout the sampling periods. All the four variants (MC – RR, LR, YR and LF) were detected and isolated in 2004 sample, while similar variants (MC – RR, LR and YR) were isolated in 2005 and 2006 with MC – LF notably not detected during these periods.

The result from Table 2 further revealed that only sites III and VI contained common variants (MC – RR, YR, LR and LF) i.e. these same variants were detected and isolated from them throughout the study period. However, all the four microcystin variants (MC-RR, LR, YR and LF) were also detected and also identified in 2004 and 2005 from site IV, whereas only two variants (MC-LR and YR) were present in 2006 in the same sample with MC- RR and LF not detected. On the other hand, sample from site V contained all the four variants (MC- RR, LR, YR and LF) which were detected in 2005 only, while two variants each were detected in 2004 (MC-LR, and LF) and 2006 (MC – LR and YR) respectively.

Table 1: Microcystin variants isolated with their retention times

Name of component	Toxin variant Retention time (RT)	Absorption spectra (nm)
MC- RR	13.73	239.2
MC -Y R	18.48	233.3
MC - LR	24.57	231.0
MC – LF	30.05	233.3

Table 2.0: Variation of microcystins content in *M. aeruginosa* identified during the study period (2004 – 2006).

	Year and variant identified		
Sample	**2004**	**2005**	**2006**
I	RR, LR, LF	RR, LR, YR, LF	RR, LR,YR
Ii	RR, LR, YR, LF	RR, LR, YR	RR, LR, YR
Iii	RR, LR, YR, LF	RR, LR, YR, LF	RR, LR, YR, LF
Iv	RR, LR, YR, LF	RR, LR, YR, LF	LR, YR
V	LR, LF	RR, LR, YR, LF	LR, YR
Vi	RR, LR, YR, LF	RR, LR, YR, LF	RR, LR, YR, LF

Result of Statistical Analysis of the data

ANOVA showed that the variance ratio (P = 0.393501) at 5% was smaller than the tabulated value of 0.602 (P <0.05). Therefore, no significant difference on the occurrence of four microcystin variants throughout the study period concerned.

Table 3: ANOVA for the variation of four microcystin variants detection during the study period.

Source of variation	SS	Df	MS	F	P – value
Between groups	3.16667	2	1.58333	1.0363636	0.393501
Within groups	13.75	9	1.527778		
Total	**16.91667**	**11**			

DISCUSSION

Toxic Algae have become an increasing world wide problem in aquatic habitats. These occurrences can be partially attributed to the gradual eutrophication of the water bodies due to inputs from house hold wastes, or sewage, industrial effluents, run off from roads. This has a considerable impact upon recreational water quality, and is also responsible for the world wide killing of wild and domestic animals, fishes, birds, human fatalities and an increased incidence of liver cancer (Carmichael, 2001). The result of this study has indicated that the *Microcystis aeruginosa* exists in all the six sewage ponds investigated.

This was in line with the work of Wirsing *et al,* (1995) and Nasri *et al* (2004) who made similar observations in Qubera pond (Algeria) and Liege pond (Belgium) respectively. The study also identified four different variants (MC- RR, YR, LR and LF) of microcystins from the algae in the six burrow pits. This agrees with the work of Nasri *et al.* (2004) who detected and isolated similar variants from *Microcystis aeruginosa* sampled from Qubera pond, located in Eastern Algeria. The results from this study are similar to the findings of Delaney *et al* (1995) and Linda, (1999) who also isolated and identified four different variants (MC- LR, YR, RR and LF) from *Microcystis aeruginosa* that were isolated from different ponds in UK. The result of this research was however found to be slightly contrary to the findings of Sagir *et al* (2002) who isolated and characterized four microcystin variants (MC-LR, YR, LR and LA) by HPLC/MS from a bloom sample of *Microcystis aeruginosa* that was collected from ponds at different localities in Bangladesh.The two results show that three variants are common (MC- LR, YR, and LR) to both except that microcystin LA was obtained in the findings of Sagir *et al.* (2002) contrary to Microcystin LF that was isolated in this work.

The difference in the microcystin variants recovered from each burrow pit during the study period (2004-2006) could be attributed to differences in the growth conditions, nutrient concentrations and light intensity. Sivonen and Jones (1999) observed that factors like growth conditions, nutrients, light and other environmental factors had an effect on the cellular microcystin and also on the quantities of microcystin variants that are produced by Microcystin-producing genera. A similar observation on the variation of microcystin variants in different *Microcystis* blooms and laboratory cultures was reported to be related to the geographical location. Geographical patterns are indicated by some survey results in which variation among the L- amino acids of microcystin has been reported in South Africa (Scott, 1991), frequent presence of dimethyl microcystins (RR, and LR) has been observed in Finnish strains (Sivonen *et al* 1995).

Microcystin LR was detected in all the sites throughout the study period. This corresponds with the results of Skulbeg, (1989), who reported that MC-LR was the most common toxin variant present among the detected variants recovered from *M. aeruginosa* from Portuguese ponds, Lakes and reservoirs.. The result was also in conformity with the work of Culvin- Aralar *et al.* (2002) who investigated the natural bloom and laboratory cultures of *Microcystis aeruginosa* collected from Laguna ponds in Philippines. Their results showed that MC-LR was the most abundant toxin variant in both samples, accounting for 77 – 85% of the total toxin variants present.

Conclusion

The result of this study has confirmed the proliferation of *M. aeruginosa* and four microcystin variants (MC-RR, LR, YR and LF) in the six burrow pits investigated. The findings suggest regular monitoring and control of the blooms to protect public health.

REFERENCES

Bold F, A. (1999). *Algal activities and phytoplankton ecology*. Univ. Wisconsin Press.

Burns, N. M, Van O. Hir (1974). Standard methods for the examination of water and waste waters*. American public health associationVol 2 pp232-240.*

Carmichael, W. W (1988). Toxins of freshwater algae. In: A. T. T.U (ed).Marine toxins and venoms. PP 121 – 147. *Handbook of Natural Toxins*. 3: New York, Marcel Dekker.

Chorus, I (2001). Cyanotoxins occurrence in freshwaters – summary of survey results from different countries. In: I. Chorus (ed*). Cyanotoxins, occurrence, causes and consequences*. Springer – Verlag Berlin Heidelberg New York. ISBN 3 –540 – 64999 – 9.

Codd G.A, Brooks W.P, Lawton L. A, Beatrice K.A (1989). Cyanobacterial toxins in European waters: occurrence, properties, problems and requirements. In: Wheeler D, Richardson M.J, Bridges, J, editors. Watershed, 89: future of water quality in Europe. Oxford: Pergamon, 2:211 – 220.

Curvin- Aralar E.F, Michael F.C (2002). Microcystins in natural blooms and laboratory cultured *M. aeruginosa* from Laguna ponds in Philippines *Toxicon* 57, 145 – 149.

Daily, C.F, and Dawson, R.M. (1974). The toxicology of microcystins. *Toxicon:* 36: 953 – 962.

Delaney, J.M. and Wilkins, R.M, (1995). Toxicity of microcystin – LR, isolated from *Microcystis aeruginosa*, against various insect species. *Toxicon* 33 (6): 771 – 778.

Edwards, C., Beattice, K.A Scrimgeour, C. and Codd, G.A. (1992). Identification of anatoxin – a in benthic cyanobacterium (blue – green algae) and in associated dog poisoning at Loch insh, Scotland, *Toxicon* 30, 1165 – 1175.

Fogg, G.E, Stewart, W.D.P, Pay, P and Walsby, A.E (1973). The blue – green Algae. Academic press, London, 459 pp.

Lawrence, J.F, Menard, C (2001). Determination of microcystins in blue – green algae , Fish and water using liquid chromatography with ultraviolet detection after sample clean – up employing immunoaffinity chromatography. *J. Chromatogr. A*. 922, 111 – 117.

Lawton, L.A, Edwards C, Codd, G.A, (1994). Extraction and high performance liquid chromatographic method for the determination of microcystins in raw and treated water. *Analyst* 119, 1525 – 1530.

Linda, A.L (1999). A bioactive modified peptide, Aeruginosamide, isolated from cyanobacterium *Microcystis aeruginosa. J. Org. Chem*. 641, 5329 – 5332.

Nasri, A.B, Bouaicha, N, Fastener, J. (2004). First report of microcystin containing bloom of cyanobacterium Microcystis spp. In Lake Quibera, Eastern Algeria. *Arch. Environ. Contam. Toxicol*. 46: 197 – 202.

Palmer, C.M, (1980). Algae in water supplies, US public health service publication No. 657, Washington.

Rapala J, Erkomaa, K, Kekkonen, J. Sivonen, K. Lahti, K. (2002). Detection of microcystins with protein phosphatases inhibition assay, high performance liquid chromatography – UV detection and enzyme – linked ImmunoSorbent assay. Comparison of methods. *Anal. Chim. Acta* 466, 213 – 231.

Rivasseau, C. Racaud, P, Deguin, A, Henion, M.C, (1999). Development of a bioanalytical phosphatase inhibition test for the monitoring of microcystins in environmental samples. *Anal. Chim. Acta*. 394, 243 – 257.

Sagir, M.A, Jens, D.A, Bern L.C. (2002). Toxic cyanobacteria bloom and the occurrence of microcystin from freshwater eutrophic ponds in Bangladesh. 5th international conference on harmful algae. Tradwinds island Grand resort and conference center, St. Pet Beach, Florida.

Scott, W.E, (1991). Occurrence and significance of toxic cyanobacteria in southern Africa. *Water. Sc. Technol*. 23: 175 – 180.

Sivonen, K, and Jones, G, (1999). Cyanobacterial toxins in: Chorus, I, Bertram, J. (Eds.) Toxic cyanobacteria in water. E and F N Spon, London, Pp. 41 – 111.

Skulberg, O.M. (1989). Cyanobacteria/cyanotoxin research – 100kingback for the future. *J. Environmental Toxicology,* 20:220 – 228.

Weidman, V.E, Walne, P.L. and Trainor, F.R (1984): A new technique for obtaining axenic cultures of algae. *Can. J. Bot*. 42: 958- 959.