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Detection and Quantification of Microscystin by Protein Phosphate Inhibition Assay From *Microcystis aeruginosa* Isolated From Burrow Pits in Kano, Nigeria

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ABSTRACT: Protein phosphatase inhibition assay (PPIA) was used to detect and quantify microcystin extracted from *Microcystis aeruginosa* isolated from burrow pits in Kano. Water samples were collected monthly between 2004 and 2006, samples were kept in refrigerator for 24 hours. Identification of the organism was achieved by using standard procedure, and its pure culture was obtained by capillary pipette technique. The pure culture was harvested and dried at 25^{o}C. These were kept in refrigerator before being subjected for the analysis of toxins. Protein phosphatase inhibition assay (PPIA) was used to detect and quantify them. PPIA extracts revealed the presence of microcystin in quantities ranging from 3.0µg/g and 12 µg/g dry weights. The results of the finding show the possibility of poisoning effect on a variety of livestock, fishes, domestic animals and human beings that use the burrow pits water for drinking.

Keywords: Protein phosphatase inhibition assay, microcystin, *Microcystis aeruginosa,* Burrow pits, Kano

Introduction

Cyanobacteria (blue –green algae) are natural inhabitants of fresh, brackish and marine waters and are of worldwide distribution. (Fogg *et al.,* 1973). The cyanobacteria provide an extra ordinary wide ranging contribution to human affairs in everyday life and are of economic important (Mann and Carr, 1992). They are important primary producers and their general nutritive value is high. The nitrogen – fixing species contribute globally to soil and water fertility (Rai, 1990). The use of cyanobacteria in food production and in solar energy conversion holds promising potentials for the future (Skulberg, 1995). However, cyanobacteria may also be a source of considerable nuisance in many situations. They have the potential to produce mass populations (blooms or scums) in natural and controlled water bodies under favourable conditions (Coddet *et al.,* 2005). These blooms cause taste and odour problems, discolours. Water which can hamper recreation by reducing water clarity (Fog *et al.,* 1973). Furthermore, cyanobacteria are well documented as being able to potentially synthesize a large number of low molecular weight, potent bioactive compounds known, as cyanotoxins like microcystin, nodulavin, anatoxin, Saxitoxin 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 pest mortalities and primary liver cancer in humans (Chorus *et al.,* 1999).

A number of analytical, biological, biochemical and immunological techniques have been developed for the detection and quantification of microcystin from water samples and laboratory grown toxin producing species (Rapala *et al.,* 2002). In recent years, methods exploiting the biochemical properties of

microcystitis have been increasingly employed in sample screening. The calorimetric protein phosphatase inhibition assay ({{PA) is commonly used to obtain a rapid detection and quantification of microcystin because it is simple to use, sensitive, cheap due to the commercial availability of the enzymes, and correlates well with HPLC for the detection of microcystins in water and cyanobacterial samples even in small quantities (Microgram e or nanogramme). (Rapala *et al.,* 2002). This assay is based on the ability of microcystin to inhibit enzymes protein phosphatases (PP_1 and PP_{2A}) and is particularly useful as indication of biochemical activity of the microcystin present in a given sample. 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 the study was aimed at the detection and quantification of microcystin from the pure culture of *Microcystis aeruginosa* isolated from Kano burrow pits by protein phosphates inhibition assay (PPIA).

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° 30′ 31.1′ E and they are designated I – VI. Site I Hauren shanu, Site II: Hauren Wanki Pond, Site III: Dan – agundi pond, Site IV: Gidan Murtala pond, Site V: Gwale pond, Site VI: Gyadi – Gyadi pond.

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 *Microcystis aeruginosa*

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

Harvesting, preservation and storage of *Microcystis aeruginosa* cultures

The pure unialgal *Microcystis aeruginosa* cultures were harvested and preserved in a refrigerator (°C) 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 0°C.

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 protein phosphatase inhibition assay.

Microcystins in extracted samples were detected and quantified using a modification calometric methods described by Carmichael (1994); Ward *et al.* (1997); Liu *et al.* (2002) and Boyer *et al.* (2004).

Results

PPIA

The PPIA test was found to be effective in confirming the presence and in quantifying microcystin concentrations that was isolated from the six burrow pits in Kano metropolis. Results of the total microcystin concentrations (µg/g) dry weight detected by PPIA assay in each sample throughout the study period (2004 – 2006) are presented in Tables 1- 6.

The results show that the total microcystin concentration in microgram per gram in these cultures ranged from 3.00µg/g – 12 .06µ/g. The highest microcystin concentration in microgram per gram in these cultures ranged from 3.00µ to 12.06µ/g was recorded in 2005 from site III (Table 3) while the lowest concentration (3.00µg/g) was obtained in 2006 from site V. The result from each site also showed variation in the amount of microcystin detected in relation to the weight of its dry cells throughout the study period (Tables 1 - 6).

Table 10 revealed that the total amount of microcystin detected in that culture ranged from 6.36 ug/g – 8. 09ug/g with the highest and the lowest concentrations recorded in 2004 and 2006. The amount of microcystin obtained from sample II was 5.00, 3.33 and 6.67ug/g in which the highest and the lowest values were obtained in 2006 and 2005 respectively (table 2.0). It was also observed that all the cultures from site III also showed variation in the amount of microcystin detected throughout the study period. It was 7.78 ug/g in 2004, then increased and reached its maximum value of 12.06 ug/g in 2005 and finally dropped to its minimum value (5.56ug/g) in 2006. The result from table 4.0 indicated that culture from that site had its maximum concentration value of 8.00 ug/g in 2005 which corresponded with a total weight lyophilized cells of 0.25g and a minimum value of 3.75 ug/g obtained in 2006. It was also observed from table 5.0 that V had a total microcystin concentration which ranged from 3.00 – 4.44 ug/g and finally increased again to 3.67ug/g in 2006.

In Table 6.0site VI, the amount of microcystin detected by this assay was 9.80ug/g, 5.41 ug/g and 8.95 ug/g in 2004, 2005 and 2006 respectively. It was however and also observed that the amount of microcystin detected in most of the cultures was found to have increased with increased weight of dry cells with the exception of few. For instance, the highest microcystin concentration (12.06ug/g) obtained in 2005 from site III corresponded with the highest mass value of 0.58g recorded from that culture. A similar pattern was also observed from sample I (Table 1.0), site III (Table 3.0), and table V site V in which the microcystin concentrations in those samples was found to increased with the increased in weight of the dry cells. But a different observation was made from sites II, IV and VI in which some cultures had a high amount of microcystin with low masses. For example in site II (Table II) the highest microcystin concentration (6.67 ug/g) obtained in 2006 corresponded with the lowest mass of 0.12g dry cells compared to the microcystin concentration detected in 2004 5.00ug/g/0.32g dry cell) and 3.33ug/g/0.21g in 2005 respectively.

Table 1: Total microcystin concentration recorded from site I during the sampling period.

Year	Weight of dry cells (g)	PPIA (µg/g dry weight)
2004	0.21	8.09
2005	0.15	8.00
2006	0.11	6.36

Table 2: Total microcystin concentration recorded from site II during the study period.

Year	Weight of dry cells (g)	PPIA (µg/g dry weight)
2004	0.32	5.00
2005	0.21	3.33
2006	0.12	6.67

Table 3: Total microcystin concentration recorded from site III during the study period.

Year	Weight of dry cells (g)	PPIA (µg/g dry weight)
2004	0.18	7.78
2005	0.58	12.06
2006	0.09	5.56

Table 4: Total microcystin concentration recorded from site IV during the study period.

Year	Weight of dry cells (g)	PPIA (µg/g dry weight)
2004	0.35	7.14
2005	0.25	8.00
2006	0.08	3.75

Table 5: Total microcystin concentration recorded from site v during the study period.

Year	Weight of dry cells (g)	PPIA (µg/g dry weight)
2004	0.09	4.44
2005	0.06	3.67
2006	0.05	3.00

Table 6: Total microcystin concentration recorded from site vi during the study period.

Year	Weight of dry cells (g)	PPIA (µg/g dry weight)
2004	0.51	9.80
2005	0.24	5.41
2006	0.19	8.95

Discussion

Increasing anthropogenic eutrophication in ponds, lakes, rivers, drinking water reservoirs and coastal waters is a world – wide phenomenon leading to the formation of blooms of toxic cyanobacteria. These pose a significant threat to wild and domestic animals, fishes, birds and human health (Carmichael, 2001). The result of this study has indicated that *Microcystis aeruginosa* exists in all the six burrow pits investigated. This was inline 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 quantities of microcystins detected in the six cultures of *Microcystis geruginosa* isolated from the burrow pits were between 3.00 and 12.06 ugly dry weight. This range corresponds with the microcystin concentrations of 3.00 – 12.00 µg/g dry weight reported by Kumar *et al* (2006) after their PPIA microcystin detection and quantitifation analysis. Nasri *et al.,* (2004) also recorded 3 - 12µg/g dry weight when they examined the amounts of microcystins present in *Microcystis aeruginosa* strain isolated from Qubera pond in Eastern Algeria. These findings are however different from the microcystin range of 3 – 25 µg/g dry weight reported by Sagir *et al.,* (2002) in a laboratory culture of *Microcystis aeruginosa.*

The variation of microcystin concentration examined among the six samples throughout the study could be attributed to the population size of *Microcystis* in each sample and also by the environmental factors. Chorus (2001) and Sivonen, (1990) reported that the amount of varying level of cellular microcystin concentration among the microcystin – producing cyanobacteria is contributed by the cell densily and the environmental factors such as pH, temperature, light and amount of nurtrients available. Thus the highest and the lowest concentrations of microcystin obtained in 2005 and 2006 from sites III and IV might be due to the variation in both microcystis population density as well as the environmental factors. These findings as present in tables 1 – 6 reveal that the amount of microcystins determined in the *Microcystis* cells

increased with increased cell biomass. This is in agreement with the findings of Kumar *et al.,* (2006) that toxin concentration in algae is expressed as a function of cell biomass (dry weight), they investigated the amount of Microcystin by PPIA from cultures of *Microcystis aeruginosa* from ponds in North India; and found that the higher concentrations of microcystins correlated with increased cell biomass. This accounts for the higher concentrations of microcystins extracted as cell biomass increased. It was however noted that the concentration of microcystin was found to be high even at low dry weight as observed in samples II, IV, and IV. This conforms with the earlier observations made by Kumar *et al.,* (2006) who reported that high microcystins in some toxigenic algae with low dry weight was related to environmental factors.

Conclusion

The result of this study has confirmed the proliferation of *M. aeruginosa* in Kano burrow pits. Investigated protein phosphatase inhibition assay (PPIA) was used in the detection and quantification of microcystin extracted from this cyanobacterium in milligramme quantities. It also gives an indication of the potential toxicity of the culture investigated. The findings suggest regular monitoring and control of the blooms to protect public health.

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