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Targeted and non-targeted LC-MS analysis of microcystins in *Clarias gariepinus* from fishponds

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ABSTRACT

Cyanotoxins produced by cyanobacteria are formidable threats to aquatic ecosystems and public health worldwide. The potential health risks associated with cyanotoxins from contaminated fishponds are becoming a growing concern, as cyanotoxin production has steadily increased over time in these aquatic environments. Therefore, this study aims to utilize targeted and non-targeted Liquid Chromatography Mass Spectrometer (LC-MS) analytical methods to detect cyanotoxins in catfish (*Clarias gariepinus*) tissue harvested from fishponds. For detecting cyanotoxins in fish tissue utilizing the non-targeted approach, high-resolution MS/MS spectra data obtained from the analysis were converted to mzML format, analyzed with the Global Natural Product Social (GNPS) Library and CANOPUS annotations for LEVEL 3 metabolite identification, and visualized as a molecular network in Cytoscape. Regarding the targeted method, the toxin identification and quantification were achieved by comparing samples spiked with known concentrations of MC-RR and YR to an authentic toxin standard. The results of the target analysis showed that microcystin variant MC-RR was not detected in the fish tissue. The MC-YR variant was detected in the intestines and gills of *Clarias gariepinus* at concentrations of 13.2−10.6 μg/g and 1.5 −13.9 μg/g, respectively. The muscle tissues across all fish ponds showed MC-YR concentrations between 10.5 and 16.06 μg/g. The highest concentration of MC-YR was found in the liver tissue in pond 6 (20.9 μg/g). The untargeted LC-MS method led to the identification of a larger number of cyanometabolites in the fish tissue, such as aeruginosins, anabaenopeptins, microginins. Non-toxic secondary metabolites like octadecadienoic acid, while phosphocholine (PC), ethanesulfonic acid, pheophorbide A, microcolins, cholic acid, phenylalanine, amyl amine and phosphocholine (PC), triglyceride (TG), phosphocholine (PC) and sulfonic acid derieved from cyanobacteria, fish and anthropogenic sources were also detected in the fish tissues. The non-targeted analysis facilitates the identification of both unexpected and unknown compounds.

1. Introduction

Cyanobacteria are present in freshwater, ponds, rivers, marine and other terrestrial environment. The unique morphological, physiological, and genetic traits of cyanobacteria make them rich sources and producers of different biological active metabolites [1,2]. Cyanobacteria metabolites (cyanometabolites) constitute a significant and rapidly expanding category of secondary metabolites, encompassing over 2000 compounds, which are synthesized by various genera of cyanobacteria [3]. Most of these secondary metabolites including different classes of cyclic peptides, phenols, lipids, alkaloids, vitamins, terpenoids, polysaccharides, and pigments identified in cyanobacteria have the potential to act as antioxidant, anticoagulant, anticancer, antiviral, antileishmanial, antibacterial, antiprotozoal, and anti-inflammatory [4,5]. Some of these secondary metabolites are widely applied in the pharmaceutical, cosmeceutical, and nutraceutical industries applications

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[5–7]. Furthermore, cyanobacteria have attracted attention as producers of novel metabolites significant in industry and medicine [8]. However, numerous cyanobacteria species that frequently form large blooms in fresh, brackish, and marine waters also produce metabolites called cyanotoxins [9]. Cyanobacteria are capable of producing potent toxins that can lead to fish mortality and bioaccumulate in fish muscle tissues [10]. These toxins are associated with acute lethal, acute, chronic, and sub-chronic poisonings in animals, and pose potential health risks to humans [9,11]. Based on their toxicological targets, the toxins are categorized as hepatotoxins, neurotoxins, and dermatotoxins [9]. According to Falconer [12], the maximum allowable concentration (MAC) for microcystins (MCs) in drinking water has been set at 1 μg per liter per day. World Health Organization (WHO) later proposed a provisional guideline for the Tolerable Daily Intake (TDI) of 0.04 μg per kilogram of body weight per day [13]. TDI refers to the amount of a potentially toxic substance that can be safely consumed daily over a lifetime [14]. Given the potential risks associated with cyanotoxins in food and water, the United States Environmental Protection Agency [15] has expanded this list by monitoring 10 cyanotoxins in public water systems. Most countries often align with international standards and guidelines set by organizations like WHO, EPA, or Food and Agriculture Organisation (FAO) to establish their own regulations or monitoring programs.

Advancements in both specific and broad-spectrum bioanalytical tools, coupled with the greater accessibility of calibration standards, have broadened the scope of toxic and bioactive metabolites that can be measured [16]. However, one of the major challenges in cyanometabolites analysis is the lack of pure standards [17,18]. Cyanometabolites standards are known to be expensive and limited to a few metabolites (mostly Microcystin and Nodularin) commercially available for purchase [18,19]. The lack of pure standards for over 2000 cyanometabolites prevents confirmation based on chromatographic properties [18]. Therefore, effective multiclass analytical methods are required to evaluate cyanotoxin in fish tissues and address these challenges [20]. The application of Mass spectrometry in combination with chromatography (Gas chromatography or Liquid chromatography) has been identified as a powerful analytical tool used for both quantitative screening and quantification of biological and environmental samples [21]. The sensitivity and selective detection of polar or ionic contaminants at trace levels using LC-MS have made it a highly favorable alternative to GC-MS [22]. The LC-MS screening can be targeted or non-targeted. The targeted analysis focuses on detecting known analytes using reference standards, but its scope is limited to the selected compounds and the availability of reference materials [22]. The non-target analysis allows for the detection of unexpected and unknown compounds by using molecular information and database searches, with identification aided by comparing MS/MS spectra to vendor-supplied libraries [22,23]. As new compounds emerge, the non-targeted approach provides more opportunities to discover previously unknown compounds. Considering the advantages and limitations of both targeted and non-targeted analytical approaches, this study aimed to identify cyanotoxin MCs in fish tissues using a combination of LC-MS targeted and untargeted methods. This offers an opportunity for non-targeted analysis and the detection of a wider range of cyanotoxins in the fish tissues.

2. Methodology

2.1. Study area and sampling sites

This investigation was carried out in commercial aquaculture fishponds mentioned in Bassey [24]. The fishponds are situated in the Vhembe District of Limpopo Province, South Africa, and in Calabar Municipality, Cross River State, Nigeria. In Nigeria, the selected sampling sites were Offiong Etim Avenue (4°59′58.92" N and 8°19′03.97" E), Essien Town (4°59′15.49" N and 8°19′40.21" E), and State Housing (4°59′6.50" N and $8^0$20′13.29" E). The aquaculture fishponds in the Vhembe District were situated in Duthuni (22°57′56.98" S and 30°23′43.96" E). The study used six fish ponds, three from each study area (Vhembe District and Calabar Municipality). Selection criteria for sampling sites included their designation as commercial fishponds, accessibility, consent from owners, and the presence of cultured fish.

2.2. Fish sampling

A total of 18 *Clarias gariepinus* (catfish) samples represented the sampling unit of six fishponds. Fishponds 4-6 were dominated by algal blooms, primarily characterized by cyanobacterial scums and greenish-colored water. Visible accumulations of blue-green algae were observed on the water surface. The fish samples were directly purchased from small-scale fishponds. The fish samples were captured using a fishing net with a mesh size of 20 mm. The age of each fish was determined using the age-length approach to select the appropriate size. *Clarias gariepinus* within the length of 38–42 cm was selected. The fish within this size range were selected for the study to protect the undersized fish population. The catch-and-release approach was used to exclude undersized fish populations during fish sampling. Afterward, the captured fish were wrapped in aluminum foiled paper and placed in an ice field cooler box before transporting to the laboratory. The selected fish samples were euthanized by suffocation in air asphyxiation and ice chilling (bath). Ethical clearance was sought from the University of Venda Research Ethics Committee. Consent from the owners of the fishpond was obtained and approved.

2.3. Targeted method using microcystin standards

2.3.1. Sample preparation, extraction, and purification

Similar procedure expressed by Manubolu [25] was employed in the present study. The collected fish samples were used to obtain muscle tissue, gills, liver, and stomach contents. Each filleted tissue was cut into smaller pieces and freeze-dried separately. Small chunks of the freeze-dried fish tissues were ground in combination with dry ice (50 % of the tissue volume) with a pestle and mortar. Afterward, each sample was divided into 1 g ($n = 3$) aliquots after processing. Each sample except for the unknown was spiked with MC-YR and MC-RR (certified analytical standard with >95 % purity). This was carried out using adding known concentrations (1 μg/L, 2 μg/L, 2.5 μg/L, and 5 μg/L) of MC-YR and MC-RR to the 1 g of wet mass fish sample. This was followed by incubation using a glass vial at room temperature for 20h in the dark. Spiking and the incubation process allowed the formation of covalently bound MC complexes [26]. This was followed by homogenization using a tissue probe homogenizer to further homogenize the samples. Homogenization was carried out using 80 % methanol. The mixture was further sonicated for 2, 5, and 10 min. This was followed by centrifugation at 3500 rpm using a centrifuge for 20 min. The extract supernatant was collected after centrifugation. Thereafter, samples were ready for solid-phase extraction (SPE) cleanup. The SPE was conducted using a 12-port SPE vacuum manifold equipped with large-volume samplers and a diaphragm vacuum pump. Oasis HLB cartridges (500 mg bed size, 6 mL capacity) were serially connected and preconditioned with 3 mL of methanol followed by 6 mL of water. A total of 30 mL of fish sample extract supernatant was slowly passed through the cartridge assembly and then rinsed with 20 % methanol. Before elution with 80 % methanol, cartridges

were vacuum-dried for 1 min. Fish samples extract supernatant were eluted with 5 mL and 25 mL of methanol, respectively. The eluates were dried under a gentle stream of nitrogen gas, and the resulting residues were re-dissolved in 1 mL of 80 % methanol. The solutions were then filtered through 0.2 μm polytetrafluoroethylene filters (Inqaba Biotec, Pretoria, South Africa) into autosampler glass vials, ready for LC-MS/MS analysis.

2.3.2. LC-MS analyses and method validation

This study employed the LC-MS method expressed in Mutoti [27]. A Liquid Chromatograph Triple Quadrupole Mass Spectrometer (LC-QqQ-MS/MS) (Shimadzu, Japan) equipped with a binary solvent delivery system and a sample manager was utilized for the analysis. Chromatographic separation was achieved using a Shim-pack Velox C18 column (2.1 × 100 mm, 2.7 μm particle size) with a serial number of 227-32009-03 (COU, MO, USA) at a flow rate of 0.4 mL/min. An injection volume of 1 μL was found to be optimal for this analysis. The mobile phase consisted of solvent A (H_2O) and solvent B (MeOH) in a gradient mode. The gradient program initiated at 95 % A (held for 1.5 min), decreased to 5 % A (from 1.5 to 2.0 min), held at 5 % A (from 2.0 to 3.0 min), and then increased back to 95 % A (from 3.0 to 4.0 min). The column oven temperature was maintained at 40 °C throughout the analysis. The limit of detection was determined before the analysis.

Identification and quantification of toxins were determined by comparison with the authentic toxin standard [28]. Linear calibration curves were generated for each microcystin (MC) congener after evaluation of measurements and calibration range using standard solutions with known concentrations (2−200 ppb) (Table 1). The precision and accuracy of the method were assessed through recovery experiments, which involved analyzing samples spiked with known concentrations of MC-LR and YR (1 μg/L, 2 μg/L, 2.5 μg/L, and 5 μg/L). The limit of detection (LOD) was determined based on a signal-to-noise (S/N) ratio of 3, estimated from the chromatograms of samples spiked at the lowest validated concentration level. The instrument response was measured for these standards to create a calibration curve, which is then used to quantify the analytes in actual samples.

2.4. Untargeted method

2.4.1. Sample preparation, extraction, and purification

Cyanotoxin extraction procedure for fish tissues was adapted from Bassey [24]. One gram (1g) of each freeze-dried fish tissue (muscle, liver, gills, and intestine) was dissolved in 10 mL of 90 % methanol. The mixture was shaken for 24 h at room temperature. This was followed by sonication for 30 min before overnight freeze-drying. The freeze-dried fish tissue residues were reconstituted in 10 mL of 90 % methanol. Ultrasonication was performed for 30 min, followed by centrifugation of 1 mL of the liquid in a microfuge at 3000 rpm for 10 min. The resulting aliquots were then filtered and transferred to a 10 mL opaque bottles. Filtrates were vortexed for 2 min and subsequently placed on a shaker for 24 h at room temperature. Finally, the mixtures were transferred to glass vials for LC-MS analysis. All the samples were prepared and analyzed in triplicates.

2.4.2. LC-MS analyses

The LC-MS procedure described by Bassey [24] was employed in the present study. A liquid chromatography-quadrupole time-of-flight tandem mass spectrometer (LCMS-9030 qTOF, Shimadzu Corporation, Kyoto, Japan) was utilized for the analysis of cyanometabolites in non-targeted analytes. Chromatographic separation was achieved using a Shim-pack Velox C18 column (100 mm × 2.1 mm, particle size 2.7 μm). The injection volume was set at 3 μL, and a binary mobile phase gradient was applied. Solvent A consisted of 0.1 % formic acid in Milli-Q water (HPLC grade, Merck Darmstadt, Germany), while solvent B was methanol (UHPLC grade, Romil SpS, Cambridge, UK) with 0.1 % formic acid. The flow rate was maintained at 0.3 mL/min over a 20-min gradient, with the following conditions: 10 % B for 3 min, a gradient from 10 % to 95 % B from 3 to 20 min, 40 % B for 7 min, and 95 % B from 10 to 15 min. The gradient returned to its initial conditions between 18 and 20 min, followed by a 3-min column equilibration. Positive-ion mode was used for mass spectrometry of all samples. Chromatographic effluents were further analyzed using the qTOF high-definition mass spectrometer, with mass spectra recorded in positive-ion mode. Mass spectrometry parameters were configured as follows: interface voltage of 4.0 kV, interface temperature of 375 °C, nebulization and drying gas flow at 3 L/min, heat block temperature of 400 °C, DL temperature of 280 °C, detector voltage of 1.8 kV, and flight tube temperature of 42 °C.

2.4.3. Data analysis

The mass spectrometry generated high-resolution MS/MS spectra data, which was converted into mzML format before uploading to the GNPS Library database. The data was analyzed using the Global Natural Product Social (GNPS) Library database (to match the compound class) in combination with CANOPUS-generated annotation from SIRIUS4 Software. Identification of metabolites was conducted using confidence LEVEL 3 annotation. The putative-identified spectra were used to construct a molecular networking visualized using Cytoscape 3.10.0. The statistical and computational analysis involved spectral similarity scoring (e.g., cosine similarity in GNPS) to match unknown spectra with reference compounds, enabling identification. Machine learning classification via CANOPUS predicted compound classes based on fragmentation patterns, improving the annotation process. Additionally, network analysis in Cytoscape visualized molecular relationships, aiding in the identification of structurally related metabolites.

3. Results and discussion

3.1. Targeted cyanotoxins (microcystins)

Chromatographic results displayed in Fig. 1 exhibited high sensitivity, selectivity, and precision in detecting MC-YR analytes from the fish tissue, but MC-RR was not detected in the fish tissues. This may indicate that the MC-RR compound is below the detection limit under current preparation conditions. The target method showed satisfactory sensitivity, with stable mass measurements aligning with previously reported LC-MS mass data from Mutoti et al. (2024). The calibration curves created by the MC-YR standard

Table 1
Determination of the standard curve regression for microcystin variants.

Reference material	Standard curve range (μg/L)	Standard curve regression fit style	Determination coefficient (r2)
MC-RR	2−200	Linear	0.997
MC-YR	2−200	Linear	0.998

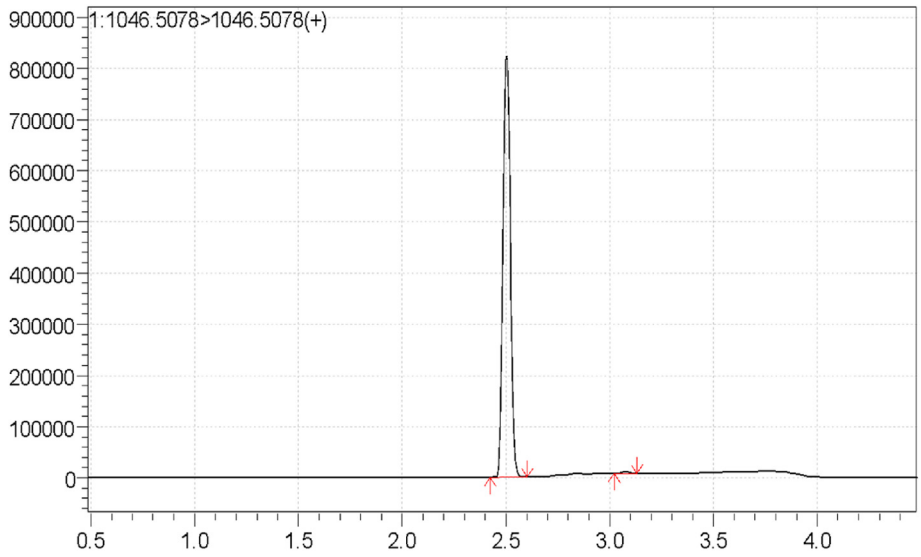

Fig. 1. LC-MS chromatograms of quantification ions for microcystins-YR standard at a concentration of 200 ppb.

were an effective approach used to compare with the analytes in fish samples presented in Fig. 2.

3.2. Method validation

The linearity of the method and measurement range were studied through analyses of standard solutions at 6 different concentrations ranging between 2 and 200 ppb. Therefore, the method was validated by assessing its correctness, and this was done by assessing samples spiked with different concentrations of stock solution of the target analytes (MC-YR and -RR) (Table 2). Additionally, blank samples were analyzed to assess the specificity of the method adopted in the present study and it was observed that no peaks or signals were appearing close to the retention time of the analytes. Finally, Table 2 further shows that four different samples were spiked and analyzed, and therefore mean recoveries were estimated and found satisfactory ranging between 81 and 97 % for the target compounds. The limit of detection was further calculated in the present study as per Mutoti [27]. These validation results therefore indicated that the method adopted was appropriate for the quantitative determination of the target compound of interest.

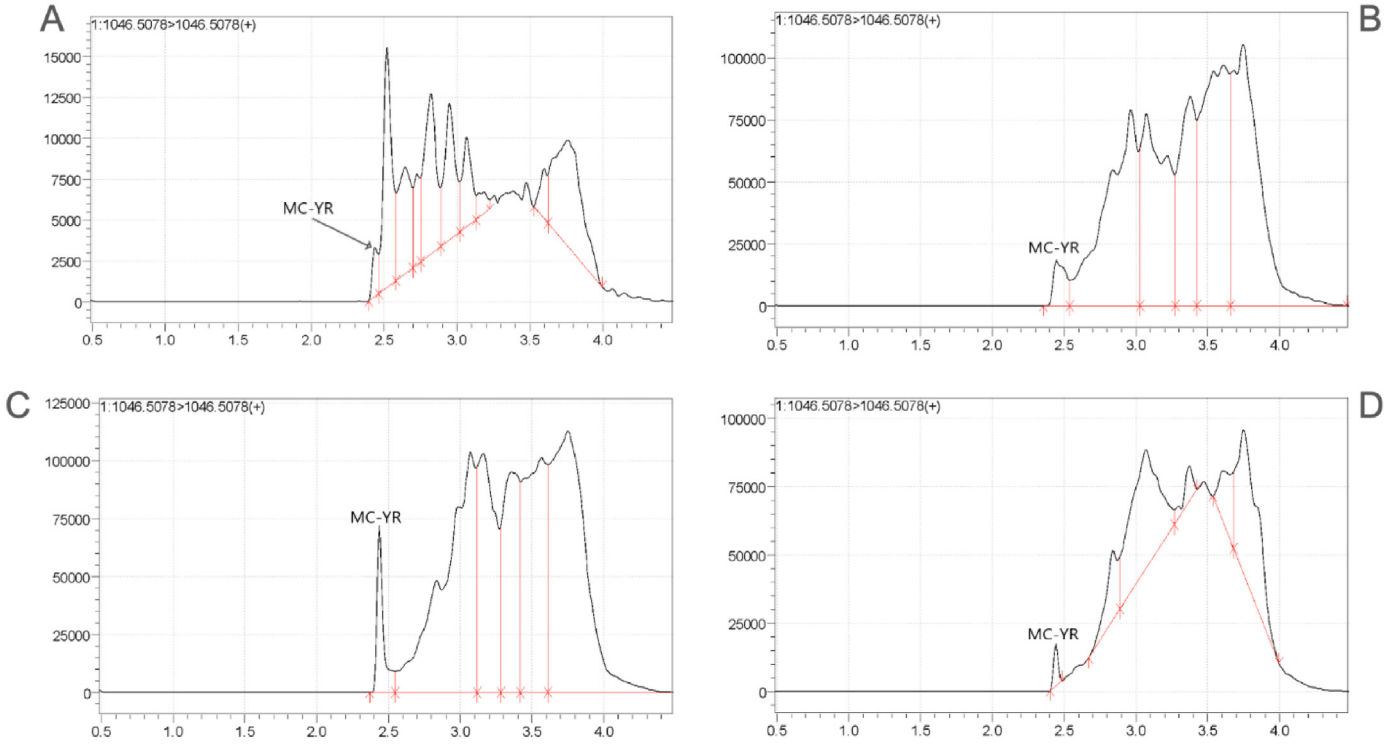

Fig. 2. LC-MS chromatograms of quantification ions for microcystins-YR for (A) liver, (B) muscles, (C) intestine, and (D) gills.

Table 2
Sample recoveries and concentration levels of spiking solutions used to spike samples for method validation.

MC variant	Intestines	Muscle	Gills	Liver	LOD (μg/L)
	1 μg/L	2 μg/L	2.5 μg/L	5 μg/L	
MC-YR	92 %	81 %	91 %	97 %	2
MC-RR	88 %	87 %	93 %	77 %	1

3.3. Method application to real samples

3.3.1. Liver

Microcystin variant MC-YR was detected in the *Clarias gariepinus* liver with concentrations ranging from 20.896 to 10.96 μg/g (Fig. 3). The concentration of MC-YR in the liver tissue from pond 6 was the highest compared to other fish tissues. Empirical studies over the past decades to date have expressed similar results that MC compounds were elevated in the fish liver compared to other fish organs in Rainbow Trout (*Oncorhynchus mykiss*), Lake Trout (*Salvelinus namaycush*) [29], *Carassius gibelio* [30] and Wild Nile and redbreast tilapia (Clarias gariepinusand *Tilapia rendalli*) [31].

These elevated concentrations of MC-YR in the liver may be attributed to the accumulation of toxins in the liver tissue. Lance [32] explained that once MCs are present in organisms, they target the liver where they interact specifically with protein phosphatases (PPases). This is followed by a covalent binding to proteins, leading to the accumulation of MCs irreversibly attached to animal tissue-bound MCs. According to Kagalou [33], the liver is a target organ for MC accumulation, corresponding to Zurawell [34] and Lance [32]. Meanwhile, Nchabeleng [35] indicated that the accumulation of MCs in fish liver may be related to the function of the liver serving as a detoxifying organ, thus exposing it to high loads of toxins. Although low MC concentrations have been detected in the liver tissue of *Talapia rendalli* [36]. Kagalou [33] suggested that the low accumulation of MCs in the liver may be due to preferential bioaccumulation in the muscle, even though the liver is typically the target organ.

3.3.2. Muscles

Analytical results showed that MC-YR was detected in *Clarias gariepinus* muscles from all the fish ponds ranging from 10.5 to 16.06 μg/g (Fig. 3), surpassing human estimated daily intake (EDI) of 0.04 μg/kg/day, set by the WHO. Similarly, Cazenave [37] observed an increase in MC concentration in the muscles of *Odontesthes bonariensis* after 24 h of exposure to a significant amount of MC-RR. In a fishpond found in Serbia, MC-RR was detected in muscle tissue at a concentration of 60 mg kg^{-1} DW [38]. Moreover, Mohamed [39] reported estimates of free MCs in tilapia fish organs at levels up to 11.8 ng/g in intestines, 8.3 ng/g in livers, and 0.38 ng/g in edible tissues. The presence of MCs in fish muscles has received substantial attention due to the concern for human health. Increased levels of MCs in fish muscle pose a health risk to consumers, as it is the most commonly consumed part of the fish by humans. Detecting elevated concentrations of MC-YR in fish muscle may indicate that the fish is unsafe for human consumption and commercial sale. It is important to highlight that neither boiling water nor cooking the fish before consumption reduces the health risks associated with MC toxins [40,41].

3.3.3. Stomach content and gills

MC-YR variant was found in the intestines and gill of *Clarias gariepinus* at the range of 13.2–10.6 μg/g and 11.52–20.78 μg/g, respectively (Fig. 3). The presence of MCs in the gills and intestinal tissues of fish is likely influenced by both the fish's diet and the routes of exposure [37,42]. Lance et al. [32] documented the trophic transfer of MCs via the food chain from zooplankton organisms with evidence of trophic transfer of MCs from the gastropod *Lymaea stagnalis* to the *Gasterosteus aculeatus* fish. The study further explained that the accumulation of MCs in organisms may be attributed to the absence of magnification metabolization and excretion of free MCs at every level of trophic transfer. Moreover, fish can absorb MCs either directly from the surrounding water through their gills. Fish gill is a major source of MCs entry when these toxins are released into the surrounding water during cyanobacterial cell senescence, death, and lysis in the aquatic environment [37,43]. This therefore explains the presence of MC-YR in gill and intestine tissues.

3.4. Untargeted secondary metabolites (cyanotoxins) in fish tissues

There was no standard required to identify the untargeted secondary metabolites (cyanotoxins). This screening method allowed the ability to screen a large list of compounds including

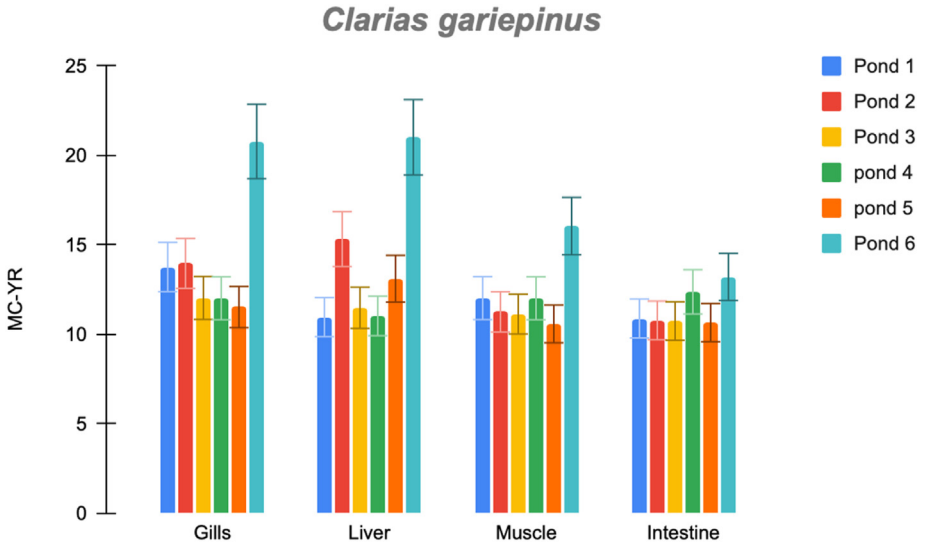

Fig. 3. Concentrations of MC-YR in fish tissues from different fishponds.

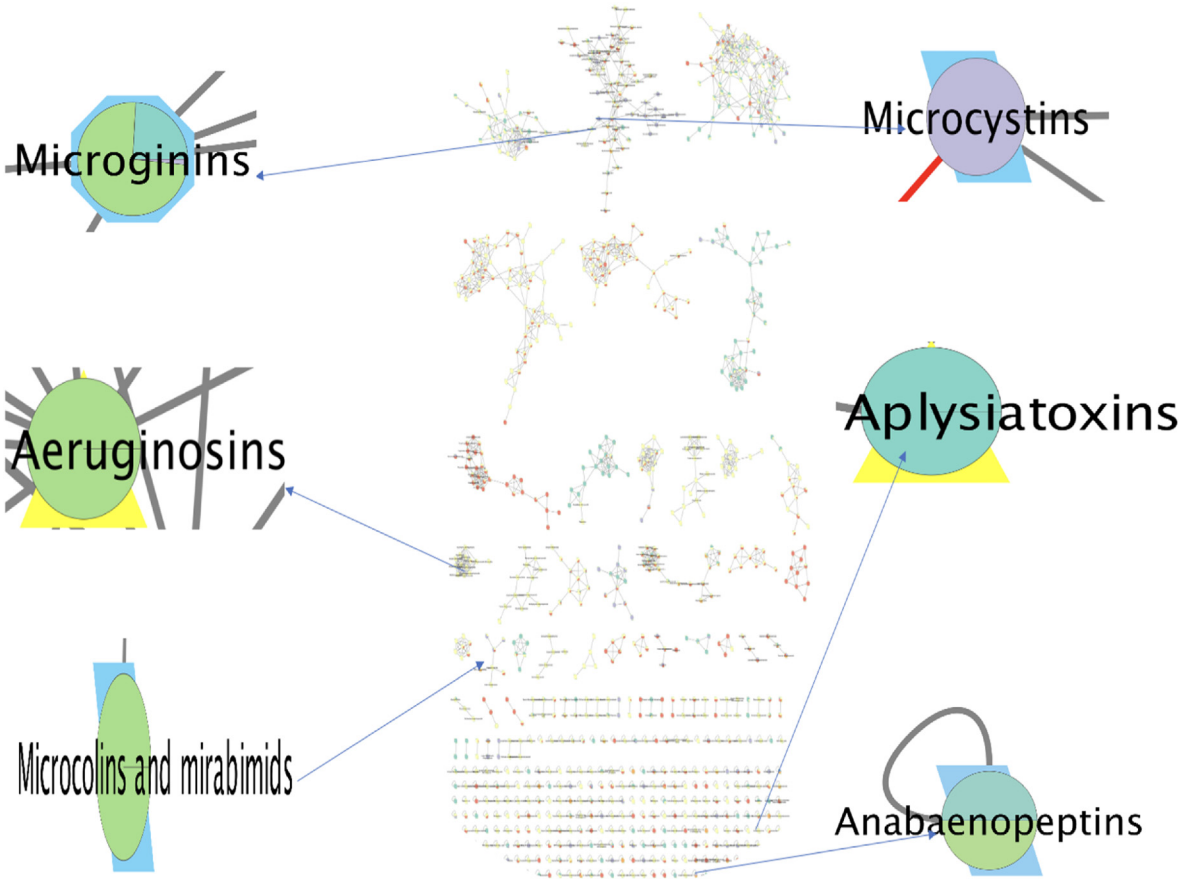

Fig. 4. Molecular network of toxic cyanometabolites: A-D representing microcystins, microginins, aeruginosins, microcolins and mirabimids, anabaenopeptins, aplysiatoxins and aeruginosins extracted from *Clarias gariepinus* fish tissue.

cyanotoxins in fish tissues. The untargeted method successfully identified cyanotoxins by comparing the putatively identified spectra with the built-in mass spectrometry (MS) spectra in the library using molecular networking displayed in Fig. 4. In fish tissues, several cyanotoxins, including aeruginosins, anabaenopeptins, MCs, and microginins were detected. MCs and microginins were identified in the liver and intestine tissues from Pond 1. Aplysaxtosin was exclusively noted in the muscle tissue of *Clarias gariepinus* from Pond 1. Microginins, anabaenopeptins, microcolins, and mirabimids were identified in the muscle tissues across all fishponds. Anabaenopeptins were exclusively identified in the gill tissues from Ponds 4 and 6. The simultaneous occurrence of cyanotoxins in fish tissues could be associated with abundances of toxic genes in fishponds specific to MCs, microginins, anabaenopeptins, microcolins, and mirabimids [44]. Marie and Gallet [45] identified harmful cyanopeptides, including MCs, cyanopeptolins, and anabaenopeptins, in the liver tissues of *Perca* and *Lepomis* species from Tri, Var, and Ver lakes. These toxins are produced by certain species of cyanobacteria, including *Microcystis, Planktothrix,* and *Anabaena* species. These classes of cyanotoxins are identified as structural variants commonly identified in cyanobacterial blooms [46]. Bassey [24] similarly reported the presence of microginins and MCs in Duthuni fishponds within the same study area, while anabaenopeptins, aplysiatoxins, and microcolins were detected across fishponds in both Duthuni and Calabar Municipality.

Non-toxic secondary metabolites associated with cyanobacterial cells and endogenous to fish metabolites were also detected in the fish tissues. Among these, pheophorbide A and microcolins were identified as cyanometabolites [47,48]. Cholic acid, phosphocholine (PC), triglyceride (TG), and octadecadienoic acid were determined to be derived from fish tissues [49–51], while ethanesulfonic acid and amyl amine were likely attributed to anthropogenic sources. The distinction between metabolites derived from fish tissue and cyanometabolites was determined using LC-MS/MS, metabolite libraries, and supporting literature. The liver tissues in all ponds were rich in compounds like octadecadienoic acid, while phosphocholine, ethanesulfonic acid, pheophorbide A, and cholic acid dominated intestines and gills. Muscle tissue was characterized by phenylalanine, amylamine, and phosphocholine, similar to South African samples. Meanwhile, in Duthuni sampling stations, the dominant compounds in muscle tissues were cholic acid, triglyceride, phosphocholine, and sulfonic acid. The prevalent compounds in the intestine and gill tissues were triglyceride and ceramide, exhibiting consistent similarities across all fish samples from South African sampling sites. These bioactive compounds are considered building blocks of cyanobacteria membranes [52]. To the best of our knowledge, this study is the first comprehensive report on cyanometabolites in fish tissues from commercial fishponds in Nigeria and South Africa, utilizing the untargeted method.

4. Conclusion

The untargeted and targeted LC-MS/MS methods were successful in detecting cyanotoxins in fish tissues. The targeted method was limited to the compounds for which the reference standard was available, while the untargeted method generated a more comprehensive dataset for retrospective interrogation.

Microcystin variant MC-RR was undetected in fish tissues, but an increased concentration of MC-YR was detected in the liver, intestines, muscles, and gills of *Clarias gariepinus* ranging from 10.66 to 20.8 μg/g. The untargeted LC-MS method identified a wide range of cyanotoxins in fish tissues, including aeruginosins, anabaenopeptins, microginins, as well as non-toxic secondary metabolites such as octadecadienoic acid, phosphocholine, ethanesulfonic acid, microcolins, pheophorbide A, cholic acid, phenylalanine, amyl amine, triglycerides, and sulfonic acid. The simultaneous presence of cyanotoxins and increased MC-YR in the fish tissues raises serious concerns for safe consumer products. However, a thorough quantitative investigation of the untargeted cyanotoxins in fishponds is necessary to determine their concentrations in fish tissues accurately.

CRediT authorship contribution statement

Odo J. Bassey: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Munyaradzi Mujuru:** Investigation. **Mulalo I. Mutoti:** Writing – review & editing, Validation, Methodology. **Adeeyo Adeyemi:** Investigation. **Farai Dondofema:** Supervision. **Jabulani Ray Gumbo:** Writing – review & editing, Supervision, Project administration, Conceptualization.

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Declaration of interest

We are delighted to confirm that there are no known conflicts of interest related to this publication or its submission to the *Emerging Contaminants Journal*. All funding sources for this study have been appropriately acknowledged, and no financial support has influenced its findings.

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