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Algal toxin profiles in Nigerian coastal waters (Gulf of Guinea) using passive sampling and liquid chromatography coupled to mass spectrometry

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ABSTRACT

Algal toxins may accumulate in fish and shellfish and thus cause poisoning in consumers of seafood. Such toxins and the algae producing them are regularly surveyed in many countries, including Europe, North America, Japan and others. However, very little is known regards the occurrence of such algae and their toxins in most African countries. This paper reports on a survey of phytoplankton and algal toxins in Nigerian coastal waters.

Seawater samples were obtained from four sites for phytoplankton identification, on three occasions between the middle of October 2014 and the end of February 2015 (Bar Beach and Lekki in Lagos State, Port Harcourt in Rivers State and Uyo in Akwa Ibom State). The phytoplankton community was generally dominated by diatoms and cyanobacteria; however several species of dinoflagellates were also identified: *Dinophysis caudata*, *Lingulodinium polyedrum* and two benthic species of *Prorocentrum*.

Passive samplers (containing Diaion® HP-20 resin) were deployed for several 1-week periods on the same four sites to obtain profiles of algal toxins present in the seawater. Quantifiable amounts of okadaic acid (OA) and pectenotoxin 2 (PTX2), as well as traces of dinophysistoxin 1 (DTX1) were detected at several sites. Highest concentrations (60 ng OA g^{-1} HP-20 resin) were found at Lekki and Bar Beach stations, which also had the highest salinities. Non-targeted analysis using full-scan high resolution mass spectrometry showed that algal metabolites differed from site to site and for different sampling occasions. Screening against a marine natural products database indicated the potential presence of cyanobacterial compounds in the water column, which was also consistent with phytoplankton analysis.

During this study, the occurrence of the marine dinoflagellate toxins OA and PTX2 has been demonstrated in coastal waters of Nigeria, despite unfavourable environmental conditions, with regards to the low salinities measured. Hence shellfish samples should be monitored in future to assess the risk for public health through accumulation of such toxins in seafood.

1. Introduction

Toxins from marine micro-algae frequently accumulate in seafood, including fish and shellfish, and maximum concentrations for such toxins have therefore been regulated at global and regional levels (DeGrasse and Martinez-Diaz, 2012; Hess, 2012; Lawrence et al., 2011; Suzuki and Watanabe, 2012). As fisheries have only limited potential to increasingly contribute to the global food supply, it is expected that any growth in seafood supply will have to come from aquaculture. Therefore, it is important to investigate the potential of coastal areas for seafood production, and also the risks associated with such production. In terms of public health risks, those originating from harmful algal blooms are particularly common in many parts of the world and must therefore be assessed relatively early on in any survey for aquaculture feasibility.

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To our knowledge, no algal toxins have been reported in coastal waters of central Western Africa, except one preliminary report on potentially toxic fish in Cameroon (Bienfang et al., 2008). The southernmost records of algal toxins in Northern Africa are from the Moroccan coastline where an official monitoring program is in place (Abouabdellah et al., 2008; Taleb et al., 2003). Lipophilic shellfish toxins were shown to accumulate in mussels, cockles, oysters and solen, causing poisoning in the Dakhla region, *i.e*. the South Atlantic Moroccan coast (Abouabdellah et al., 2011). Toxins of the okadaic acid (OA) group, *i.e*. OA and dinophysistoxins (DTXs) and their associated esters were the agents responsible for those shellfish poisoning events, attributable to the presence of several potentially toxic species of *Dinophysis*. Taleb et al. (2006) also were the first to report the presence of azaspiracids in mussels, in Morocco.

In southern parts of Africa, regular monitoring is in place in South Africa and Namibia. Production of saxitoxin (STX) off the west coast of South Africa has been attributed to *Alexandrium catenella* (Pitcher and Calder, 2000; Pitcher et al., 2001). Fawcett et al. (2006) have developed and deployed a bio-optical buoy for monitoring HABs in the southern Benguela Current region off South Africa. These buoys have proved their efficiency in providing both real-time and time-series data, giving interesting information on the occurrence of Prorocentrum triestinum in the region. The northernmost records of algal toxins in the southern African region are from Angola (Blanco et al., 2010; Vale et al., 2009).

Phytoplankton surveys in Nigeria by one of the authors have reported non-toxin producing as well as potentially toxic algae including *Prorocentrum micans*, *Protoperidinium depressum*, *Prorocentrum mite*, *Dinophysis caudata*, *Peridinium gatunense*, *P. cinctum*, *Gymnodinium fuscum* and an array of *Ceratium* species (Kadiri, 1999, 2001, 2002, 2006a, b, 2011). Previous studies by other authors also showed sporadic occurrences of *D. caudata*, *P. depressum*, *P. diabolus*, *P. micans*, *Noctiluca scintillans* in Lagos Lagoon (Nwankwo, 1991, 1997). A recent report additionally recorded *Lingulodinium polyedrum*, *Prorocentrum minimum*, *P. sigmoides* and *Scrippsiella trochoidea* in Lagos, Cross Rivers and Delta States (Ajuzie and Houvenaghel, 2009).

As potentially toxic algae have repeatedly been reported from Nigerian coastal waters this study attempted to verify whether algal toxins actually do occur in Nigerian waters. Since there was no algal culturing facility available on site, and as many dinoflagellates are difficult to bring into culture, in particular *Dinophysis*, we have opted for an indirect approach based on passive sampling of algal toxins in Nigerian coastal waters. This approach had been introduced for monitoring of toxins by MacKenzie et al. (2004). We have focussed on regulated lipophilic toxins known to cause problems in terms of public health but have also used in parallel an approach for untargeted analysis based on high-resolution mass spectrometry as previously described (Zendong et al., 2015).

2. Materials and methods

2.1. Chemicals, reagents and sorbent materials

Certified standard solutions of okadaic acid (OA), domoic acid (DA), dinophysistoxins (DTX1, DTX2), 13-desmethyl spirolide C (13-desmeSPX-C), pectenotoxin 2 (PTX2), gymnodimine A (GYM-A), azaspiracids (AZA1,-2 and -3), yessotoxin (YTX) and homo-yessotoxin (homo-YTX) were obtained from the National Research Council in Halifax, Canada. HPLC grade methanol and acetonitrile as well as ammonium formate and formic acid (98%) were acquired from AtlanticLabo (Bordeaux, France) and Sigma Aldrich (Steinheim, Germany). Deionized water was produced in-house to 18 MΩ cm^{-1} quality, using a Milli-Q integral 3 system (Millipore). For analyses with the high resolution mass spectrometry instrument, acetonitrile and water of LC/MS grade were obtained from Fischer Scientific (Illkirch, France). For passive sampler devices, Diaon® HP-20 polymeric resin was purchased as bulk resin from Sigma–Aldrich and 12 mL capacity polypropylene 2 frits-Reservoirs were from Agilent Technologies.

Brucine-sulfanilic acid reagent was prepared by dissolving 1 g brucine sulphate [$(C_{23}H_{26}N_2O_4)_2$ H_2SO_4, $7H_2O$] and 0.1 g sulfanilic acid ($NH_2C_6H_4SO_3H$, H_2O) into 70 ml of hot distilled water. Concentrated hydrochloric acid (3 mL) was further added and this mixture was cooled, mixed and then diluted to 100 mL with distilled water. The final mixture was stored in a dark bottle at 5 °C. For ascorbic acid, the ready-made PhosVer 3 Hach™ was used.

2.2. Study area

The study area (Fig. 1), *i.e*. the Nigerian coastal area, is situated in the Guinea Current Large Marine Ecosystem, in the Gulf of Guinea. There are two main seasons in the deploying sites: the rainy (wet) season spanning from May to October and the dry season from November to April. The area is influenced by coastal upwelling which occurs seasonally along the northern and eastern coasts. There are two (major and minor) upwelling seasons. Those seasons occur annually with differing duration and intensities off Ghana and Cote d'Ivoire, in the central part of the large marine ecosystem. The major upwelling season occurs from June to September and transient upwelling events are from January to March (Ibe and Ajayi, 1985).

The coastline of Nigeria is approximately 853 km long between latitude 4°10′–6°20′ N and longitude 2°45′–8°35′ E. The Nigerian coastal area is low-lying of not more than 3.0 m above sea level, generally covered by fresh water swamp, mangrove swamp, lagoonal mashes, tidal channels, beach ridges and sand bars (Dublin-Green et al., 1997).

The Nigerian coast is composed of four distinct geomorphological units namely: the Barrier–Lagoon complex; the Mud coast; the Arcuate Niger delta; and the Strand coast (Ibe, 1988). The vegetation of the Nigerian coastal area is characterised by mangrove forests, brackish swamp forests and rain forests. The coastal zone is richly endowed with a variety of mineral resources, including oil and gas. The four selected sites are located in the Gulf of Guinea (Atlantic Ocean), two in the Bight of Bonny to the East (Arcuate Niger delta) and two in the Bight of Benin to the West (outside the Barrier-lagoon complex).

Seawater sampling for nutrients and for phytoplankton analysis, as well as passive sampling were carried out at sites and dates as listed in Table 1.

2.3. Physico-chemical parameters and water sampling for analysis of nutrients and phytoplankton identification

Water samples (1 L) were obtained for analysis of nutrients at an integrated depth of 10 m to the surface of the ocean, with a lund tube of 2.5 cm diameter. Temperature was measured with a mercury-in-glass thermometer. Dissolved oxygen was measured using a Milwaukee NW 600 probe and salinity was measured with a Hach™ Salinity/Conductivity probe (Hach Company, USA).

Nutrients were analysed according to ASTM (1980). For the determination of nitrate, brucine sulphanilic acid reagent (1 mL) was added to standard solutions as well as to samples (10 mL). The resultant mixtures were mixed thoroughly and allowed to stand for 15 min. Then 10 mL of H_2SO_4 solution were carefully added to 10 mL of distilled water and the resulting solution was added to each of the beakers containing the nitrate standard solutions and the water samples, respectively. This was allowed to stand for 20 min in the