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Full Length Research Paper

Assesment of *hepatotoxins* and *neurotoxins* from five *Oscillatoria species* isolated from Makkah area, KSA using HPLC

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Abstract

Cyanobacteria (blue-green algae) form massive water blooms and surface scums in eutrophic waters. They are found in a variety of habitats; live in terrestrial, fresh, brackish, or marine water. Fresh water cyanobacteria produce wide range of toxins among them hepatotoxic and neurotoxic. Five species of *Oscillatoria* (*O. agardhii*, O. *brevis*, *O. limnetica*, O. *rubescens*, and *O. tenuis*) were used in this study. They were identified and collected from sixten different sites in Makkah area, KSA. Cyanotoxins were detected and determined using High Performance Liquid Chromatography HPLC. The HPLC analysis of the five species of *Oscillatoria* extract demonstrated that *O. tenus* has the highest percentage (35. 267%) of neurotoxins and the lowest percentage (0.249%) of hepatoxins. On the other hand, *O. rubescens* showed the highest percentage of hepatotoxins (37.657%) and the lowest percentage (0. 62%) of neurotoxins. The results revealed that neurotoxins and hepatotoxins are produced by species and stains of *Oscillatoria* but the amount of these toxins are different.

Keywords: Hepatotoxins, neurotoxins, *Oscillatoria*, high performance liquid chromatography (HPLC).

INTRODUCTION

Cyanobacteria are found in a variety of habitats; live in terrestrial, fresh, brackish, or marine water. They usually are too small to be seen individually, but sometimes can form visible colonies (Mankiewics et al., 2003; Briand et al., 2003; Mazur et al., 2003). Cyanotoxins are toxic, secondary metabolites produced by about 40 species of cyanobacteria (Westrick et al., 2010). It appears likely that cyanotoxins are produced and contained within the actively growing cyanobacterial cells (i.e. they are intracellular or particulate). Release to the surrounding water, to form dissolved toxin, appears to occur mostly, if not exclusively, during cell senescence, death and lyses, rather than by continuous excretion (Lehtimäki et al*.,* 1997; Negri et al*.,* 1997; Rapala et al*.,* 1997).

Toxic cyanobacterial blooms in eutrophic lakes and reservoirs have been reported in many countries (Skulberg et al., 1984; Gorham and Carmichael, 1988; Carmichael 1992). These toxic blooms have caused death to livestock and wildlife, in addition to cases of illness and death in humans (Billings, 1981; Falconer, 1989; Carmichael et al*.,* 1996). Toxins from freshwater cyanobacteria are classified functionally into two groups, hepatotoxins and neurotoxins (Park, 1988). Most scientists have been more concerned about the cyclic peptide hepatotoxins than neurotoxic alkaloids (Falconer and Humpage 2005; Msagati et al., 2006).

Neurotoxins include anatoxin-a, anatoxin-a(s) and saxitoxin, and are commonly produced by the *Anabaena* and *Oscillatoria* species. Consumption of large amounts of these toxins by animals or humans can result in muscle cramps, twitching, paralysis and cardiac or respiratory failure. Hepatotoxins include microcystin and cylindrospermopsin, and are produced by the *Microcystis* and *Cylindrospermopsis* species. These toxins produce symptoms including nausea, vomiting and acute liver failure (Falconer, 1996; Codd et al., 1999; Sivonen and Jones, 1999; Mazur-Marzec et al., 2003, 2008).

The aim of this study was to investigate the amounts of hepatotoxins and neurotoxins in five species of *Oscillatoria* (*Oscillatoria agardhii*, *Oscillatoria brevis*, *Oscillatoria limnetica*, *Oscillatoria rubescens*, and *Oscillatoria tenuis*) using High performance Liquid Chromatography (HPLC); because HPLC is currently the most widely used for detection of known cyanotoxins.

MATERIALS AND METHODS

Site of collection

Sixten different sites in Makkah area, KSA, were chosen for collecting the samples during the morning hours. Sampling was carried out in 1-liter acid- washed polyethylene bottles. Water was collected from depth 25 cm up to 3 m.

The underground water used for irrigation are stored in concrete pools, the remaining water in these pools and the open ducts after the irrigation are directly exposed to sunlight, which in turn, support extensive cyanobacterial growth.

Dominant genera

Five species of *Oscillatoria* genus, the most dominant species at all sites were selected from seventeen cyanobacterial organisms isolated from Makkah area. The collected samples were examined using light microscope in a preliminary study of genus identification. Further identifications for species were carried out at National Research Center, Cairo, Egypt according to Strebel and Krauter (1978). Five species of *Oscillatoria* were identified and used in this study due to their dominance These species are 1) *O*. agardhii 2) *O*. brevis 3) *O*. limnetica 4) *O*. rubescens and 5) *O*. tenuis.

Toxin purification

Toxins from the five *Oscillatoria* species were purified using the method of Jamel Al-layl *et al.* (1988).

The toxic fractions were separated by HPLC 6.0 x 150 clc OSD column. The HPLC had two delivery pumps model (LC-9A) Shimadzu liquid chropmatography with a manual injector and a photodiode array UV-VIS detector Shimadzu SPD-M6A at 230nm. Two solvent systems were used as mobile phase according to the nature of the toxin to be purified as follows: 26% (v/v) Acetonitrile/TFA (0.05M)) for hepatotoxins and 48% (v/v) Methanol/TFA (0.05M)) for neurotoxins. The column was run at a flow rate of 1ml/min for both toxins.

RESULTS AND DISCUSSION

The HPLC analysis of the five species of *Oscillatoria* extract s howed some peaks corresponding to the retention time of neurotoxins and hepatotoxins as presented in (Tables 1 and 2). These results revealed that the number of peaks detected differed from species to species. According to neurotoxins the highest numbers of peaks detected were 8 peaks while seven peaks were detected for hepatotoxins.

Taking into consideration the results obtained for HPLC separation, it could be observed that the highest number of peaks (8 peaks) was recorded in *O. brevis* while the least number of peaks were recorded in *O. agadhii* and *O. rubescens*. At the same time 7 peaks were recorded in *O. limnetica* and *O. tenuis*. However, peak number one represented the high percentage of neurotoxin above the other detected peaks. Among these results of peak number one; *O. tenuis* showed the highest percentage (35.267%). Consequently the least percentage was 0.62% which detected in *O. rubescens* and represented by peak number six. In this context; Neurotoxins are produced by species and strains of *Oscillatoria* (Sivonen et al*.,* 1989; Skulberg et al*.,* 1992a) but their main toxins were different (Sivonen et al., 1992).

The five dominant species of the genus *Oscillatoria* which isolated from Makkah are differed slightly in the number of hepatotoxins peaks. The results obtained showed the presence or absence of these toxins peaks. Two species of *Oscillatoria* (*O. limnetica* and *O. tenuis*) represented 7 peaks (the highest number of peaks) and other two species (*O. brevis* and *O. rubescens*) represented the least number of peaks (5 peaks) while one species (*O. agadhii*) represented 6 peaks. These results revealed that the highest percentage of hepatotoxins (37.657%) was detected in *O. rubescens* which presented by peak number five. Acute hepatotoxicosis involving the hepatotoxins (liver toxins) is the most commonly encountered toxicosis involving cyanobacteria. These toxins are produced by strains of species within the genera *Microcystis, Anabaena, Nodularia, Oscillatoria* and *Nostoc* (Skulberg *et al*., 1992b).

According to Sivonen *et al.* (1989) a simultaneous occurrence of neurotoxin and hepatotoxin was found in natural blooms and strains in which these toxins apparently often coexist. The occurrence of *Oscillatoria* sp. Blooms in ponds, lakes or rivers that produce hepatotoxic microcystins is a problem, especially if the water is utilized as a drinking supply and/or for recreational purposes. Epidemiological investigations have demonstrated that microcystins cause stomach and intestinal inflammation, liver cancer and disease of the spleen in humans who drink water containing microcystins (McDemott et al*.,* 1998; Ding et al., 2000; Zhou et al., 2002; Ahmed et al., 2010)

All *Oscillatoria* strains produced only one major toxin at a time, in contrast to the strains of *Anabaena* spp. (Sivonen *et al*., 1992) or Microcystis spp. (Watanabe, 1988), which usually produced two to four main toxins simultaneously. The toxins in *Oscillatoria* strains varied

Table 1. HPLC separation of neurotoxins of the five *Oscillatoria* species (results detected as % of total).

Peak No.	*O. agardhii*	*O. brevis*	*O.* limnetica	*O.* rubescens	*O.* tenuis
1	28.313	34.153	34.873	34.203	35.267
2	27.677	29.921	25.214	28.868	22.475
3	24.791	24.215	24.356	29.198	27.921
4	7.998	2.237	2.092	5.386	4.967
5	7.603	4.498	6.415	1.725	4.755
6	3.619	2.765	3.919	0.620	3.655
7	-	1.060	3.131	-	0.961
8	-	1.152	-	-	-

Table 2. HPLC separation of hepatotoxins of the five *Oscillatoria* species (results detected as % of total)

Peak No.	*O. agardhii*	*O. brevis*	*O.* limnetica	*O.* rubescens	*O.* tenuis
1	20.646	22.370	6.566	13.855	16.887
2	27.057	20.686	12.259	13.568	15.628
3	13.994	14.915	10.160	10.345	12.498
4	17.960	21.124	11.843	24.574	18.352
5	17.129	20.906	24.211	37.657	35.312
6	3.215	-	33.785	-	1.075
7	-	-	1.176	-	0.249

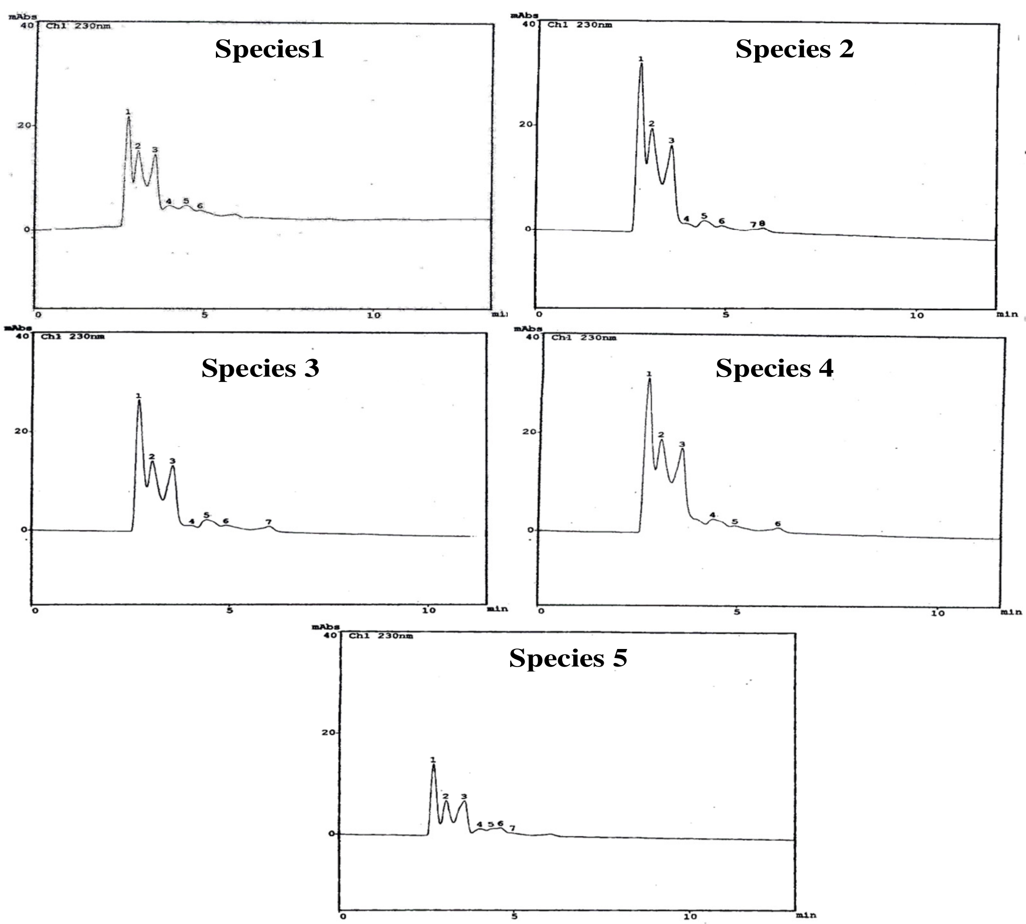

Figure 1. HPLC chromatogram separation of neurotoxins of the five*Oscillatoria* species.

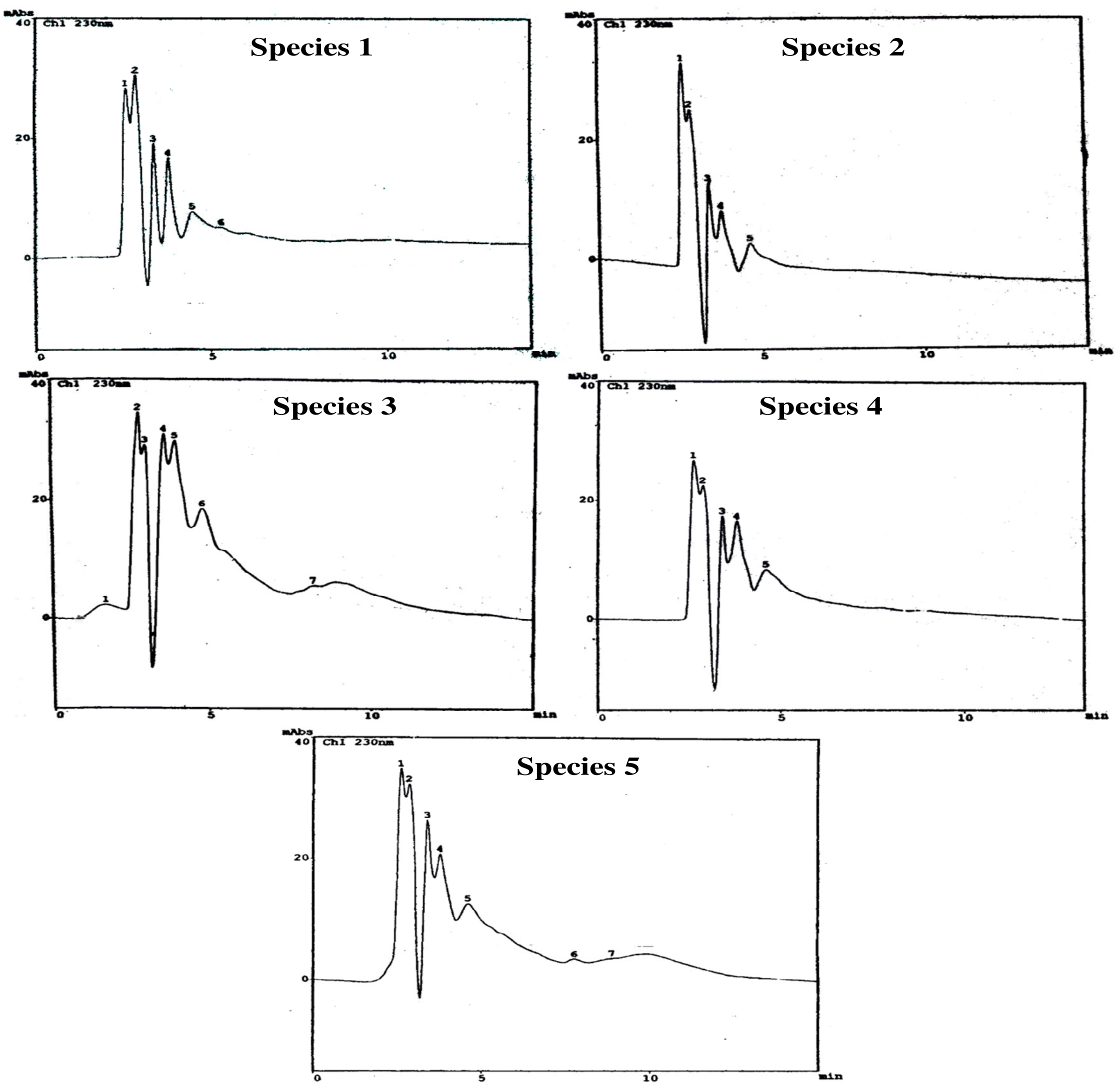

Figure 2. HPLC chromatogram separation of hepatotoxins of the five*Oscillatoria* species.

less structurally than the toxins in *Anabaena* spp. (Sivonen *et al.*, 1992) obtained from the same geographical area. For example, 17 different microcystins were identified from seven strains of *Anabaena* spp. (Sivonen *et al.*, 1992), compared with 8 microcystins from *Oscillatoria sp.*

Temporal and spatial measurements of cyanotoxin and toxic species composition in lakes and reservoirs are necessary to assess the risks for the health of humans, aquatic animals, livestock, and wildlife (Park et al. 1998). So, the effect of cyanobacteria blooms on human through direct exposure or food chain in waters remains to be identified. Moreover, it is necessary to have regular monitoring on abundance of cyanobacteria in waters for public health safety. Also, determining the biogenesis of these compounds will require future research.

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