Evaluation of Cyanotoxins in the Farmington Bay (Summer 2007) of Great Salt Lake, Utah

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April, 2008

INTRODUCTION

Recently, severe eutrophication has been observed in Great Salt Lake, Utah Lake and other surface water bodies. It is speculated that the eutrophication is primarily due to the presence of excess nutrients discharged by sewage effluents and agricultural runoffs. Great concern is being raised over the consequences of eutrophication and issues are being discussed on the potential presence of cyanobacteria among the state water quality board personnel and the local water treatment districts. One region of primary concern in the Great Salt Lake is the Farmington Bay.

Farmington Bay, located in the southeast corner of the Great Salt Lake (Utah), receives the majority of municipal and industrial wastewater from the Salt Lake City metropolitan area, as well as non-point source pollution from agriculture and urban runoff (Marcarelli et al., 2003). Nutrients from seven wastewater plants flow into Farmington Bay, either directly or through wetland complexes, and the bay also receives industrial effluents. Extreme turbidity, foul odor production, and heavy sewage inflow to Farmington Bay have prompted the water quality board and many water treatment districts in the state to come together to solve the grave problem of eutrophication.

Previous studies have shown the presence of *Nodularia spumigena* as the dominant cyanobacteria in the Farmington Bay of the Great Salt Lake, contributing to cyanotoxins in the lake (Wurtsbaugh et al., 2006). This study also claimed Nodularin, a commonly found cyanotoxin in brackish waters and believed to be produced by *Nodularia spumigena,* to be present at high concentrations. Although the study by Wurtsbaugh et al., 2006 was important and certainly demonstrated the presence of *Nodularia spumigena,* this study was incomplete (based on the report posted at CDSD web page) in some sense due to the following reasons.

1. It was mentioned that *Nodularia spumigena* was the dominant Cyanobacteria contributing cyanotoxin into the bulk water. However, no molecular identification methdology targeted to identify *Nodularia spumigena* was presented in the materials and methods section of the report.
2. No methodology on representative sample collection was discussed.
3. It was mentioned that the concentrations of Nodularin could not be reported because the concentrations were too high. If the concentrations were too high, the extracted samples could have been diluted to fit in the calibration curve or analysis range.
4. From materials and methods section, it is not clear that the Nodularin was measured in the cell mass or in the bulk water. When cyanobacteria decays, the intercellular toxins are released into the bulk liquid. Hence, it would be necessary to measure total cyanotoxins (bulk liquid plus cell mass) and then to normalize that value over a given volume of water in the lake represented by that particular sampling event.

In light of the research work done so far on the identification and quantification of cyanotoxins, more systematic research is needed to quantify cyanotoxins and that is the overall goal of this research. *The specific objectives of the research are outlined below.*

- Quantify seasonal variations of concentrations of Cyanotoxins in Farmington Bay.
- Identify the different species of Cyanobacetria in Farmington Bay.
- Correlate the seasonal variations of toxins with cynaobacteria species type and concentration.

MATERIALS AND METHODS

Water Sampling

Bulk water samples containing bacterial cells were collected from six different locations in the Farmington Bay region of the Great Salt Lake in the summer of

2007 on monthly basis. The locations were chosen in such a way that either they were receiving effluent discharges or surface runoff directly or indirectly.

To collect representative samples, a hollow plastic tube of approximately 20 cm diameter and 2.5 ft in length was placed (figure 1) on the lake sediment to create a water column. Care was taken as not to disturb the lake sediments to the extent possible. The water in the column was mixed gently manually with the help of a plastic scoop attached to a wooden stick. Samples were collected from the top, middle and bottom of the column and were transferred into a 1-liter amber colored glass bottle. The bottles were stored on ice and were immediately transported to the lab for analysis. Sediment samples were also collected from the same sampling locations for phosphorus speciation analysis.

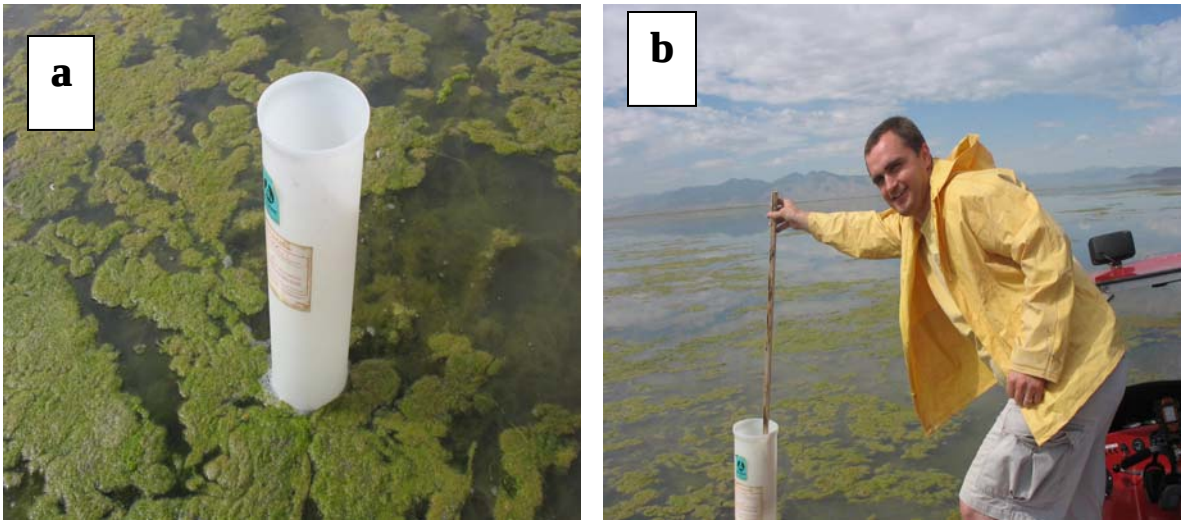

Figure 1: Water sampling at the Farmington Bay; (a) sampling column and, (b) Student collecting water samples.

Sample preparation for cyanotoxins

200 ml of each sample was transferred to a clean Erlenmeyer flask and cell lysis to release toxins in the solution was accomplished through 5 to 6 cycles of freeze and thaw using liquid nitrogen (-195 ^{o}C) and 70°C hot water bath. The samples were then filtered and filtrate was collected for further extraction. The remaining unused water samples (~ 800 mL) were stored in glass bottles at -20 ^{o}C. For

relatively more turbid samples, bead beating and ultrasonic disruption were also employed along with freeze and thaw.

Calibration Curves for cyanotoxins

Pure compounds of Microcystin-RR, Microcystin-LR, Microcystin-LA, Microcystin-YR and Nodularin were purchased from Sigma-Aldrich, and standard solutions of known concentrations were prepared in HPLC grade methanol. The samples were analyzed in LC/MS in triplicates. The standard chromatographs for each of the five toxins are shown in Figure 1

Cyanotoxin Recovery Tests

Control tests were conducted to check the recovery of cyanotoxins through SPE extraction methods. To accomplish this, 200 ml of ultra pure water was spiked with known concentration of standard toxin solutions. The spiked samples were subjected to the same extraction procedures described above.

LC-ESI-MS/MS analysis

The samples were analyzed using a 250×4.6 mm Luna C18 column with a 4-µm particle size (Phenomenex, Torrance, CA). The solvent used for the mobile phase were 0.05% (v/v) Trifluoroacetic acid (TFA) water solution (solvent A) and 0.05% TFA acetonitrile (solvent B) at a flow rate of 1 ml/min. The gradient was as follows: 15% solvent B held for 3.5 min, increased linearly to 70% in 20 min and held for 3 min, and stepped to 100% and held for 8 min. A 9-min equilibration step at 15% solvent B was used at the beginning of each run to bring the total run time per sample to 38.5 min. An injection volume of 20 µl was used for all analyses. MS analysis was performed using Micromass Quattro II - Triple Quadrupole Mass Spectrometer with electospray ionization in positive mode.

Other Analyses

The water samples were also analyzed for dissolved phosphorus, NH_3-N, NO_3-N and NO_2-N using Hach Ascorbic acid (8048), Chromotropic acid (10020), Salicylate (10031), and Ferrous sulfate methods respectively. Total phosphorus was also analyzed using ascorbic acid method as per Standard Methods (APHA-AWWA-WPCF 1985).

Phosphorus speciation

1g sediment sample was weighed and dried at 100^{o}C for 2 hours. The weight of the sample was measured after drying in order to determine the moisture content. Total phosphorus was determined in the sediment sample as soluble reactive phosphorus after wet oxidation (APHA, AWWA, WEF, 1988).

To conduct P speciation, approximately 1 g sediment sample was taken and a 5 step sequential extraction method was followed, to extract different forms of phosphorus from the lake sediment. Phosphorus speciation analysis was done for loosely sorbed soluble reactive P, iron and manganese bound P, Calcium bound P, polyphosphates and residual P. The method was developed in our lab based on the available literature.

RESULTS AND DISCUSSIONS

Extraction and detection of Cyanotoxin

The water samples were analyzed for phosphorus, ammonia, nitrate and nitrite, and the results are shown in Table 1. Sample locations 2 and 3 consistently showed high concentrations of phosphorus and nitrite, irrespective of the time of sampling. From this data it was speculated that these locations might have higher concentrations of cyanotoxins present.

However, concentrations of cyanotoxins measured in all the samples at all locations were below detection limit. Control experiments, in which case the DI water was spiked with 5~10 μg/L toxins and was subjected to the same extraction protocol, always showed detectable peaks of all toxins in the chromatograph. Control experiments illustrate that our extraction protocol and analysis method using LC/MS were accurate and competitive enough to detect toxins. The retention times of pure toxins are shown in Table 2, and a typical chromatogram obtained from LC-MS analysis is shown in Figure 1. The lowest concentration used for the detection and making calibration curve was 50 μg/L.

Generally, Nodularin is the primary hepatotoxin found in brackish waters. We spiked DI water with pure compound of Nodularin bought from Sigma Aldrich. Figure 2 shows triplicate chromatographs obtained from the LC/MS when the Nodularin spiked sample was subjected to our extraction protocol and triplicate injections were made. To confirm the extraction procedure that was employed for the actual water samples, recovery tests were also conducted. Known concentrations of toxins were spiked in ultra pure water, and were extracted in the same way as the samples. The recovery was in the range of 70~80 %.

Figures 3 though 8 shows chromatographs for all five toxins obtained for water samples for the months of May, June, July, August, September and October respectively in 2007. It is evident from these chromatographs that none of the toxins corresponding to the retention times presented in table 2 is present. Simultaneous control and recovery tests conducted on pure toxins and analysis on actual water samples form Farmington Bay strongly suggests that cyanotoxins were either not present in the water samples or were present at concentrations well below our method detection limits. As illustrated above, the smallest concentration used in the calibration curve was 50 μg/L. The concentration factor (200 mL samples was concentrated to 0.5 mL) employed in all samples was 400. When we further apply a recovery factor of 0.75 for toxins, the original water sample should contain 0.167 μg/L ($=\frac{50\mu g/L}{400*0.75}$) of any of the five toxins to be quantified using our method. In other words, this study could not detect any

toxins in the samples analyzed in the summer of 2007 and we hereby conclude that the toxins were either absent completely (rare possibility) or were present at a concentration below 0.167 μg/L.

Simultaneous analysis for nutrients on water samples showed the presence of ammonia, nitrate and nitrite nitrogen and orthophosphate (table 1). These data implies that the sampling locations were not nutrients limited. The total phosphorus in the sediments was estimated to be at an average value of 1.1 mg P/gram of sediments. Table 3 shows P speciation results on a sediment sample collected from a site where algae growth was observed physically.

Table 1. Nutrient concentrations at different sampling locations in the Farmington Bay

Month of May						Month of June					
	PO4-P (mg/L)	Total P (mg/L)	NH4-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	#	PO4-P (mg/L)	Total P (mg/L)	NH4-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)
1	1.61	3.57	0	0.89	1.28	1	0.33	2.89	0.38	1.58	3.01
2	3.91	12.38	1.85	1.05	7.17	2	7.59	18.96	1.02	0.86	4.52
3	5.27	17.06	1	0.2	4.22	3	5.58	9.08	0.95	0.77	2.63
4	1.23	3.38	1.34	0.68	2.67	4	1.26	3.33	0.87	0.35	1.73
5	0.93	2.82	0.77	0.43	3.56	5	0.24	4.97	1.22	0.71	2.64
	0.25	3.83	1.46	0.59	1.93	6	0.14	4.09	1.83	0.28	3.52

Month of July						Month of August					
#	PO4-P (mg/L)	Total P (mg/L)	NH4-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)	#	PO4-P (mg/L)	Total P (mg/L)	NH4-N (mg/L)	NO3-N (mg/L)	NO2-N (mg/L)
1	2.02	N/A	0.46	0.79	1.58	1	2.83	N/A	1.53	0.49	1.35
2	11.94	N/A	0.23	1.05	3.69	2	13.48	N/A	1.02	0.79	1.04
3	8.73	N/A	0.05	0.63	2.61	3	7.32	N/A	0.69	0.41	4.95
4	3.64	N/A	0.21	1.73	1.03	4	4.69	N/A	0.98	0.88	2.42
5	1.27	N/A	1.04	0.53	1.94	5	3.55	N/A	1.38	1.74	1.93
6	1.03	N/A	0.68	0.79	3.44	6	1.63	N/A	1.73	1.35	1.04

Table 2: Retention Times of Cyanotoxins as obtained from LC-MS analysis

Name of Compound	Retention Times (minutes)
Microcystin –LA	20.28
Microcystin –LR	18.88
Microcystin –YR	18.11
Microcystin –RR	17.39
Nodularin	17.54 (old method) and 14.76 (modified)

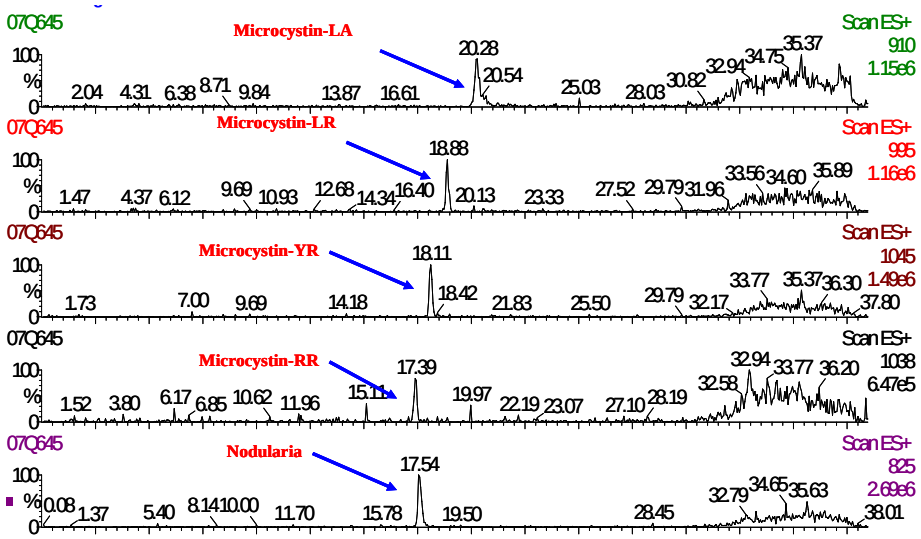

Figure 1: Typical Chromatographs of Cyanotoxins (50µg/L concentration)

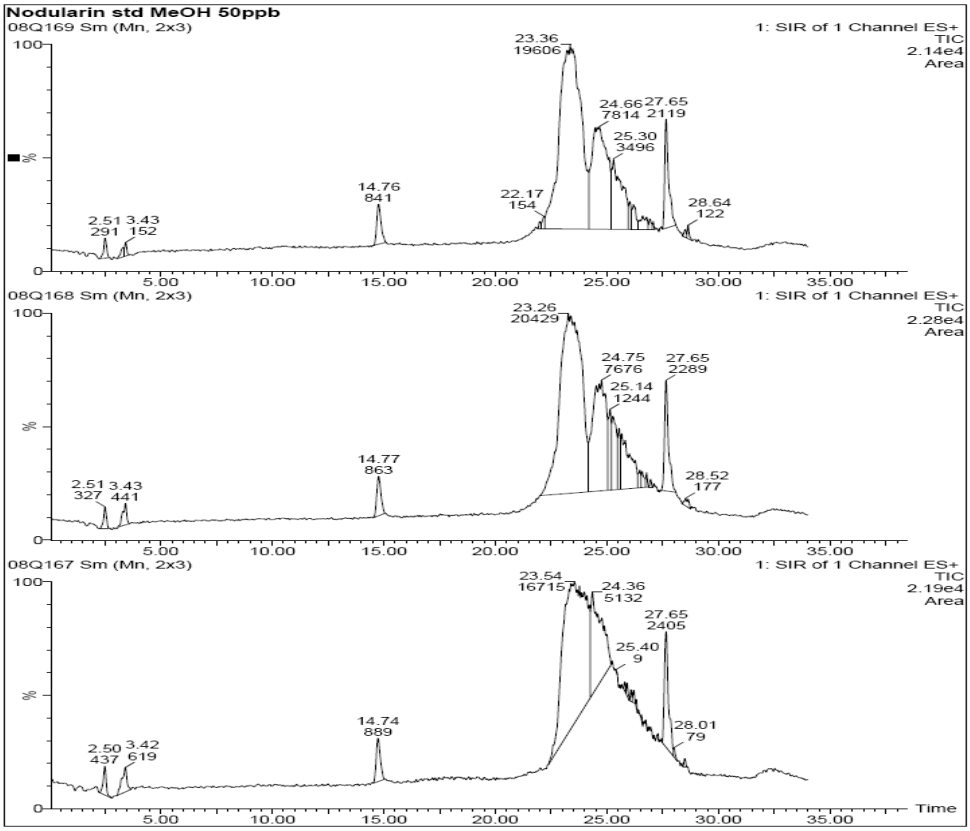

Figure 2. 50 ppb Nodularin standard solution chromatographs, run in triplicate. All the three runs showed consistent and well detectable peaks of Nodularin

standard. Note a changed retention time (14.74 min) because of changed instrument conditions to improve analysis sensitivity

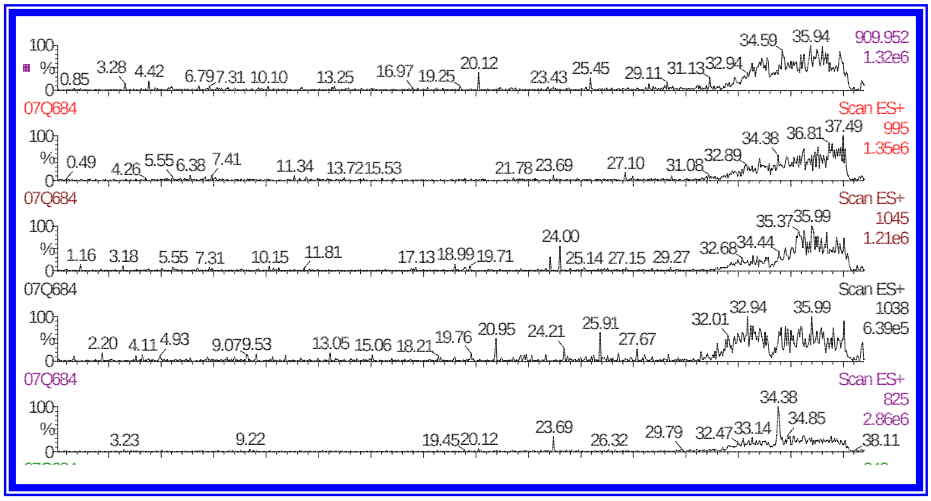

Figure 3. LS chromatograph for the extracted sample for May

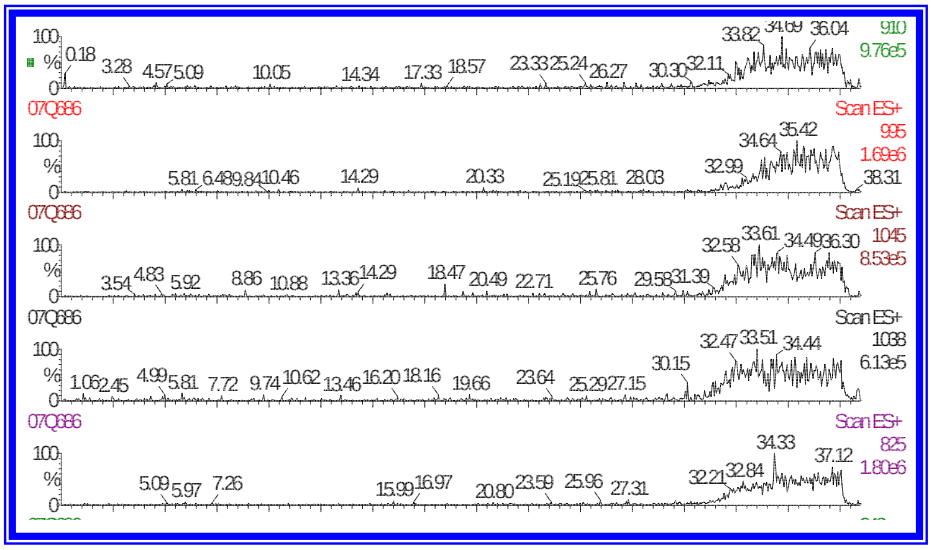

Figure 4. LS chromatograph for the extracted sample for June

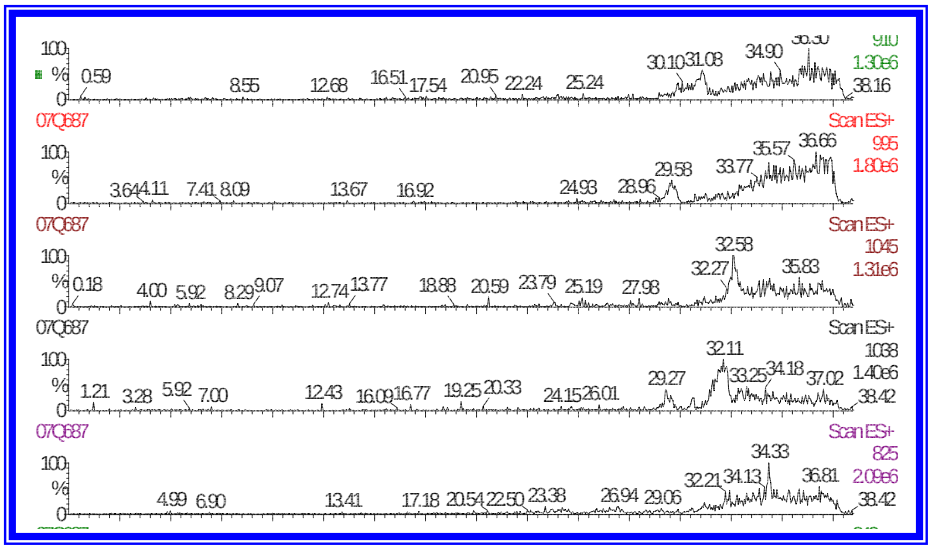

Figure 5. LS chromatograph for the extracted sample for July

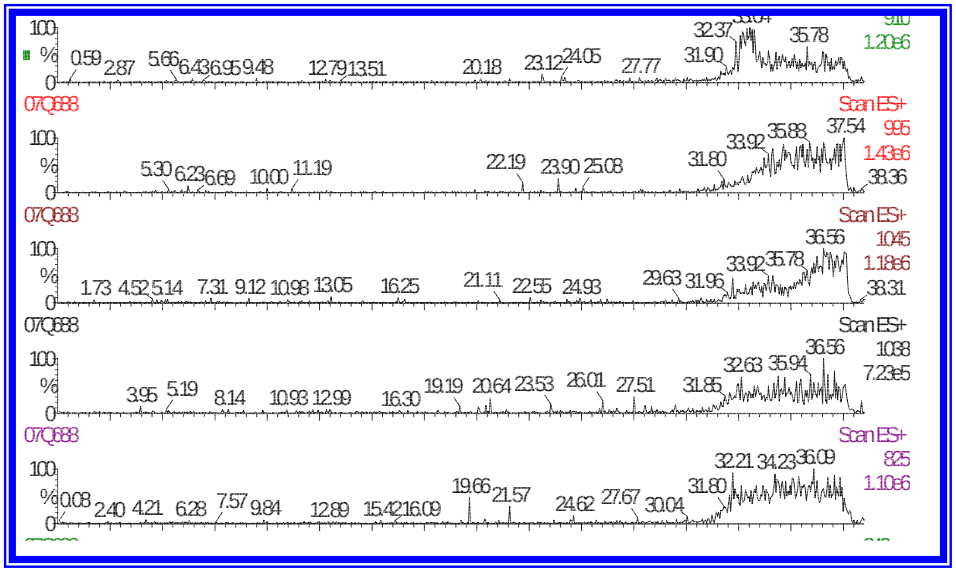

Figure 6. LS chromatograph for the extracted sample for August

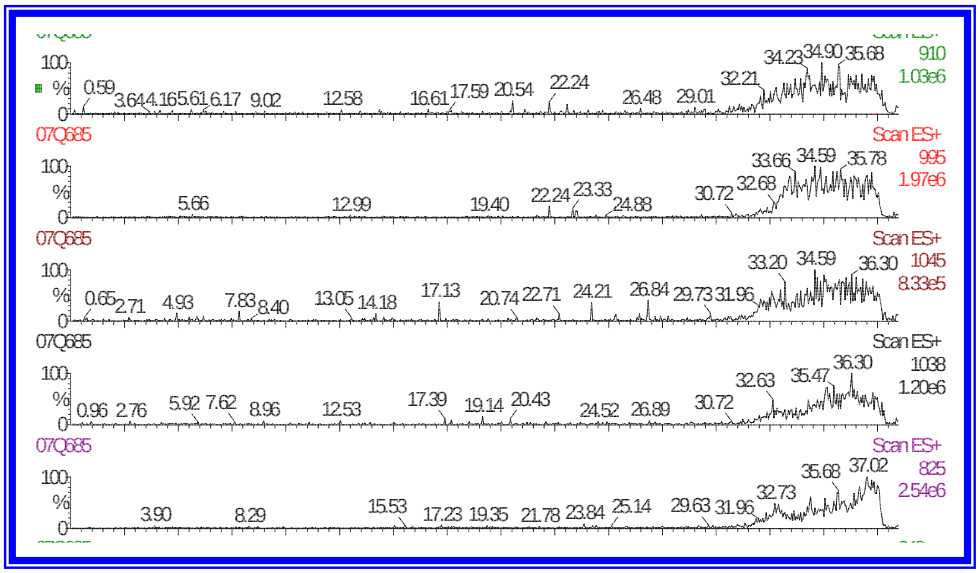

Figure 7. LS chromatograph for the extracted sample for September

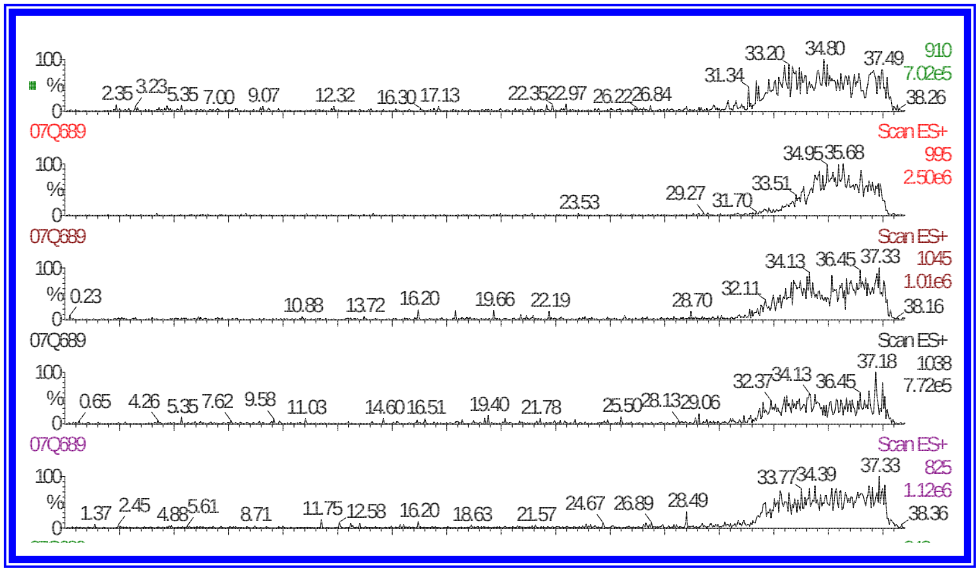

Figure 8. LS chromatograph for the extracted sample for October

Table 3: Sequential extraction of P from sediment samples of Great salt lake, Utah

	Wet wt of sediment (g)	SRP conc (mg/L)	SRP (g dry weight/specific volume)	SRP (g/L dry weight)	SRP Dry weight (mg/g)
Soluble Reactive phosphorus					
	1.09	1.37	0.623	12.22	0.112
FE and Mn bound phosphorus					
	1.092	1.07	0.623	5.77	0.185
Calcium bound P					
	1.092	1.01	0.623	7.99	0.126
Polyphosphates					
	1.092	7.19	0.623	12.46	0.576
Residual P					
	1.092	8.03	0.623	6.233	1.288

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