Biological and Chemical Investigations of Water Quality and Contamination Study After a Suspicious Algal Bloom in a Coastal Lagoon, Jeddah, Saudi Arabia

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Abstract. The General Authority of Meteorology and Environmental Protection (GAMEP) started an investigation about suspicion of microcystis bloom with toxin production in lagoon near Obhour, Jeddah. Water samples were collected from different spots covering all lagoon directions. The samples were analyzed for salinity, coliforms, toxin producing microalgae using MPN, and microscopic examination. LC/MS-MS analysis was used to identify and quantify the extra-cellular microcystins (MCs) in the lagoon water. The results revealed complete absence of the toxic algae or its secreted toxins. The results revealed the presence of *Dunaliella salina* with rich β -carotene production responsible for brown or pink color in the lagoon.

Keywords: Fish coastal lagoon, Algal bloom, *Dunaliella salina*, Jeddah, LC/MS-MS.

1. Introduction

Jeddah is known as the bride of the Red Sea because of its magnificent beaches and coastline which spans for about 30 km (Rasul *et al.*, 2015). The recreational coast of Jeddah includes a number of famous semi-closed water bodies such as Al-Arbaeen, Al-Shabab, and Al-Salam lagoons (Al-Lihaibi, 2000). During the last three decades, as the city was developing and expanding, these three lagoons started to face environmental pressures and showed some signs of environmental degradation. Poor environmental management of municipal and industrial wastewater in addition to torrents streams was always among the top list root causes of Jeddah coast environmental deterioration (Jastaniah, 2010; Kitto and Sambhu, 2012).

In addition to the above mentioned lagoons, environmental issues emerged in other smaller lagoons recently, the lagoon with fish statue (Samaka) fetched public attention through social media with pink color and odor. The lagoon is located in the southern district of Obhur in Jeddah and was named after the presence of an aesthetic metal sculpture in the shape of a large fish (statue) in the middle of the artificial coastal lagoon (Fig. 1a). The area of the lagoon is estimated at 11,160 m² and its depth is close to 2-3 meters (personal communications). The lagoon lacked the ideal biodiversity due to poor water exchange between its water and Sharm Obhur sea water. Despite its small size, the lagoon attracted media and community attention because of its elevated pollution. In mid-November 2017, environmental activists and media reports indicated the appearance of red or pinkish tide

due to algae blooms that produce toxic chemicals. The media reports were based on descriptive laboratory observations that identified these algae by belonging to the family of cyanobacteria (unpublished data). The lagoon was blamed to be highly contaminated with a toxic algae.

Microcystins (MC) is a group of peptide toxins that are normally produced by the cells of the cyanobacterial blooms in fresh water. This type of monocyte is multiplying in lakes, ponds and stagnant water. MC consist of a number of congeners characterized by the presence of leucine (L), arginine (R) and tyrosine (Y) as L-amino acids in positions 2 and 4. Based on acute toxicity of MC variants were classified as MC-LR, MC-LA, MC-RR, MC-YR, MC-YA, MC-AR and MC-M(O)R (Zurawell *et al.*, 2005). The most commonly occurring MC congeners were MC-LR, MC-YR and MC-RR, where MC-LR was reported to be highly toxic among the three congeners (Li *et al.*, 2017). Once the cyanobacterial cell dies, the toxins get released and solubilized in the surrounding waters (White *et al.*, 2005., Ye *et al.*, 2017). The environmental half-life of MC was reported to be 90-120 days in natural water and a maximum of 50 days in sediment. The environmental persistence of MC is dependent on different environmental factors such as light penetration, algal bloom, oxic and anoxic conditions (Welker and Steinberg, 2000, Holst *et al.*, 2003., Zastepa, 2014). Exposure of MCs (either acute or chronic phase) leads to severe liver damage and results in tumor formation (Grosse *et al.*, 2006, Li *et al.*, 2011., Fujiki and Suganuma, 2011). MCs were also reported as potential human carcinogens (Lone *et al.*, 2015). The liver is the target organ of MCs in exposed organisms including fish, mammals and birds. Humans could also be exposed to such toxins through swimming and/or the involving in some marine sport activities (Drobac *et al.*, 2013).

MCs may also bio-accumulate in food chain and hence humans could be exposed to trace levels via the ingestion of contaminated food as well (Larson *et al.*, 2014; Greer *et al.*, 2018). Mortality cases of animals such as dogs, cows, and birds were linked to the consumption of water contaminated with such toxins (Hilborn and Beasley, 2015.; Backer and Miller, 2016). Symptoms of human exposure to MCs include skin rashes, ear and eye irritation, asthma, allergic reactions, sporadic dry cough, vomiting, diarrhea, abdominal pain, pneumonia. MCs were also reported to cause liver damage and acute illness such as myalgia, severe headache, vertigo and blistering in the mouth (Stewart *et al.*, 2006.; Drobac *et al.*, 2013). The World Health Organization (WHO) considered oral ingestion as the main root to MCs in humans and thus estimated a tolerable daily intake (TDI) value of 1.5 mg L^{-1} via drinking water and 0.04 mg kg^{-1} body weight (WHO, 2004).

In response to public concerns the General Authority for Meteorology and Environmental Protection (GAMEP) and Jeddah Municipality signed a cooperation agreement to deal with the pollution of the coastal lagoon (fish statue). According to the agreement and as a precaution measure to protect the general public, it was assumed that the change in the lagoon color was due to the growth of a toxic algal bloom. Accordingly, the site of the lagoon was monitored and the public was prevented from approaching, and each party exercised its functions according to the provisions of the convention document. As the agreement emphasized, GAMEP was in charge of conducting a comprehensive study of the lagoon to identify the root causes of its pollution and provide Jeddah Municipality with a scientific report illustrating the methods of control, treatment and optimal rehabilitation of the lagoon. As part of the comprehensive study, the main aim of this paper was to: i)

2. Materials and Methods

A total number of 25 lagoon water samples were collected in different occasions during the study time frame (November 11 2017 to February 01, 2018) for both biological and chemical analysis. For biological characterization, the water samples mixed with salt flakes, algae and sediments were collected in sterile Schott Duran bottles (250 mL) from all four directions of the lagoon. A total of sixteen (eight/sampling) samples were collected during first and second sample collection. Nine samples were collected in the third sample collection which includes the center of the lagoon (Fig. 1b). The samples were labeled appropriately based on the directions (North, South, East and West) with

glass marker. The samples were collected by following appropriate safety protocol such as wearing the safety mask and hand gloves. The samples were collected using the sampling stick fitted with 1L sampling mug. For microbiological analysis, pre-sterilized (autoclaved at 121 °C at 15 lbs for 15 min) Schott Duran bottles (250 mL) were used to collect the samples. The temperature of the lagoon was recorded as (25 °C ± 2 °C). All the samples were stored in the sampling box with ice pack and transported from the sampling site to the Center of Excellence in Environmental Studies (CEES) laboratories at King Abdulaziz University (KAU). For chemical analysis, a total of 30 grab sample were collected from the coastal lagoon in plastic bottles with 2L capacity (Table 1). Samples were transported from the field in ice box to the department of Environmental Sciences laboratories at KAU. Upon arrival, all samples were wrapped in tin foil and refrigerated at 4 °C until ready for further processing.

Fig. 1. A) Shows a general overview of the Fish Lagoon within Obhur Bay, B) Sampling locations from the Fish Lagoon and the coast of Obhur Bay.

Table 1. Average physicochemical parameters data for the whole lagoon water (n=25).

Parameters	GAMEP ambient red sea water quality standard level	Lagoon water quality (Average)
Physicochemical parameters		
Temperature	-	25 °C
pH	-	8.18
Salinity (%)	3.9 to 4	11
Dissolved oxygen (mg/L)	>5	8.57
Electrical conductivity (mS/cm)	-	117.3
Total Dissolved Solids (TDS)-mg/L	37000 to 45000	117000

2.2 Sample Preparation and Analysis

Initial physico-chemical parameters such as pH, temperature, electrical conductivity, dissolved oxygen, salinity and total dissolved solids were analyzed immediately after sample collection (Table 1). Biological parameters such as bacterial cell count, total coliform, fecal coliform, *E. coli* (Harrigan, 1998) and identification and quantification of algae were performed. Pour plate technique with nutrient agar was used for total viable bacterial count (Harrigan, 1998). All plates including control were incubated at 37 °C and viable colony count as colony forming unit (cfu/mL) was performed after 24 - 48 h of incubation. Coliform analysis was performed by using most probable number (MPN) technique as per standard methods detailed in APHA (2005). Algal samples collected in algal net and mounted under light microscope for qualitative and quantitative analysis. Based on the morphology of the algal species the identification was executed. The slide used for algal quantification consist of one hundred boxes with the holding capacity of 1 mL sample, where around ten boxes will be counted on each direction. Average of the ten boxes was calculated for 1 mL of sample.

Sample preparation steps to determine the concentration of the extracellular MC in the lagoon water by liquid chromatography-tandem mass spectrophotometry (LC-MS/MS) were completed within a week after sample collection. All samples were filtered using

Whatmann filter paper Grade (0.45 µm) and extracted by solid phase extraction cartridges (SPE: Oasis HLB 20cc). The SPE columns were conditioned with 12 mL methanol followed by 12 mL MilliQ water and then samples were loaded on the SPE cartridges using a vacuum manifold system at a flow rate of 8 mL/min. After the extraction was completed, the SPEs were vacuum dried for 30 min prior to shipment to the laboratory of natural and synthetic environmental toxins (LANSET) (University of Ottawa, Canada) for chemical analysis by LC-MS/MS.

In LANSET, all SPE cartridges were eluted with 6 ml of LC/MS grade methanol, centrifuged at 3000 RPM and filtered through 0.22 µm PTFE (Polytetrafluoroethylene) syringe filters. Chemical analysis was accomplished according to Zastepa *et al.* (2015). Briefly, separations were performed on a Shimadzu LC20 series high performance liquid chromatography hyphenated with a 4000 QTRAP MS system (AB SCIEX, Concord, ON, Canada). Reversed phase chromatography was performed on a Phenomenex Luna® PFP (2) LC Column (100 mm × 2.0 mm I.D., particle size 3 µm). The column thermostat was maintained at 40 °C. The sample volume of 5 µL was injected for analysis. The flow rate was maintained 0.6 mL/min with an initial mobile phase composition of 90% A (water with 0.1% formic acid) and 10% B (acetonitrile with 0.1% formic acid), linearly changing to 100%

B in 8 min, followed by a hold at 100% B for 2 min. Starting conditions were re-established over an additional 3 min. Analyst software (version 1.6.3) was used for data acquisition. Source conditions were: temperature 350 °C, voltage +4500 V, curtain gas (N₂) pressure 20 psi, and the collision-induced dissociation gas (N₂) set as medium. The authentic MC standards (>95% purity) were analyzed in this study included MC-LR (LR; CAS: 101043-37-2), MC-7dmLR (7dmLR; 134842-07-2), MC-RR (RR; 111755-37-4), MC-YR (YR; 101064-48-6), MC-WR (WR; 138234-58-9), MC-LA (96180-79-9), MC-LY (LY; 123304-10-9), MC-LW (LW; 157622-02-1), and Nodularin (NOD; 118399-22-7). The instrument detection limit was 0.05 ng (on column load). Stock solutions were prepared at 1 mg/mL and diluted serially to 10 ng/mL. Quantitation was attempted based on area under the peak using external calibration curves (0.05-5 µg/ml).

3. Results and Discussion

3.1 Microbiological Analysis of the Lagoon Water Samples

The microbiological analysis covered the bacterial cell count, total coliform, fecal coliform, *Escherichia coli* (*E. coli*), and identification and quantification of algae.

3.1.1 Coliform analysis

The results of coliform analysis revealed that total coliform load was high in south east (SE) and south west (SW) regions of the lagoon where a sewage leak was found (Fig. 2). The presence of fecal coliform and *E. coli* in this region solidly confirmed the sewage contamination at this spot (SE and SW). Southern region of the lagoon was highly contaminated with the coliforms to a highest level of 5022 MPN/100 mL (Table 2).

The coliform analysis confirmed the presence of total and fecal coliform in the lagoon strongly due to disposal or leak of

sewage waste into the lagoon. The results of coliforms were in correspondence with the bacterial cell count. The coliform contamination reached the center of the lagoon, which was confirmed by the presence of total, fecal coliforms and *E. coli* in the samples obtained from center of the lagoon. This sewage contamination leads to bad odor problem in the lagoon.

3.1.2 Identification and quantification of algal strains in the lagoon

The study on algal analysis established the presence of marine algae *Dunaliella salina* which was the sole responsible for the brownish color of the lagoon. Production of β carotene by *Dunaliella salina* gave brownish and pink color shade to the lagoon. Apart from *Dunaliella salina*, algal species such as *Nitzschia*, *Holopedium*, and *Spirulina* were found in the lagoon samples (Table 3). The algal analysis results revealed the absence of microcystin producing fresh water algae such as *Microcystis*, *Oscillatoria*, *Planktothrix*, *Nostoc* and *Anabena*. Abundant amount of β -carotene producing *Dunaliella salina* (400 cells/mL of sample) was present in the contaminated coastal lagoon (Table 3). *Dunaliella salina* is a halophilic microalgae present in the high saline condition (salt evaporation ponds) with β -carotene and glycerol production (McGenity and Oren, 2012).

The carotenoids produced by the algae were used in pharmaceutical, cosmetics and dietary products (Niranjana *et al.*, 2015; Sathasivam *et al.*, 2018) β carotenes were reported to be precursors for vitamin A (Xu *et al.*, 2018). A 100 years of research was performed on *Dunaliella salina* reported the survival under hyper saline condition (Liska *et al.*, 2004; Oren, 2005). The enhancement of β carotene production by *Dunaliella salina* in the high saline coastal lagoon could be

considered as a novel solution after the proper rehabilitation of the lagoon and an investment opportunity for Jeddah municipality.

The next dominant algal strain present in the saline coastal lagoon was identified as *Spirulina* (80 cells/mL of sample). The bio-products pertained from *Spirulina* also consist of several health benefits such as protein rich superfood, enhance cell regeneration, fights aging, anti-fungal, anti-bacterial and also fight against cancer (Deng and Chow, 2010.; Mishra *et al.*, 2014). *Spirulina* was also reported as food material with 70% protein and remaining part consist of vitamins (B12 and β carotene) and minerals (iron).

Lack of cellulose cell walls in *Spirulina* make them feasible for easy digestion (Dillon *et al.*, 1995). *Spirulina* act as antioxidants in comparison with blue barriers (Hampson, 2017).

Therefore, the present study revealed that the coastal lagoon is characterized by its richness of two marine algae, which could have economical values.

3.2 Influence of Salinity on MC Producing Algae

The salinity of the lagoon ranged from 41 to 160 g/L, NaCl during the three sampling sessions with an average of 110 g/L (Table 4). The northern part of lagoon recorded high salinity ranging from 120 to 160 g/L, NaCl and followed by Middle East (ME) with 110 to 150 g/L, NaCl. High salinity of the lagoon was due to accumulation of salt from the sea water and evaporation of water. Salinity was found to be less in the areas where the sewage contamination occurred due to improper disposal and leak in the discharge tube of sewage. Thus, the leak in the sewer line diluted the salinity in southern (SW and SE) part of the lagoon (41 and 70 g/L). The results of salinity also confirmed the absence of the toxic algae. The harsh environmental condition in the lagoon water exceeded the tolerance

limit for the microcystis algae (Martin-Luna *et al.*, 2015).

S1-Sampling 1, S2-Sampling 2 and S3 – Sampling 3

Overall average salinity of all the lagoon samples was 109.56 g/L (110 g/L)

3.3 Microcystin (MC) Analysis in the Lagoon Water

The chemical analysis performed by a highly sensitive LC-MS/MS method revealed that none of the nine MC congeners (MC-LR, MC-7dmLR, MC-RR, MC-YR, MC-WR, MC-LA, MC-LY, MC-LW, and NOD) was detected in any sample (n = 30) above 0.05 ng (on the column load). The analysis showed the absence of any extracellular MC levels in the Fish Lagoon water samples, which further confirmed the absence any harmful microcystis algal bloom during the time course of this study. The pinkish to brownish pigmentation of the lagoon water was due to the presence of highly populated *Dunaliella salina* algal strain (Fig. 3a, 3b and 3c) as illustrated above. The tolerance limit for the MC producing algae (*Microcystis aeruginosa*) was reported to range from 0.5 to 17 g/L of NaCl (Martin-Luna *et al.*, 2015). Furthermore, positive correlation between salinity and MC toxin release was suggested (Preece *et al.*, 2017). In the current study, the lagoon water was characterized by very high salinity ranging from 40 to 160 g/L, NaCl which could easily lyse the cyanobacterial cells and hence increase the water levels of MC (Preece *et al.*, 2017). A typical water concentration of MC during microcystis blooming event could range from the detection limits up to 1800 $\mu\text{g/L}$ (WHO, 1999) and a maximum concentration of MC (150,000 $\mu\text{g/L}$) was reported in a lake at Kansas by United states geological survey (Graham *et al.*, 2012). Accordingly, if presence of pink to red color seen in the lagoon water was due to the

blooming of toxin producing cyanobacterial cells, MC would persistence for a minimum duration of three months (Welker and Steinberg, 2000; Holst, *et al.*, 2003.; Zastepa, 2014), which was not the case in this study since MC was not detected even when the pink water samples (Fig. 3c) were collected during or just after the claimed blooming. Furthermore, since the coastal lagoon contained no fish or any other marine species it was difficult to link any mortalities to any suspected harmful algal bloom during the lagoon coloration. A simple risk assessment of human exposure to MC from the lagoon would require setting some assumptions. Since the lagoon ecology was not suitable for fishing or

any other sport activities, the only recreational activity that may result in human exposure would be setting or walking close to the lagoon. Through such route, MC contaminated aerosols may affect humans. However, the duration of such activities was usually no more than few hours with negligible probable health threats. In the present study the results clearly revealed no contamination of MC in the coastal lagoon. The study also detailed the sewage contamination in the coastal lagoon which is still a threat to the environment. Thus, GAMEP proposed to stop sewage leak and rehabilitation of the coastal lagoon for bioenergy production from the dominant algal strains.

Fig. 2. Coliform contamination in the Fish Lagoon. Level of contamination represented in different colors and codes, 1: Lowest polluted area, 2: Medium polluted, 3: highly polluted, 4: Extremely polluted.

Table 2. Comparison of the lagoon microbial water quality results with the existing water quality standards by GAMEP (General Authority of Meteorology and Environmental Protection).

Parameter	Lagoon water quality (n=25)	GAMEP ambient red sea water quality standard level
Total Bacterial Cell count (cfu/mL)	10 ⁶ (Sewage contaminated regions- 2, 3, and 4) 10 ³ to 10 ⁵ (Other part of lagoon)	<500
Total coliform (MPN/100mL)	809 (4.5 to 5022)	<200
Fecal coliform (MPN/100mL)	260 (4.5 to 986)	<200
<i>E.coli</i> (MPN/100mL)	139 (4.5 to 679)	<500

Table 3. Quantitative analysis of algal strains in the coastal water sample.

Sample Directions	Algae/ Sampling	Algal Count (cells/mL)			
		First	Second	Third	Average
North	<i>Dunaliella Salina</i>	50	160	80	96.6
	<i>Nitzschia</i>	30	50	30	36.6
	<i>Synedra</i>	20	20	10	16.6
	<i>Cocconeis</i>	20	0	0	6.6
	<i>Holopedium</i>	10	0	10	6.6

West	<i>Dunaliella Salina</i>	50	40	60	50
	<i>Nitzschia</i>	20	20	20	20
	<i>Navicula</i>	10	20	30	20
	<i>Diploneis</i>	20	10	20	16.6
Middle west	<i>Dunaliella Salina</i>	30	60	60	50
	<i>Nitzschia</i>	20	20	20	20
	<i>Cocconeis</i>	20	0	0	6.6
	<i>Holopedium</i>	0	60	60	40
	<i>Skeletonema</i>	10	0	10	6.6
South West	<i>Dunaliella Salina</i>	50	400	260	236.6
	<i>Nitzschia</i>	20	80	60	53.3
	<i>Cocconeis</i>	20	50	30	33.3
	<i>Skeletonema</i>	10	20	0	10
	<i>Spirulina</i>	30	0	20	16.6
	<i>Synedra</i>	10	0	0	3.3
	<i>Dunaliella Salina</i>	0	100	90	63.3
	<i>Nitzschia</i>	20	10	20	16.6
	<i>Navicula</i>	50	40	30	40
	<i>Spirulina</i>	30	40	40	36.6
Middle East1	<i>Dunaliella Salina</i>	100	90	110	100
	<i>Nitzschia</i>	0	100	60	53.3
	<i>Spirulina</i>	80	80	80	80
	<i>Navicula</i>	70	0	40	36.6
Middle East2	<i>Dunaliella Salina</i>	80	80	90	83.3
	<i>Spirulina</i>	0	40	20	20
	<i>Nitzschia</i>	50	50	40	46.6
North East	<i>Dunaliella Salina</i>	60	90	180	110
	<i>Nitzschia</i>	40	140	80	86.6
	<i>Spirulina</i>	0	30	10	13.3
	<i>Holopedium</i>	30	0	10	13.3
	<i>Diplonosis</i>	30	0	0	10
Center	<i>Dunaliella Salina</i>	150	110	100	120
	<i>Nitzschia</i>	20	20	30	23.3
	<i>Spirulina</i>	60	50	50	53.3
	<i>Navicula</i>	20	10	20	16.6
	<i>Cocconeis</i>	10	0	10	6.6

Table 4. Salinity analysis of the coastal lagoon water samples.

Sample ID	Salinity (g/L)			
	S1	S2	S3	Average
S1-N	160	120	130	136.6
S2-W	150	120	120	130
S3-MW	120	100	110	110
S4-SW	90	70	68	76
S5-SE	70	41.1	42	50.3
S6-ME1	120	110	120	116.6
S7-ME2	140	120	120	126.6
S8-NE	150	120	120	130
Center	110	110	110	110

Fig. 3. **A)** Typical *Dunaliella salina* under fluorescence microscope obtained from MedSalt (<https://medsalt.eu/fig-20-5a-bigger/>), **B)** Picture of *Dunaliella salina* taken at CEES under light microscope using digital camera, **C)** A representative filter paper during sample preparation for MC analysis showing the fish lagoon water color (samples were collected November 20th, 2017).

4. Conclusions

The results of the chemical and microbiological analysis of the coastal lagoon water samples confirmed the absence of toxic MCs. Still the lagoon was contaminated with sewer line leakage which resulted in color and odor problem. GAMEP asked Jeddah municipality to inspect the sewer leakage in the coastal lagoon and to stop the release of the sewage water with appropriate sealing technology. GAMEP proposed the following ways to rehabilitate the coastal lagoon by i) Dilution of the lagoon with low saline water to reduce the salinity, ii) Growing halophytes in this region for bioenergy production and iii) enhancing the cultivation of *Dunaliella salina* and *Spirulina* for production of β -carotene (Precursor to vitamin A) and single cell protein which generate revenue to the ministry.

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التقصي البيولوجي والكيميائي لجودة المياه ودراسة التلوث بعد ازدهار طحالب مشته فيها في بحيرة ساحلية

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المستخلص. الاشتباه في تلوث بحيرة السمكة بالقرب من بحر على خط جدة الساحلي بالطحالب المنتجة للسموم المعروفة بالميكروسستن، وذلك بسبب تكوين اللون البني أو الوردي في مياه البحيرة، أثار قلق نشطاء البيئة ووسائل الإعلام. تلقائياً، بدأت الهيئة العامة للأرصاد وحماية البيئة (GAMEP) بالتحقيق في المشكلة البيئية في البحيرة. تم جمع عينات مياه من البحيرة من مناطق أخذ عينات مختلفة تغطي جميع الاتجاهات. تم تحليل العينات لتقدير الملوحة والكوليفورم والطحالب المنتجة للسموم باستخدام MPN (الرقم الأكثر احتمالاً) والفحص المجهرى و MS-MS / LC. أظهرت النتائج غياب تام للطحالب السامة. والنتائج كشفت عن وجود دنيليا ساليينا (*Dunaliella salina*) بكمية كبيرة (٤٠٠ خلية / مل من عينة المياه) كسلالة طحلبية رئيسية في مياه البحيرة، منتجة للبيتا كاروتين، ومسؤولة عن اللون البني أو الوردي في البحيرة.

كلمات مفتاحية: بحيرة شاطئية ساحلية، ازدهار الطحالب، دنيليا ساليينا، جدة.