MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Linear Alkylbenzene Sulphonate (LAS) Accumulation and Toxicity on Fish Tilapia, *Oreochromis niloticus*, in Burullus and Edku Lakes, Egypt

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Received:20/10/2021
Accepted:5/3/2021

Abstract: The aim of the present study is to assess the accumulation of detergents residues; Linear Alkylbenzene Sulphonate (LAS) in Burullus and Edku water and muscles of Nile Tilapia (Oreochromis niloticus) and investigate hazards effect on fish. The data showed a significant accumulation of LAS in Burullus and Edku water and fish muscles compared to the reference site. A significant decrease in serum superoxide dismutase (SOD) and catalase (CAT) activities, and glutathione reductase (GSH) concentration was shown in Burullus and Edku fishes compared to those in the control fish.

keywords: Linear Alkylbenzene Sulphonate, Burullus Lake, Edku Lake, Oxidative Stress Biomarkers.

1.Introduction

Surfactants are one of the petrochemical derivatives that are widely used in cleaning processes due to their low cost and great ability to foam, whether in acidic or hard water. The main component of detergents is surfactants which are responsible for the cleaning action of detergents. The other components such as foam stabilizers, perfume, bleach, builders, optical brighteners, soil-suspending agents, fillers, enzymes, and dyes which enhance the action of the surfactant. Synthetic detergents were first used in the 1930s, but it was not commonly known until the 1950s and later that they became popular with large quantities entering the aquatic ecosystem, Recently the world annual production of detergent and surfactants exceed 10 and 15 million tons, respectively (Isyaku and Solomon, 2016). Detergents and surfactants are extensively utilized at home as well as in industry, but unfortunately, they have been considered the most harmful pollutant causing water pollution due to their low degradability. Therefore, large quantities of detergents and their components are accumulated and sustained in the environment for several years causing disturbances in soil, water, and aquatic life. The emission of synthetic detergents into lake ecosystems by domestic sewage harms aquatic animals due to increased foam in water bodies that causing oxygen depletion. Furthermore, these compounds are rich in polyphosphates which increase artificial eutrophication that threatens aquatic life. Linear alkylbenzene sulfonate (LAS) is the most widely used synthetic anionic surfactant which used for over 40 years. Its global consumption reached 18.2 million tons in 2003 compared to consumption of amphoteric, cationic, nonionic, anionic, soap, and other surfactants which were 0.1, 0.5, 1.7, 4.5, 9, and 2.4 million tons, respectively. Although, it poorly degrades in rivers, lakes, ponds, and even in soils causing toxicity to aquatic fauna by inducing severe damage to vital organs and disturb hematological, hormonal, and enzyme performance (Mousavi and Khodadoost, 2019).

Aquatic ecosystems are susceptible to being contaminated by detergents residues which precipitated in the Environmental compartment (surface waters, sediment, biota…) causing changes in these systems. Furthermore, the stability behavior of surfactants resulted in an increased concentration of insoluble or soluble-water pollutants. Low concentrations of surfactants even to 0.1mg/L causing the appearance of foam insulating layer which hinders oxygen exchange between the water body and gas atmosphere, leading to reduction of dissolved oxygen. This produces hypoxia condition which dies many micro-organisms, resulting in deterioration of water bodies (Rand et al., 2020). The northern delta lakes fisheries play an important role in the Egyptian economy provides about 77% of the harvested fish. Fishing is the main economic activity and source of livelihood for tens of thousands of fishermen and their families settled around these lakes, however, they are threatened by problems of eutrophication and expansion of aquatic vegetation combine also to reduce fish production. The discharge of toxic industrial and agricultural wastewater has been responsible for the disappearance of much high-valued fish (Hegab et al., 2020).

Burullus Lake is the second largest of the delta lakes, its importance lies in its great yield of fish which reaches 42.5 % of the total fish production in Egypt. It has been listed in 1998 as a Ramsar site because it provides feeding, refuge, and breeding for waterbirds, it covers an area of 410 km2. However, the lake's open water surface had reduced from 1092 Km^2 in 1801 to 434.6 Km2 in 1972 and 220 Km^2 in 2015 with shrinkage of about 80.0 % of the water area. The loss in the water area of the lake returns to the infringement of large areas of the southern and southwestern parts of the lake for reclamation activities and transformation of the lakes to fish farms along their southern regions. Moreover, discharging of the drained wastewater from the drains into the lake leads to an increase in the number and area of islands inside the lake and to the progressive growth of aquatic plants (Alprol et al., 2021). Edku Lake is the third largest wetland area in the northern delta which supports a fishery and both wintering and breeding waterbirds. Its area has decreased from 28.5x 10^3 to about $12x10^3$ Fadden's (Okbah et al., 2020)

Fish are served as a perfect tool in assessing the quality of the aquatic environment, monitoring environmental pollution because they are the major targets of toxicants. The Nile tilapia, *Oreochromis niloticus* considers one of the most marketable freshwater species satisfied selection criteria for the environmental assessment due to its efficiency in adapting for diet variation, high growth, and reproduction rates, high resistance to diseases, good tolerance to a wide variety of environmental stress and its economic importance which supporting the fishing industry of inland and lake water in Egypt (Biswas, 2020).

Exposure to water pollutants established stress conditions in fish which induce the production of reactive oxygen species (ROS) which danger its life. Therefore, fish resorting to response to overcome this stress with the utilization of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GPx) protect tissues against this oxidative stress condition. And from here these enzymes are important biomarkers in evaluating water quality and in environmental assessment. Antioxidant defense response in fish may be induced and varied depending on the type and intensity of several factors such as: metabolic, reproductive status, age, and environmental conditions such as water temperature, salinity, photoperiods, level of oxygen, availability of food, and presence of toxins and pathologies that either strengthen or debilitated this defense system (Gonçalves et al., 2021)

2. Materials and methods

Study area:

Burullus Lake extends along the Deltaic Mediterranean coast of Egypt. It lies between longitudes 30º 30´ and 31º 10´ E and latitudes 31º 21´ and 31º 35´ N. It has an oblong shape that extends for 47 km along the NE-SW axis with width varies between 4 and 14 Km. The depth of the lake ranges between 0.40 and 2.0 m. It receives approximately 4 billion m^3 of drainage water per year from the Nile Delta agricultural lands, the remaining 3% is precipitation and groundwater (A Elsayed et al., 2019). Many drains connected directly to the lake including (Burullus east Drain, El-Khashaa Drain, Terra, Zaghloul Drain, and Brullus west Drain); in addition to Brimbal (Brinbal) canal that was transferred Nile water from Rosetta Branch to Burullus Lake. Edku Lagoon is one of the Nile Delta lakes which are in the northern delta of Egypt and is connected to the

Mediterranean Sea. It is located about 30 km east of Alexandria between at longitude 30° 8′ 30″ and 30° 23′ 0.0″ E and latitude 31° 10′ 30″ and 31° N. It receives huge amounts of drainage water from four main drains, namely, Edku, Bousaly, El-Khairy, and Berseek, which open into the eastern basin of the lake, the drainage water contains unspecified quantities of urban, industrial, agricultural and chemicals from Beheira Governorate and beyond (A Shetaia et al., 2020). The River Nile is one of the largest rivers of Africa and the longest river in the world. Its basin has an area of 2.9 .106 km^2 extending from latitude 4° south to latitude 31 N and flows northward into the Mediterranean Sea. It is used in the present study as a reference site.

Sampling:

Water and fish samples were collected from autumn 2019 to summer 2020. Sampling stations in Burullus and Edku Lakes and River Nile are shown in Map (1). In Burullus Lake, Station I at longitude 31º04´32´´ and 2 º E and latitude 31º33´28´´ and 7º N. Station II at longitude 31º05´13´´ and 6 º E and latitude 31º31´26´´ and 9º N. Station III at longitude 31º03´59´´and 3 º E and latitude 31º 0´4´´and 77º N. Station IV at longitude 30º57´45´´and 1º E and latitude 31º25´29´´1ºN. Station V at longitude 30º44´11´´and 8ºE and latitude 31º22´52´´and 1ºN. In Edku Lake, Station I at longitude 30º12´16´´ and 1 º E and latitude 31º15´47´´ and 5 º N. Station II at longitude 30º14´0´´ and 2º E and latitude 31º15´23´´ and 1º N. Station III at longitude 30º12´53´´ and 2 º E and latitude 31º14´9´´ and 1ºN. Station IV at longitude 30º09´50´´ and 6 º E and latitude 31º13´26´´ and 4ºN. Station V at longitude 31º15´18´´ and 1 º E and latitude 31º15´18´´ and 6 º N. In **River Nile**, the selected station located in Sherbeen, Dakahlia governorate, 20 km north of Mansoura City at longitude 31° 31′ 18″ and 2° E and latitude 31° 11′ 19″ and 6° N.

Estimation of Linear Alkylbenzene Sulfonate (LAS) Concentrations:

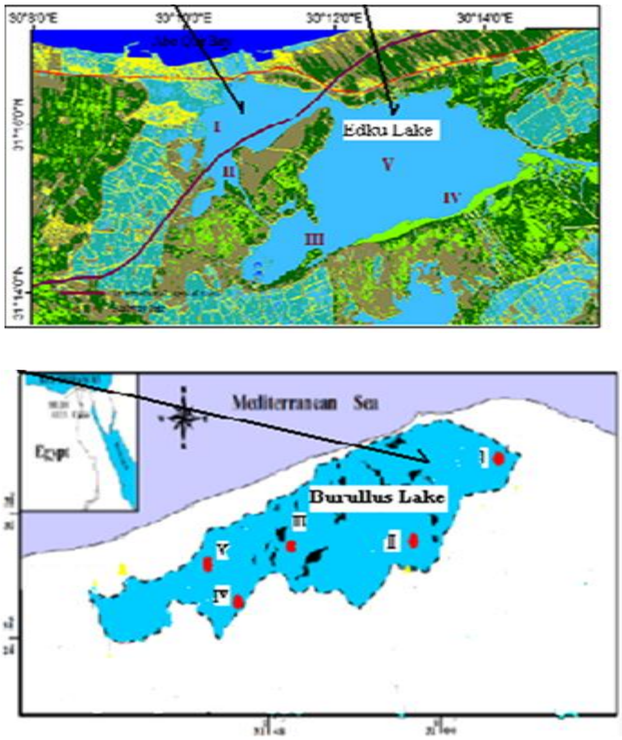
To calibrate the analytical measurements made in one location should be consistent with those made elsewhere. Taking into account these principles, the analysis by HPLC of LAS has been validated in Lake's water and fish muscles samples, following the general requirements of the UNE-EN ISO/IEC 17025:2005 standard. According to the mentioned European Standard, the first requirement is setting the concentration range of considered analytes. In our case, we established a range of LAS concentrations from 0.5 to 100 ppm. For that purpose, we validated two calibration curves, one from 0.5 to 10 ppm named as the low range calibration curve (LRCC), and the other one from 5 to 100 ppm named as the high range calibration curve (HRCC). Finally, the full analytical method involves spiking real samples (Lake's water and fish muscles) with LAS and further determinations of the LAS added.

.1In Water Samples

LAS concentration in water was determined spectrophotometrically by Koga et al. (1999) using methylene blue (MB) at 654 nm .

.2In Fish muscles

After dissection of fish, a weight of 100 mg of fish muscle from each station was extracted according to Knepper et al. (1999) with 30 ml methanol for 16 h in extraction vessels which evaporated to dryness then the dry residues were dissolved in 100 ml of warm deionized water in a water bath, the dissolved solutions were used to determine levels of surfactants by the procedure described in APHA (1995) and Zaporozhets et al. (1998.(



Map 1: Sampling Station

Fish Investigations:

Blood samples were obtained from three fish, per each site for each season from the caudal vein of each fish and were incubated at room temperature for one hour at 3000 rpm for 10 minutes. Catalase (CAT) activity in serum was determined by the colorimetric method of Aebi (1984). Superoxide dismutase (SOD) activity in serum was evaluated by the procedures of Nishikimi et al. (1972) at 560 nm. Reduced glutathione (GSH) was estimated in serum by the colorimetric method of Beutler et al. (1963) at 405 nm.

Statistical Analyses:

The recorded data were represented as (Mean ± SE). Variations in LAS concentrations in the water and fish muscles were tested statistically using the One-Way ANOVA test on the SPSS package. Furthermore, Multiple Range Comparisons (PostHoc tests: Tukey HSD) was employed to detect differences between different study stations and different seasons ($p \leq 0.001$). Probability values ≤ 0.001 were very highly significant, those ≤ 0.01 as highly significant, those ≤ 0.05 as significant, and those > 0.05 as non-significant

3.Results and discussion

Linear Alkylbenzene Sulfonate (LAS) Concentrations:

1. In Water Samples

In Burullus water, the annual mean of LAS concentration ranged between 266.00 ± 89.07 µg l^{-1} at station IV and 336.08 ± 74.85 µg l^{-1} at the station I. While in Edku water, it extended from 310.11 ± 77.33 µg. l^{-1} at the station I and 453.74 ± 92.16 µg. l^{-1} at station III. These results show a clear increase compared to the annual mean of LAS concentration in the River Nile which was 153.74 ± 44.42 µg. l^{-1} (Table 1). Statistically, the One-way ANOVA test revealed that there was a very high significant difference in LAS concentration in water between different study stations ($p \leq 0.001$). Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected very high significant differences between Edku Lake and River Nile ($p \leq 0.001$) and highly significant differences between Brullus Lake and River Nile ($p \leq 0.01$).

Wastewater containing surfactants from the utilization of synthetic detergents discharged into environment causing the appearance of bubbles which not disappeared easily forming water persistent foams which acting as the insulating layer which weakens gas exchange between the water body and atmosphere leading to the deterioration of water bodies, reduction of dissolved oxygen causing the death of microorganisms causing inhibition of toxic substances degradation, as well as decreasing the surface tension of water which increase the concentration of insoluble or soluble-water pollutants in the water (Chandanshive, 2014). Increased concentrations of LAS in the investigated stations of both Burullus and Edku Lakes compared to that in River Nile may be due to continuous discharge of sewage and domestic wastes to the water surface of both lakes, these results agree with Shukla and Trivedi (2018).

2. In Fish muscle

In Burullus fish muscles, the annual mean of LAS concentration varied from 106.44 ± 9.72 µgkg^{-1} at station I to 123.08 ± 13.19 µgkg^{-1}at station III. In Edku fish muscles, the annual mean of LAS concentrations fluctuated between 164.72 ± 11.20 µgkg^{-1} at station II and 175.83 ± 14.28 µgkg^{-1} at station III compared to that in River Nile which was 52.82 ± 2.04 µgkg^{-1} (Table 1). According to the One-way ANOVA, there was a very high significant difference in LAS concentration in fish muscles between different study stations ($p \leq 0.001$). Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected very high significant differences between all investigated stations ($p \leq 0.001$). Increased concentrations of LAS in muscles of both Burullus and Edku fish compared to that in River Nile may be due to elevated concentration of LAS in Burullus and Edku water which passes into fish through food and skin penetration and accumulated in its tissues, these results agree with Latef and AL-Azawey (2020)

Table 1: The Ranges and Annual Means of LAS Concentrations in Burullus and Edku Lakes and River Nile Water and Fish Muscles Samples.

<table>
<tr><th colspan="2">Stations</th><th>LAS in Water Samples ($\mu g.\ l^{-1}$)</th><th colspan="2">Stations</th><th>LAS in Fish Muscles ($\mu g kg^{-1}$)</th></tr>
<tr><td rowspan="5">Burullus Lake</td><td>I
Annual mean</td><td>202.45 - 518.26
336.08 ± 74.85</td><td rowspan="5">Burullus Lake</td><td rowspan="5">I
Annual mean
II
Annual mean
III
Annual mean</td><td rowspan="5">96.96 - 133.31
106.44 ± 9.72
95.93 - 147.58
118.65 ± 13.01
92.66 - 148.43
123.08 ± 13.19</td></tr>
<tr><td>II
Annual mean</td><td>169.55 - 467.61
330.44 ± 70.65</td></tr>
<tr><td>III
Annual mean</td><td>211.16 - 515.03
307.86 ± 74.91</td></tr>
<tr><td>IV
Annual mean</td><td>135.68 - 501.81
266.00 ± 89.07</td></tr>
<tr><td>V
Annual mean</td><td>237.29 - 518.58
330.52 ± 69.51</td></tr>
<tr><td rowspan="5">Edku Lake</td><td>I
Annual mean</td><td>162.13 - 488.26
310.11 ± 77.33</td><td rowspan="5">Edku Lake</td><td rowspan="5">I
Annual mean
II
Annual mean
III
Annual mean</td><td rowspan="5">154.74 - 209.57
174.21 ± 12.11
138.56 - 192.89
164.72 ± 11.20
150.34 - 213.02
175.83 ± 14.28</td></tr>
<tr><td>II
Annual mean</td><td>212.45 - 752.13
442.61 ± 131.46</td></tr>
<tr><td>III
Annual mean</td><td>258.58 - 636.32
453.74 ± 92.16</td></tr>
<tr><td>IV
Annual mean</td><td>224.71 - 696.65
422.53 ±115.77</td></tr>
<tr><td>V
Annual mean</td><td>215.03 - 519.55
337.94 ± 74.03</td></tr>
<tr><td colspan="2">River NileAnnual mean</td><td>69.23 - 256.97
153.74 ± 44.42</td><td colspan="2">River NileAnnual mean</td><td>48.42 - 57.35
52.82 ± 2.04</td></tr>
</table>

Fish Investigations:

Serum SOD in fish collected from Burullus Lake showed the highest value 344.00 U/L at station I during spring and the lowest value 206.87 U/L at station III during autumn with an annual mean ranged between 249.72 ± 3.34 U/L at station III and 265.16 ± 1.76 U/L at station II. While that in fish sampled from Edku Lake showed fluctuation between179.88 U/L at station II during winter and 306.00 U/L at station I during spring with variation in the annual mean between 250.11 ± 2.64 U/L at station II and 270.95 ± 2.54 U/L at station I. In River Nile, the minimum value was 249.53 U/L during winter and the maximum value was 376.50 U/L during spring with an annual mean of 302.99 ± 2.71 U/L (Table 2). According to the One-way ANOVA test, there was no significant difference in SOD concentration in fish serum between different study stations ($p \geq 0.05$).

Serum CAT activity in fish sampled from Burullus Lake varied from 74.76 U/L at station III during summer to 223.32 U/L at station III during spring with annual mean oscillated between 142.56 ± 2.12 U/L at station II and 153.28 ± 1.51 U/L at station III. While in Edku Lake showed fluctuation between 76.45 U/L at station II during summer and 200.17 U/L at station III during spring with an annual mean between 143.858 ± 2.19 U/L at station II and160.05 ± 2.90 U/L at station I. In River Nile, the highest CAT activity was 246.33 U/L during winter and its lowest was 97.80 U/L during summer with an annual mean of 192.608 ± 3.30 U/L (Table 2). According to the One-way ANOVA test between different study stations, there was no significant difference in CAT concentration in fish serum between different study stations ($p \geq 0.05$).

The concentration of serum GSH showed oscillation in fish sampled from Burullus Lake between 11.64 mg/dl at station III in summer and 43.57 mg/dl at station III in spring with annual mean varied from 23.57 ± 0.78 mg/dl at station II to 26.72 ± 2.32 mg/dl at station III. Whilst that in fish from Edku Lake ranged between 11.58 mg/dl at station III in winter and 36.32 mg/dl at station III in winter with variation in an annual mean between 21.86 ± 1.08 mg/dl at station II and 22.86 ± 0.45 mg/dl at station III. In River Nile, the lowest serum GSH concentration was 28.70 mg/dl in summer and its highest was 74.85 mg/dl in winter with an annual mean of 44.94 ± 1.38 mg/dl (Table 2). According to the One-way ANOVA test between different study stations, there was a very high significant difference in GSH concentration in fish serum between different study stations ($p \leq 0.001$).

Statistically, the One-way ANOVA test revealed that there was a very high significant difference. Multiple Range Comparisons (PostHoc tests: Tukey HSD) detected a very high significant difference in SOD activity and GSH concentration in Burullus fish serum while CAT activity showed high significant difference in Edku and River Nile fish serum between different seasons ($p \leq 0.001$).

Accumulation of water pollutants produces an increasing amount of (ROS) in fish by generating free radicals which damage cell structures and alter the cell membranes leading to different pathologic processes. These pathologic processes were scavenged by these antioxidants which providing protection against these oxygenated compound's toxicity (Paithankar et al., 2021). According to the previous results, decreases in the activity of CAT and SOD and concentration of GSH in both Burullus and Edku fish compared to River Nile due to increased LAS concentration in Burullus and Edku water that causes oxidative stress leading to high disruption followed by cytotoxicity resulted in increased production of ROS which exploited SOD, CAT, and GSH in detoxification, these results are agreement with Miranda et al. (2020)**.**

Table 2: Seasonal Variation of Serum SOD, CAT, and GSH of Nile Tilapia from Brullus, Edku Lakes and River Nile during The Period from Autumn 2019 to Summer 2020

Parameters	Stations		Autumn	Winter	Spring	Summer	Annual Mean
SOD(U/L)	**Burullus Lake**	I	238.29	239.19	344.00	238.50	265.00 ± 2.34
		II	275.86	226.54	312.48	245.76	265.16 ± 1.76
		III	206.87	241.36	337.89	212.76	249.72 ± 3.34
	Edku Lake	I	290.30	212.30	306.00	275.19	270.95 ± 2.54
		II	279.77	179.88	276.45	264.34	250.11 ± 2.64
		III	297.43	197.45	296.53	279.02	267.61 ± 2.77
	River Nile		300.00	249.53	376.50	285.91	302.99 ± 2.71
CAT(U/L)	**Burullus Lake**	I	140.60	185.33	202.00	82.80	152.183 ± 2.97
		II	153.65	203.79	122.15	90.65	142.56 ± 2.12
		III	137.46	177.58	223.32	74.76	153.28 ± 1.51
	Edku Lake	I	190.50	157.30	198.80	93.60	160.05 ± 2.90
		II	183.45	141.54	173.99	76.45	143.858 ± 2.19
		III	195.88	133.68	200.17	95.32	156.263 ± 2.37
	River Nile		196.00	246.33	230.30	97.80	192.608 ± 3.30
GSH (mg/dl)	**Burullus Lake**	I	11.89	31.53	36.60	15.20	23.81 ± 0.05
		II	16.67	25.74	33.29	18.56	23.57 ± 0.78
		III	13.23	43.57	38.43	11.64	26.72 ± 2.32
	Edku Lake	I	19.90	12.13	35.96	20.31	22.08 ± 1.00
		II	22.87	16.99	30.18	17.38	21.86 ± 1.08
		III	16.96	11.58	36.32	26.58	22.86 ± 0.45
	River Nile		33.51	74.85	42.70	28.70	44.94 ± 1.38

Conclusion and recommendations

The current investigation revealed that both Burullus and Edku Lakes receive massive quantities of detergents residues which resulted in serious harm on inhabitant fishes including disturbance in enzymes performance. Thus, great efforts must be exerted to control the existence of detergents in aquatic environments and making strict laws that restricted the dumping of wastes within the water. Also, treatment of wastes must be continuously performed and controlled by laws.

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