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Study of Linear Alkyl Benzene (LABs) as molecular marker of sewage pollution in Bivalves Mollusk *"AnodontaCygnea"* in Anzali lagoon

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ABSTRACT: Tracking and determining the amount and types of urban sewage that is being releasing to the environment is important in planning for wastewater treatment systems and water quality control. In this research, in four stations of Anzali Lagoon, (by means of a gas chromatograph device with mass detector spectrometer) wastewater pollution has been measured and evaluated with molecular indicator Linear Alkyl Benzene(LABs) in bivalve "Anodonta cygnea". Average concentrations of LABs were calculated 520 µg/kg dry weight in samples of station 1, 1245 in station 2, 2144 in station 3, and 1746 in station 4. Results from this study indicate severe LABs pollution in studied stations in Anzali Lagoon's sugesing that urban wastewater has reached these stations.

Key words: *Sewage pollution; molecular indicator; Linear Alkyl –Benzene; Anodonta Cygnea; Anzali Lagoon*

INTRODUCTION

In recent years, with population growth, and extensive urbanization urban wastewater has reached to the rivers and coastal environment, causing many risks to human health and also for other organisms. Also today environmental issues especially those relating to aquatic ecosystems, and pollutions caused by human activities or their consequences which already have left many damages

and are going on yet, are from the most main concerns of researchers and scientists. (Isobe *et al.*, 2002)

Anzali Lagoon (Guilan Province) is one of the valuable lagoons of southwest Caspian Sea which has special importance due to specific ecological, economical, and social conditions and diversity of different species of aquatic plants and animals. Existence of various urban, industrial and agricultural activities in around Anzali Lagoon or rivers leading to it, has caused large amounts of various pollutants enter into the wetland and eventually into the sediments of it's bed and affect benthos communition (Paydar, 2001).

Mollusks, particularly bivalves, have the possibility to accumulate contaminants in their body because of their filter feeding system, so a large number of bivalves have been identified as biological indicators (Beon *et al*., 2003). Also the meat of bivalve mollusk is edible for human consumption. Subfamily of Anodontinae (toothless) includes different types of freshwater bivalves which have been formed from large number of genera and species. One important species *Anodonta cygnea*, may be found in Anzali Lagoon (Ashja Ardalan, 2006).

Usage of detergents has increased in Iran especially in the past few decades have caused new types of pollutions enter to the environment. (Irani and Yarndi, 1993). Linear Alkyl Benzene (LABs) is an organic compound based on crude oil and typically containing alkyl C10_C13. In the petrochemical industries, Linear AlkylBenzene Sulfonate (LAS) are produced from

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LABs and since 1960s, LAS is the original anionic surfactant of detergents. Approximately 1-3% of the LABs produced of LAS synthesis process remain as impurities (Takada and Ishiwatari., 1987; Eganhouse *et al.,* 1983).

Since the LAB in detergents and household purifiers is in impure form, and considering its very extensive use, it enters through the sewage into the environment, particularly affecting the aquatic ecosystems, as pure LAB release (Eganhouse *et al*., 1983)., and due to it's slow microbial decomposition compared to LAS, it is known as a molecular indicator of sewage pollution and is used to track urban wastewater (Takada and Ishiwatari., 1991).

LABs isomeric distribution can also provide information of various abandoned sewage water in the environment (Tsutsumi *et al*., 2002). Tracking sewage pollution is very necessary to control water quality. Thus in developed countries, using molecular indicators in domestic outputs have been increasingly used. Establishment of urban wastewater treatment facilities, can reduce release of sewage into the environment. However in many countries, statistical data in sewage refining systems are not available and therefore it is very important to specify the amount and types of contaminated wastewater releasing to the environment. For example, in Asian countries, use of this way to improve urban sanitation has been estimated very low. In recent years, urban sewage pollution has been reviewed in the world by the new method in which determining the molecular indicator of LABs are used as biological indicator of urban wastewater contamination rather common method, i.e. determining the bacterial population of Coli form.(Isobe *et al*., 2004).

As direct and unrefined wastewater from some northern cities of Iran reach into Anzali wetland through some major rivers such as Mahrouzeh and Samandag, this methodology may be used to evaluat swage contamination leading to Anzali. Studying composition of LABs in bivalve *"Anodonta cygnea*" in Anzali Lagoon is important to determine the amount and types of LABs and estimating the scale of sewage contamination of this aquatic ecosystem.

MATERIALS AND METHODS

Based on distance of wastewater discharging facitities and also considering distribution of bivalve "*Anodonta cygnea*" in Anzali Lagoon, four stations were selected for sampling (Fig. 1).

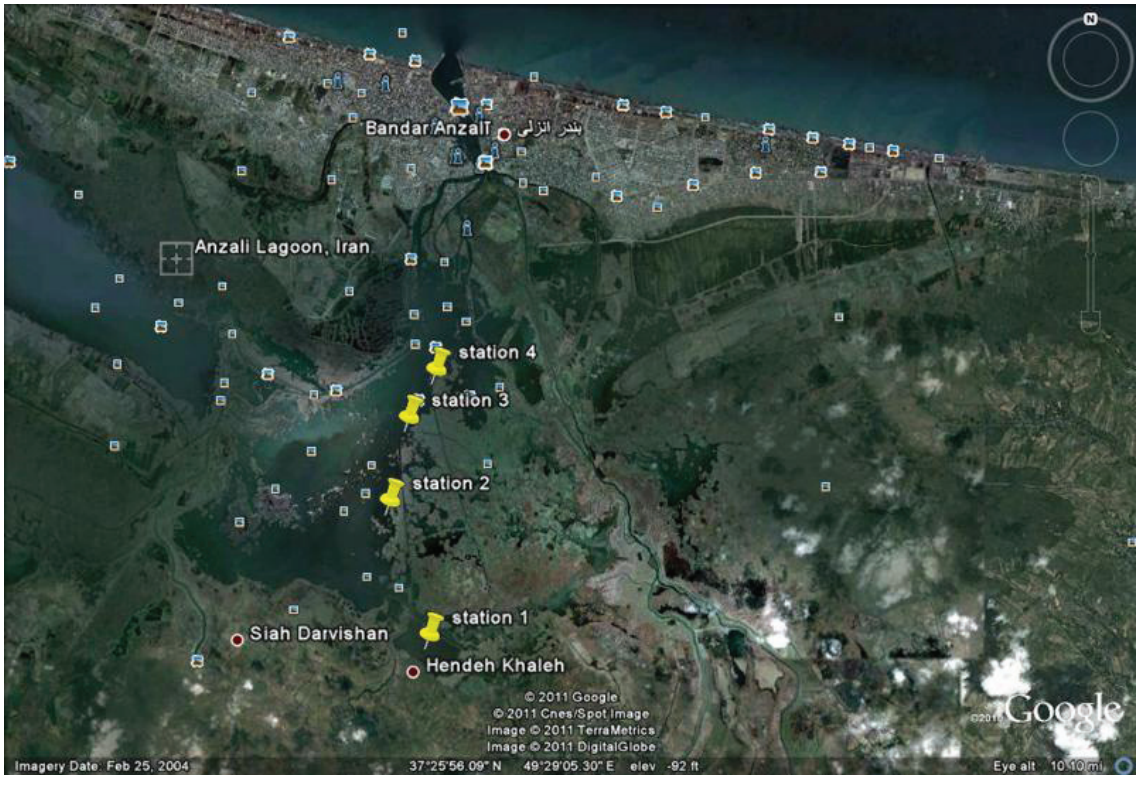

Fig. 1: Location of sampling stations in Anzali Lagoon

With three replications at each station, totally 12 samples of bivalves were collected. After samples were extracted from water, they were rolled inside aluminum foil, coded, and placed inside an icebox, then they were moved to the freezer until they were transfered to the laboratory.

In the laboratory, samples of bivalve "*Anodonta cygnea*" initially were left frozen and then were brought to ambient temperature, then their biometry and weight were measured. (Table 1)

In order to obtain the wet weight of bivalves, soft tissues was extracted and re-weighed, then for drying the tissues, during 24 hours they were placed inside special balloons of freeze dryer machine

CHRIST (Alpha 1-4 LD plus) at temperature of (53)°C and pressure of (-0.065) mbar (MOOPAM., 1999)

To obtain dry weight of samples, they were re-weighed in dry state, Water percentage of the soft tissues was obtained from the following equation (Table 1):

$$\text{Water percentage} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}}$$

Dried samples were powdered, 5g of powdered samples was taken from stations 1, 2 and 3, and 0.8g of station 4 samples -due to their low weight were transfered into the thimbles of soxhelt device, then 250 ml of normal solvent 1-1 dichloromethane- hexane was added to each thimble and during 8 hours extraction was performed at 3 rounds/1 hour speed (MOOPAM., 1999).

Solution obtained by soxhlet apparatus was concentrated at temperature (30)°C and pressure (1/2) bar by rotary machine (Heidolph-LABOROTA 4001) and was completed to about 5 ml (MOOPAM., 1999).

for preparing the chromatographic column, a chromatographic column having height of 30cm and 1cm thick with silica gel (grade 62 with a size of 200-60) were placed in the oven for 5 hours at a temperature of 200°C to be activated and then equal to 5% of it's weight, distilled water was added to it during 3 days they were shook up two times in a day until the distilled water penetrated between the silica grains equally. 10g of prepared silica gel and 25ml of normal solvent 1-3 dichloromethane- hexane were added to each column and the valve of column was opened to passes the solvent between silica particles, in this stage solvent was discarded so column was prepared for sample purification step. (Takada and Ishiwatari.,

Table 1 : Biometric profile of the samples containing bivalve *Anodonta cygnea*

Water percentage	Thickness (mm)	Width (mm)	Length (mm)	Station & Repetition
81.05	54.28	69	140.22	1-1
81.15	52.44	68.24	133.52	2-1
82.17	56.38	72.36	147.76	3-1
83.63	54.66	77.48	145.2	1-2
81.81	55.92	78.32	146.58	2-2
84.40	54.9	72.64	144.41	3-2
81.53	54.58	62.74	131.78	1-3
77.01	53.08	75	141.01	2-3
76.18	46.68	72.86	143.72	3-3
91.07	34.76	56.51	97.62	1-4
90.96	33.82	57.02	96.08	2-4
90.53	33.54	55.38	95.36	3-4
83.41	48.75	68.13	130.27	Total average

1991). After adding the concentrated samples to chromatographic column, 25 ml of normal solvent 1-3 dichloromethane- hexane was added to it, then the column's valve opened and the output solution was collected in a glass container with cap (Takada and Ishiwatari., 1991).

Collected solution was concentrated again by the rotary device (Heidolph-LABOROTA 4001) and was completed to 1 ml volume (MOOPAM., 1999).

In order to determining the concentration of LABs, 2 microliter of this solution with LABs were injected into gas chromatographic device (Agilent 5975 C) with a mass spectrometer detector (Agilent 5975 C), while during the process the device was in SIM mode (MOOPAM., 1999). Ultimately for each station, average of three replications was calculated (Table 2).

Table 2 : Studying the trend of change of LABs concentration in different stations (µg/kg dry weight)

LABs		Average Station1	Average Station2	Average Station3	Average Station4	Total average
LAB10	Isomer 1	39	70	119	109	84.25
	Isomer 2	38	71	177	112	99.5
	Isomer 3	38	74	166	119	99.25
	Isomer 4	35	70	130	129	91
Total LAB10		151	285	592	470	374.5
LAB11	Isomer 1	28	69	98	82	69.25
	Isomer 2	28	69	101	86	71
	Isomer 3	48	73	130	119	92.5
	Isomer 4	29	71	108	98	76.5
Total LAB11		134	282	437	386	309.75
LAB12	Isomer 1	29	71	111	107	79.5
	Isomer 2	21	60	96	70	61.75
	Isomer 3	21	60	94	70	61.25
	Isomer 4	21	60	98	73	63
	Isomer 5	21	62	97	75	63.75
Total LAB 12		113	313	497	394	329.25
LAB13	Isomer 1	23	65	113	95	74
	Isomer 2	18	58	95	63	58.5
	Isomer 3	18	58	95	64	58.75
	Isomer 4	18	59	106	67	62.5
	Isomer 5	19	58	89	68	58.5
	Isomer 6	26	67	119	139	87.75
Total LAB 13		122	364	617	496	399.75
total LABs		520	1245	2144	1746	1413.75

RESULTS AND DISCUSSION

In this study concentration of 10, 11, 12 and 13 carbonaceous LABs from bivalve samples of four stations in Anzali Lagoon, were exracted and measured. Each of these 10, 11, 12 and 13 carbonaceous groups had 4, 4, 5, and 6 isomers respectively; concentration of all compounds and their isomers are shown in Table 2.

The results of this study indicate that the total concentration of LABs in stations one to four, were respectively, 520, 1245, 2144 and 1746 µg/kg dry weight, and according to these results, among all studied stations, station 3 (River Samandag) was the most contaminated (2144 µg/kg dry weight). These results show severe LABs and consequently urban wastewater pollution, in station 3 and then in station 4 (Mahrouzeh River) and station 2 ("Sorkhanhol" Wildlife Refuge). The least amount of pollution was calculated in station 1 ("Salakeh" Wildlife Refuge).

Minimum total concentration of 10, 11, 12, and 13 carbonaceous LABs was observed in station 1, and maximum concentration of these compounds was obtained in station3.

It should be mentioned that among the studied LABs, the highest concentration was related to total concentration of 13 carbonaceous LABs in station 3, and the lowest was related to total concentration of 12 carbonaceous LABs in station 1 with respectively 617 and 113 µg/kg dry weight.

CONCLUSION

Today, in developed countries tracking urban wastewater contamination using molecular indicators such as LABs is studies of (Zeng *et al.,* 1995) and his colleages about the validity of LABs as an indicator of urban wastewater pollutants, clearly confirmed usage of these compounds in tracking contamination of urban sewage and defined that technique as an accurate and reliable method (Zeng *et al.*, 1998).

In 1987, Takada *et al* studied distribution and sources of LABs in sediments, river suspended particles, and wastewater inside and around Tokyo. These results show that detergents used in Tokyo, release into the LABs aquatic environment. LABs in urban river sediments significantly stem from domestic wastewater and from the city (Takada and Ishiwatari., 1987).

In years 1998 to 2003, some river and coastal environments of South and South-East Asia, were studied by Isobe and his colleagues who analyzed and studied 89 samples of water from Malaysia, Vietnam, Tokyo, Japan and also 160 samples of sediments from Tokyo,3 Thailand, Malaysia, Philippines, Cambodia, Indonesia and India in order to track sewage for LABs pollution. The amount of LABs in sediments were measured (<0,002 – 42,6) µg/kg dry weight (Isobe *et al.*, 2004).

In this study, bivalve "*Anodonta cygnea*" is used as a filter feeder organism which is able to accumulate pollutants in its body and may be used to study the status of LABs compounds in the Anzali Lagoon ecosystem (Beone *et al.*, 2003).

Lowest LABs contamination was observed in station 1 (Salakeh Wildlife Refuge) in the eastern part of the lagoon, which is further away from human settlements (520 µg/kg dry weight), and the most polluted station was observed in station 3 (Samandag river) with severe LABs contamination. (2144 µg/kg dry weight)

In 2007 in Asia, by performing the program of "evaluating contaminants by bivalves" (mussel watch), the amounts of LABs pollution have been reported between 17 to 1638 in India, 14-1612 in Indonesia, 11- 807 in Malaysia, 22- 1478 in Philippines, 12- 17 in Cambodia, 14- 16 in Vietnam, and 33- 349 (µg/kg dry weight) in Thailand. It is clear, that pollution level in Anzali Lagoon is in the range 520 to 2144 µg/kg dry weight, which is significant compared to results reported above from Asian countries. such as India and Malaysia, had LABs pollution because of their more extensive use of detergents and hygienic material than countries such as Vietnam (Isobe *et al.*, 2007).

In a study unduoted in Spain by (Bayona *et al* 1991), the fate of organic contaminants such as LABs, PCB, PAH in urban wastewater were tracked and evaluated in coastal areas. To do this samples of seawater, suspended particles in the water column and some tiny organisms of were studied. Amount of the polluting organic LABs concentration in "Polychaeta" was 715, in Macropipus was 30 and in Mytilus gallop was 2950 ng/g wet weight; compared with data obtained in bivalves of Anzali Lagoon, these achieved concentrations in bivalve Mytilus are a little more (Bayona *et al.,* 1991).

The concentration of LABs in studied samples from the Anzali Lagoon show that distance stations to cities is factor contributing to pollution; and according to values resulted from this studi, sewage contamination in Anzali Lagoon is severe.

One of the most effective ways to prevent this kind of pollution is removal and decomposition processes in the wastewater treatment facilities. In 2004, Mahvi *et al.* evaluated removal efficiency of these compounds in the wastewater treatment facilities to approximately 93%, and it was determined that amount of detergents in treated effluent was below standard levels and needed no further treatment (Mahvi *et al.*, 2004). Also, Akbarpour in his study on 2005 impact of biodegradation by bacterium Pseudomonas Aeruginosa on decomposition of LAS in wastewater treatment facilities, concluded that this bacterium is able to affect, analyse and mineralize effluent containing LAS alone more effectively than mixed bacterial culture; these bacterium can break down other surfactants, up to 70% which are not in general biodegradable (Akbarpour, 2005). This study showed that comparing with other parts of the world, concentration of LABs compounds in Anzali lagoon is relatively high, and it represents relatively high amount of detergents and urban wastewater pollution of this ecosystem, and in

order to control and reduce the pollution, necessary administrative measures should be done.

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