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RESEARCH ARTICLE

Distribution of linear alkylbenzenes as a domestic sewage molecular marker in surface sediments of International Anzali Wetland in the southwest of the Caspian Sea, Iran

Alireza Riyahi Bakhtiari[1] **· Islam Javedankherad**[1] **· Jahangard Mohammadi**[2] **· Roholla Taghizadeh**[3]

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Abstract

Due to directly receiving high volume of untreated urban and industrial sewage and in turn transferring the pollutants to fish and back to humans, the International Anzali Wetland has been considered to be urgently registered in the Montreux Record. Hence, the present study was aimed to determine the spatial distribution of the linear alkylbenzenes (LABs) in surface sediments of the wetland and its sewage contamination situation. The surface sediments (sampling stations = 167) were collected from the western, eastern, southwest, and central regions of the wetland. The samples were extracted, fractioned, and then analyzed using gas chromatography–mass spectrometry (GC-MS). The concentration of LABs in the sediment samples revealed a range from 394.12 to 109,305.26 ng g^{-1} dw. The concentrations of ΣLABs in the eastern region were significantly higher than that in the other regions. The occurrence of low ratio of internal to external isomers (I/E ratio) of LABs (from 0.65 to 1.30) and D% (from − 0.07 to 24.13) implied effluent row or poorly untreated sewage into the wetland. No correlation was observed between the detected LAB concentrations with total organic carbon (TOC) and grain size. Taken together, regional anthropogenic inputs are the controlling factors for the observed spatial distributions of ∑LABs in the International Anzali Wetland. The findings suggested that LABs are powerful indicators to trace anthropogenic sewage contamination and also highlighted the necessity of sewage treatment plants to be founded around the International Anzali Wetland, especially in the vicinity of the eastern and central regions.

Keywords Linear alkylbenzenes · I/E ratio · International Anzali Wetland · Sewage · Sediment

Introduction

The aquatic ecosystems, especially coastal areas and wetlands, have historically been selected to dispose of human wastes. Even today, sewage is one of the main problematic and chronic sources of contaminants in aquatic environments because of some limitations in sewage treatment plants in coastal areas (Islam and Tanaka 2004). Tracking anthropogenic pollutants is of high importance to evaluate the type, risk, concentration, and ultimate fate of organic contaminants discharged into the aquatic environment and their impacts on these ecosystems and human health (Venkatesan et al. 2010). Anthropogenic materials transported to the aquatic environments can be deposited into sediments. Hence, the investigation into sedimentary history could be useful to discover the terrestrially originated materials and in turn would help evaluate the manmade impacts on the coastal ecosystems (Long et al. 1996; Vails et al. 1990; Chalaux et al. 1995).

During the past decades, the deposition of chemical anthropogenic pollutants, such as herbicides and linear alkylbenzenes (LABs), has significantly been increased and accumulated in the bottom sediments (Martins et al. 2010; Masood et al. 2016). LABs are the materials with a C_{10}–C_{14} normal alkyl

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✉ Alireza Riyahi Bakhtiari
triahi@modares.ac.ir

[1] Department of Environmental Science, Faculty of Natural Resources and Marine Sciences, Tarbiat Modares University, P.O. Box 64414-356, Noor, Mazandaran, Iran

[2] Department of Soil Science, College of Agriculture, Shahrekord University, P.O. Box 88186-34141, Shahrekord, Iran

[3] Faculty of Agriculture and Natural Resources, University of Ardakan, P.O. Box 89516-56767, Yazd, Iran

chain. These materials are industrially sulfonated to produce linear alkylbenzene sulfonates (LAS), one of the worldwide anionic surfactants extensively used to manufacture detergents. During LAS synthesis, about 1–3% of LABs escape sulfonation, thereby releasing LABs into domestic wastewater and in turn discharging into aquatic environment (Isobe et al. 2004).

LABs consist of external and internal isomers ($n = 26$) with different susceptibility to aerobic microbial degradation (i.e., the external isomers with substitutional positions near the terminal of the alkyl chain are more biodegradable than the terminal ones). Therefore, isometric distribution of LABs provides information on their biodegradability and also can be applied to determine the type of sewage (untreated sewage vs. secondary effluents) discharged into the aquatic environments (Martins et al. 2010; Raymundo and Preston 1992; Takada et al. 1992). Due to their high hydrophobicity, LABs settle down on surface sediments and under anaerobic condition residue without any microbial degradation for many years and thus are considered as molecular markers for tracking municipal wastewater (Martins et al. 2014; Wei et al. 2014).

The International Anzali Wetland, with approximately 200 km^2 and watershed area of about 374,000 ha, is located in the southwest of the Caspian Sea and consists of distinctly eastern, western (Abkenar), southern (Siakeshim), and central regions. Due to its high ecological importance (e.g., annually hosting more than 150 species of overwintering migrant birds), the wetland was registered as an international lagoon in Ramsar convention in 1970 and also as an "Important Bird Area" by Bird Life International (Evans 1994). However, due to directly receiving high volume of raw and poorly treated wastewaters from surrounding cities, including Rasht, Bandar-e Anzali, Rezavanshahr, Shaft, Sumesara, Masal, and Fuman, this wetland has been considered to be urgently registered in the Montreux Record (Rezaitabar et al. 2017; Sajedipour et al. 2017). This paper is the first report on sewage pollution monitoring using LABs as molecular markers in sediments in Iran. Hence, the present study was aimed to assess the current anthropogenic impacts on the aquatic environment of the International Anzali Wetland by examining the spatial distribution, compositional profile, and molecular indices of sediment LABs as well as demonstrates the efficiency of sewage treatment plants in this area.

Material and methods

Sampling

From October to November 2016, superficial sediment samples (upper 5 cm (to indicate modern input of target contaminants), sampling station = 167) were collected across the International Anzali Wetland (i.e., from its western, eastern, southern west, and central regions). The sampling was according to a systematic-random design in order to cover the whole wetland and represent different types of discharging domestic sewage into the wetland (Fig. 1). The sediment samples were obtained using a stainless steel Van Veen grab and placed on clean aluminum foil bags, and then kept in a cooler box containing dry ice and stored at − 20 °C until further analysis.

Extraction and analysis of LABs

Sample preparation and the extraction and analysis of LABs were conducted according to the previous published studies and protocols with some modifications (Dauner et al. 2015; Hartmann et al. 2000; Martins et al. 2010, 2014). Briefly, sediment samples (10 g) were Soxhlet extracted for 8 h using hexane (95% n-hexane) and dichloromethane (1:1). The extraction process was conducted through an activated copper treatment to eliminate elemental sulfur. The obtained extract was reduced to 2 ml following rotoevaporation and then submitted to a clean-up procedure in a chromatographic column, using 5% deactivated alumina and silica. The extractions were eluted with 10 ml of n-hexane to obtain LABs (first fraction). The LAB fraction was then transferred to an amber vial (2 ml) and evaporated via a gentle stream of nitrogen. The remained semi-dried substance was resolved in 100 μl of 1 mgl-1 p-terphenyl-d$_{14}$, an injection external standard, and finally, 1 μl of the dissolved substance was injected into GC-MS (Agilent Technologies, Avondale, PA, USA). All the authentic standards for LABs were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All the solvents used for analyses were of chromatographic grade from Merck.

Instrumental analysis

The instrumental analysis of 26 LABs was conducted by GC-MS using a 7890A Series gas chromatograph interfaced with a C5975 MSD split/splitless injector. A 30-m fused silica capillary column of 0.25-mm internal diameter (i.d.) and a DB-5MS capillary column of 0.25-μm film thickness were used. Helium was used as the carrier gas at a constant flow rate of 1.2 ml/min. The injection port was maintained at 310 °C, and the sample was injected in the splitless mode followed by a purge 1 min after the injection. The column temperature was held at 70 °C for 2 min, programmed at 30 °C/min to 150 °C and then 4 °C/min to 310 °C, and held for 10 min. The hydrocarbons were identified with comparison of the retention times with those of the known standards of LABs.

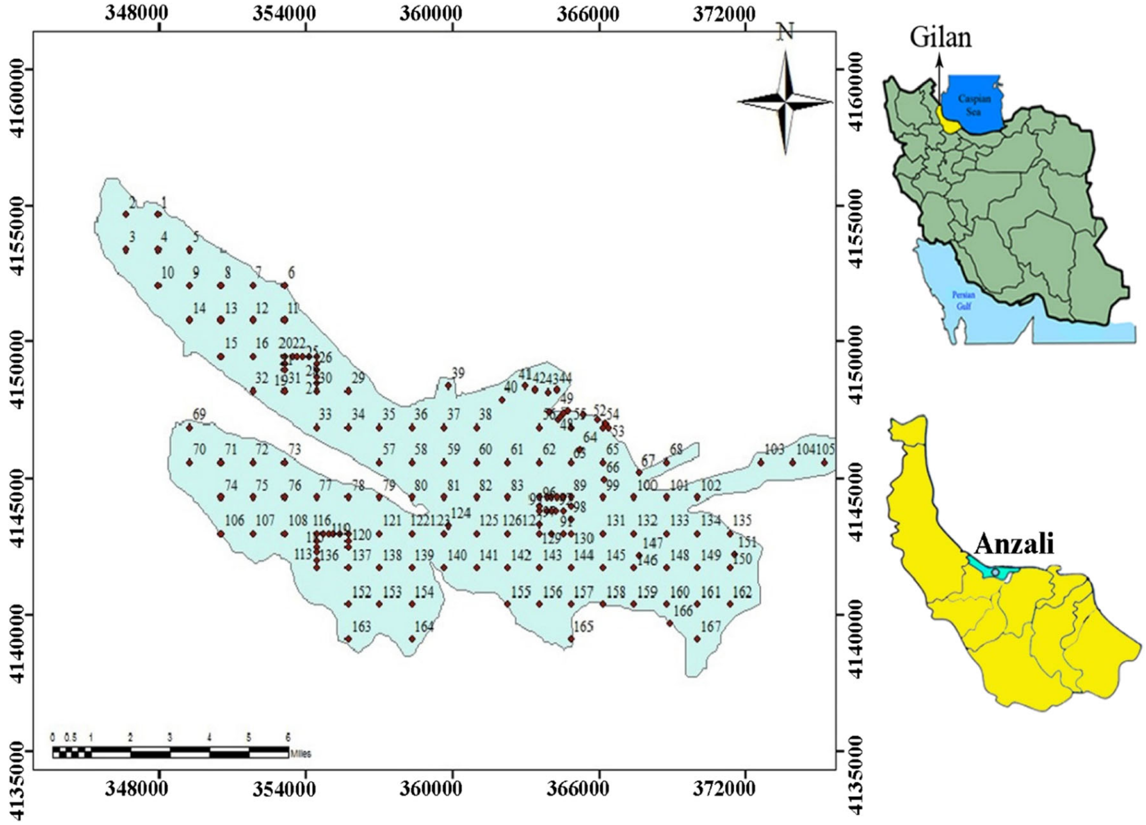


Fig. 1 Map of the study area and locations of sediment sampling sites in International Anzali Wetland

Twenty-six individual alkylbenzenes were quantified by comparing the integrated peak area by the summed selected ion monitor ($m/z = 91 + 105$) with the peak area of the injection external standard (p-terphenyl-d_{14}; $m/z = 244$) (see supplementary data Fig. S1).

Blanks were arranged periodically with each batch of the samples (10 samples/batches) to determine contamination, and the values were always less than the detection limit. Recoveries were calculated by spiking a known concentration of SIS (phenanthrene-d10) into the sample followed by

Table 1 The concentrations of linear alkylbenzenes (ng g^{-1}) in the surface sediments of the International Anzali Wetland, Guilan Province, Iran

	West	Siakeshim	Center	East
∑-C10 LABs	25.27–322.28	12.51–192.89	23.13–8,791.70	72.16–5,458.71
∑-C11 LABs	127.85–760.33	42.78–352.88	56.09–16,658.95	131.05–1,899.22
∑-C12 LABs	280.22–965.53	106.53–498.74	89.01–29,343.88	204.83–26,876.47
∑-C13 LABs	262.38–1,474.76	154.32–722.12	180.54–33,423.11	280.71–32,126.18
∑-C14 LABs	39.60–1,065.95	50.78–446.92	94.61–21,087.62	184.50–14,992.50
∑-LAB[a]	1,003.15–3,964.95	394.12–2,205.12	504.60–109,305.23	925.59–91,353.08
I/E[b]	0.66–1.30	0.67–1.11	0.0.65–0.95	0.66–1.05
D%[c]	0.37–24.13	0.75–18.76	− 0.07–13.24	0.39–16.79
C13/C12[d]	0.77–2.38	1.01–2.10	0.84–2.40	1.08–2.49
L/S[e]	0.56–6.70	1.33–6.30	1.40–12.46	1.91–6.81

[a] ∑LAB = sum of the 26 LAB congeners

[b] I/E = (6-C_{12}LAB + 5-C_{12}LAB) / (4-C_{12}LAB + 3-C_{12}LAB + 2-C_{12}LAB)

[c] LAB degradation (%) = 81 × log (I/E ratio) + 15

[d] C_{13}/C_{12} = (6-,5-,4-,3-, 2-C_{13}) / (6-, 5-, 4-, 3-, 2-C_{12}LAB)

[e] L/S = (5-C_{13}LAB + 5-C_{12}LAB) / (5-C_{11}LAB + 5-C_{10}LAB)

performing the entire analytical procedure. The surrogate recoveries in all samples ranged from 81 to 110% and used for the recovery correction.

Calculations

Reproducibility was determined by five replicate analyses of the sediment extracts, which showed acceptable relative standard deviation (RSD) values (1.1–9.31 for LAB compounds).

Limits of detection (LOD) of the analytical method were 0.05–0.20 ng/g for the for LAB series.

In addition, total organic carbon (TOC) in the sediment was determined by ignition of the dried sediments at 450 °C for 6 h (Dean 1974; Veres 2002; Santisteban et al. 2004).

Statistical analysis

The descriptive statistics and other statistical analyses were performed using SPSS (version 17.0). The distribution of the data was tested for normality by Kolmogorov–Smirnov (K-S) test. Analysis of variance (ANOVA) was undertaken to evaluate the significance of the differences between LAB

Table 2 The concentrations of LABs and I/E ratios in surface sediments around the world compared to our results from International Anzali Wetland

Location	∑ LABs[a] (ng g^{-1})	I/E ratio[b]	Reference
Coastal shelf of China	5.6–77	0.5–1.2	Wei et al. (2014)
Dongjiang River	1.5–410	0.6–1.4	Zhang et al. (2012)
Outfalls of paper mills	1,160–3,270	0.8–1.3	Zhang et al. (2012)
Chaohu Lake	19–5,720	0.8–2.1	Wang et al. 2012)
Coastal zone off South China	11–160	0.5–1.2	Liu et al. (2013)
Pearl River Estuary	5.8–26	0.6–1.5	Luo et al. (2008)
Northern South China Sea	2.5–23	0.2–0.9	Luo et al. (2008)
Zhujiang River	59–2,330	0.9–1.5	Luo et al. (2008)
Dongjiang River	97–566	0.7–1.9	Luo et al. (2008)
Xijiang River	21–69	0.6–1.0	Luo et al. (2008)
Sumidagawa River	560–12,110	1.1–1.7	Takada and Ishiwatari (1987)
Tamagawa River	10–15,790	1.3–1.9	Takada and Ishiwatari (1987)
Arakawa River)	720–1,720	1.7–2.0	Takada et al. (1992)
Victoria Harbor	410–23,500	1.8–2.6	Hong et al. (1995)
Santos Bay and Estuary	16.9–431		Medeiros and Bıcego (2004)
Santos Bay	< DL–117	1.1–2.9	Martins et al. (2008)
Barcelona Harbor	1,200–53,100	0.6–5.5	Díez et al. (2006)
Jakarta Bay	235–86,700	0.9–2.9	Rinawati et al. (2012)
Jakarta City	1,559,373	1.3	Alkhadher et al. (2015)
Admiralty Bay	< DL–46.5	0.8–0.9	Martins et al. (2012)
Santa Monica Bay	3–9,342		Venkatesan et al. (2010)
Tokyo	3.0–5,860	1.2–6.0	Isobe et al. (2004)
Thailand	3.0–14,100	0.7–5.9	Isobe et al. (2004)
Malaysia	4.0–8,590	0.7–4.8	Isobe et al. (2004)
Philippines	56–13,000	0.6–2.9	Isobe et al. (2004)
Vietnam	3.0–8,650	0.6–2.2	Isobe et al. (2004)
Cambodia	< 3.0–4,200	0.8–1.7	Isobe et al. (2004)
Indonesia	< 3.0–42,600	0.9–2.1	Isobe et al. (2004)
India	2.0–4,450	0.5–2.1	Isobe et al. (2004)
Southern California Bight	1.7–93	0–4.6	Macıas-Zamora and Ramırez-Alvarez (2004)
Tokyo Bay	1,000–3,000	1.3–3.1	Takada et al. (1992)
Anzali Wetland	394.09–109,305.24	0.66–1.32	This study

[a] ∑LAB = sum of the 26 LAB congeners

[b] I/E = $6\text{-}C_{12}LAB + 5\text{-}C_{12}LAB/4\text{-}C_{12}LAB + 3\text{-}C_{12}LAB + 2\text{-}C_{12}LAB$

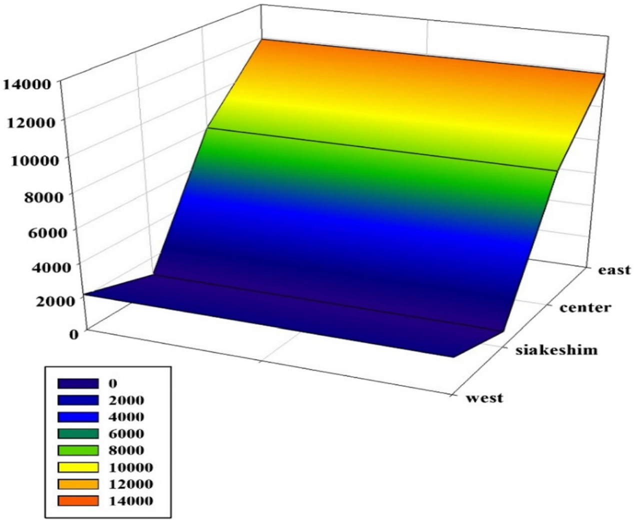


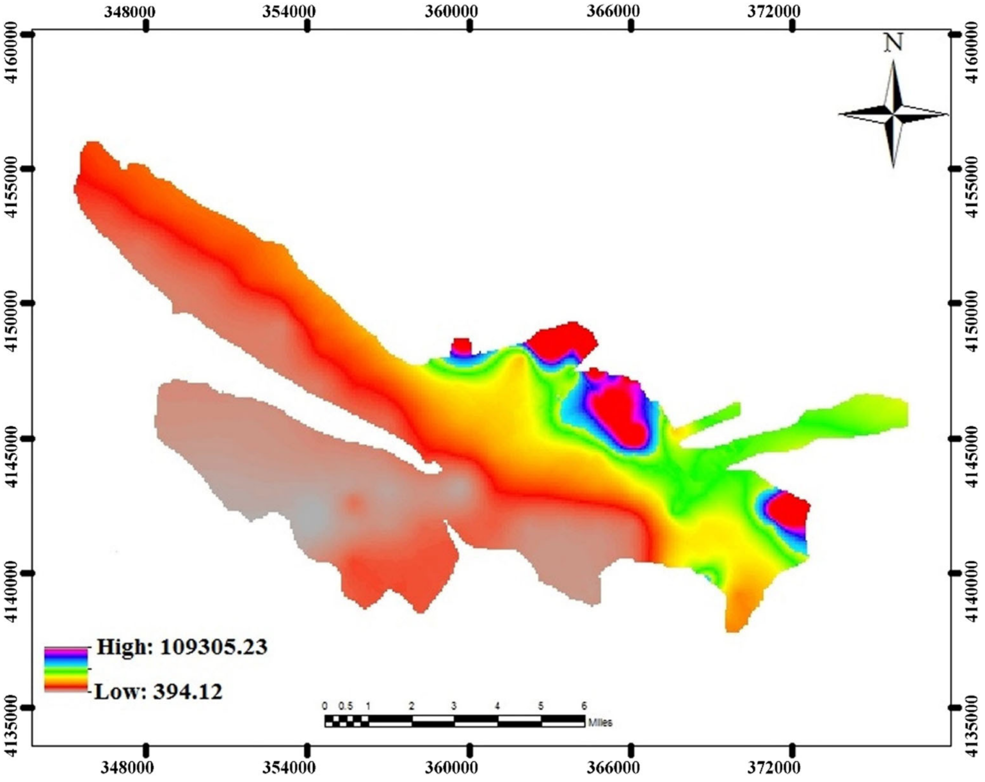
Fig. 2 3D spatial distributions of LABs of the sediments in International Anzali Wetland

concentrations in four distinct regions of the International Anzali Wetland, i.e., eastern, Siakeshim, central, and western ones. Pearson's correlation analysis was performed to test the relationship between the total LAB concentrations with TOC and grain size fractions. The ordinary kriging interpolation method was carried out with ArcGIS 9.3 (ESRI, Redlands, CA, USA) to characterize the spatial distribution of LABs in sediments and also to assess sewage contamination.

Results and discussion

Twenty-six congeners of LABs were quantified in all of the Anzali Wetland surface sediments. The total LAB concentrations showed a large scale of different concentrations, with an arithmetic mean concentration of 5930.25 ng g^{-1} dw and a range of 394.12 (station 112) to 109,305.2 ng g^{-1} dw (station 41) (Table 1). These detected total LAB concentrations were much lower than those reported in the Jakarta City (Alkhadher et al. 2015), but comparable or greater than those in the coastal shelf of China, Dongjiang River, outfalls of paper mills, Chaohu Lake, coastal zone off South China, Pearl River Estuary, northern South China Sea, Zhujiang River, Dongjiang River, Xijiang River, Sumidagawa River, Arakawa River, Victoria Harbor, Santos Bay and Estuary, Santos Bay, Barcelona Harbor, Jakarta Bay, Admiralty Bay, Santa Monica Bay, Tokyo, Thailand, Malaysia, Philippines, Vietnam, Cambodia, Indonesia, India, Southern California Bight, and Tokyo Bay (Díez et al. 2006; Isobe et al. 2004; Koike et al. 2012; Liu et al. 2013; Luo et al. 2008; Macıas-Zamora and Ramırez-Alvarez 2004;Martins et al. 2008, 2012; Medeiros and Bıcego 2004; Rinawati et al. 2012; Takada and Ishiwatari 1987; Takada et al. 1992; Venkatesan et al. 2010; Wang et al. 2012; Wei et al. 2014; Zhang et al. 2012) (Table 2). Hence, the sedimentary LAB concentrations demonstrated that the amount of discharged sewage into the International Anzali Wetland is fairly high and considerable.

Fig. 3 Spatial distribution of LABs in International Anzali Wetland



ΣLAB concentrations showed significant levels ($p < 0.05$) between the regions of the wetland and demonstrated different ranges: west, from 1003.15 to 3964.95; center, from 504.6 to 109,305.26; east, from 925.59 to 91,353.08; and Siakeshim, from 394.12 to 2025.12 ng g^{-1} dw (Table 1 and Fig. 2), and overall, its order was eastern > central > western > Siakeshim, respectively (Figs. 2 and 3). The observed spatial distribution of LAB concentration in the sediments could be attributed to higher urbanization, industrialization, and tourist attraction of Anzali and Rasht Cities, surrounding the eastern and central regions, than the other ones, and thereby discharging larger amount of poorly treated or untreated domestic sewage and in turn more LABs into these regions. Further cause of spatial difference in LAB concentration in the sediments could be related to release of domestic wastewater from boats following intensive fishing activities that occurred in the eastern and central regions.

Based on observational information, the eastern and central parts of the wetland daily receive untreated domestic wastes in which pathogenic microorganisms, steroid hormones, pharmaceuticals, and personal care products could exist, and these wastes are from hot spot areas such as hospitals, hotels, laundries, schools, governmental institutes, restaurants, parks, and shopping centers. As the household detergents are the main source of LABs in the environment, the population may be a major factor in distribution of LABs in the aquatic environment (Ni et al. 2008).

The lowest detected concentration of LABs in the Siakeshim (station 112) and western parts represent lower human activities and lesser utilization of synthetic detergents as well as higher agricultural activities in the their surrounding area. In addition, due to being located far from the densely populated urban areas, these two regions illustrated lower LAB concentrations.

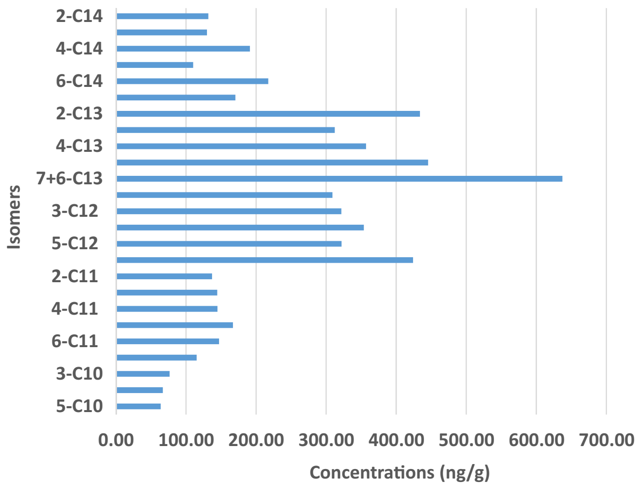


Fig. 5 The concentrations of individual LAB isomers

The isometric composition and chain length distribution of LABs are shown in Table 1 and Fig. 4, respectively. In most of the sampling stations, C_{12} and C_{13} homologs (6-C_{12}, 6-C_{13}, and 5-C_{13}) demonstrated the highest relative abundances (about 30 and 40%, respectively), whereas C_{10} (5-C_{10}) showed the lowest percentages (Fig. 5). Support for these data have come from studies of LABs in bottom sediments at the near-outfall and mid-shelf sites of Southern California, Santos Estuary of southeastern Brazil, Babitonga Bay Brazil, and Perlis River of Peninsular Malaysia, in which sediments were enriched with C_{12} and C_{13} homologs, while the proportions of C_{10} homologs were found to be lower (Magam et al. 2016; Martins et al. 2010, 2014; Phillips et al. 2001). It has been suggested that the abundance difference between these homologs could be attributed to selective degradation or higher solubility of the short homolog groups (e.g., C_{10}) during settling of effluent particles in the environment. However, LABs with longer alkyl chain have lower vapor pressure and are less volatile compared to those with shorter alkyl chain (Zhang et al. 2012). In addition, given that the relative abundance of LAB isomers in commercial detergents and, in turn, in raw

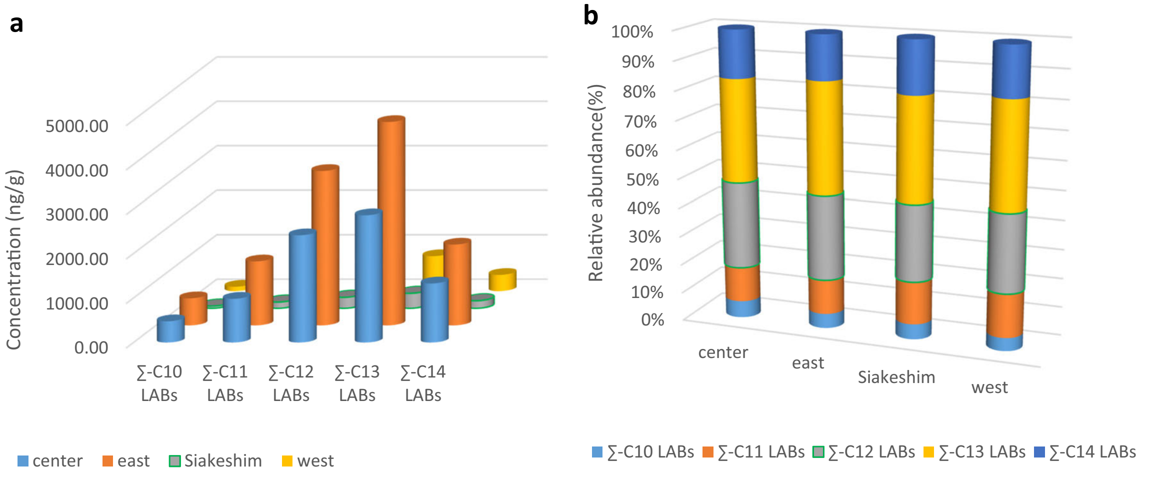


Fig. 4 Composition of C10–C14 LABs (ng g^{-1} dw) (**a**) and the relative abundance compositional profiles of LABs (**b**) in the sediments of International Anzali Wetland

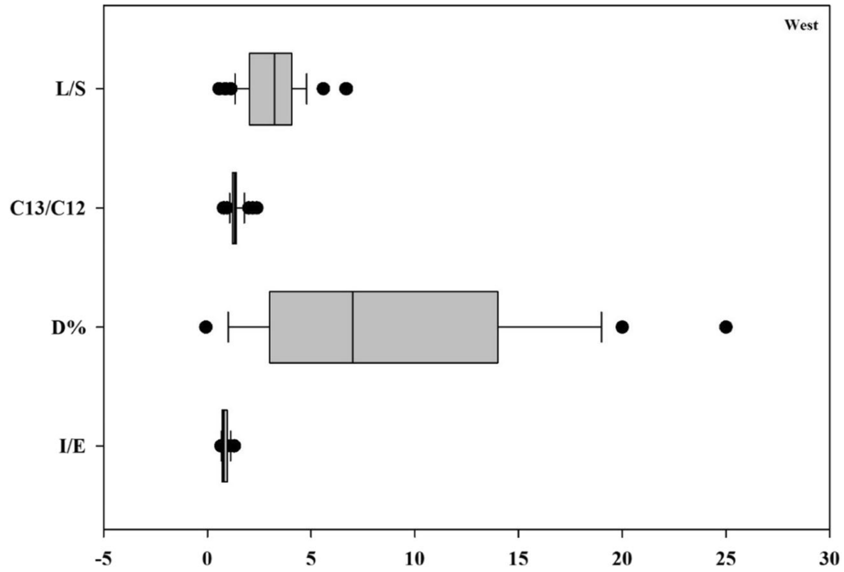


Fig. 6 I/E, L/S, and C_{13}/C_{12} ratios in sediments collected from the west section of International Anzali Wetland

sewage are stable (Takada and Ishiwatari 1987), the observed significant changes in isomeric composition in the treated wastewater and coastal sediment could be ascribed to selective biodegradation (Isobe et al. 2004).

The (bio)degradation of LABs mainly occurs in aerobic conditions (Takada et al. 1992). This phenomenon is broadly evaluated using some indicators such as I/E ratio (6-C_{12}LAB + 5-C_{12}LAB/4-C_{12}LAB + 3-C_{12}LAB + 2-C_{12}LAB), C_{13}/C_{12} = ΣC_{13}-LAB/ΣC_{12}-LAB, and L/S (5-C_{13} + 5-C_{12})/(5-C_{11} + 5-C_{10}) (Gustafsson et al. 2001; Luo et al. 2008). The estimated values for all of the sediment samples showed a range of 0.65 to 1.30 (with an average of 0.80; see Table 1, supplementary data Fig. S2, and Figs. 6, 7, 8, and 9). The estimated low I/E value for the International Anzali Wetland could be mainly due to receiving poorly treated and untreated sewage effluent as well as anaerobic condition in/on the sediments. This value is similar to those recorded previously for commercial detergents in the southern China (Luo et al. 2008) and significantly lower than those found in sludge and suspended particles from wastewater (Luo et al. 2008; Takada and Ishiwatari 1987).

In a study conducted by Takada and Ishiwatari (1990), it was suggested that D (%) = 81 × log (I/E ratio) + 15 ($r^2 = 0{:}96$) exponentially increase following increase in I/E. Similarly, owing to the selective degradation of alkylbenzenes with long chains relative to those with short chains, the values of ΣC_{13}-LAB/ΣC_{12}-LAB and L/S in detergent and untreated sewage were significantly lower than those in river water (Ni et al. 2008) and sediment (Luo et al. 2008). The average D% value was 6.88 (ranging from −0.07 to 24%). These results

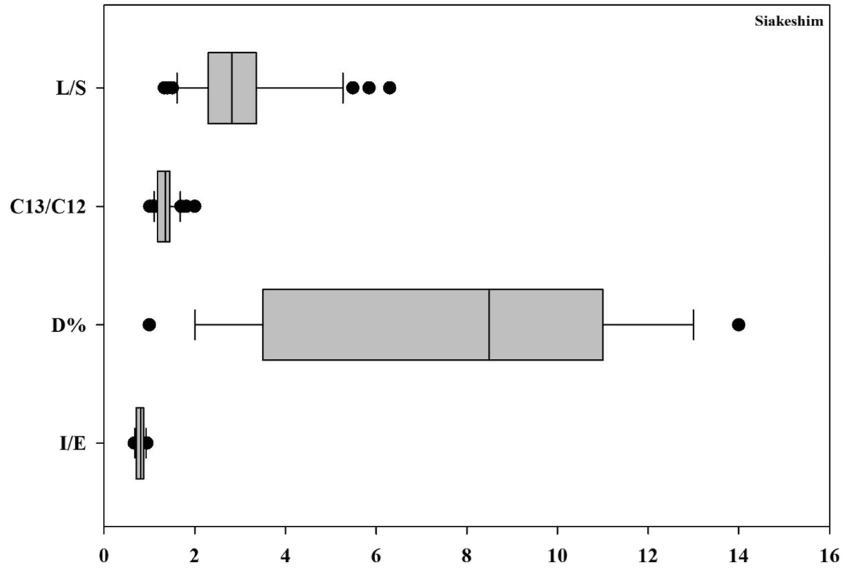
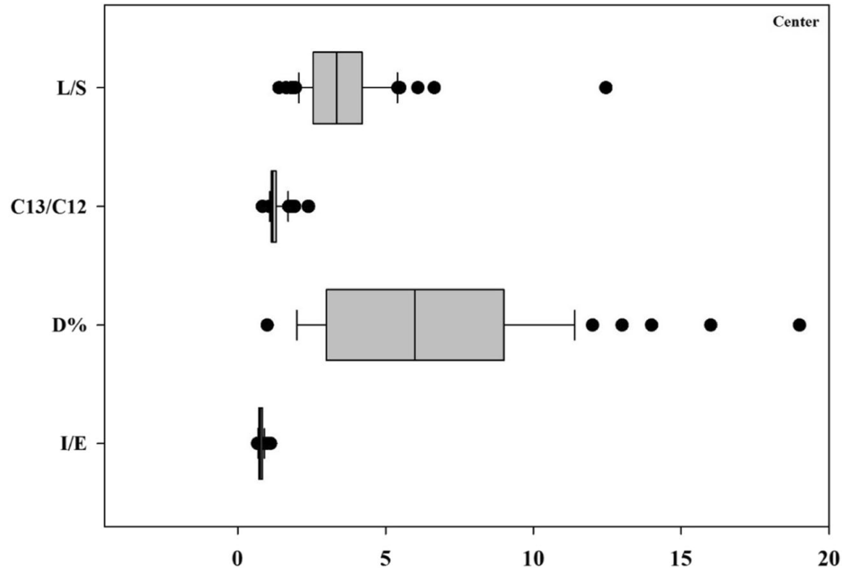


Fig. 7 I/E, L/S, and C_{13}/C_{12} ratios in sediments collected from the Siakeshim section of International Anzali Wetland

Fig. 8 I/E, L/S, and C_{13}/C_{12} ratios in sediments collected from the center section of International Anzali Wetland

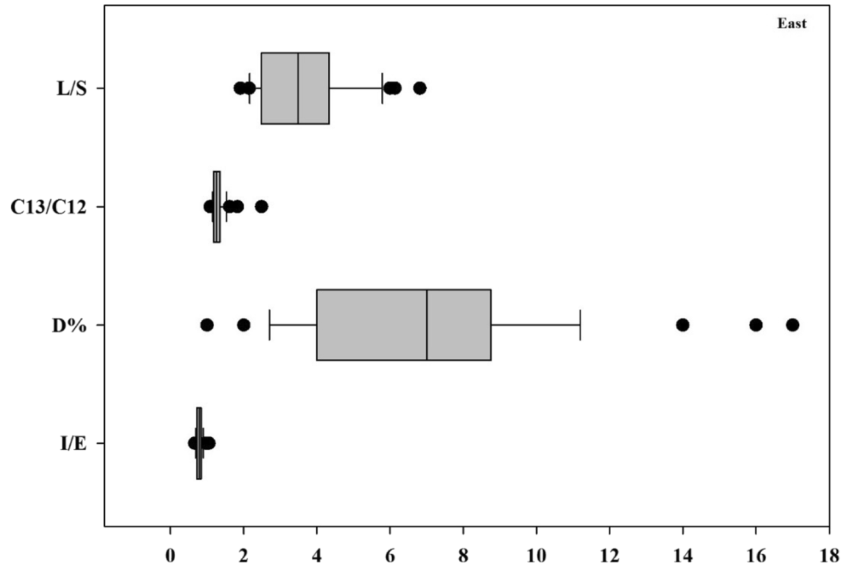


suggested weak biodegradation of LABs or discharging raw sewage into the International Anzali Wetland.

The ΣC_{13}-LAB/ΣC_{12}-LAB and L/S values ranged from 0.77 to 2.49 (mean = 1.33) and from 0.56 to 12.46 (mean = 3.42), respectively (Table 1 and Figs. 6, 7, 8, and 9). The results suggest that long-chain alkylbenzenes were largely maintained in the sediment of International Anzali Wetland relative to short-chain alkylbenzenes. In addition, these indices were slightly higher than those found in commercial detergents (Luo et al. 2008; Ni et al. 2008) but significantly lower than those in riverine runoff from the Pearl River Delta (Ni et al. 2008).

Pearson correlation demonstrated a weak positive correlation between TOC and grain size (%silt and %clay) with ∑LABs (Fig. 10), although the spatial variability of ∑LAB concentrations in sediments can be a result of both TOC content and input intensities; that is, a good positive correlation between LABs and organic matter content is expected because of the hydrophobicity of LAB molecules (KOW ranging from~7 to ~9). These data corroborate the findings of other researchers (Hassanzadeh et al. 2014; Mortazavi et al. 2012; Wang et al. 2012; Yancheshmeh et al. 2014) who reported weak correlations between total DEHP, OP, LABs, PAHs, and TOC, and thus implying that TOC and grain size could not be considered as a significant factor in distribution of LABs in International Anzali. Therefore, the observed spatial variability of ∑LAB concentrations can be attributed to direct inputs and to different transport processes for TOC and ∑LABs as well as to different sources of LABs and TOC inputs (Vaezzadeh

Fig. 9 I/E, L/S, and C_{13}/C_{12} ratios in sediments collected from the east section of International Anzali Wetland



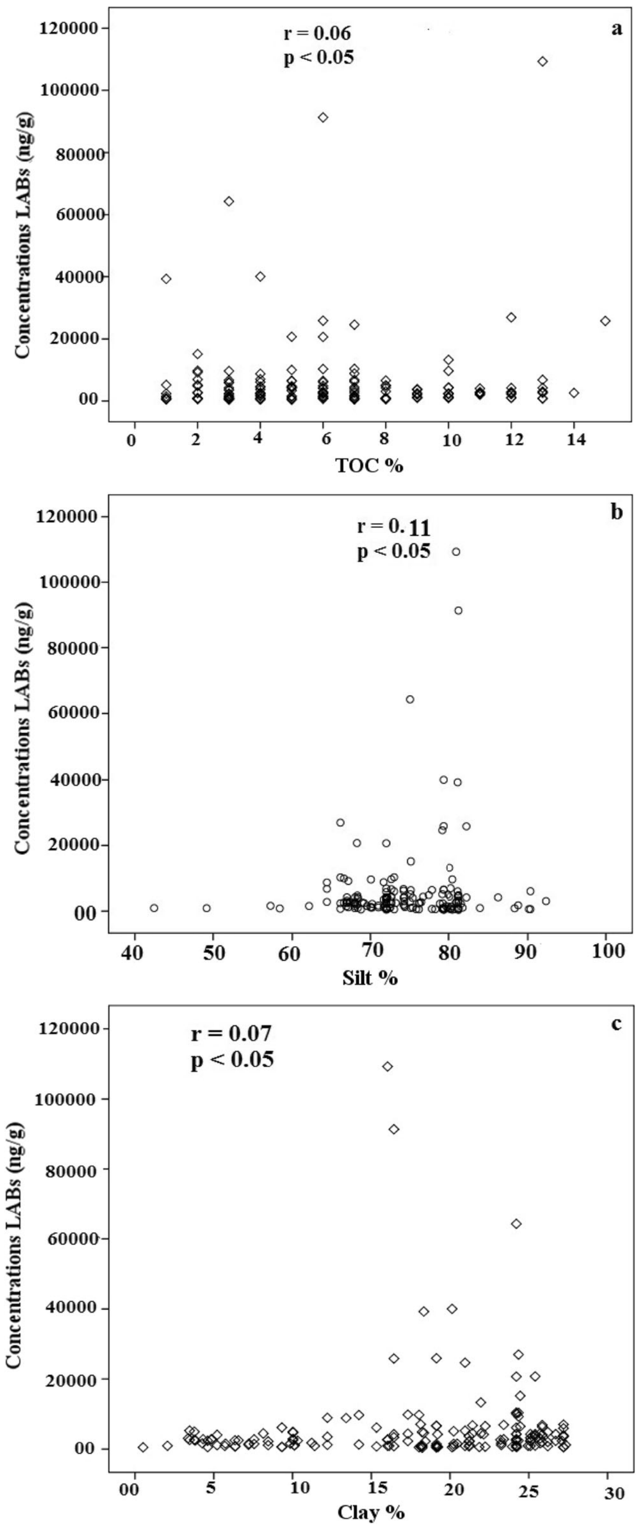


Fig. 10 Correlation of the ∑ LAB (ng g^{-1}) concentrations with the proportions of organic carbon (TOC %) (**a**), silt (**b**), and clay (**c**) in sediments from the International Anzali Wetland

et al. 2015), suggesting regional anthropogenic inputs as controlling factors for the observed spatial distributions of ∑LABs in the Anzali Wetland.

Conclusion

This study provides the first data on the contamination levels of 26 ∑LABs in the sediments of the International Anzali Wetland, Guilan Province, Iran, and also, it could be a baseline for future monitoring and management of LAB compounds in the wetland. In addition, the data demonstrated that LABs are powerful indicators to trace anthropogenic sewage contamination and highlighted the necessity of sewage treatment plants to be founded around the wetland (especially around of eastern and central regions). The LAB concentrations in surface sediments of International Anzali Wetland were high as compared to other countries around the world and thus indicating high ecological risk on the wildlife and may endanger human health in the region. Moreover, LABs displayed no correlation with TOC concentration and grain size (%silt and %clay), suggesting that TOC and grain size are ineffective factors to control spatial distribution of ΣLABs in the International Anzali **W**etland sediments.

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