

«Original Article»

## Survey on karun river water contamination by alkylphenolic compounds as endocrine disrupting contaminants

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### Abstract

**Background:** Alkylphenol polyethoxylates (APnEOs) and their metabolites disposal from sewage treatment plants (STP) are of particular concern due to their persistence, toxicity to aquatic organisms, and a potential endocrine disruptor. In this work, we focused on the determination of nonylphenol (NP) and nonylphenol polyethoxylate (NPnEOs) in water were collected from Karun River.

**Material and methods:** In this study, Sample collections for Karun River water were carried out on rainy and dry seasons from 10 hydrometric stations according to standard methods. The water samples were prepared using solid phase extraction procedure and analyzed by HPLC–FLD.

**Results:** According to this study, NP and NPnEOs concentrations in Karun river water ranged from 0.118–1.369 µg/L and 0.130–0.365 µg/L, respectively. This study showed that the NP concentration in water samples of Karun River was higher than the other alkylphenolic metabolites. The data showed significant difference between mean concentrations of these compounds in the upstream and downstream stations of Ahvaz megacity (p-value <0.05).

**Conclusion:** According to the classification of water resources in terms of concentration levels of nonylphenol and its ethoxylates, the Karun River in upstream and downstream of Ahvaz city can be considered to be of low pollution content and polluted, respectively. But in terms of toxicity effects, concentration of nonylphenol and its ethoxylates was acceptable. Due to the significant contributions of NP to the estrogenic activities, assessing the risk of estrogenic activity is necessary and future efforts should be focused on its impacts on biota in Karun River.

**Keywords:** Nonylphenol polyethoxylate (NPnEOs), Nonylphenol (NP), Endocrine disruptor chemicals, Karun river, Water

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## Introduction

Recently, contamination of the surface and groundwater resources to a variety of the persistent organic compounds has an extensive growth due to rapid development of chemical and petrochemical industries. Discharge of industrial and municipal wastewater into the environment has caused environmental and health problems, because the ineffective elimination of the persistent organic compounds in the conventional treatment systems. Among these compounds, Alkylphenol ethoxylates (APnEOs) used in the manufacture of antioxidants, lubricating oil additives, pesticides, paints, cosmetics and the production of NPnEOs nonionic surfactants which is its major use (65%) (1-3). Among APnEOs, nonylphenol polyethoxylates (NPnEOs) are the most commonly used, accounting for more than 80% of the world market (4). These compounds are an important group of non-ionic surfactants which have been used in producing industrial and domestic detergents. Once NPnEOs are present in water bodies and sewer systems, they are biodegrade by removal of ethoxy groups, yielding relative stable small metabolites, particularly nonylphenol (NP). Additionally, NP is a raw material for the production of NPnEOs (5-8). Unfortunately, this small metabolite of NPnEOs is xenobiotic, toxic and furthermore has endocrine disrupting activities. Therefore, some developed countries have banned the use of alkylphenols, but in many developing countries, they are still used because APnEOs are inexpensive and have superior cleaning properties (2, 9).

NP, which has numerous isomers, shows estrogenic activities at very low concentrations (ppb level), and their feminizing effect on fish is a serious problem in terms of ecological system conservancy (10). Currently NP risks have

been accepted by EPA, and have prepared a guideline for natural water quality that recommends NP concentrations be below 6.6  $\mu\text{g/L}$  and 1.7  $\mu\text{g/L}$ , in freshwater and saltwater, respectively (5, 8, 9).

Since the alkylphenolic compounds cannot be produced naturally, therefore, the main source of alkylphenolic compounds in the environment appears to be closely related human activities with the discharge of effluents and sludge from wastewater treatment works due to incompletely degradation of its parent compounds, APnEOs, mainly by anaerobic digestion and application of pesticides (5, 11). The environmental occurrence and effect of APnEOs and particularly metabolites of NPnEOs have been studied worldwide (12, 13) and contamination of the surface waters and sediments by NP are reported as well (12).

NP has been found with a concentration of as low as 0.6 ng/L and up to 644  $\mu\text{g/L}$  in surface waters (5, 14). Lou et al. (2012) found that the concentration of NP in river water ranged from  $8.54 \pm 1.23 \mu\text{g/L}$  (Qiantang River) to  $65.77 \pm 3.69 \mu\text{g/L}$  (Tiesha River) using the HPLC-FLD method (15). According to Tao et al. (2011), the concentrations of NP and OP in Nanming river water ranged from 40 to 1582 ng/L (16). Martinez & Peñuela (2012), analysis of 4n-nonylphenol in Colombian reservoir water by GC/MS, where 4n-NP was not found in any of the water samples (17). According to Babaei et al. (2013), NP was detected in Karun River water and Ahvaz wastewater samples with 0.17-1.83  $\mu\text{g/L}$  and 15.27-21.79  $\mu\text{g/L}$ , respectively (18).

The Karun River is the largest river in Iran in terms of annual discharge ( $2.2 \times 10^{10} \text{ m}^3$ ) that flows to the Persian Gulf. It is estimated that  $2.1 \times 10^8 \text{ m}^3/\text{annum}$  and  $3.15 \times 10^8 \text{ m}^3/\text{annum}$  domestic and industrial wastewaters discharged into Karun River,

respectively. More than 90% of the wastewaters are not treated effectively prior to discharge into Karun River. In recent decades, the Karun River contamination is increasingly serious with the explosive increase of population and heavily industrial development. The purpose of the present study was to evaluate the concentration levels of NPnEOs and NP in Karun River at Khuzestan plain as well as to prepare zoning maps.

### **Material and methods**

#### **Chemicals and instruments**

NP, NPnEOs (99.5%) and 4-n-NP (d4-ring d8) as internal standard were purchased from Dr. Ehrenstorfer (Audgsburg, Germany). Methanol (MeOH), acetonitrile (ACN) and water were all of HPLC grade and formic acid was of analytical grade obtained from Merck. C18 cartridges (Capital Company, UK) were used as solid phase extraction (SPE). The Knauer HPLC system (Knauer, Germany) coupled with a RF-10AXL fluorescence detector (FLD) were employed in this study. HPLC separations were performed using a Eurospher 100-5 C18 column (4.6 mm×250 mm, 5 µm) from Knauer (Knauer, Germany) thermostatted at 30 °C and injection volumes of 20 µl. ACN and water, named solvents A and B, respectively, were used as mobile phase for NP and NPnEOs analysis. Gradient elution was carried out with a program from 100% A and 0% B to 60% A and 40% B in 15 min, then to 100% A and 0% B in 20 min with a flow rate of 1.0 mL min<sup>-1</sup>. Analyte were monitored by fluorescence detection (ex: 222 nm, em: 305 nm) and quantified by external calibration using peak area measurements.

#### **Sampling sites and dates**

Sample collections for Karun River water were carried out from 10 hydrometric station

(Vali-Abad (S1), Arab-Asad (S2), Bamdezh (S3), Band-e-Ghir (S4), Veys (S5), Zergan (S6), Koot-e-Amir (S7), Darkhoveyn (S8), Haffar (S9) and Toreh-Bokhah (S10)) (Fig. 1) on January to March 2011 as rainy season and June to September 2011 as dry season using a semi-composite sampling procedure. Briefly, water samples were taken at three points in the river cross-section with 6 hours interval in each sampling campaign. Afterward, the samples were mixed together based on river flow and three 2.5 L composite sample were taken. The water samples were stored in amber glass bottles that were previously cleaned and heated to 450 °C for 8 h after adding the formic acid up to pH 2. A total of 40 water samples each with 3 replicate were collected and all samples were directly transported to the laboratory, stored at 4 °C and analyzed within 24 h using SPE and HPLC-FLD.

#### **Sample preparation and measurements**

Initially, an appropriate volume of water samples (1 L) was filtered through pre-ashed 0.7 µm pore size GF/F filters (Whatman, UK). The retained particulate material and filter was washed with 3×10 ml of methanol and reduced to 2 ml which was added to the filtrate. NP and NPnEOs were extracted from water samples by using SPE C18 cartridge (LH), 500 mg/6ml (Capital, UK). Briefly, the filtered water samples were loaded onto cartridges previously conditioned with 2×6 mL of MeOH and 6 mL of MeOH/water (70:30 v/v). Cartridges were washed with ultrapure water, dried under vacuum for 1h and analytes were eluted with 3×3 mL of MeOH and 1×3 mL of ACN. Finally, sample extracts were evaporated to dryness under a nitrogen stream, redissolved in 1 mL ACN containing 50 ng internal standard, and analyzed by HPLC-FLD.

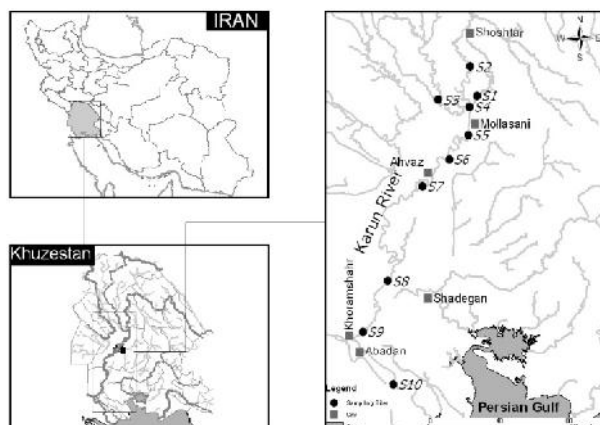


Fig 1. The sampling sites of Karun River in Khuzestan Plain, Iran

### Sample preparation and measurements

Initially, an appropriate volume of water samples (1 L) was filtered through pre-ashed 0.7  $\mu\text{m}$  pore size GF/F filters (Whatman, UK). The retained particulate material and filter was washed with  $3 \times 10$  ml of methanol and reduced to 2 ml which was added to the filtrate. NP and NPnEOs were extracted from water samples by using SPE C18 cartridge (LH), 500 mg/6ml (Capital, UK). Briefly, the filtered water samples were loaded onto cartridges previously conditioned with  $2 \times 6$  mL of MeOH and 6 mL of MeOH/water (70:30 v/v). Cartridges were washed with ultrapure water, dried under vacuum for 1h and analytes were eluted with  $3 \times 3$  mL of MeOH and  $1 \times 3$  mL of ACN. Finally, sample extracts were evaporated to dryness under a nitrogen stream, redissolved in 1 mL ACN containing 50 ng internal standard, and analyzed by HPLC-FLD.

## Results

### Method Validation

**Linearity;** A linearity regression function was set up based on calibration measurement. There were good linearity in the detected range, and correlation coefficients ( $R^2$ ) were 0.995 and 0.999 for NP and NPnEOs, respectively.

**Precision;** Table 1 shows the results of instrumental and method repeatability. Seven successive injections were performed in different three days on spiked matrix at five concentration levels ranged from 0.05  $\mu\text{g/L}$  to 1  $\mu\text{g/L}$ . The relative standard deviations (RSDs) were satisfactory for the spiked matrix, which were 12.2–20.8% and 4.8–12.2% for NP and NPnEOs, respectively.

**Recovery of method;** Water samples were spiked with specific amount of NP and NPnEOs to determine the recovery. The average recoveries (R%) and the RSD in water were shown in Table 1. The average R% were 71.6–92.6% (NP) and 67.5–95.3% (NPnEOs) and RSDs were 6.6–11.9 (NP) and 4.7–10.0 (NPnEOs) indicating acceptable accuracy of the analytical procedure.

**Limits of detection and quantification;** The results of the extraction and preparation of spiked water samples with 0.05  $\mu\text{g/L}$  of the NP and NPnEOs are according to Table 2. In this study, the limit of detection (LOD) and limit of quantification (LOQ) of the method were 0.017  $\mu\text{g/L}$  and 0.055  $\mu\text{g/L}$  for NP, and 0.015  $\mu\text{g/L}$  and 0.048  $\mu\text{g/L}$  for NPnEOs.

**Table 1. Recoveries (R%) and relative standard deviations (RSDs) obtained for NP and NPnEOs at different concentration levels**

Spiking level ( $\mu\text{g/L}$ )	Compounds			
	NP		NPnEOs	
	R%	RSD	R%	RSD
0.05	92.6	11.9	95.3	10.0
0.1	85	9.4	84.9	8.7
0.25	79.3	6.0	74.8	5.8
0.5	72.4	6.9	73.1	4.4
1.0	71.6	6.6	67.5	4.7

**Table 2. Results of measurement of various compounds in the Karun River water at spiked level 0.05  $\mu\text{g/L}$**

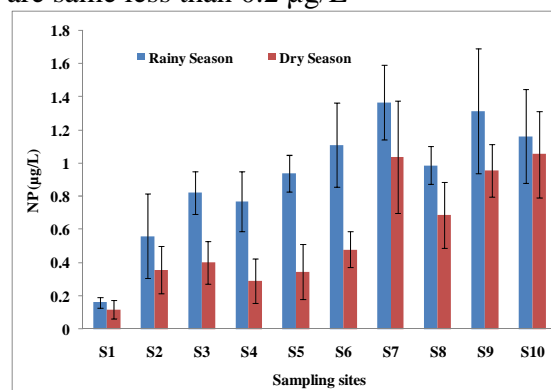
Compounds	Mean ( $\mu\text{g/L}$ )	SD ( $\mu\text{g/L}$ )	LOD ( $\mu\text{g/L}$ )	LOQ ( $\mu\text{g/L}$ )
NP	0.046	0.0055	0.017	0.055
NPnEOs	0.048	0.0048	0.015	0.048

### Distribution of NP and NPnEOs in Karun River water

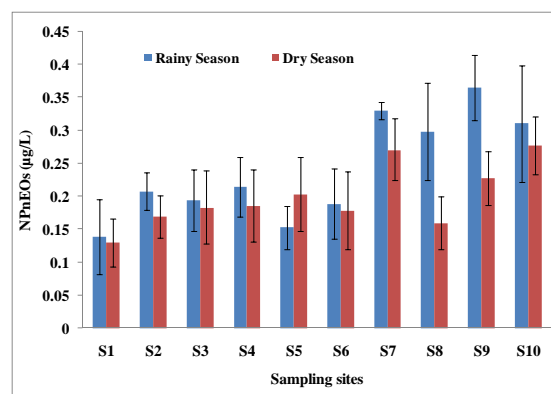
Results of the analyses of NP and NPnEOs in water samples taken from 10 hydrometric stations along the Karun River in Khuzestan plain are summarized in Figures 2 and 3, respectively. According to the results, the NP concentrations of Karun river water, in the rainy and dry seasons were 0.161–1.369  $\mu\text{g/L}$  and 0.118–1.055  $\mu\text{g/L}$ , respectively. The results showed that the concentrations of NPnEOs of Karun River water in the rainy and dry seasons were 0.138–0.365  $\mu\text{g/L}$  and 0.130–0.277  $\mu\text{g/L}$ , respectively.

The average concentrations of the NP and NPnEOs compounds in Karun River water were shown on the GIS maps (Figures 4 and 5). These maps specify the concentration range of studied compounds between sampling stations. According to the Figure 4, the average concentrations of the NP in Gargar (S1 to S4) and Shoteyt branches (S2 to S4) of Karun River is less than 0.5  $\mu\text{g/L}$  but in Dez branch (S3 to S4) is 0.5–0.75  $\mu\text{g/L}$ . As shown in Figure 5, the average concentrations of the NPnEOs in Gargar branch (S1 to S4), Shoteyt branch (S2 to S4)

and Dez branch (S3 to S4) of Karun River are same less than 0.2  $\mu\text{g/L}$



**Fig 2. Concentrations of nonylphenol (NP) in the water of Karun River**



**Fig3. Concentrations of nonylphenol polyethoxylates (NPnEOs) in the water of Karun River**

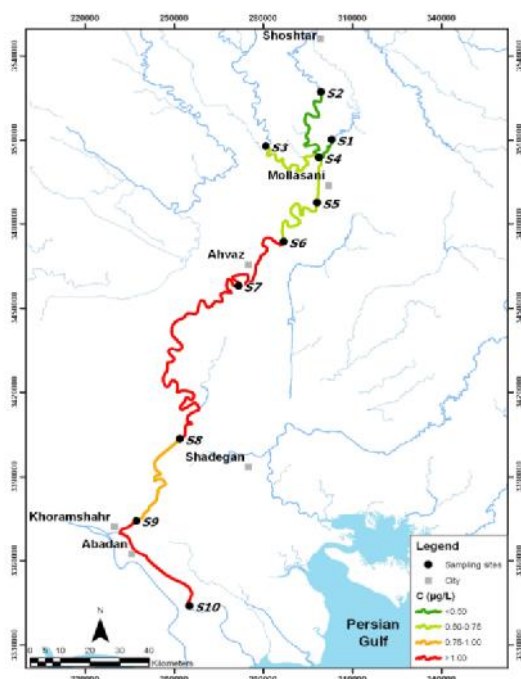


Fig 4. The GIS map of the mean concentrations of nonylphenol (NP) in the water of Karun River ( $\mu\text{g/L}$ )

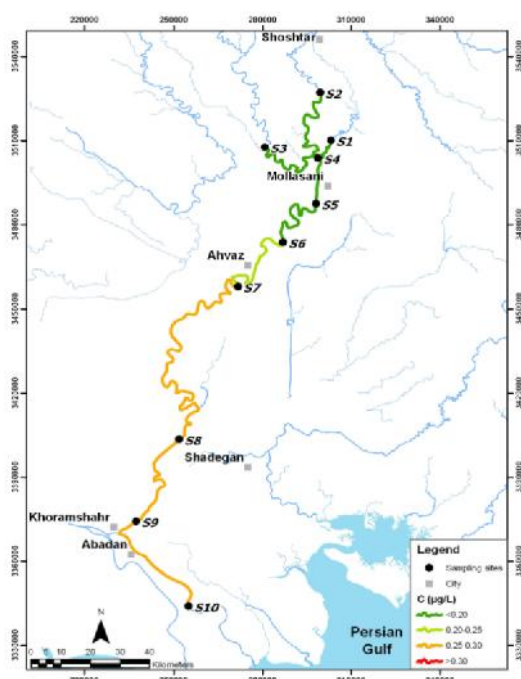


Fig 5. The GIS map of the mean concentrations of nonylphenol polyethoxylates (NPnEOs) in the water of Karun River ( $\mu\text{g/L}$ )

## Discussion

According to the results, the concentration of NP in Karun River water is more than NPnEOs not only in all studied stations but also in both rainy and dry seasons. This is because NP has been shown as final and persistent metabolite of NPnEOs in the wastewater and sludge usually. According to the studies, 60–65% of the total nonylphenolic compounds finally enter natural water environments via various pathways, such as municipal or industrial wastewater discharges, and sewage treatment plant (STP) that the majority of these compounds (25%) is NP (5,9). According to the Ahel *et al.*, nonylphenolic compounds in the digested sludge includes 95% NP and 5% NP1EO+NP2EO, which implies conversion of NPnEOs to NP during anaerobic digestion of sludge (6).

Comparison of the average concentration of APnEOs derivatives between sampling stations showed a significant difference between the upstream and downstream stations of the Ahvaz city ( $P$ -value<0.05). The increasing concentration of APnEOs derivatives over the Karun River in Khuzestan plain is primarily attributed to untreated and treated industrial and municipal wastewater discharges. The APnEOs are used in the formulation of household detergents and cleaners like as washing powders, dishwashing liquids, shampoos, etc according to the excellent cleansing properties, therefore the widespread application of detergents in homes and industries tends to discharge APnEOs to the environment via wastewater that biological shortening of the ethoxylates chain in particular, yielding relative stable small metabolites, such as NP and other metabolites (5,19). Babaei *et al.* (2013) reported that NP level of raw wastewater in Ahvaz city was ranged from 15.27 to 21.79  $\mu\text{g/L}$ (18). According to Babaei *et al.* (2010), NP concentration of influent and effluent of

Ahvaz wastewater treatment plant was on average  $23.3 \pm 2.98 \mu\text{g/L}$  and  $5.38 \pm 0.97 \mu\text{g/L}$ , respectively. They also reported the NP concentration in the industrial wastewater of an industrial park located along Karun River was  $98.87 \pm 11.11 \mu\text{g/L}$  (20).

There were some reports on the concentrations of alkylphenolic compounds in river water worldwide (Table 3). Thus, it was found that concentration of alkylphenol ethoxylates and derivatives in the Karun River water were lower compared to countries such as the UK (21, 22), Canada (23), China (24, 25), Italy (26), Portugal (27) and Spain (28), *vice versa* higher than countries such as Turkey (2), South Korea (29), Switzerland (30), Germany (31), Japan (32), United States (33, 34) and Canada (35) (Table 3).

**Table 3. Summary of NP concentration in surface water from various countries in the world**

Country	Concentration ( $\mu\text{g/L}$ )	Ref.
Turkey	N.D.	(2)
UK	<0.1–53	(21)
Canada	1.16–96.9	(23)
China	1.9–32.8	(24)
	1.55–7.33	(25)
Italy	8.8–158	(26)
Portugal	<10	(27)
Spain	15	(28)
South Korea	0.0232–0.1876	(29)
Switzerland	0.015–2.25	(30)
Germany	<0.010–0.770	(31)
Japan	0.051–1.08	(32)
USA	<0.1	(33)
	0.1–0.5	(34)
Canada	<0.01–0.92	(35)
Iran (Karun River)	0.274–1.533	This study

According to the classification of water resources based on the NP levels, Karun River is among the low contaminated (<1  $\mu\text{g/L}$ ) and contaminated (1–10  $\mu\text{g/L}$ ) water resources in the upstream and downstream of Ahvaz city, respectively (36). In accordance with the European Union (EU) regulations, the concentrations of NP in Karun River water is exceeds the limit of 0.01  $\mu\text{g/L}$ , therefore risk assessment in terms of estrogenic activity is necessary (19). Although based on the USEPA ambient water quality criteria levels of NP for freshwater (<6.6  $\mu\text{g/L}$ ) and saltwater (<1.7  $\mu\text{g/L}$ ), the concentration of the NP in Karun River water was acceptable (10). In conclusions the present work, the occurrence of APnEOs and their metabolites in Karun River water was investigated. The endocrine-disrupting chemicals NP and NPnEOs were detected at relatively high levels in surface water of the Karun River. The concentrations of nonylphenol in Karun River water were higher than other APnEOs derivatives in all studied hydrometric stations in both rainy and dry seasons. NP levels of the Karun River are acceptable based on toxicity effects on aquatic lives. A significant difference was observed between the concentration of the APnEO derivatives in the upstream and downstream of Ahvaz city. Increasing concentrations of EDCs in Karun River water through Khuzestan plain is related to municipal and industrial wastewater discharges to this river. Further research is required to study the environmental fate of NP and NPnEOs and their real estrogenic risk to organism in aquatic environment of Karun River.

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