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ORIGINAL PAPER

Occurrence of nonylphenol an endocrine disrupter in Karun River, Khuzestan Province, Iran

A. A. Babaei · A. H. Mahvi · R. Nabizadeh · A. Mesdaghiniai · Z. Nazari · E. Ahmadpour

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Abstract The important Iranian Karun River has never been investigated for the presence of potentially endocrine-disrupting chemicals, nonylphenol (NP). In this study, concentrations of NP were measured in water from Karun River and five wastewater discharge points into this river, collected during April to July 2010. The analytes were extracted by solid-phase extraction, and quantitative analyses were performed by HPLC–FLD. NP was detected in water and wastewater samples with 0.17–1.83 and 15.27–21.79 μg/L, respectively. The results showed that the NP content of aqueous phase of all wastewater samples higher than particulate phase, which were detected in the aqueous and particulate phases with mean concentrations of 12.8 ± 2.4 and 5.2 ± 1.2 μg/L, respectively. These data suggest that the NP levels in Karun river water are likely attributable to untreated municipal wastewaters discharged directly into the river. To our knowledge; this is the first study to evaluate NP concentrations in water and wastewater in Iran.

Keywords Nonylphenol · River · Water · Wastewater · Contamination

A. A. Babaei
Environmental Technologies Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

A. A. Babaei
Department of Environmental Health Engineering, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

A. H. Mahvi · R. Nabizadeh · A. Mesdaghiniai
Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Z. Nazari
Department of Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

E. Ahmadpour (✉)
Department of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
e-mail: eahmadpour386@gmail.com

Introduction

Public awareness and concern have been growing about exposure to chemicals that have the potential to interfere with the endocrine system (Hecker and Hollert 2011). Nonylphenol (NP) is an endocrine disrupter, toxic, and xenobiotic compound used in the manufacture of antioxidants, lubricating oil additives, pesticides, paints, cosmetics, and the production of NP polyethoxylates nonionic surfactants which is its major use (65 %) (Ahel et al. 1994a; Uguz et al. 2003). NP shows estrogenic activities at very low concentrations (ppb level), and its feminizing effect on fish is a serious problem in terms of protection of ecological systems (Inumaru et al. 2004; Shue et al. 2009).

The main source of NP in the environment appears to be closely related to the discharge of effluents from wastewater treatment works due to incompletely degradation of its parent compounds, NP ethoxylates mainly by anaerobic digestion (Ahel et al. 1994b; Fries and Püttmann 2004; Langford et al. 2005; Sabik et al. 2003; Mauricio et al. 2006; Zhou et al. 2009; Isobe et al. 2007).

NP has been found with a concentration of as low as 0.6 ng/L and up to 644 μg/L in river waters (Sole et al. 2000; Soares et al. 2008) and even more than 3,520 mg/kg in sediments of natural waters (Ahel et al. 1994b). Lou et al. (2012) found that the concentration of NP in the river water ranged from 8.54 ± 1.23 (Qiantang River) to

65.77 ± 3.69 μg/L (Tiesha River) using the HPLC–FLD method. According to Tao et al. (2011), the concentrations of NP and OP in Nanming river water ranged from 40 to 1,582 ng/L. The concentration of 4-NP in river water of Germany was reported by Fries and Püttmann (2004), which is ranged from 28 ng/L (Elbe River) to 1,220 ng/L (Oder River). Martinez and Peñuela (2012) carried out an analysis of 4n-NP in Colombian reservoir water by GC/MS, where 4n-NP was not found in any of the water samples.

Currently, NP risks have been accepted by EPA and has prepared a guideline for natural water quality that recommends NP concentrations to be below 6.6 and 1.7 μg/L, in freshwater and saltwater, respectively (Soares et al. 2008; Brooke and Thursby 2005).

The Karun River is the largest river in Iran in terms of annual discharge (2.2×10^{10} m^3). It consists of the Dez River, the Gargar River, and the Shoteit River and flows to the Persian Gulf. It is estimated that 2.1×10^8 and 3.15×10^8 m^3/annum domestic and industrial wastewaters are discharged into Karun River, respectively. More than 90 % of the wastewaters are not treated effectively prior to discharge. In recent decades, the Karun River contamination is increasingly serious with the explosive increase of population and heavily industrial development. Although many studies focused on the identification of NP in aquatic environments and its side effects around the world, but there has not been any report about NP in Iran. In this study, the NP concentrations in water samples collected from upstream and downstream of Karun River around the Ahwaz metropolis and wastewater discharges into this river were measured.

Materials and methods

Sampling sites and dates

Sample collections from Karun River water were carried out from April to July 2010 period using a semi-composite sampling procedure. Briefly, water samples were taken at three points in the river cross-section with 6 h interval in each sampling campaign. Afterward, the samples were mixed together based on river flow and a 3 L composite sample were taken. Twenty-four-hour composite samples of raw wastewater were taken from discharge points during two sampling campaigns in April and July 2010 from five wastewater discharge points along Karun River on both banks in Ahwaz city area. The geographic location of the Karun River points where water and wastewater samples were collected are shown in Fig. 1. For NP measurements, 3 L water and 1 L wastewater samples were collected in amber glass bottle that were previously cleaned and heated to 450 °C for 8 h. Water and wastewater samples were preserved by the addition of formic acid to pH ≤ 2. A total of 28 water samples and 20 wastewater samples were collected, and all samples were directly transported to the laboratory, stored at 4 °C and analyzed within 24 h with 3 replicate.

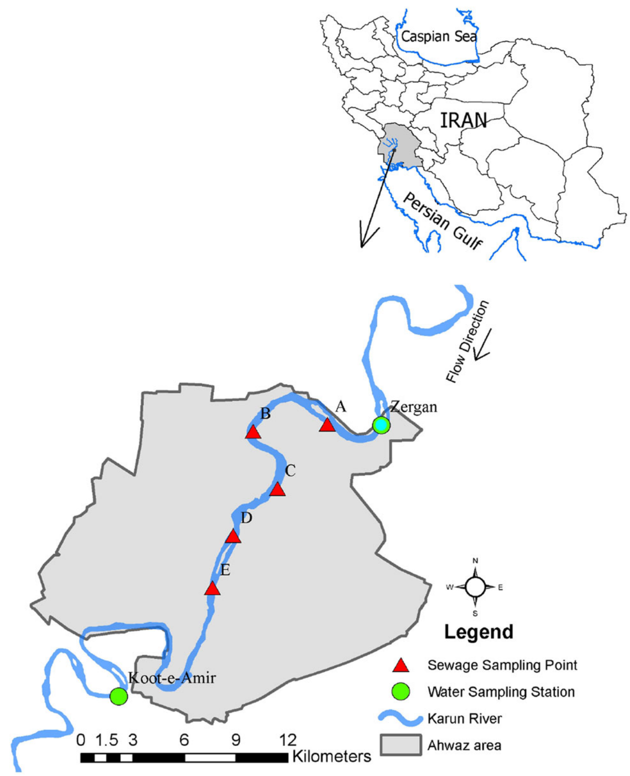

Fig. 1 sampling locations in Karun River and wastewater discharges

Instruments and chemicals

The Shimadzu 10Avp Series HPLC system (Shimadzu, Japan) coupled with a RF-10A_{XL} fluoresces detector was employed in this study. We used HPLC grade organic solvents obtained from Merck (Dormstad, Germany). NP (99.5 %) and 4n-NP-d8 were purchased from Dr. Ehrenstorfer (Audgsburg, Germany). HPLC separations were performed using a Kromasil 100 C18 column (4.6 × 150 mm, 5 μm) from Eka Chemicals AB (Bohus, Sweden) thermostatted at 30 °C, injection volumes of

20 μl, flow rate of 1 mL/min, and isocratic elution with 40 % water and 60 % acetonitrile (AcN) during 25 min. Analyte was monitored by fluorescence detection (λ_{ex}: 222 nm, λ_{em}: 305 nm) and quantified by external calibration using peak area measurements.

Sample preparation and NP measurements

Initially, each water and wastewater samples sub-divided into three parts and an appropriate volume of water and wastewater (100 mL of wastewater and 1 L of river water) was filtered through pre-ashed 0.7-μm-pore-size GF/F filters (Whatman, UK), and for water samples, the retained particulate material and filter were washed with 3 × 10 mL of methanol and reduced to 2 mL which was added to the filtrate. NP was extracted from water and wastewater samples by using SPE C18 cartridge (LH), 500 mg/6 mL (Capital, UK) described by Martinez and Peñuela (2012). Briefly, the filtered water or wastewater samples were loaded onto cartridges previously conditioned with 6 mL of acetone, 6 mL of methanol (MeOH), and 6 mL of ultrapure water acidified to pH 2. Cartridges were washed with ultrapure water, dried under vacuum for 30 min, and analyte was eluted with 3 × 3 mL of dichloromethane (DCM). Finally, sample extracts were evaporated to dryness under a nitrogen stream, redissolved in 500 μL mobile phase H_2O/AcN (40/60) containing 50 ng internal standard, and analyzed by HPLC-FLD. For the determination of NP in the wastewater particulate phase, the retained solids and filters were oven-dried in 30 °C until constant weight which were placed in Soxhlet apparatus and extracted with 100 mL methanol for at least 24 h. The extracts were evaporated to 2 mL and then diluted to 100 mL with ultrapure water and treated as aqueous phase described previously.

A linearity regression function was set up based on calibration measurement in a range of 25–1,000 μg/L. There was a good linearity in the detected range, and correlation coefficients (R^2) were 0.9989.

Method precision was evaluated in terms of instrumental repeatability and reproducibility of the method. For instrumental and method repeatability, seven successive injections were performed on a standard of 25 μg/L and standard solutions in the matrix at concentration level of 50 μg/L, respectively. The %RSD were satisfactory for the standard and spiked matrix, which were 5.4 and 10.3 %, respectively.

The recoveries of target compound in water, wastewater, and particulate phase of wastewater were more than 83 %, about 81 and 77 %, respectively, indicating acceptable accuracy of the analytical procedure. The obtained method detection level (MDL) ranged from 0.03 (Karun River water samples) to 0.038 μg/L (particulate phase of wastewater samples).

Results and discussion

Analyses of 28 water and 20 wastewater samples showed that NP is present in Karun River water and wastewater discharges, although its concentrations in Karun River water samples were low (Table 1). The NP level of Karun River water samples ranged between 0.15 and 1.97 μg/L, with mean values (±standard deviation) of 0.24 ± 0.06 and 1.57 ± 0.3 μg/L in Zergan (upstream) and Koot-e-Amir (downstream) sampling stations, respectively (Fig. 2). The NP level of Koot-e-Amir station ranged from 1.12 to 1.97 μg/L and was higher than Zergan station, which was 0.15–0.32 μg/L. This relatively high NP concentration at the Koot-e-Amir might be discharge related and can probably be attributed to raw wastewater discharges and effluents of local wastewater treatment works. The concentrations were comparable with those found in river water of Japan (up to 1.08 μg/L) (Isobe et al. 2001) and Switzerland (up to 2.25 μg/L) (Ahel et al. 1994b), but higher than those found in Colombian reservoir water (was not found) (Martinez and Peñuela 2012), rivers of Germany (0.0007–0.0044 μg/L) (Bester et al. 2001), Korea (0.0232–0.1876 μg/L) (Li et al. 2004), and Canada (<0.092 μg/L) (Sabik et al. 2003). The water intake of southeast region of Khuzestan Province, with a capacity of 450,000 m^3/day, was located in Koot-e-Amir area, and therefore, NP entering risks into water supply systems is predictable and very important.

Results of the analyses of NP in wastewater samples taken from the five wastewater discharge point along the Karun River in Ahwaz city are summarized in the Table 1 and Fig. 3. In points A–E, NP level of wastewater samples in April and July was ranged from 16.73 to 20.36 and 15.27 to 21.79 μg/L, respectively. The maximum concentrations of NP in wastewater samples were observed in D and E discharge points with values of 20.36 and 21.79 μg/L in the first and second sampling campaigns, respectively. The results showed that the NP content of aqueous phase of all wastewater samples higher than particulate phase, which were detected in the aqueous and particulate phases with mean concentrations of 12.8 ± 2.4 and 5.2 ± 1.2 μg/L, respectively. The concentrations of NP identified in wastewater samples from different discharge points were consistent with the

Table 1 Occurrence of NP in Karun River water and wastewater discharges (in μg/L) included in this study

Sampling points		Sampling campaigns (2010)				Mean
		April	May	Jun	July	
Karun River	Zergan	0.17 ± 0.02	0.28 ± 0.04	0.29 ± 0.04	0.23 ± 0.02	0.24 ± 0.06
	Koot-e-Amir	1.32 ± 0.22	1.70 ± 0.22	1.83 ± 0.19	1.59 ± 0.23	1.57 ± 0.30
Wastewater discharges	A	18.54 ± 2.51	–	–	15.85 ± 1.99	17.20 ± 2.51
	B	17.47 ± 1.14	–	–	17.77 ± 2.30	17.62 ± 1.63
	C	19.28 ± 0.46	–	–	15.27 ± 2.13	17.27 ± 2.60
	D	20.36 ± 0.92	–	–	17.08 ± 0.85	18.72 ± 1.97
	E	16.73 ± 1.99	–	–	21.79 ± 1.58	19.26 ± 3.20
	Mean	18.48 ± 1.89			17.55 ± 2.84	18.01 ± 2.42

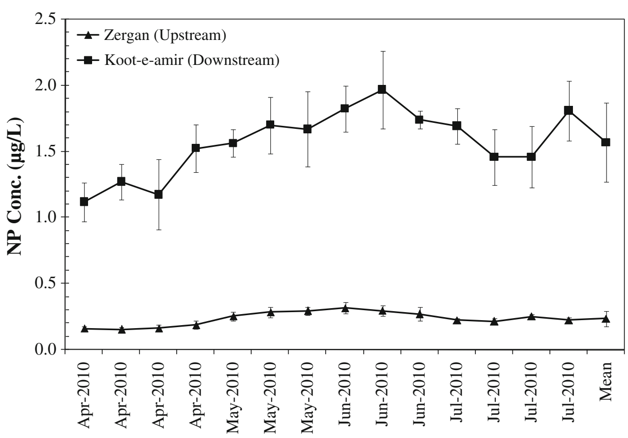

Fig. 2 Concentrations of NP in water samples from the Karun River

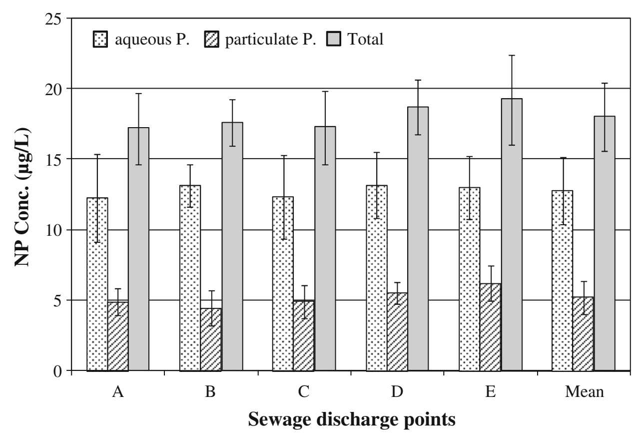

Fig. 3 NP levels of aqueous and particulate phase in different wastewater discharge points included in this study

reported levels in Italy (Petrovic and Barceló 2004) and China (Lian et al. 2009), but were at high level compared to those observed in the European and Asian countries, such as Spain (<1.5 μg/L) (Farre et al. 2002), UK (<0.02–0.1 μg/L) (Sheahan et al. 2002), Japan (0.1–0.9 μg/L) (Nakada et al. 2006), and China (0.015–0.101 μg/L) (Zhou et al. 2009), indicating that the contamination in Ahwaz seemed heavy than abroad, probably due to high consumption of NPnEOs surfactants or poor sewer conditions.

Conclusion

NP concentrations in Karun River water and wastewater discharges of Ahwaz city were surveyed during April to July 2010. This study showed that the NP was ubiquitous in Karun River water and wastewater discharges. At downstream of Ahwaz city (Koot-e-Amir hydrometric station), total NP concentrations of Karun River water were increased by a factor of 6.65 compared to upstream of Ahwaz city (Zergan hydrometric station). In Ahwaz, only a minor fraction (about 10 %) of wastewater can be treated before discharging. As Ahwaz is a densely populated city, NP detected in Karun River water is probably closely related to the domestic activities of urban dwellers.

The detection of NP in the all wastewater discharges suggests anaerobic transformation of its parent compounds, NPnEOs, in the sanitary sewers due to septic conditions in Ahwaz sewer and low dissolved oxygen levels in raw wastewaters. On the one hand, about 2.1×10^8 m^3/annum of untreated domestic wastewater effluent into Karun River could discharge about 3,780 kg/year of potentially endocrine-disrupting chemicals (EDCs), NP.

With the importance of Karun River as main water supply source in Khuzestan Province and a place for living of biota, the information on occurrence and fate of EDCs such as NP is particularly important because of their potential effects on the aquatic biota. Future research on the temporal and spatial variation of the EDCs such as alkylphenol ethoxylates and their derivatives in water body and especially sediments of Karun River and other water resources in Iran are needed and recommended. Due to the significant contributions of NP to the estrogenic activities, future efforts should be focused on its impacts on biota in Karun River and other water resources in Iran.

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