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Determination of polycyclic aromatic hydrocarbons (PAHs) in water, sediment and tissues of five sturgeon species in the southern Caspian Sea coastal regions

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ABSTRACT

Concentration levels of 16 polycyclic aromatic hydrocarbons (PAHs) consisting of naphthalene, acenaphthylene, acenaphthene, fluorine, phenantherene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd) pyrene and benzo(g,h,i)perylene were measured in water and sediment samples collected from the southern Caspian Sea coasts at four stations of Noshahr, Freydoonkenar, Sari and Amirabad during autumn, winter and spring of 2005-2006. Also, tissue samples from five sturgeon species including stellate sturgeon, Persian sturgeon, beluga, Russian sturgeon and ship sturgeon were obtained. Samples were analyzed by gas chromatography after the extraction process and the obtained data were statistically analyzed using One-Way ANOVA analysis at confidence level 95%. The mean concentrations of PAHs in water and sediment samples were 0.004-2.946 mgl^{-1} and 0.024-2.336 $\mu g\ g^{-1}$, respectively. No significant difference was found in the mean concentrations of PAHs among stations and seasons of the examined samples. Also, mean concentration of PAHs in the liver, muscles, gills, kidney and gonads of five sturgeon species was 0.81-1.34 μgg^{-1}. The results of this study show that the levels of PAHs in water, sediment and sturgeon organs were below the acceptable levels of PAHs proposed by USEPA and WHO.

Keywords: *Polycyclic aromatic hydrocarbons, water, sediment, sturgeon, fish.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) have become widespread environmental contaminants due to their occurrence in petroleum, coal, soot, air pollutants and cutting oils (Reynaud and Deschaux, 2006). These chemicals are carcinogenic for humans (Kannan and Perrotta, 2008) and may induce hepatic lesions, physiological and biochemical disorders in fish (Ribeiro *et al.* 2005). The United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) have identified 16 PAHs as priority pollutants, some of these, e.g. benzo(a)anthracene, chrysene and benzo(a)pyrene are considered to be potential human carcinogens (Oliva *et al.*, 2010).

Despite the rapid occurrence of industrialization and urbanization in the coastal areas of the Caspian Sea particularly during the last decades, minimum data on the environmental distribution of PAHs is available in the Iranian side of the region. This is particularly true because nowadays the the Caspian Sea region is becoming increasingly polluted with massive loads of contaminants discharged from various anthropogenic sources (Mohammadi Zadeh et al., 2010). For instance some studies show that 160,000 tons of hydrocarbon materials are annually released into the Caspian Sea (Pak and Farajzadeh. 2007)

As a land-locked system, pollutant discharges into the Caspian Sea remain trapped within the basin. Offshore oil production and land-based sources, notably the Volga River, are considered to be the main sources of the pollution (Stephen et al., 2004). Also, sea currents probably transport and circulate the entrapped pollutants along the Iranian coast of the Caspian Sea (Mohammadi Zadeh *et al*., 2010). If so, the major hydrophobic PAHs in water strongly adsorb to the particles and generally become more resistant to the bacterial degradation (Beyer *et al*, 2010). Water sampling in different parts of the Caspian Basin has shown a detectable contamination of phenols, oil products, and other sources (Dahl and Kuralbayeva, 2001, Tolosa *et al*., 2004). Other studies on water of Caspian Sea have also determined the presence of PAHs (Nasrollahzadeh et al., 2002; Habibi *et al*., 2008).

PAHs and their halogenated forms are also chemically stable, and due to their lipophilic nature they can easily penetrate biological membranes and accumulate in organisms (Oliva *et al*., 2010). Many studies have shown that PAHs are toxic to fish and other aquatic organisms (Anderson and Lee, 2006; Buryskova *et al*., 2006; Lee and Anderson, 2005; Wurl and Obbard, 2004; Kurunthachalam *et al*., 2008; Salazar-Coria *et al*., 2007, Olive *et al*., 2010).

The accumulation of such pollutants in the fish can be transferred to the human food chain (Mohammadi Zadeh *et al*., 2010). Sturgeons are an important source of food and 80–90% of the world's sturgeon catch is obtained from the Caspian Sea basin, mainly from the Volga River. However, as a threatened species, Caspian sturgeon have been included in the Red List by the International Union for Conservation of Nature and Natural Resources (IUCN) because of overharvest, habitat loss, and serious exposure to chemical pollution (Khodorevskaya et al., 1997; Kajiwara et al., 2003). Moreover, since the late 1980s, high levels of tumors, abnormalities in gonad development and gametogenesis, and disturbances in the morphogenesis of organs have been noticed in Caspian sturgeons (Kajiwara et al., 2003).The long life of sturgeons may leave them particularly vulnerable to the effects of bioaccumulative pollutants. As opportunistic bottom feeders, these fish frequently come in contact with sediments that could contain sediment-adsorbed hydrophobic pollutants (Billard and Lecointre, 2001; Kajiwara *et al*., 2003; Hosseini *et al*., 2008). Also these spieces consume molluscs, shrimp and amphipods (Silvestre *et al*., 2010), that may accumulate large amounts of pollutants over time (Moore *et al*., 2003).

Therefore, in this study, concentrations of 16 USEPA priority PAHs were measured in muscle, liver, gill, kidney and gonad of five species of sturgeons including stellate sturgeon (*Acipenser stellatus*), Persian sturgeon (*Acipenser persicus*), beluga (*Huso huso*), Russian sturgeon (*Acipenser gueldenstaedtii*) and ship sturgeon (*Acipenser nudiventris*) in the Iranian coasts of Mazandaran province. Also due to the increasing pollution in the Caspian Sea, PAHs distribution was monitored in water and sediment samples during the autumn, winter and spring seasons.

MATERIALS AND METHODS

Samples of water and sediment were obtained from four stations at Noshahr, Freydoonkenar, Sari and Amirabad port, in Mazandaran province during 2005-2006. The samples were obtained using Kalsico sampler. In each station three samples were collected from different depths of 1 to 5 m during autumn, winter and spring.

The water samples were first fixed using CH_2Cl_2 as solvent and then transported to the laboratory of the Ecology Institute of Caspian Sea in Sari. The sediment samples were also collected using a Van Veen grab and stored at 4^{o}C in aluminum containers. After the extraction process the mean PAHs of water and sediment samples were determined using gas chromatography (Shimadzu CR6A) coupled with flame ionization detector (GC-FID) method according to ASTM (1995) and Russian standard method (1992).

During autumn, 15 sturgeon individuals (3 of each species) were obtained from local fishermen in each station and samples of liver, muscles, gills, kidney and gonads were obtained. All samples were dried using freeze drying. Soxhlet, saponify, extraction by n-hexane were used for the extraction process. The extracted elution was concentrated to 1 ml by vacuum rotary evaporation and clean-up method. For hydrocarbon analyses, the extract was passed through a prepared silica/alumina column. Silica and alumina were first activated at 200^{o}C for 4 h and then partially deactivated with water (5% w/w). Samples

were analyzed using (GC-FID) (Moopam, 1999).
The data were tested for homogeneity of variances at a significance level of 0.05 using One-Way ANOVA test.

RESULTS

Distribution fractions of mean levels of PAHs in water samples at four stations in three seasons are shown in Table 1. Maximum of mean concentrations of PAHs in water samples was seen for naphthalene, which is more soluble than the other fractions. During autumn the maximum and minimum of mean concentrations of PAHs were observed in Freydoonkenar (2.946mgl^{-1}) and in Sari stations (0.128mgl^{-1}), respectively. All mean acceptable levels of PAHs declared by USEPA and WHO were detectable in water samples during the winter sampling (Table 1.2). No significant differences were seen in the mean concentrations of PAHs in water samples in the four sampling stations during different seasons.

Table 1. The concentrations of PAHs (mgl$^{-1)}$ in water samples obtained from the southern Caspian Sea during different seasons.

Table 1.1 Autumn

Type of PAHs	Station			
	Amirabad	Sari	Freydoonkenar	Noshahr
Naphthalene	0.068	0.038	0.096	0.14
Acenaphthylene	0	0	0	0
Acenaphthene	0	0	0	0
Fluorine	0	0	0.088	0
Phenantherene	0.056	0.033	0.052	0.25
Anthracene	0	0	0.058	0.02
fluoranthene	0.028	0.022	0.0432	0.12
Pyrene	0.044	0.036	0.22	0.3
Mean	0.196	0.128	2.946	0.83

Table 1.2 Winter

Type of PAHs	Station			
	Amirabad	Sari	Freydoonkenar	Noshahr
Naphthalene	0.003	0.003	0.003	0.003
Acenaphthylene	0.0054	0.0091	0.0081	0.132
Acenaphthene	0.003	0.011	0.003	0.003
Fluorine	0.003	0.003	0.003	0.003
Phenantherene	0.003	0.0081	0.003	0.003
Anthracene	0.003	0.0051	0.003	0.003
fluoranthene	0.0082	0.003	0.003	0.0212
Pyrene	0.003	0.003	0.0059	0.003
benzo(a)anthracene	0.003	0.003	0.003	0.003
Chrysene	0.0242	0.003	0.003	0.0518
benzo(b,k)fluoranthene	0.0161	0.003	0.003	0.0509
benzo(a)pyrene	0.0135	0.003	0.003	0.003
dibenzo(a,h)anthracene,	0.0082	0.003	0.003	0.0212
indeno(1,2,3-cd) pyrene	0.015	0.003	0.003	0.003
benzo(g,h,i)perylene	0.015	0.003	0.003	0.003
Mean	0.1266	0.0663	0.053	0.1883

Table 1.3. Spring

Type of PAHs	Station			
	Amirabad	Sari	Freydoonkenar	Noshahr
naphthalene	0	0	0	0.054
fluorene	0.086	0.45	0	0
phenantherene	0.15	0	0	0
anthracene	0.13	0	0.39	0.223
pyrene	0.18	0	0.26	0.39
benzo(a)anthracene	0	0	0	0.043
chrysene	0	0	0	0.042
benzo(b,k)fluoranthene	0.036	0	0	0.073
benzo(a)pyrene	0.13	0	0	0
Mean	0.0044	0.041	0.011	0.036

Results of PAHs concentrations in the sediment samples are shown in Table 2. The mean concentrations of PAHs in the sediment samples ranged from 0.072 to 2.63 µg g^{-1}. Maximum mean concentrations of PAHs fractions in the sediment samples were seen for phenantherene and naphthalene, respectively in autumn (Table 2.1).

In winter, PAHs were detectable in the sediment samples with the highest concentration measured for benzo(a)pyrene (0.451 µg g^{-1}) (Table 2.2). During spring, the highest concentration of PAHs was measured for pyrene (Table 2.3). Mean concentrations of PAHs did not show any significant differences in sediment samples in the 4 stations during different seasons (P>0.05).

Table 2. The PAHs levels ($mgl^{-1)}$) in sediment samples obtained from the southern Caspian Sea during autumn, spring and winter.

Table 2.1. Autumn

Type of PAHs	Station			
	Amirabad	Sari	Freydoonkenar	Noshahr
Naphthalene	0	0.628	0.008	0.02
Fluorine	0	0.3	0	0.050
Phenantherene	0.012	0.808	0.036	0.064
Anthracene	0	0.416	0	0.012
fluoranthene	0.008	0.092	0.008	0.044
Pyrene	0.004	0.092	0.02	0.036
benzo(a)pyrene	0.0392	0	0	0
Mean	0.0632	2.336	0.072	0.024

Table 2.2. Spring

Type of PAHs	Station			
	Amirabad	Sari	Freydoonkenar	Noshahr
Naphthalene	0	0	0	0.054
Fluorine	0.086	0.45	0	0
Phenantherene	0.15	0	0	0
Anthracene	0.13	0	0.39	0.223
Pyrene	0.18	0	0.26	0.39
benzo(a)anthracene	0	0	0	0.043
Chrysene	0	0	0	0.042
benzo(b,k)fluoranthene	0.036	0	0	0.073
benzo(a)pyrene	0.18	0	0	0
Mean	0.712	0.45	0.65	0.825

Table 2.3. Winter

Type of PAHs	Station			
	Amirabad	Sari	Freydoonkenar	Noshahr
Naphthalene	0.1	0.1	0.1	0.1
Acenaphthylene	0.1	0.1	0.1	0.1
Acenaphthene	0.1	0.1	0.1	0.1
Fluorine	0.0056	0.1	0.1	0.1
Phenantherene	0.1	0.27	0.1	0.1
Anthracene	0.1	0.1	0.1	0.1
fluoranthene	0.1	0.1	0.0319	0.1
Pyrene	0.1	0.1	0.299	0.1702
benzo(a)anthracene	0.1	0.1	0.1	0.1
Chrysene	0.1	0.1	0.067	0.1
benzo(b,k)fluoranthene	0.1	0.509	0.324	0.1
benzo(a)pyrene	0.1	0.0512	0.065	0.451
dibenzo(a,h)anthracene,	0.1	0.1	0.15	0.1
indeno(1,2,3-cd) pyrene	0.1	0.1	0.15	0.1
benzo(g,h,i)perylene	0.1	0.1	0.051	0.1
Mean	1.4056	1.5721	1.8379	1.9212

Results of PAHs levels in the tissues of five sturgeon speies are shown in Table 3. PAHs were detectable in the liver and muscles of all sturgeon species examined. Mean PAHs concentrations of 0.81, 0.63, 0.66, 1.34 and 1.1 µg g^{-1} were measured in the tissues of stellate sturgeon, Persian sturgeon, beluga, Russian sturgeon and ship sturgeon, respectively.

Table 3. Mean concentrations of PAHs in different tissues of five sturgeon species (Mean ±S.E.* µg/g dry weight, NT= not detectable)

Sturgeon species	PAHs (µg/g dry tissue)					
	Liver	Muscle	Gill	Kidney	Gonad	Mean(µg/g)
Stellate sturgeon	0.31 ± 0.037	0.71 ± 0.30	0.39±0.129	NT	NT	0.81
Persian sturgeon	0.29 ± 0.008	0.21±0.28	0.28±0.093	NT	NT	0.63
Beluga sturgeon	0.64 ± 0.02	0.79±0.24	NT	0.58±0.19	NT	0.66
Russian sturgeon	0.71 ± 0.35	1.82±0.38	NT	1.08±0.35	NT	1.34
Ship sturgeon	0.61 ± 0.14	0.57±0.16	NT	NT	0.95±0.32	1.10

DISCUSSION

Results of this study showed that minimum and maximum levels of PAHs in water samples were 0.011 and 2.946 mgl^{-1}, respectively which are higher than that described by Nasrollahzadeh *et al* (2002) who reported a range of 0.024-1.44 mgl^{-1} in water samples in 2002.

Also, in a study by Tolosa *et al*. (2004), total hydrocarbons levels detected in the sediment samples ranged from 29 to 1820 μgg^{-1}, with the highest levels in Azerbaijan region and the lowest in the north Caspian Sea.

In this study the mean concentrations of PAHs in the sediment samples ranged from 0.024 to 2.336 µg g^{-1} with the highest levels detectable for phenantherene and naphthalene during autumn, benzo(a)p-yrene (0.451 µg g^{-1}) in winter and pyrene (0.39 µg g^{-1}) in spring in Noshahr station. However, no significant difference was found in mean concentrations of PAHs in sediment samples in 4 stations and during these three seasons.

One possible reason for such differences in the levels of PAHs between the north and south parts of the Caspian Sea may be in part due to the lack of any oil or gas producing activities in the southern parts of the Caspian region, so far. However, with an increase in the oil production and oil transit in the northern part of the Caspian Sea as well as the directions of the water currents from the north to the south, the possibility of oil pollution in the south parts is increasing (Effimoff, 2000; Korotenkoa *et al*., 2004).

The distribution of total PAHs in fish tissues has been reported in several regions such as in Hiroshima Bay, Japan (0.23-13.6 μgg^{-1}); Puget Sand, Maryland (160 μgg^{-1}); Port Philip Bay, Australia (55.7 μgg^{-1}) and the Arabian Sea (118 μgg^{-1}) (Nasrollahzadeh *et al*, 2002). In this study, the detectable levels of PAHs in sturgeons were generally lower than the values reported in available literatures, and therefore, reflecting a cleaner environment in the south Caspian Sea at the time of sampling.

Also, in this study the highest and the lowest levels of PAHs were detectable in Russian sturgeon (1.34 μgg^{-1}) and Persian sturgeon (0.81 μgg^{-1}), respectively. Such differences might be due to the difference in food habits among the sturgeons . Although most sturgeons are generally benthic feeders, food composition may be different between the fish species. For example, predominant food items of stellate sturgeon are crustaceans and worms (*Nereis Diversicolor, Hipania invalida, Hipaniola kavalewski*), while the preferred itemsfor Russian sturgeon is smaller bivalve mollusks which may be a source of local pollution as well. Also, beluga with body length of more than 40 cm feeds on benthic and pelagic fishes (Billard and Lecointre, 2001; Agusa *et al*., 2004). However, correlation between the PAHs composition in the sturgeon tissues and the fish feeding grounds is beyond this study and therefore calls for further investigation.

Also, the difference in intensity of specific species of sturgeon may depend on the difference in their migration habits. For example, during winter, Russian sturgeon intensify at the near western shelf of the middle Caspian Sea, which is impacted by oil production and heavily polluted with hydrocarbons including PAHs (Moore *et al*., 2003), while Persian sturgeon mainly intensify in the southern part of the Sea, the habitat (Khodorevskaya *et al*., 1997) where there is lack of much prevalent offshore oil exploration and production.

PAHs in fish organs are not only directly responsible for fish kills, but also may induce a key step in the mechanism of malignant transformation through the formation of adducts between DNA and reactive electrophilic metabolites (Deb *et al*., 2000). Although the effects of PAHs have not been extensively investigated here, the detected levels of PAHs in liver, muscles, gills, kidney and gonads has raised serious concern for the natural ecosystem of these valuable species in the Caspian Sea. This is particularly true in the case of sturgeons because they mature after several years and can survive for a long time. Therefore, the gradual accumulation of pollutants including PAHs can seriously damage their life.

In the case of PAHs accumulation in muscle tissue which is the edible part for humans, it can directly influence human public health (Espinoza-Quin˜ones *et al*., 2010). Therefore, the results of this study raised an issue of risk of human health.

In conclusion, the present study found that the levels of PAHs in water, sediment and sturgeon organs were relatively low during autumn, winter and spring time. However, because of recent increase in oil and gas production activities in the Caspian Sea, regular studies are required to monitor and reveal the possible adverse effects of oil substances in the Caspian Sea aquatic animals including sturgeons, as part of restoration programs of sturgeon populations as well as the public health particularly the consumers of sturgeon meat and caviar. Although the present study found that the levels of PAHs in water, sediment and tissue samples were relatively low, with regard to the increasing oil contamination in the Caspian Sea, further studies are needed to reveal the possible adverse effects of harmful substances on sturgeons, as part of restoration programs of sturgeon populations. The contamination problems with PAHs outlined in this study are not unique to the Caspian Sea, but have become intensified due to its land-locked nature. Therefore, a close cooperation made by all bordering countries are required to improve and prevent future pollution of the Caspian Sea.

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اندازه‌گیری میزان هیدروکربنهای آروماتیک (PAHs) در آب، رسوب و عضله پنج گونه ماهی خاویاری در سواحل جنوبی دریای خزر

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چکیده

میزان غلظت ۱۶ هیدروکربن آروماتیک (PAHs) شامل نفتالین، اسنافتین، اسنافتیلن، فلوئورین، فنانترن، آنتراسین، فلوئورانتین، پایرین، بنزو (آلفا) آنتراسین، کراسین، بنزو (بتا) فلوئورانتین، بنزو (کا) فلوئورانتین، بنزو (آلفا) پیرین، دی بنزو (آلفا و اچ) آنتراسین، ایندینو (۱و ۲و ۳-cd) پایرین، بنزو (جی، اچ و آی) پریلین در نمونه های آب و رسوب جمع آوری شده از سواحل جنوبی دریای خزر در چهار ایستگاه نوشهر فریدونکنارساری و امیرآباد در فصول پاییز، زمستان و بهار ۲۰۰۵-۲۰۰۶ اندازه گیری شد. همچنین نمونه های بافت پنج گونه ماهی خاویاری شامل ازون برون، تاس ماهی ایرانی، فیل ماهی، تاس ماهی روس و شیپ تهیه شد. نمونه ها بعد از فرآیند جداسازی با دستگاه گازکروماتوگرافی مورد تجزیه و تحلیل قرار گرفت و داده های حاصله با استفاده از روش ANOVA یک طرفه، در سطح معنی داری ۵٪ تجزیه و تحلیل شد. میانیگین غلظت PAHs در نمونه های آب و رسوب به ترتیب در محدوده ۰,۰۰۴-۲,۹۴۶mgl^{-1} و ۰,۰۲۴-۲,۳۳۶ μgg^{-1} به دست آمد. میانگین غلظت PAHs در نمونه های مورد آزمایش با ایستگاهها و فصول مختلف اختلاف معنی‌دار نداشته است. همچنین متوسط PAHs در کبد، عضله، آبشش، کلیه و گناد پنج گونه ماهی خاویاری در محدوده ۰,۸۱-۱,۳۴ μgg^{-1} بود. نتایج این مطالعه نشان داد مقدار PAHs در آب، رسوب و اندام های ماهی خاویاری در سواحل جنوبی دریای خزر، پایین تر از میزان قابل قبول بود.

*مولف مسئول