

# Investigation on Polycyclic aromatic hydrocarbons (PAHs) in Anzali Lagoon (Siah-Kashim) by HPLC: role of octanol-water partition coefficient

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## Abstract

There is a little published information on pattern of Polycyclic aromatic hydrocarbons (PAHs) pollution on marine protected areas. 36 sacrificial sediment samples were collected from four stations in Siah-Kashim Lagoon on a temperate season (autumn). The samples were frozen and transported to laboratory and kept in -35 freezer till analysis. 16 Environmental Protection Agency (EPA) priority PAHs were extracted, cleaned up and quantified by fluorescence detector in a high performance liquid chromatography (HPLC) apparatus. An aliquot of the samples were also treated for total organic matter (TOC) determination. The results showed that within low level of pollution with respect to PAHs in the studied area, the concentration (i.e. availability and environmental toxicity) of PAHs in Siahkashim Lagoon is governed first by their water solubility and then their Octanol-Water partition coefficient ( $K_{ow}$ ).

**Keywords:** Polycyclic aromatic hydrocarbons (PAHs), sediment, accumulation, Siahkashim, Anzali Lagoon

تعیین هیدروکربنهای حلقوی آروماتیک (PAHs) در تالاب انزلی (منطقه سیاه کشیم) با استفاده از روش HPLC: بررسی نقش ضریب تفکیک اکتانول / آب ( $K_{ow}$ )

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## چکیده:

اطلاعات اندکی در مورد الودگی PAHs در مناطق حفاظت شده (نظیر سیاه کشیم) وجود دارد. در این تحقیق ۳۶ نمونه رسوبی از چهار ایستگاه در منطقه سیاه کشیم تالاب انزلی در یک محدوده دمایی (فصل پاییز) جمع آوری شده و سپس نمونه ها فریز شده و به آزمایشگاه منتقل گردیدند و تا زمان شروع آزمایشات نمونه ها در دمای ۳۶- درجه سانتی گراد، نگهداری شدند. پس از آن بر روی نمونه ها آزمایشات شیمیایی و آماده سازی ها آغاز شده و شانزده PAH خطرناک اولویت EPA، پس از طی مراحل استخراج و خالص سازی و با استفاده از آشکارساز فلوروسانس و روش HPLC تعیین مقدار شدند. در مرحله بعد مقادیر TOC هم در نمونه های رسوب تعیین گردید.

نتایج حاکی از آن است که مقادیر PAHs در منطقه مورد مطالعه در حد پایینی بوده همچنین در این تحقیق برای اولین بار غلظت (دسترسی و سمیت زیست محیطی) PAHs در منطقه سیاه کشیم با استفاده از محاسبه ضریب تفکیک اکتانول به آب ( $K_{ow}$ ) بررسی گردیده است.

## واژگان کلیدی

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## Introduction

Environmental quality of Anzali Lagoon (37° 28' N, 49° 25'E) is of utmost importance for several reasons. People rely on this Lagoon as a source for their food and economy. Additionally it is one of the most important habitats for many migrating animals and as an important spawning and nursery ground for the economical fishes. Anzali Lagoon is one of the international Lagoons in Iran this is one of the most important habitats for fishes, bivalves and birds in the south of the Caspian Sea. Anzali Lagoon is connected with Caspian Sea and so it is affected from this sea. Many fishes of Caspian Sea and adjacent rivers spawn in this Lagoon; so if concentration of contaminants increases in this Lagoon, it is probable that they transfer to food chain. Urban and industrial waste has an important role in pollution of Anzali Lagoon. Some of the rivers pass in industrial Rasht city which this phenomenon may transfer contaminated and polluted wastes to Anzali Lagoon (FAO, 1990). This Lagoon represents an internationally important wildlife reserve and some other reports have been published on increase of pollutants in this area (Talebi, 1998; Tavakkoli & Sabet Raftar, 2003). Polycyclic Aromatic Hydrocarbons (PAHs) are among the most ubiquitous pollutants which their concentration is increasing due to the dependency of human communities to the fossil fuels (Men *et al.*, 2009). Solubility characteristics of these pollutants in aquatic environments cause a different way of habits for these pollutants which ultimately result in their transfer to food chain which can make a risk for people residing in vicinity of the region. Increase of urban and industrial waste in recent decades had resulted in increase of PAHs contaminants in the environment. PAHs are organic compounds containing two or more adjacent aromatic rings. They are reactive to organic matter in the environment and absorb the particulate matters as soon as they enter the aquatic environment due to their high hydrophobicity, low solubility and aromaticity while they are categorized in "persistent organic pollutants" (POPs) because of their chemical structure stability and resistance to chemical and biological degradation (Malmstrom, 2009). In this way they can accumulate in marine sediments of industrial or petroleum producing countries (Eghtesadi *et al.*, 2002). Some of the PAHs are highly toxic and can cause mutations and cancer and can form covalent bonds to biological macromolecules such as DNA and form DNA adducts that change the genetic code (Malmstrom, 2009). There are two types of PAH origin classification: 1-Petrogenic paths derived from petroleum products as, they are released into the environment. 2. Pyrogenic paths derived from the products of combustion released into the environment by chemical reactions.

One of the most important determining factors on PAHs toxicity in marine environments are their octanol-water partition coefficient ( $K_{ow}$ ). Increasing toxicity of PAHs with increasing value of  $\log K_{ow}$  was showed by Sverdrup *et al.* (2002) to the point of  $\log K_{ow} \leq 5.2$ .

The objective of present study was to assess the PAHs levels and TOM in sediments on autumn season in Siah-Kashim Lagoon as a marine protected area (MPA).

## Material and methods

We chose four stations in Siah-Kashim Lagoon for determination of 16 EPA priority PAHs; total organic matter (TOM) were also determined in the sediments of these sites. It is important that depth of water in Lagoon changes in the seasons; therefore to

compensate the seasonal changes, the samples were collected in a temperate season (autumn).

36 surface sediments samples were collected in aluminum containers previously washed thoroughly with Hexane to remove any organic constituent. The sampling was done by Van Veen grab from four stations in Siah-Kashim Lagoon (Fig. 1, Table 1) with three replicates. The samples were frozen and transported to laboratory and kept in -35 freezer till analysis. Then they were thawed by taking out from freezer and 16 EPA priority PAHs were quantified in them as follows. 10 g wet samples were weighted accurately and then it was mixed well with  $\text{Na}_2\text{SO}_4$  till the sediment changed to powder shape. This powder was transferred to 250 ml balloon and 150 ml dichloromethane were added and kept for an hour in 30 °C temperature in ultrasonic bath. Then it was removed from the bath and kept for 24 hours in 30°C and then again were kept for an hour in the ultrasonic bath.

Clean up: Silica gel (Merck®; mesh 60; 0.063-0.200 for column chromatography art# 1.07737.1000) was activated through incubation in 105 °C for 24 hours. Then 10g Silica gel was added to 50 ml dichloromethane and transferred to glass column of inner diameter 1 cm, previously filled in its bottom with asbestos. When the column was filling, it was knocked to the column to fill better and equally. After transferring completed, 1 or 2 cm dry  $\text{Na}_2\text{SO}_4$  was added and then the column washed with 40 ml Pentane (Merck® grade HPLC). When the silica gel touched  $\text{Na}_2\text{SO}_4$  in the column, extraction solution transferred to column by pipette and with 2ml Cyclohexane, transferring was completed. Afterwards the column was washed with 25 ml Pentane and it was left out of this portion.

After solution touched with  $\text{Na}_2\text{SO}_4$ , solution was changed to 25 ml Pentane and dichloromethane (with rate 2:3) and this portion collected to a balloon and it was reduced to 5 ml after evaporation in vacuum. Then samples were kept in hot bath and were reduced by the  $\text{N}_2$  flow.

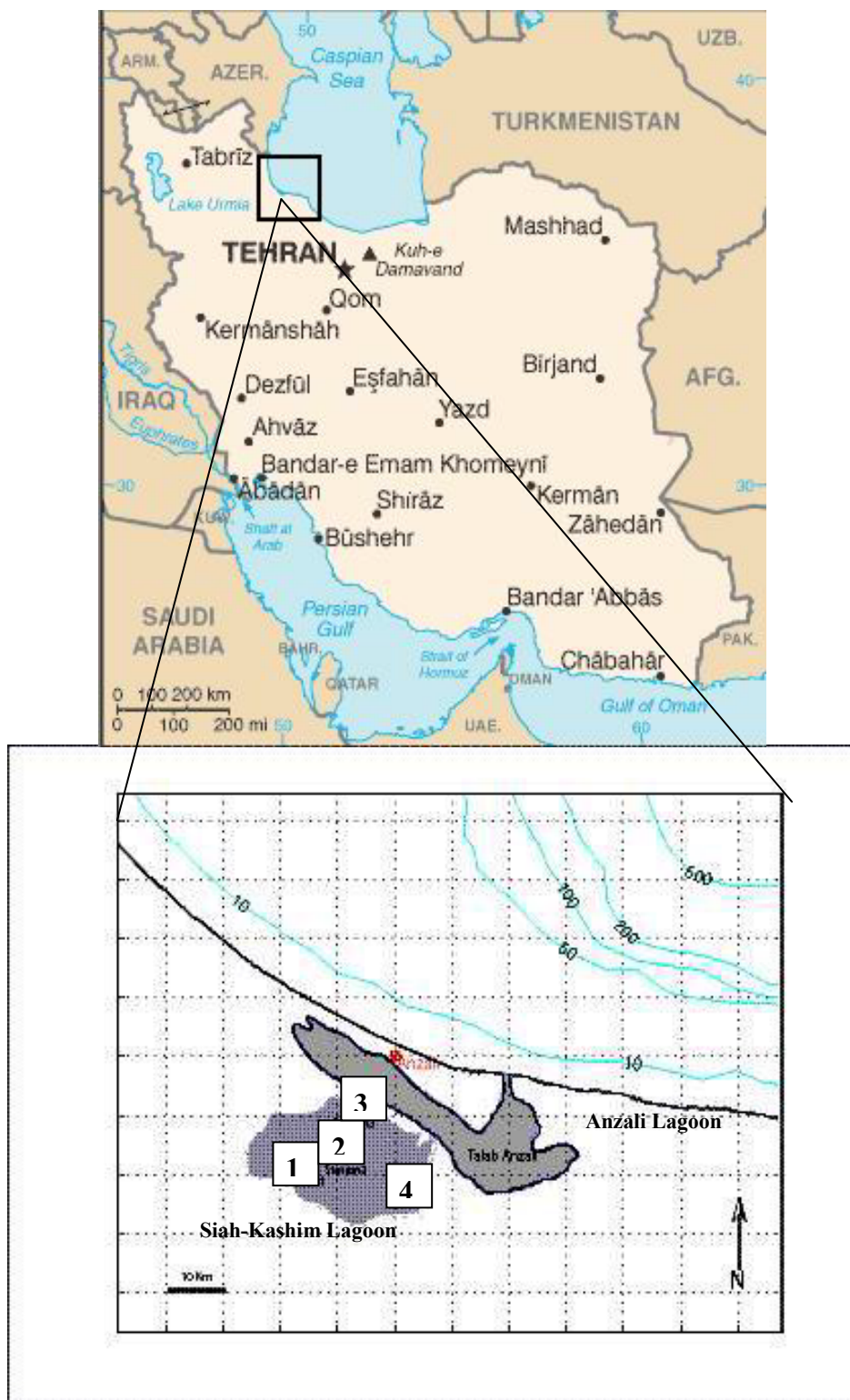


Fig. 1: Sampling stations in Siah-Kashim Lagoon (Anzali)

volume was reduced to 1ml by adding Acetonitrile (HPLC grade) and then it was ready to be injected to HPLC instrument. After clean up, PAH content of extracted samples were measured by HPLC (Agilent 1100). The column type was RESTEK, Pinnacle II PAH (150 × 3.2 mm, 5 μm) and detector was fluorescence (EX: 280 nm, Em: 389 nm), temperature was 25° c (with thermostat). In this analysis injection volume of 20 μl (loop) was used and mobile phases were water and Acetonitrile. Gradient of the program is in Table .2.

**Table1. Geographical Position of sampling stations**

Station	Geographical Position
1	N3725251 E04920758
2	N3725372 E04921446
3	N3725356 E04921659
4	N3724591 E04923070

**Table 2. HPLC gradient of the program**

Time	%B	Flow (ml/min)
0	10	1
10	100	1
18	100	1

Dry weight Determination: 5g wet sample was weight and kept in oven (105°C) for 24 hours and after than the sample weight once more and water percentage was determined by below mentioned formula:

$$\text{Water weight (g)} = \frac{\text{weight of wet sample} - \text{weight dry sample}}{\text{weight wet sample}}$$

Determination of TOM: Total organic matter of sediments was determined by percent of dry weight. Samples were transferred to vials and kept to oven for 24 hours at 70 °C. After 24 hours and cooling the samples, their weight was determined. Then samples transferred to incinerator at 550 °C for 4-6 hours. Organic matter was burned and the samples were weighted again. The percent of organic matter was calculated by below formula:

$$100 \times \frac{A-B}{A-C} = \text{TOM}$$

C is weight of vial, A is weight of vial and dry sediment (after oven) and B is weight of vial and dry sediment (after incinerator).

**Quality control and quality assurance:** The instrument was calibrated daily with calibration standards and the relative percent difference between the five-point calibration and daily calibration was less than 10%. For every set of samples, a procedural blank consisting of all reagents was run to check for interference and contamination. The samples were analyzed in duplicate and the Certified Reference Material (CRM) IAEA-417 was analyzed to check the method efficiency. All the obtained values for PAHs in CRM were in the reported range. The method detection limits (MDLs) of PAHs were determined with a signal-to-noise ratio (S/N) of 3. The

MDLs were between 0.1-1 ng/g. PAHs recovery studies were undertaken to demonstrate the efficiency of the method. Recoveries were between 68% (naphthalene) and 110% (Benzo[g,h,i]perylene). The PAH concentrations were corrected for recovery efficiency and also water content. The relative standard deviation (RSD) of the method was in the range of 4-11%.

## Results

In this study 16 EPA priority PAHs were analyzed in 12 (three replicate samples in each of the four stations) samples collected from Siah-Kashim Lagoon in autumn. Concentration of three individual PAHs were higher than the other ones which include Phenanthrene, Pyrene and Benzo(b)fluoranthene which are shown in figures 2, 3 and 4 respectively. Lowest level of

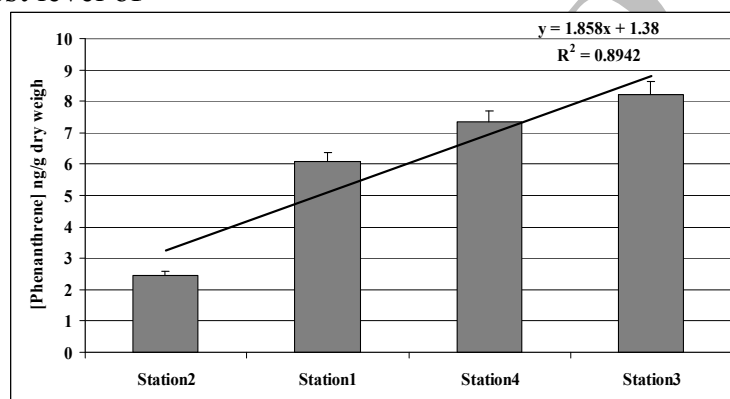


Figure 2: Phenanthrene concentration in sediments at different stations of Siahkashim Lagoon

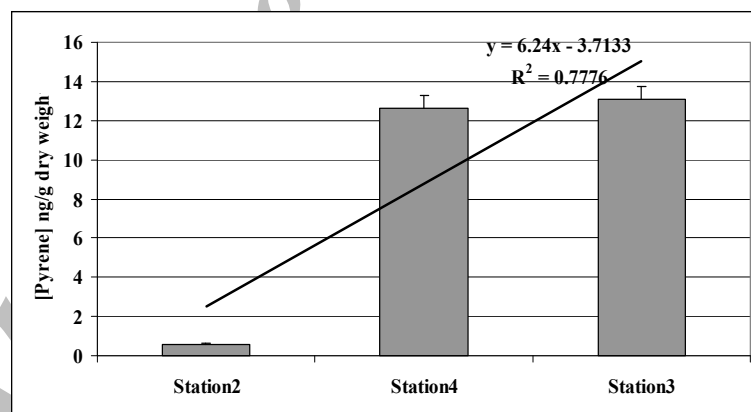


Figure 3: Pyrene concentration in sediments at different stations of Siahkashim Lagoon

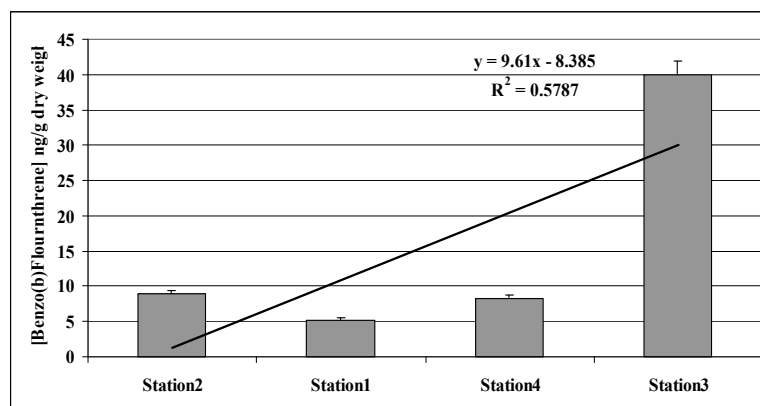


Figure 4: Benzo(b)Fluoranthene concentration in sediments at different stations of Siahkashim Lagoon

individual concentration for PAHs were observed to be 0.6 ng/gr dry weight sediment (ng/g dry wt sed) for Pyrene in station 3 and highest level was observed to be 39.98 for Benzo(b)Fluoranthene. The overall trend for increase of these three PAHs is from west to east and from south to north. Therefore northeastern stations (station 4 and 3) pose highest PAHs concentrations. Total organic matter (TOM) and total organic carbon (TOC) also showed highest scores in autumn and organic matter percentage was also high in autumn in every site (data not shown). Total PAH concentration in each sites varied between 11.32 (in station 1) to 63.74 ng/g dry wt in site 3 (Fig. 5).

The results have been shown that they significantly correlated with TOC (data not shown) which is in agreement with previous results e.g. (Batarseh et al., 2003).

On the other hand low molecular weight PAHs (LMWPAHs) such as Naphthalene are found at below detection limit concentrations while some medium molecular weight PAHs (MMWPAHs) such as Phenanthrene and Benzo (b) Fluoranthene exhibit higher concentrations in site 3 (Fig. 2 and Fig. 4), Pyrene which is a representative of higher molecular weight PAHs (HMWPAHs) shows concentration increase in all 4 sites (Fig. 6).

For total organic matter (TOM) spring and summer data is lower than autumn and winter data (results not shown) and this has been the result for selection of autumn as the representative season for investigation of PAHs in the sediments.

The individual concentrations for PAH which is shown in figure 6 indicated that the highest concentration is observed for Benzo(b) Fluoranthene (Fig. 6).

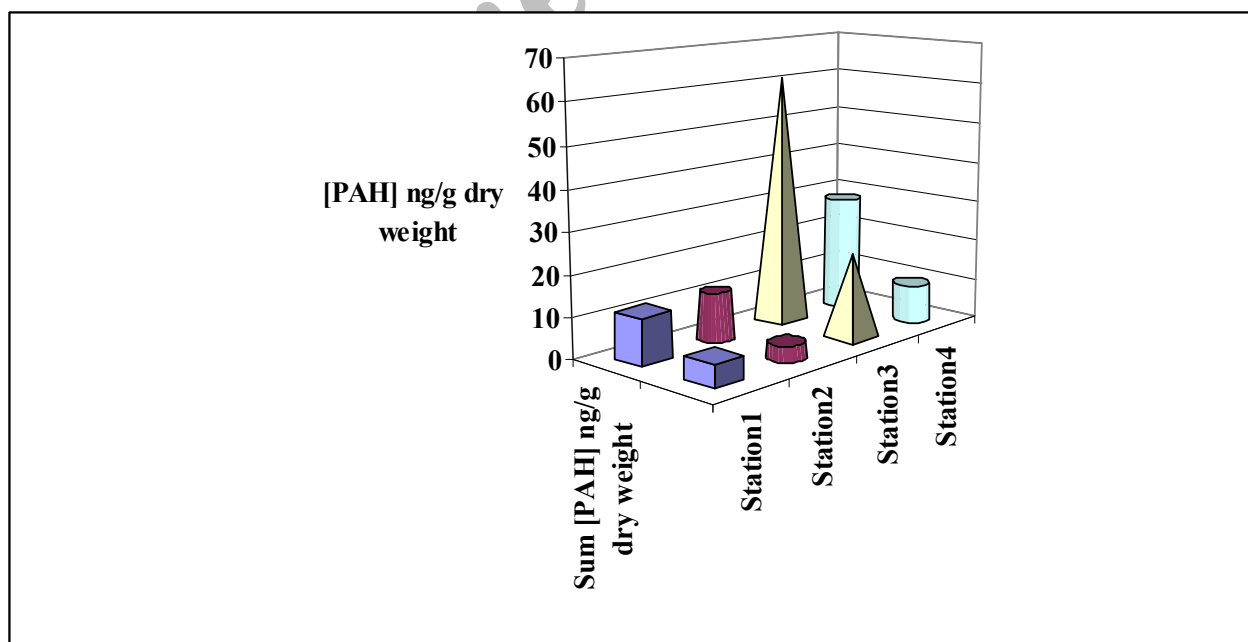


Figure 5: Spatial variation of PAHs in the sampling stations for total PAH concentration in the sites (1<sup>st</sup> row horizontal axis) and average PAH concentration (2<sup>nd</sup> row horizontal axis)

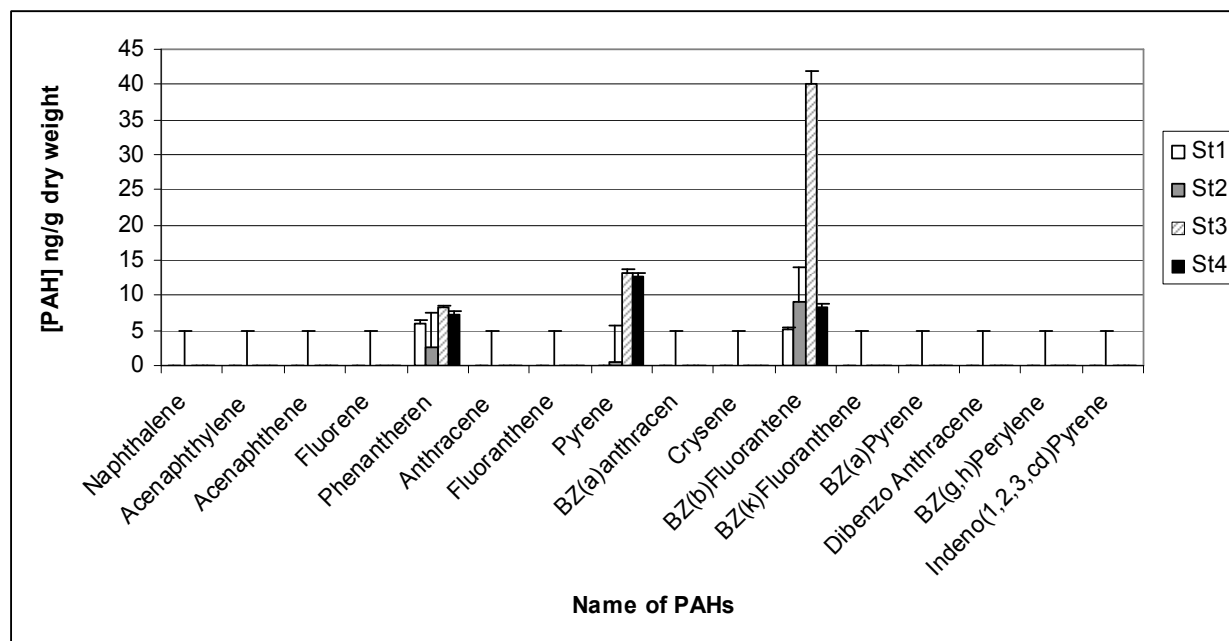


Figure 6: Average concentration of individual PAHs in Siahkashim Lagoon four sampling locations

## Discussion

Generally, concentration of PAH compounds level were much lower than other coastal and marine areas of the world (table 3). Therefore it can be concluded that in the aspect of PAHs the pollution situation in the studies area is not as bad that it has been reported by environmental authorities previously.

The amount LMWPAHs such as Naphthalene and Anthracene were found at low concentrations (Fig. 5) because they have short resident time (Bunce & Dryfhout, 1992). For naphthalene as a prototype PAH, the half-lives were of the order of several hours, but varied by more than an order of magnitude with season and geographical location.

In site 3 Phenanthrene and Benzo(b)Fluoranthene showed higher amounts than the other MMWPAHs (Fig. 2 and 4 and 6). It seems site 3 is more polluted than the other sites. This is probably due to vicinity of this site to urban runoff through rivers passing from Rasht city which is near to the disposal industrial and municipal waste products. In fact Phenanthrene is observed to be very high in PAH petrogenic pollution (similar reports has been seen in e.g. Webster, 2007; Xu, 2007; Yim *et al.*, 2007 and Zhu *et al.*, 2007, but lower in pyrolytic contamination cases such as Fluoranthene and Pyrene are often associated during natural matrices analyses (Socolo, 2000) and were considered as typical pyrogenic products derived from high-temperature condensation of lower molecular weight aromatic compounds. Therefore according to figure 3 when it is observed that Pyrene is increased on winter in sites 3 and 4 we can conclude that associated Pyrene cannot evaporate or dissolve in autumn by low temperature. Therefore the increase could be attributed to temperature decrease in the studied area. The average of PAHs indicates BZ(k) Fluoranthene is the highest (Fig. 6) and it can be resulted the BZ(k)Fluoranthene is a pyrogenic product that was derived from lower molecular weight aromatic compounds in high-temperature.



TOM on spring and summer data was lower than autumn and winter data (results not shown). We know that when Caspian Sea water level increases, Siahkashim Lagoon water level also increases and this process effect on biological activity. On the other hand, temperature is added in spring and summer and this is a parameter which increases primary production; so primary production increased by algae and plants in Siahkashim Lagoon, following it, organic matter is used rapidly, therefore TOM decreases in spring and summer. On the contrary, TOM increased in autumn and winter because primary production and use of organic matter decreased by low temperature of environment, so organic matter is higher than spring and summer. It is important that around Siahkashim Lagoon, there are a lot of macrophyte plants who can decompose during the sedimentation in the autumn and winter that caused TOM concentration increased.

Total PAH concentration in the each sites varies between 11.32 (in station 1) to 63.74 ng/g dry wt in site 3 (Fig. 5). This is higher than total [PAHs] concentrations reported for Volga delta on 1993 and North of the Shetland Islands But less higher than many cases reported in Table 3 e.g. Boston Harbor (USA) or Lazaret Bay (France), or Arcachon Bay in France (Tolosa *et al.*,2004).

Table 3.  $\Sigma$ PAHs concentrations in sediments from different locations

Area	Survey year	Concentration (ng g <sup>-1</sup> dry weight)	References
Boston Harbor, USA	1999	7300–358,000	Wang et al. (2001)
Lazaret Bay, France, (Mediterranean Sea)	1991	1600–48,090	Benlahcen et al.(1997)
Arcachon Bay, France, (Mediterranean Sea)	1996	900–4100	Baumard et al. (1998b)
Western Coast, Australia	1991	1–3200	Burt and Ebell (1995)
Coastline, Black Sea	1995	7–638	Readman et al. (2002)
Coastal area of the Adriatic Sea	1996	24–501	Magi et al. (2002)
Victoria Harbour, Hong Kong	1992	350–450	Hong et al. (1995)
Gironde estuary, (France -sandy sediments)	1993	19–252	Budzinski et al. (1997)
Balearic Islands, (Spain, Mediterranean Sea)	1996	mean 30	Baumard et al. (1998a)
Volga delta	1993	40	Winkels et al. (1998)
North of the Shetland Islands		≤40	Webster et al. (2001)
Caspian Sea, Azerbaijan	2000	338–2988	Tolosa et al. (2004)
Caspian Sea, Iran	2001	94–1789	Tolosa et al. (2004)
Caspian Sea, Russia	2000	6–345	Tolosa et al. (2004)
Caspian Sea, Kazakhstan	2001	7–294	Tolosa et al. (2004)

All of the observed sediment PAHs in this study belongs to MMWPAHs which their  $\log K_{ow}$  is between 2 to 5. As reported before, in order to be transferred in marine environments,  $\log K_{ow}$  should be between 2 to 5 (Incardona, *et al.*, 2004) or below 5.2 (Sverdrup, *et al.*, 2002); because the PAHs with higher values of  $K_{ow}$  failed to be directly toxic in marine environments due to their lower water solubility (Lee, *et al.*, 2004; Sverdrup, *et al.*, 2002). Although a insignificant correlation ( $[PAH] = 8.0126\log K_{ow} - 32.241$ ;  $R^2 = 0.7927$ ,  $p > 0.3$ ) could be observed between  $\log K_{ow}$  and the available PAHs (Fig. 7), the correlation coefficient is high enough to let us make this hypothesis that the concentration of PAHs in the studied area is a function of their octanol-water partition coefficient ( $K_{ow}$ ). Therefore we believe that within the capability of this study and our previous experiences, the concentration (i.e. availability and environmental toxicity) of PAHs in Siahkashim Lagoon is governed first by their water solubility and then their  $K_{ow}$ .

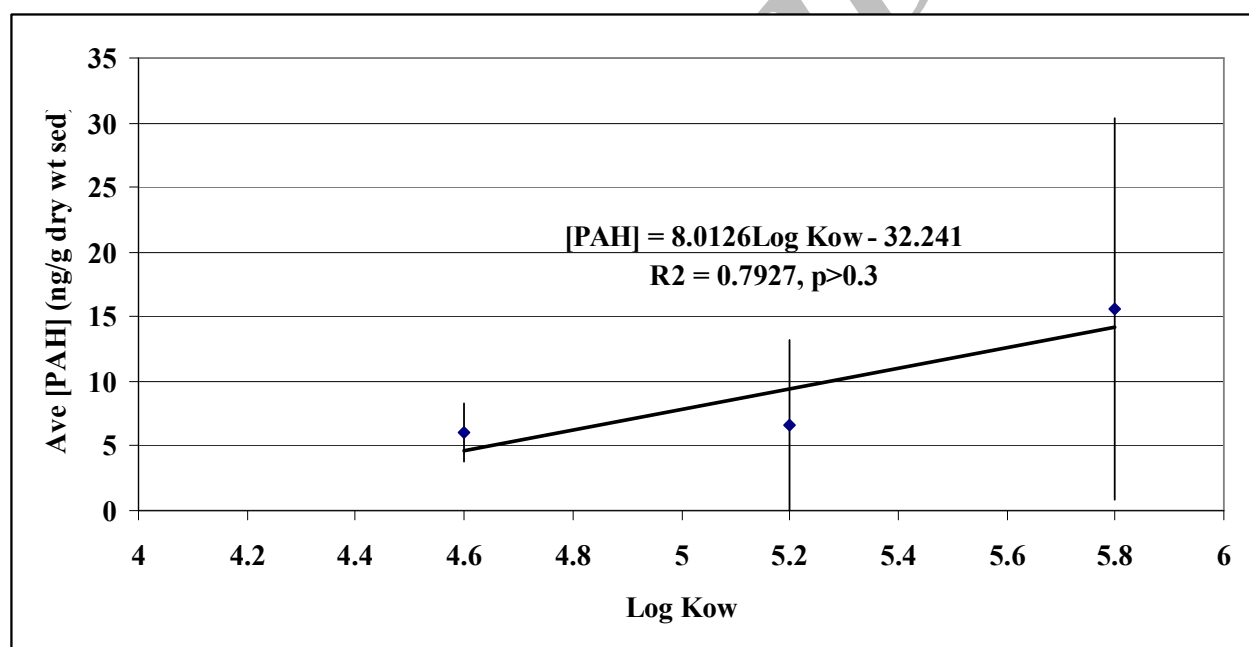


Figure 7. Increase of PAH concentration as a function of their Octanol-water partition coefficient ( $K_{ow}$ )

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