# Evaluation of Ark clam (Barbatia helblingii) as Biomonitor Agent for PAHs Contamination in Coastal Area of Bushehr

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Received: May, 2010 Accepted: Nov., 2010

**Introduction**

Polycyclic aromatic hydrocarbons (PAHs) are a major group of organic contaminants consisted of two or more benzene rings. Many of these compounds are potentially toxic for both human and aquatic life. Therefore, many studies have been carried out to investigate their toxicity in PAHs exposed organisms. Bushehr coastal area is impacted by PAHs compounds derived from human activities related to oil exploitation and export. In addition, it is also influenced by contaminations originated from industrial and municipal discharges. Since all mentioned contaminants are major sources of PAHs input in to the Bushehr marine environment, PAHs contamination of water and aquatic organisms in the area is an expected issue. The bivalve's metabolism system is not well developed to remove PAHs content from their bodies rapidly. These are filter feeder organisms with low mobility. These characteristics make them eligible candidates for biomonitoring of PAHs compounds in coastal ecosystems. Since Ark clam (*Barbatia helblingii*) is well distributed in Bushehr coasts, this investigation is carried out to evaluate the potential of this specie as a biomonitoring agent for PAHs contamination.

**Materials and Methods**

Seawater and clams were sampled from five different stations of intertidal zone, including Rafael, Sheghab, Abshirinkon, Industrial zone and Helyleh (stations 1 to 5) along Bushehr coasts (Figure 1). PAHs were extracted from seawater using hexane as solvent and separation funnel as apparatus. The extract was passed throughout anhydrous sodium sulphate followed by florsil column. In order to digest PAHs and extract them from clam's tissues, about 5 g of homogenised and freeze-dried soft tissue was digested in soxhlet apparatus with 200 ml of methanol and 20 ml of 2M KOH. Then PAHs were extracted by separation funnel containing 90 ml hexane and 30 ml water. The extracts were passed through clean-up column containing 10 mg Alumina, 10 mg silica and 10 mg anhydrous sodium sulphate. When the whole solvent was evaporated, 1 ml of acetonitril was added to each sample and the samples were transferred into the vials. PAHs concentration was detected by HPLC system, equipped with reversed-phase $C_{18}$ (4.5×250 mm) column and UV detector. The liquid gradient used was a mixture of acetonitril (60%) and water (40%), which was shifted to 100% acetonitril within 30 minutes.

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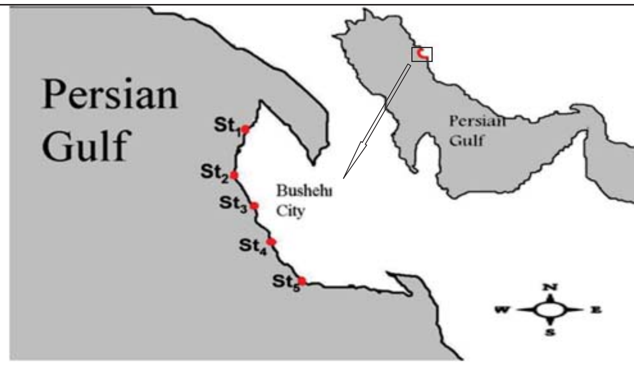


**Fig.1: Locations of the sampling stations.**

**Results and discussion**

Results showed that the PAHs concentrations in seawater samples taken from Rafael, Sheghab, Abshirinkon, Industrial zone and Helyleh stations were 31.03, 20.85, 4.04, 17.58 and 12.29 µg/l respectively (Figure 2-a). These levels are below the PAHs levels reported from Bandar Anzali lagoon (116.5), coastal waters in Bahrain (16-89) and China (4.70-67.70), while they were higher than the PAHs concentrations in the water samples from Chesapeake Bay (0.02-0.06), northwest of Baltic Sea (0.03-0.05) and Daya Bay in China (4.22-29.32) reported by previous studies.

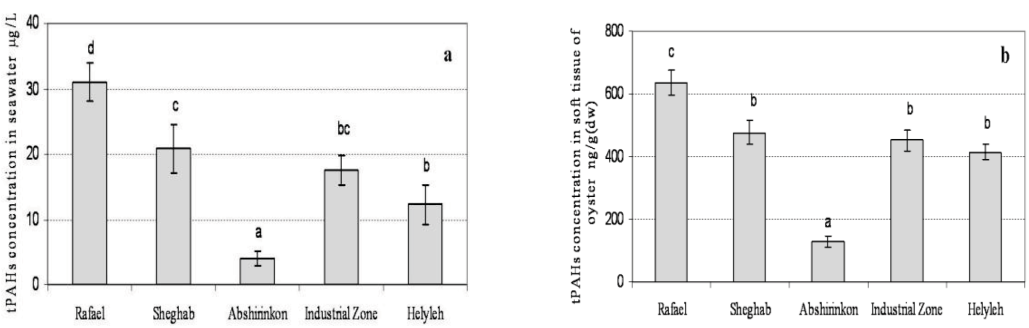


**Fig. 2:Total PAHs (tPAHs) concentration in seawater (a) and soft tissues of *B.helblingii* (b)**

Concentration of tPAHs in soft tissue of *B.helblingii* from the same stations was 634.75, 476.66, 129.54, 452.47 and 414.96 ng/g (dw) respectively (Figure 2-b). Significant differences were found between tPAHs concentrations in both seawater and clam samples collected from different stations ($P<0.05$). The maximum PAHs concentration in seawater was measured in Rafael station, while the minimum was observed in Abshirinkon. The Rafael station is exposed to municipal and harbor wastes. Low PAHs concentration in Abshirinkon station might be attributed to its long distance from urban area and harbors. The molecular composition of PAHs in seawater and clams were different to some extent. Generally 3-ringed PAHs molecules were dominant in water samples, while 5+6-ringed PAHs molecules were scarce. The same pattern of molecular composition was also observed in clam's soft tissue. The only exception was found in the soft tissues collected from Helyleh station in which 4-ringed PAHs were dominant compared to 3-ringed compounds (Table 2). Generally it seems that the amount of clams accumulated by low molecular weighted PAHs (3 and 4-ringed) was more than high molecular weighted compounds (5 and 6-ringed).

**Table 2: PAHs concentration in soft tissues of Ark clam (*B.helblingii*) and seawater sampled from Busher, the Persian Gulf**

| Stations | Rafael | Sheghab | Abshirinkon | Industrial Zone | Helyleh |
|---|---|---|---|---|---|
| *Seawater (µg/l)* | | | | | |
| Σ3 ring PAHs | 25.09±2.33 | 12.45±2.33 | 3.60±0.99 | 13.12±1.60 | 8.34±2.30 |
| Σ4 ring PAHs | 5.94±0.66 | 8.40±1.26 | 0.48±0.17 | 2.81±0.29 | 3.54±0.60 |
| Σ5+6 ring PAHs | - | - | - | 1.54±0.18 | 0.41±0.08 |
| tPAHs | 31.03±2.99 | 20.85±3.59 | 4.04±1.16 | 17.58±2.29 | 12.29±3.05 |
| *B.helblingii ng/g(dw)* | | | | | |
| Σ3 ring PAHs | 352.24±23.78 | 224.56±16.19 | 68.24±8.27 | 247.96±20.38 | 153.37±10.37 |
| Σ4 ring PAHs | 243.77±16.62 | 223.54±17.07 | 44.01±8.15 | 136.54±10.02 | 261.59±14.92 |
| Σ5+6 ring PAHs | 38.74±3.02 | 28.56±4.47 | 17.28±1.69 | 67.97±2.80 | - |
| tPAHs | 634.75±43.43 | 476.66±37.74 | 129.54±18.10 | 452.47±33.14 | 414.96±25.30 |

A significant linear correlation was found between tPAHs concentrations in seawater and clam's tissues ($P<0.05$). The same correlation was also found for 3-ringed, 4-ringed, and 5+6-ringed PAHs in seawater and clams. Correlations between PAHs concentrations in seawater and soft tissues of clams are shown in Figure 3. Although it seems that the value of Pearson correlation coefficient between PAHs concentrations in water and clam's tissues is negatively influenced by the number of aromatic rings existing in PAHs molecules, all correlations were positive and significant ($P<0.05$).

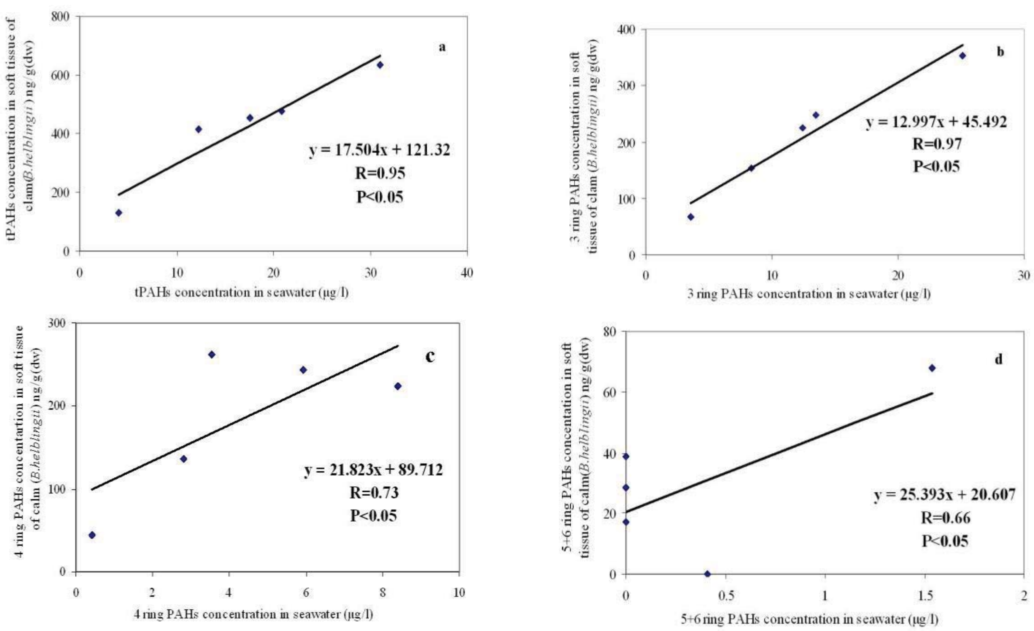


**Fig. 3: Correlation between PAHs concentrations in seawater and soft tissues of clams (P<0.05), a: tPAHs, b: 3-ringed PAHs, c: 4-ringed PAHs and d: 5+6- ringed PAHs.**

The clam *B.helblingii* is well distributed in the north Persian Gulf. It could be sampled easily and provides sufficient amounts of soft tissues for PAHs analysis. According to all mentioned advantages it is concluded that *B.helblingii* could serve as suitable biomonitoring agent for PAHs contamination in Persian Gulf.

**Key words**
Biomonitoring, PAHs, Ark clam (*B.helblingii*), Bushehr coasts, Persian Gulf