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Direct Determination of 1-Hydroxypyrene in Fish from Coastal Water by Synchronous Fluorimetry

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Polynuclear aromatic hydrocarbons (PAHs) are ubiquitous contaminants in marine environments as a result of fossil fuel utilization, river transportation, surface runoff and atmospheric deposition. Due to the carcinogenic nature of certain PAHs, great attentions have been concerned. Based on their physicochemical properties, PAHs are distributed among different biotic and abiotic ecosystems (Utvik *et al.*, 1999). Although most of PAHs are likely to bind to sediment particles in the aquatic environment, they are still being bioavailable to marine organisms (Brookes *et al.*, 1979). This implies that PAHs could be harmful to human beings by consumption of fish accumulating PAHs through food chain.

During the last decade, many efforts have been put to develop approaches for studying on the input, fluxes and fate of PAHs in marine environment. However, in order to assess their effects on marine ecosystem, it is very essential for marine environmental scientists to know the ecotoxicological effects of those compounds that can be taken up by aquatic biota (Escartin *et al.,* 1999). Routine monitoring of PAHs levels in the aquatic environment usually involves the determination of concentration of parent PAHs in seawater, sediment samples, and in marine organism tissues. However, it is known that only the bioavailable fraction of PAHs cause ecotoxicological effects. It was reported that even though fish caught at highly polluted area often showed too trace level of biovailable PAHs to be measured in the tissues, due to their ability to metabolize these compounds (Varanasi *et al.*, 1989). PAHs within the organisms are subjected to oxidation and conjugation reactions that will facilitate their excretion. Thus, alternative techniques, such as the determination of PAHs excreted through the bile as conjugated metabolites, have been developed for assessing PAHs exposure in fish. Several reports have demonstrated that the presence of PAHs metabolites in fish bile was well correlated with levels of exposure (Escartin *et al.,* 1999). This trend has been also corroborated in a number of field studies (McDonald *et al.*, 1995). Therefore, the biomonitoring of PAHs uptake should be concerned on the

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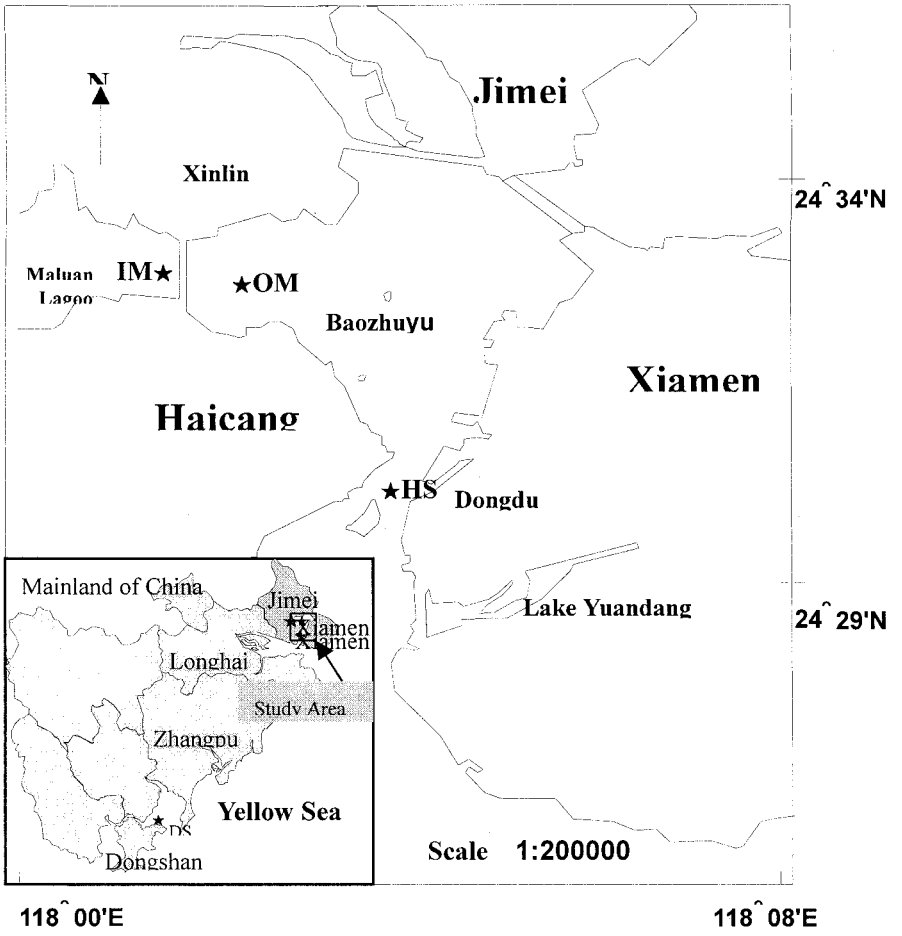

Figure 1. Map of sampling stations (★) at Xiamen Harbor and Dongshan island.

determination of PAHs metabolites in excreta (Krahn *et al.*, 1993; Ariese *et al.*, 1993;Hollender *et al.*, 2000). Compared to the available methods for determination of 1-hydroxypyrene (1-HP) in fish bile, synchronous fluorimetry (SF) was proposed as a method of analysis that offers the advantages of excellent linear ranges, limits of detection at ng/mL level and operation benefit (Warner *et al.*, 1996).

In this study, SF has been used to determine 1-HP in raw fish bile. Two species of fish mostly cultured in Xiamen, *Pagrosomus major (Pm)* and *Nibea miichthys (Nm),* which were cultivated for 56 d during experimental period at four sites of maricultural area in Yellow Sea, were chosen and examined to assess the degree of exposure to PAHs at Xiamen Harbor.

MATERIALS AND METHODS

Analytical grades of ethanol (A.R, Beijing Reagents Co.), 1-HP (Aldrich) and Milli-Q water have been used throughout the experiment. A fluorescence spectrophotometer (Hitachi-850) was used for all spectra measurements. Both excitation and emission slits were fixed at 5nm and the scan speed was set at 120nm/min. A 1cm pathlength quartz cell was stored in ethanol and rinsed three times with Milli-Q water before use. A synchronous fluorescence scanning was carried out with $\Delta\lambda$=37nm. Based on the relationship between concentrations of 1-HP in ethanol aqueous solution (1:1,V/V) and their SF intensity, standard calibration curve was obtained and the calibration equation was formulated.

Living fishes of *Pm* and *Nm* were taken from the control station near Dongshan (DS) island where known to be relatively unpolluted from PAHs, and were cultivated at four stations including DS as showed in Fig.1, near Xiamen Harbor from March to May, 2000. At each station, about 40 to 50 fish of each species were placed in two cages with a dimension of 3 (length) x 3 (width) x 8 (height) m. After 56 d, six fish of each species from each station were killed by severing the spinal cord, the gall bladder were immediately dissected and placed into an Eppendrof tube and then stored in a liquid nitrogen container. After being transported to the laboratory, all raw fish bile of the same species from each station were mixed and diluted 500 to 2000 times with ethanol aqueous solution. The concentrations of 1-HP in the raw fish bile samples were determined directly by SF.

RESULTS AND DISCUSSION

The bile of PAHs-exposed fish contains a multitude of oxygenated PAHs derivatives, usually in the form of glucurnide, sulfate, or glutathion conjugates. It is well known that a complete analysis of all detectable PAHs metabolites would be rather difficult, costly and time consuming (Kuijt et al., 2001). However, if one was merely interested in monitoring the relative PAHs stress at a particular location, it would be advantageous to introduce a single parameter that is indicative of the overall PAHs uptake. Up to now, three different methodologies were emphasized on different aspects, (1) levels of fluorescent aromatic compounds in raw bile samples, (2) levels of fluorescent aromatic compounds in hydrolyzed bile samples, and (3) determination of selected hydroxylated metabolites by GC-MS spectroscopy (Aas et al., 2000). According to the above reported methods, a fast scanning of SF was established to determine levels of fluorescent aromatic compounds representing the unconjugate 1-HP in raw fish bile samples in the laboratory.

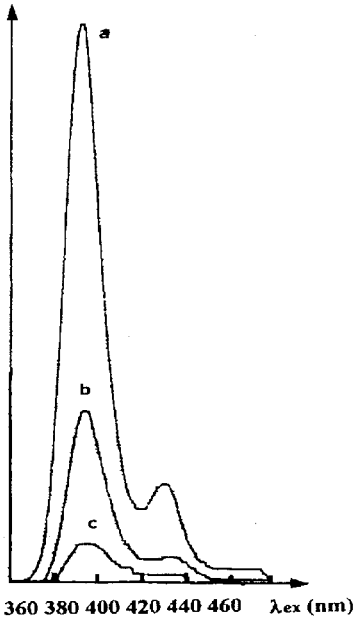

Figure 2. Synchronous fluorescence spectra of 1-HP in ethanol-water (V/V=1:1) solution. Concentrations of 1-HP: a=60ng/mL; b=20ng/mL; c=5ng/mL.

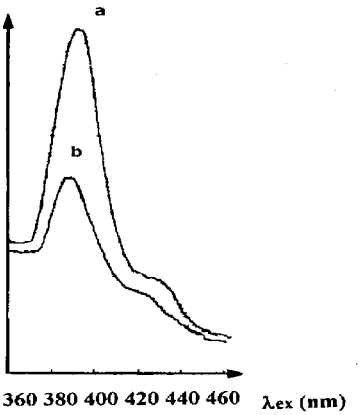

Figure 3. Synchronous fluorescence spectra of 1-HP in fish bile prepared from different sampling stations: a, inside of Maluan dam(IM), b, outside of Maluan dam(OM).

Using this method, main aim was focused on the fast scanning of the difference of PAHs exposure at different stations in Fig.1. The excitation and emission spectra of 2.5×10^{-4} mol/L 1-HP in ethanol aqueous solution were scanned. It was found that maximal wavelength of excitation and emission was located at 315nm and 410nm, respectively. Fig.2 showed SF spectra of 1-HP obtained with $\Delta\lambda = 37$ nm. The maximal SF peak of 1-HP was located at 395nm. At the same time, it could be found that the SF height at 395nm was increased proportionally depending on the increment of concentrations of 1-HP.

Under the optimal conditions, calibration curve of 1-HP was obtained. The graph was linear in the range of 0 - 87 ng/mL and the regression equation was $y = 0.0542x - 0.0253$ ($r^2 = 0.9996$), where x is the concentration of 1-HP in ng/mL. The detection limit was found to be 0.69 ng/mL with the relative standard deviation less than 2.46% (n=6).

Fig.3 showed the SF spectra of raw fish bile collected from two different

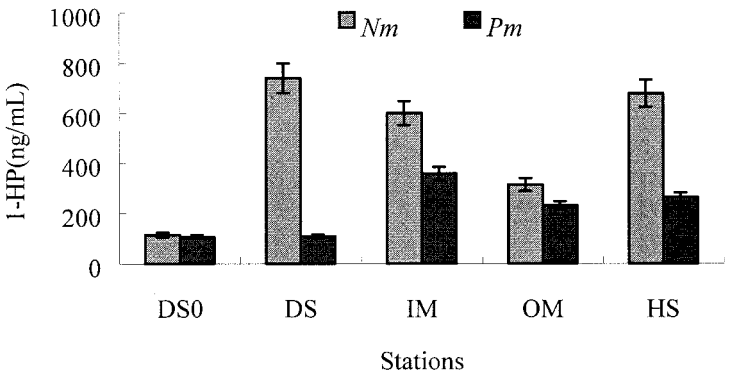

Figure 4. 1-HP concentrations in *Nm* and *Pm* cultivated at four stations DS, IM, OM and HS after 56 d of exposure. DS0 was determined before exposure.

sampling stations at the same day. The shape and λ_{max} were almost as same as those of standard 1-HP. The only difference between spectra a and b was their peak height. This might imply a very useful, fast and easy monitoring method to make a judgment on the difference of bioavailability of PAHs in coastal environments where the fish were cultivated. For this reason, all of the raw fish bile samples, which were collected from the four designed stations, were examined by the established method. Experimental results were showed in Fig.4.

These data demonstrated that the stations IM and HS were more polluted than station OM with PAHs. Zhou et al. (2000) and Maskaoui et al. (2002) studied on the concentrations of total PAHs including pyrene in surface water at Xiamen harbor during the consecutive two yr. Their experimental results showed that total PAHs concentrations varied form 106 to 945ng/L and from 909 to 1330ng/L, pyrene concentrations varied from <1 to 481ng/L and from 220 to 1840ng/L among different stations in 1998 and 1999, respectively. Among these data the concentration of pyrene at stations 4 and 5 were 56 and 10ng/L (Zhou et al., 2000). It was evident that there was severe seasonal fluctuation of total PAHs or pyrene in seawater. The locations of HS and OM stations in the present work were as same as the stations number of 4 and 5 in Zhou's work, respectively. Our experimental results represented that 1-HP concentrations from *Pm* and *Nm* fish bile at HS, 263 and 676ng/L, were higher than those concentrations at OM, 231and 314ng/L, respectively. *Nm*'s results were higher than those of *Pm* from all four stations. One plausible explanation was that *Nm* is likely to live in the upper layer of water than *Pm*. Compared to cited both results, an identical tendency was confirmed and it was demonstrated again that the presence of PAHs metabolites in fish bile was correlated with levels of PAHs pollution at HS and OM stations. On the other hand, as a biomonitoring parameter, direct monitor 1-HP concentration

in raw fish bile showed a more reliable way to evaluate the bioavailablity of PAHs pollution in the research area. Because the sampling period lasted 56 d, the experimental results could not be greatly affected by some accidental events. Unexplainably, *Nm* from control station (DS) showed relatively higher concentration of 1-HP after 56 d. However, the initial concentrations of 1-HP (DS0), 107 and 114 ng/L, were relatively low and it also could not be found difference between *Pm* and *Nm* raw fish bile at the beginning of our experiment. So, it was believed that *Pm* gave more reliable result than that of *Nm* when 1-HP in raw fish bile was directly determined by SF indicating the difference of bioavailability of PAHs pollution in maricultural area. From these results it could be concluded that SF method, as one of less laborious, time saving and cost benefit methods, showed potential to assess the degree of exposure to PAHs in the coastal zone.

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