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COASTAL POLLUTION BY POLYNUCLEAR AROMATIC HYDROCARBONS (PAHs) IN THE EASTERN MEDITERRANEAN

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ABSTRACT

PAHs in the northeastern Mediterranean have been monitored throughout the decade. The surface waters have maintained an average concentration of 0.24 µg PAH/L; however, average concentrations of PAH in the underlying surface sediment have increased from 0.5 to 3.7 µg/g. Fish flesh contains 1-15 µg PAH/g. Several discharges of domestic and industrial waste cause localised pollution of up to 4.5mg PAH/L. Comparison of Grey Mullet (Mugil sp.) inhabitting two harbours showed those in the more polluted harbour to be the larger and to have proportionately larger livers containing more protein. Measurements of enzyme activities showed that the greater pollution induced greater metabolism of xenobiotics. Nevertheless, ^{32}P postlabelling demonstrated that in 1994-1996 grey mullet livers in the more polluted harbour possessed 50 to 250 DNA-adducts per 10^8 nucleotides whereas livers from grey mullet in the less polluted harbour possessed only 3 to 20 DNA-adducts per 10^8 nucleotides.

KEYWORDS

DNA-adducts; eastern Mediterranean; enzymes; grey mullet: Mugil sp.; PAHs; pollution; Polynuclear Aromatic Hydrocarbons; ^{32}P postlabelling.

INTRODUCTION

METU has now monitored PAH concentrations in the eastern Mediterranean environment for more than 10 years as part of the MED POL programme. The region includes busy, moderately sized ports, two small oil refineries and several discharges of domestic and industrial waste. In the vicinity of the discharges PAH concentrations are high. The long term toxicological effects of the steady concentration of PAHs resulting from their persistent spillage and seepage into the marine environment are difficult to assess (Nishimoto, et al., 1991). It is well established that many PAHs at large in the marine environment are carcinogenic (Carmichael et al., 1990; Gupta et al., 1982; Hawkins et al., 1990; Heinrich et al., 1985) and the stages by which fish develop carcinogenesis involve;
1- assimilation of PAHs from the marine environment,
2- metabolic oxidation of the assimilated PAHs , ultimately to give dihydrodiol epoxides (Sims and Grover, 1981; UNEP, 1992).
3- the formation of aromatic DNA-adducts by the covalent addition of the dihydrodiol epoxides to cellular DNA,
4- the partial amelioration of adduct formation by the repair of the DNA (Bailey et al., 1987, 1988; Brookes et al., 1985; Hawkins et al., 1990; Kurukawa et al., 1990; Malins et al., 1990; Miller and Miller, 1981).

The induced activity of enzymes metabolising xenobiotics and the detection of DNA-adducts in fish may therefore be a means of monitoring their exposure to carcinogens (Hawkins et al., 1990). Indeed, the measurement of the concentrations of specific adducts could provide a key to the determination of the biological effects of the genotoxic agents since the adducts are the direct consequence of the assimilation of the individual agent from the environment (Kurukawa et al., 1990; Maddock et al., 1986).
Here we summarise the concentration of PAHs in the eastern Mediterranean during the past decade and record the biochemical effects of PAH concentrations observed in Grey Mullet (Mugil sp.)- species of pelagic/benthic fish common in the Mediterranean as well as elsewhere. Grey mullet are essentially herbivorous but possess a gizzard and ingest sediment. The species satisfy all save two of the eight characteristics suggested by Uthe, (1994), as necessary for selection as a monitoring organism. The species are not sedentary and in the present study two harbours have been selected which confine the region in which the fish can swim.

MATERIAL AND METHODS

Surface water, sediment and biota were sampled for subsequent analysis of PAHs during seasonal cruises by R/V Bilim throughout the eastern Mediterranean. The location of sampling sites and the standard methods of extraction and determination of PAHs have been given elsewhere (Sakarya, 1985; UNEP, 1992, 1994, 1995; Yilmaz et al., 1991, 1997).

Grey mullet (Mugil sp) were sampled at two north eastern Mediterranean sites, the harbour of METU's Institute of Marine Sciences, which is relatively unpolluted and was therefore used as a control and the harbour of Mersin port where sources of obvious pollution, of concern to the municipality, include a discharge with a high PAH content. Table 1 summarises the characteristics of the sites.
Fish were caught and killed. The length, weight and sex of the fish were noted. Samples of liver were separated from fresh specimens and were then labelled and refrigerated until their DNA could be extracted. Fish livers were homogenised in ice cold 1-1.5% KCl and protein concentrations were determined by Lowry's (1951) procedure. Aliquots of the crude homogenate were centrifuged at 19,000g at 4C. The supernatants were retained for the following assays: Glutathione peroxidase (GSH-P) (Beutler, 1984), Glutathione reductase (GSSG-R) (Beutler, 1984), Superoxide dismutase (SOD) (Randox, 19ab) and Malondialdehyde (MDA) (Bus and Gibson, 1979; Okhawa et al., 1979). DNA was isolated from fish liver according to the procedure of Gupta (1984). DNA adducts were determined by the 32P postlabelling technique discussed in detail by Gupta, (1993); Phillips and Castegnaro (1993), and Reddy (1993).

Table 1. Characterisation of sampling sites for grey mullet.

Site	Annual Salinity (‰)	Annual T°C	Dissolved Oxygen (µg/L)	PAH (µg/L)
Mersin	32-29	23-25	9.5-9.7	0.4-355*
METU	38	15-30	7.1-7.3	1.0

*in the vicinity of a discharge of waste.

RESULTS AND DISCUSSION

Table 2 shows eastern Mediterranean water to have remained unpolluted throughout the decade. Twelve discharges of domestic and industrial waste, monitored regularly, introduce concentrations of PAH varying widely from zero to 4500 µg/L into the eastern Mediterranean but these inputs are localised. During the decade PAH concentrations in sediments have been rising from 0.51 to 3.7 (µg/g dry wt); accordingly eastern Mediterranean sediments are moderately contaminated (UNEP, 1994). One hypothesises that the coastal waters are being kept clean by perpetual scrubbing by sediments (cf. Bouloubassi and Saliot, 1991).

Table 2. Average PAH concentrations in the northeastern Mediteranean, 1985-1996

Samples	Year	Year	Year	Year
Surface sea water µg/L	0.24±0.05			
Surface sediment: (µg/g dry wt.)		1985-1986	1995-1996	
		0.51±0.10	3.7±1.0	
Fish flesh (mixed species) (µg/g dry wt.)	1987	1991	1995	1996
	10-14 (n=10)	1.1-1.6 (n=8)	2.8±0.5 (n=15)	6.4-8.7 (n=23)

n: Number of sample

Flesh of eastern Mediteranean fish have contained from 1 to 14 µg of PAH /g dry wt. Table 3 examines the consequences of this in Grey mullet inhabitting METU and Mersin harbours in 1994. Table 3 shows the concentrations of DNA-adducts found in liver samples from these reference and polluted areas. In terms of adduct formation there was a homogeneous Gaussian fish population in the reference harbour with a low level of adduct formation.The average concentration of adducts in the fish from the polluted region was 2.5 times greater than in the fish from the reference harbour and the difference was significant at the 95% level. The distribution of adducts was non Gaussian. One is obviously concerned for the future health of the fish population in the polluted regions.

Table 3. Reference and polluted areas, 1994: DNA-adducts (per 10*8 nucleotides) Grey Mullet livers.

Sample No.	Reference area (METU)	Polluted area (Mersin)
1	28	109
2	22.4	9.2
3	32.9	92.3
4	23.1	4.2
5	13.8	36.4
6	17.4	54
7	24.6	12.4
8	18.2	66.5
9	13.6	6.3
10	20.3	6.7
11	15.2	12.8
12	10.7	140.1
13	27.2	102.5
14	17.3	108.4
15	-	24.5
Mean±SE	20±2	52±12

Table 4 shows a further comparison of the mullet in the two harbours in 1995/1996. The species of grey mullet in Mersin harbour were defined and DNA-adduct concentrations were complemented by studies of the regulation of xenobiotics. All the fish examined were superficially healthy. The Table shows that the mullet observed in Mersin harbour were bigger, had proportionally larger livers and had more protein in the livers. The higher GSH-P and GSSG-R activities observed in the livers of mullet from Mersin harbour testify to the greater metabolism of xenobiotics induced by the pollution. The higher concentration of MDA, an end product of the oxidation of lipids, in livers from the Mersin mullet is also consonant with greater metabolic activity in removing hydrocarbon pollutants. Even the observed reduction in SOD activity can be associated with the presence of increased organic pollution (Bagnasco et al., 1991). It is clear that the mullet living in the richer but more polluted Mersin harbour grew larger and grew larger livers which endeavoured to metabolise and mitigate the intrusion of PAHs. Ultimately, however, the stress on the livers as expressed by DNA-adduct formation was much greater than in the reference harbour.

CONCLUSIONS

Although PAH concentrations in the surface of the northeastern Mediterranean remain low, the underlying surface sediments are now moderately polluted and one suggests that the water column remains clean because it is continuously scrubbed by sediment. This is consistent with the rather localised pollution (and its apparent rapid dispersal) observed in the vicinity of discharges of waste.

Fish flesh in the region now contains from 1 to 15 μg of PAH/g. Fish can frequently be observed in the neighbourhood of discharges and our investigation of grey mullet in Mersin harbour is important in establishing the physiological impact of the pollution on fish livers. The fish appear to enjoy increased nutrient; the xenobiotics induce remedial enzyme activity but nevertheless, the fish livers suffer concentrations of DNA-adducts in their liver which presage carcinogenesis.

Table 4. Reference and polluted areas, 1995/1996: Enzyme activities (IU/g liver) and DNA-adducts (per 10^8 nucleotides) in grey mullet livers.

Species	Lisa ramada	Oedalechilus labeo	Mugil sp.
Harbour	Mersin	Mersin	METU
TFW(g)	453±25	453±25	301±35
(n)	(n=27)	(n=27)	(n=16)
g liv/g fish	0.018	0.018	0.013
g pro./g liv	0.35±0.03	0.35±0.03	0.23±0.015
GSH-P	9.4±0.5	8.7±0.7	3.7±0.4
(n)	(n=15)	(n=11)	(n=14)
GSSG-R	2.3±0.1	2.8±0.1	1.4±0.1
(n)	(n=15)	(n=11)	(n=15)
SOD	4.2±0.4	4.4±0.5	6.1±0.25
(n)	(n=13)	(n=11)	(n=15)
MDA	0.45±0.07	0.43±0.05	0.2±0.015
(n)	(n=12)	(n=10)	(n=14)
DNA-adducts	130±37	258±21	3.3±2.3
(n)	(n=14)	(n=6)	(n=10)

TFW: Total fish weight
g pro/g liv:g protein/g liver
The means of samples from Mersin and METU are always significantly different; $p<0.025$ (single tailed Student's t test)

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ABSTRACTS

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