CYP1A and other biomarker responses to effluents from a textile mill in the Volta River (Ghana) using caged tilapia (*Oreochromis niloticus*) and sediment-exposed mudfish (*Clarias anguillaris*)

Bernard Kwaku-Mensah Gadagbui and Anders Goksøyr

The ecotoxicological effects of a textile mill effluent were investigated by caging tilapia (*Oreochromis niloticus*) in the Volta River, Ghana, and by exposing mudfish (*Clarias anguillaris*) to sediment collected from the same river. Tilapia were caged for 3 weeks at three locations (0.6, 4, and 8 km) downstream from the effluent outlet. Mudfish were exposed in the laboratory for 2 weeks to sediment collected from the vicinity of the effluent outlet and 8 km downstream. Upstream reference locations 2 km (tilapia) and 10.2 km (mudfish) were included. Liver cytochrome P4501A (CYP1A) monooxygenase activity (measured as activity of 7-ethoxyresorufin *O*-deethylase, EROD, and CYP1A protein level) and two conjugation enzymes, UDP-glucuronosyl transferase (UDP-GT) and glutathione *S*-transferase (GST), were analysed. A distance-related decrease in EROD activity and CYP1A protein level was observed. EROD activity was 21-fold higher in tilapia caged at the site nearest the effluent outlet and 25-fold higher in mudfish exposed to sediment collected from the vicinity of the outlet, compared with the respective reference values. UDP-GT and GST levels increased significantly by 70 and 27%, respectively, in tilapia while the respective levels in mudfish were 73 and 28%, compared with reference values. The results clearly indicate that the textile mill effluent contains some highly potent inducers of biotransformation enzymes. This first assessment of the biological effects of organic pollutants in the Volta River demonstrates the utility of the CYP1A system as a valuable early warning biomarker of industrial effluents and also as a biomarker to detect exposure of aquatic resources to environmental chemical contamination in tropical waters.

Keywords: *textile mill effluents, caging, pollution monitoring, biotransformation enzymes, cytochrome P450 1A.*

Introduction

Production of textiles requires use of considerable volumes of water during scouring, bleaching, dyeing, rinsing and other finishing processes. Hence, textile mills are sited so as to ensure an adequate supply of water and a means of effluent disposal. Textile wastewaters are generally characterized by high concentrations of chemical oxygen demand (COD), biological oxygen demand (BOD), suspended solids, extreme pH, and elevated temperatures. The effluents can contain a cocktail of bleaches, detergents, dyes, fluorescent whitening agents, resins, fire, rot and waterproofing agents, pesticides and dispersants and many other organic and inorganic chemicals, including some metals (EPA 1978, Chen 1989, Rutherford *et al.* 1992).

Foaming from detergents and dyes may cause aesthetic problems and preclude downstream use of the water. Carrier chemicals used in dyeing may impart unpleasant tastes and odours to water and fish. Oil and grease and acidic or alkaline wastes can also be harmful to aquatic biological systems. In addition, many of the dyes used in textile mills are known to be mutagenic and some are suspected of being carcinogenic. Untreated textile effluents may therefore pose a hazard to aquatic life and possibly to human health. The observed effects on fish include significant reduction in oxidative enzymes and tissue respiration (Sakthivel *et al.* 1991), histopathological (Murugesan and Haniffa 1992) and haematological changes (Haniffa and Vijayarani 1989), mortality, reduction in food intake, growth rate and conversion efficiency (Sakthivel and Sampath 1989).

The induction of the cytochrome P450 1A (CYP1A) system in different fish species has been widely used as a biomarker of contamination in the aquatic environment (Goksøyr and Förlin 1992, Stegeman 1993, Bucheli and Fent 1995). UDP-glucuronosyl transferases (UDP-GTs; ECC 2.4.1.17) and glutathione S-transferases (GSTs; EC 2.5.1.18), the two most widely studied phase II (conjugation) enzymes, may indicate the ability of fish to adapt to polluted environments (George 1994) and are being investigated for their usefulness as possible biomarkers.

In field studies, catching feral fish is often problematic in terms of catching the right number, size and species of fish, establishing a cause–effect relationship as well as preventing non-sedentary organisms from migrating away from pollutants or into the area. An alternative to this approach is caging. Here, a genetically homogeneous group of fish can be subdivided and placed at different locations in a suspected pollution gradient or receiving locale (Goksøyr 1995). Caging has been employed in several biomarker studies in fish (e.g. Derksen 1991, Haasch *et al.* 1993, Goksøyr *et al.* 1994, Beyer *et al.* 1996). Studies undertaken in freshwater systems have indicated the induction of CYP1A in caged fish exposed to organic pollutants in industrial effluents (Lindström-Seppä and Oikari 1989, 1990a, b, Haasch *et al.* 1993, Soimasuo *et al.* 1995).

The objective of this study was to investigate the effects of a textile mill effluent on biochemical responses associated with xenobiotic metabolism in fish. These responses were measured after caging exposure of farm-reared tilapia (*Oreochromis niloticus*) in the Volta River, Ghana, at three different distances from the effluent outlet of a textile mill, and after laboratory exposure of farm-reared mudfish (*Clarias anguillaris*) to sediments collected from two locations in the same river. The responses in tilapia were compared with corresponding responses in a reference group caged at a presumed clean site and those in mudfish to a reference group exposed to sediment

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from a presumed clean site. Tilapia and mudfish were chosen because they are pelagic and benthic species, respectively. Mudfish were exposed to sediment because caging on site failed. In this study, we measured liver biotransformation enzymes (CYP1A and phase II-associated enzymes) and fluorescent bile metabolites of polycyclic aromatic hydrocarbons (PAHs) (metabolites were measured instead of the parent compounds because PAHs are readily metabolized, resulting in low tissue parent compound levels). In addition, levels of polychlorinated biphenyls (PCBs), DDT and some non-DDT pesticides were analysed in the liver. In an earlier study, these fish species were found to be responsive to typical organic xenobiotic and model CYP1A inducers such as β-naphthoflavone (BNF) and a commercial PCB mixture, Clophen A50 (Clo A50) (Gadagbui *et al.* 1996). The present study served as field assessment to determine the utility of several biomarkers as valuable early warning systems for exposure of aquatic fish resources to contamination in tropical waters.

MATERIALS AND METHODS

Study area

The Volta River (Figure 1) originates in Burkina Faso, West Africa, spans the entire length of eastern Ghana, and enters the Gulf of Guinea at 0° 42′ E and 5° 56′N. At Akosombo, 100 km from the coast, the Volta Hydroelectric dam has created one of the largest man-made lakes in the world, covering an area of about 8316 km² (Obeng 1975). For the first 12 km below the dam, the river is in effect a very narrow natural lake—no wider than a river but vastly deeper—blocked at the lower end by the rocky channels of the Senchi rapids.

The study area stretches from Site C1 (reference site in the lake) to Site S4. Sites C2 to S4 comprise the semi-stagnant stretches of the river water below the dam. Land use around the study area is mainly agricultural. Industry is not a significant activity with the exception of a textile mill located about 4 km from the dam which discharges its effluent into the river through a single outlet. About 2 km upstream of the mill outlet is located a community sewage outlet which empties its contents into the river after biological treatment. The sites were chosen so as to determine a possible pollution gradient in the river. Caging sites are indicated as C2, S2, S3 and S4 while the sites from which the sediments were collected are represented as C1, S1 and S4. S1 is also the point at which the waste water is released into the river. Sites C1 and C2 refer to caging and sediment-exposed sites presumed to be clean.

Fish and experimental design

Farm-reared male tilapia, *Oreochromis niloticus* (44–95 g and 15–19 cm) and mudfish, *Clarias anguillaris*, of both sexes (395–750 g and 38–50 cm), were supplied by the Institute of Aquatic Biology of the Centre of Industrial and Scientific Research (CISR), Ghana. Tilapia were randomly divided into four groups and mudfish into three groups and held in concrete tanks containing tapwater for acclimation to the ambient temperature of 28–29 °C for 7 days. A half-volume change of water was performed every other day and the water was aerated throughout the acclimation period. Fish were fed once a day with a mixture of wheatbran and groundnutbran (2:1), by-products from local flourmill and oilmill industries, respectively.

Tilapia were transported in plastic containers with oxygenation to four selected sites in the Volta River (Figure 1) and kept in floating net cages (n = 30) for 3 weeks (April–May, 1994). A floating surface cage was used, measuring 1.5 × 0.7

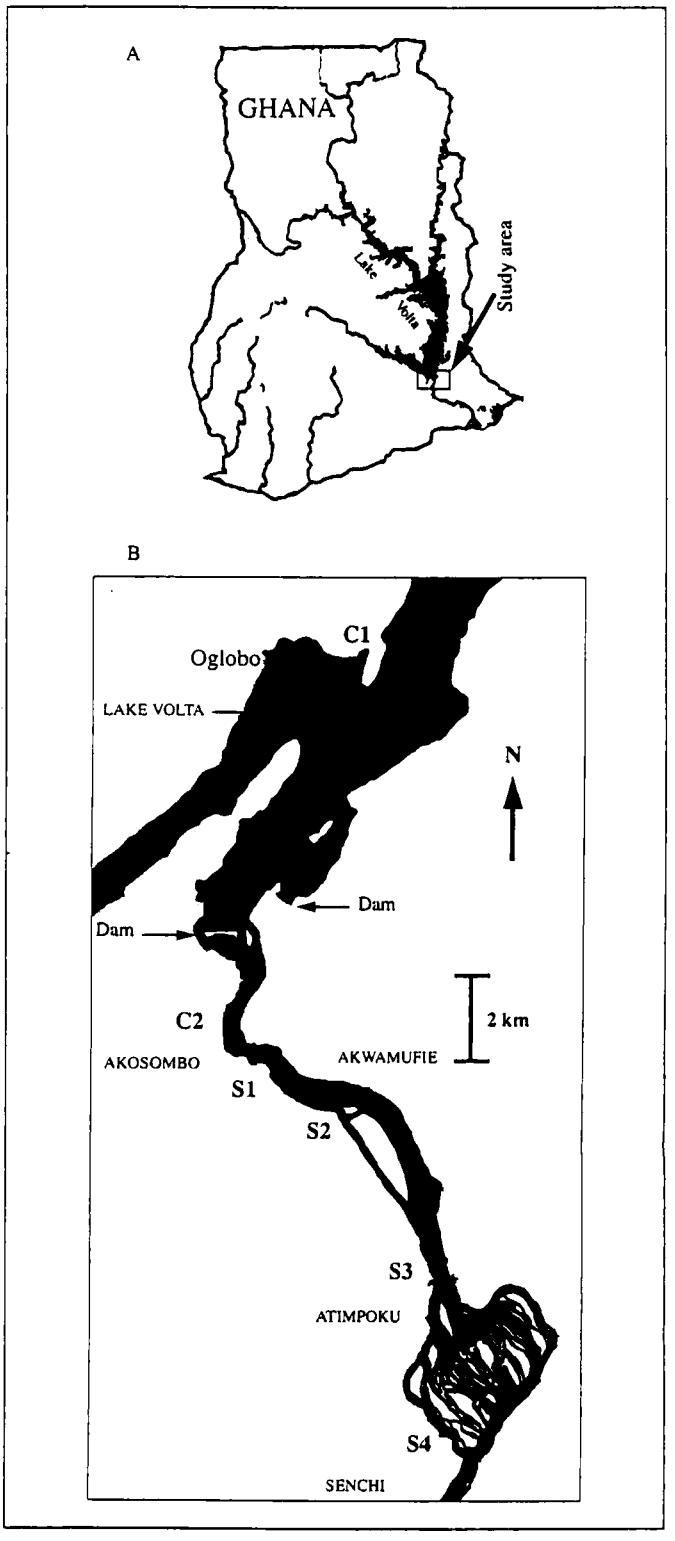

Figure 1. Map of Ghana showing the study area. Caging sites are indicated as C2, S2, S3 and S4 while the sites from which the sediments were collected are shown as C1, S1 and S4; the site numbers correspond to those in tables and figures. S1 is also the point at which the waste water is released into the river. C1 and C2 are reference sites.

× 0.7m, with a wooden frame and covered with multifilament twine net of 1/2-inch mesh size which permitted water exchange. The cages were kept afloat using bamboo tied around the top of the cage and kept stationary by being anchored to the bottom. The top of the net, constructed so as to be closed using a padlock, was also tied to the shore to prevent drifting. The cages were exposed at sites in water 3–7 m from the bottom. Tilapia were caged at sites designated S2 (0.6 km), S3 (4.7 km) and S4 (8 km) downstream of the effluent outlet, with C2 (2.4 km, upstream) serving as the reference site. With mudfish, laboratory exposure was undertaken after attempts to cage them as above failed owing to their aggressive behaviour which led to the death of all the 10 fish exposed at each site. Mudfish were exposed to sediments collected by divers at C1 (10.2 km, upstream of effluent outlet, serving as the reference site), S1 (approx. 20 m from the outlet) and S4 (km, downstream). Concrete tanks were bedded with the sediments. The sediments were then flooded with chlorine-treated pipe-borne water to a height sufficient to cover the mudfish and to prevent them from flying out of the tanks. After initial flooding, the sediments were allowed to settle for 2 days. Mudfish were randomly assigned to each tank (9–10 fish/tank); each tank was aerated and the water was not changed in the course of the experiment. Fish were not fed during the sediment or caging exposure. The water temperature at the beginning of the caging exposure was 28–29 °C, while that in the tanks was at 28 °C.

Sampling of fish

After the exposure, a maximum of 10 tilapia were randomly selected from each cage and brought to the sampling station in containers. Mudfish (n = 9–10) were also sampled from each tank. The fish were killed by a blow to the head, their length and weight measured. Bile was carefully collected from the gall bladder using a small hypodermic needle and frozen in liquid nitrogen for metabolite analysis. The livers were then removed, washed briefly in ice-cold homogenization buffer (0.1 M Na-phosphate buffer containing 0.15 M KCl, pH 7.4), blotted dry and weighed. Liver samples were then immediately frozen in liquid nitrogen and transported in liquid nitrogen (together with the bile samples) to Bergen, Norway, where liver microsome preparations and analyses were done. The body length and weight and liver weight were used to calculate the condition factor (CF, body weight (g)×100/(length, cm)^3 and liver somatic index (LSI, the percentage weight of detached liver of the total fish weight) (Bagenal and Tesch 1978).

Chemicals

7-Ethoxyresorufin, resorufin, NADPH, o-phenylenediamine dihydrochloride (OPD) tablets, β-NADH and uridine 5′phosphoglucuronic acid (UDPGA) were purchased from Sigma Chemical Company (USA). 1-Chloro-2,4-dinitrobenzene (CDNB) was from Aldrich (Germany); reduced glutathione (GSH) and para-nitrophenol (pNP) were from Merck (Germany). Goat anti-rabbit IgG (H + L) horseradish peroxidase conjugate (GAR,HRP), Tween-20 and sodium dodecyl sulphate (SDS) were obtained from Bio-Rad (USA). All other chemicals were of the highest commercially available grade.

Microsome preparation

Microsomes were prepared according to Förlin et al. (1994) and as described by Gadagbui et al. (1996). Essentially, individual livers were homogenized in 0.1 M Na-phosphate buffer (as above) in a volume four times the liver weight, using a Potter-Elvehjem glass Teflon homogenizer and centrifuged at 12 000 × g for 20 min at 4 °C. The postmitochondrial supernatant was recentrifuged at 100 000 × g for 60 min at 4 °C. The microsome pellet was resuspended and rehomogenized in 0.1 M Na-phosphate buffer (pH 7.6) containing 1 mMEDTA, 1 mM dithiothreitol, and 20% glycerol, 1.0 ml g^{-1} liver. Microsomes and cytosolic fractions (100 000 × g supernatant) were stored at –80 °C until use.

Measurement of protein

Microsomal and cytosolic protein concentrations were measured by the Bradford method (Bradford 1976) using bovine serum albumin as standard. Measurements were simplified using a Titertrek Multiscan Plus MkII (Flow Laboratories) for absorbance readings.

Enzyme assays

7-Ethoxyresorufin O-deethylase (EROD) activity was determined fluorometrically at room temperature essentially as described by Andersson et al. (1985). A known amount of resorufin was added as internal standard to each reaction series, and quantified using an extinction coefficient of 73.2 mM^{-1} at 572 nm (Klotz et al. 1984). Fluorometric analysis was performed on a Perkin-Elmer LS-5 Luminescence Spectrophotometer using an excitation wavelength of 535 nm and an emission wavelength of 586 nm for the detection of resorufin formation. The assay was performed in 0.1 M Na-phosphate at pH 6.8 (tilapia) or 7.5 (mudfish) and in the presence of 0.6 μM (tilapia) or 0.8 mM (mudfish) of 7-ethoxyresorufin (Gadagbui et al. 1996).

Glutathione S-transferase (GST) activity was measured spectrophotometrically in the cytosolic fraction according to Habig et al. (1974) and as described by Gadagbui et al. (1996) using 1-chloro-2,4-dinitrobenzene as substrate. Essentially, the assay was carried out in a reaction mixture of 0.1 M Na-phosphate buffer (pH 7.4 for both species), 1 mM EDTA and 20 μg cytosolic protein. After incubation for 3 min at 34 °C (tilapia) or 36 °C (mudfish), 0.8 mM (tilapia) or 1.2 mM (mudfish) CDNB (solubilized in ethanol) was added to a final cuvette volume of 3 ml to start the reaction. Increases in absorbance at 340 nm were recorded for 1 min in a Perkin Elmer UV–visible Spectrometer (Lamda 16) and GST activity was quantified using an extinction coefficient for glutathione-CDNB of 9.6 mM^{-1} cm^{-1}.

UDP-glucuronosyl transferase (UDP-GT) activity towards pNP was determined as described by Koivusaari (1983), but with some modifications (Gadagbui et al. 1996). The incubation was carried out in 0.15 ml of a mixture of 0.1 M Na-phosphate buffer, 6.9 mM UDP-glucuronic acid (UDPGA) and 0.53 mM pNP at pH 7.0 (tilapia) or 7.2 (mudfish). The incubation was started by adding 50 μl microsomes (0.25 – 0.3 mg protein, without digitonin-activation) and lasted for 20 min at 25 °C (for both species). A blank was prepared without UDPGA. The reaction was stopped by the addition of 0.9 ml cold 3% trichloroacetic acid (TCA, w/v). The mixture was then centrifuged at 4000 rpm for 15 min, and 0.75 ml of the supernatant was added to 0.1 ml of 5 M NaOH. The absorbance was immediately measured in microcuvettes at 400 nm in an Ultrospec II UV–visible spectrophotometer.

Fluorescent aromatic compounds (FACs) in bile

Polyaromatic hydrocarbon (PAH) uptake by fish was analysed by the measurement of fluorescent metabolites in the bile using direct synchronous scanning as described by Beyer et al. (1996). Bile samples were diluted (1:1600) in 48% ethanol and analysed for metabolites of benzo[a]pyrene (BaP) at fluorescent wavelength pairs (excitation/emission) of 379/425 nm, pyrene at 341 nm/383 nm and phenanthrene at 275/383 nm, i.e. five-, four- and three-ring PAHs, respectively.

Chlorinated hydrocarbons in fish samples

Fat content and chlorinated hydrocarbons were measured in pooled liver samples of tilapia and mudfish according to Brevik (1978), but with some modifications as described by Bernhoft and Skaare (1994). Internal standard PCB-112 was added to the samples prior to analytical treatment. The chlorinated hydrocarbons measured were β-hexachlorocyclohexane (HCH), heptachlor, hexaclorobenzene (HCB), p,p′-dichlorodiphenyltrichloroethane (p,p′-DDT) and its metabolic derivates p,p′-DDE and p,p′-DDD (denoted together as DDTs), and PCBs (PCB-56, -66, -101, -105, -114, -118, -128, -132, -138, -153, -156, -170, -180, -187, -189, and -194).

Immunochemical studies

Microsomal CYP1A levels were quantified immunochemically using an enzyme-linked immunosorbent assay (ELISA) according to Goksøyr (1991), using rabbit anti-cod CYP1A IgG and goat anti-rabbit IgG horseradish peroxidase conjugate (GAR–HRP) as primary and secondary antibodies, respectively. Western blotting was performed with pooled microsomes by the general procedure of Towbin *et al.* (1979), as described previously by Goksøyr *et al.* (1991) using the same antibodies as in the ELISA assay. Visualization of immunoblot with chemiluminescence (ECL-Western blotting kit, Amersham) was also used to enhance sensitivity. Primary antibodies were diluted 1:100.

Statistical analysis

Testing of statistical differences between control and treated groups and correlation analyses were performed on log-transformed data using the JMP Software for Statistical Visualization (SAS Institute), version 3.0.4. for Macintosh. Tests that variances were equal between control and treated groups were performed before other analyses. When variances were shown to be equal, an ANOVA was performed to detect treatment effects, and Dunnett's test was performed to detect whether treated groups were different from their respective control groups. Where variances were not equal, a Welch ANOVA was performed. A p value < 0.05 was considered as statistically significant.

Results

General observation of the material and study area

Visual observation showed high colour intensity of the river water at the effluent outlet with the intensity decreasing with distance downstream; no coloration was observed at the sites upstream. This indicates a decreasing concentration of dye with distance. A high mortality was observed at S2 in the caging experiment (22 out of 30 tilapia died), while at the other sites all fish survived except at S3 where 26 out of 30 escaped through an opening in the cage. No mortality was observed in the sediment-exposed mudfish. The high mortality at S2 could not possibly be due to cage stress but rather to contaminants and other environmental factors (e.g. oxygen level and pH in the water) since all fish at the reference and other downstream sites survived. If cages become fouled with extensive algal growth, there is the tendency to preclude water exchange resulting in insufficient supply of oxygenated water. Fouling, however, did not appear to be a great problem in this experiment, probably because of the tendency of tilapia to browse on algae, thus acting as its own ecological anti-foulant (Coche 1976). The browsing behaviour of tilapia and the size of the mesh used in relation to the body size of tilapia could provide a suitable surface area, allowing for satisfactory water exchange and food supply. On the other hand, mudfish could experience starvation during the 2-week period. Water temperature was constant both in the river and tank water while pH of the water at the site nearest the sewer outlet was slightly higher than at the other stations (pH 7.8 vs 6.8–7.2).

No significant sex differences in any of the biomarker responses studied were observed in mudfish at any of the sites, hence the data were treated without reference to sex.

Biological data

The condition factors for both species from the exposed sites were not significantly different from control values (Table 1).

Site	N[a]	Length	Weight	C F[b]	LSI[c]
Tilapia					
C2	9	16.0±0.0	63±5	1.54±0.12	1.18±0.14
S2	8	15.8±0.8	55±11	1.39±0.15	1.34±0.32
S3	4	18.5±0.6	91±4	1.44±0.08	0.86±0.18*
S4	8	17.1±0.8	70±4	1.40±0.08	0.94±0.19*
Mudfish					
C1	10	44.5±4.5	540±128	0.61±0.09	0.76±0.17
S1	9	44.6±5.8	555±160	0.62±0.10	1.56±0.28*
S4	10	45.5±3.7	610±142	0.64±0.10	0.78±0.11

Table 1. Biological data on tilapia (*O. niloticus*) and mudfish (*C. anguillaris*) used in the caging and sediment exposure studies, respectively, in the Volta River.

[a]Total number of individuals; [b]condition factor; [c]Liver somatic index (see Materials and Methods section for further information). All values are represented as mean ± SD; * significantly different from control values ($p < 0.05$). C1 and C2 are the reference sites for tilapia and mudfish, respectively.

The liver somatic index was significantly higher only at S1 in the mudfish compared with the control values, whereas in the tilapia, we observed slightly but significantly lower LSI at S3 and S4.

Chemical contaminants in the liver and bile

The fat content and concentrations of chemical contaminants detected in the liver samples of both species are presented in Table 2. Concentrations of PCBs, DDTs (representing the metabolized derivatives DDE and DDD) and non-DDT pesticides were higher in mudfish exposed to sediments than in tilapia exposed by caging. In both species, the levels were generally higher (up to three-fold) in samples from the sites in the vicinity of the mill than in those farther away, showing more or less a pollution gradient in the river. Overall, however, these levels indicate only low contamination with organochlorine compounds.

Bile analysed for fluorescent aromatic compounds (FACs) displayed low fluorescence values at all sites, indicating that PAH compounds are unlikely to contribute significantly to contamination of the area (results not shown).

CYP1A analyses

CYP1A-mediated enzyme (EROD) activity was affected by both caging- and sediment-exposure (Tables 3 and 4). Compared with reference values, EROD activity in both fish species was significantly induced ($p < 0.001$). In the caging-exposure, we observed 21-, 10- and 3.3-fold inductions of this activity in tilapia at S2, S3 and S4, respectively (Figure 2(A)). In the sediment-exposure with mudfish, 25- and 3-fold induction of EROD activity was observed at S1 and S4, respectively (Figure 3(A)).

CYP1A protein level, measured as ELISA absorbances (CYP1A-ELISA) showed 4.4-, 3.3- and 1.9-fold induction in tilapia at S2, S3 and S5, respectively (Figure 2(B)). In the mudfish, there was only 4.4-fold induction at S1 (Figure 3(B)).

Immunochemical analysis of pooled tilapia and mudfish liver microsomes, using polyclonal anti-cod CYP1A IgG in Western blotting, revealed the presence of a highly induced single immunoreacting protein band in the CYP1A region

	Tilapia			Mudfish		
Site:	C2	S2	S4	C1	S1	S4
% fat:	3.42	4.74	3.66	4.67	7.35	3.49
Pesticides						
HCB	n.d.	n.d.	n.d.	n.d.	1.47	*0.15*
β-HCH	*1.13*	2.10	n.d.	n.d.	2.29	*1.09*
Heptachlor	3.83	3.84	5.90	n.d.	1.46	n.d.
p,p-DDE	5.57	8.61	9.33	6.95	19.10	4.15
o,p-DDD	1.32	2.44	3.46	n.d.	n.d.	1.74
p,p-DDD	*0.73*	1.35	2.42	n.d.	6.29	*1.06*
ΣDDTs[a]	7.62	12.40	15.21	6.95	25.4	6.95
PCBs						
PCB-56	n.d.	n.d.	n.d.	n.d.	*1.03*	n.d.
PCB-66	n.d.	n.d.	n.d.	n.d.	*1.04*	n.d.
PCB-101	*0.93*	*1.72*	n.d.	n.d.	2.30	n.d.
PCB-105	*0.77*	n.d.	n.d.	0.82	1.50	*0.75*
PCB-114	n.d.	n.d.	n.d.	0.66	*0.59*	n.d.
PCB-118	*0.80*	*1.49*	*1.07*	0.85	2.20	*0.78*
PCB-128	*0.63*	n.d.	n.d.	0.67	*0.60*	n.d.
PCB-132	n.d.	n.d.	n.d.	n.d.	*0.82*	n.d.
PCB-138	*0.77*	1.35	*0.97*	2.63	5.39	1.88
PCB-153	*0.68*	*1.26*	*0.91*	2.39	4.83	1.54
PCB-156	n.d.	n.d.	n.d.	n.d.	*0.76*	n.d.
PCB-170	n.d.	n.d.	n.d.	n.d.	1.30	n.d.
PCB-180	*0.65*	*1.21*	*0.86*	1.86	2.30	*0.63*
PCB-187	n.d.	n.d.	n.d.	0.41	0.37	n.d.
PCB-189	n.d.	n.d.	n.d.	n.d.	*0.56*	n.d.
PCB-194	n.d.	n.d.	n.d.	n.d.	*0.99*	n.d.
ΣPCBs	5.2	7.0	3.8	10.3	26.5	7.6

Table 2. Concentrations (ng g^{-1}, wet weight) of contaminants measured in pooled liver samples of tilapia (*O. niloticus*) caged in the Volta River for 3 weeks and of mudfish (*C. anguillaris*), exposed, for 2 weeks, in the laboratory to sediment from the same river.

Samples from S4 were not analysed owing to small sample size. Values *in italics* are below detection limit and are given as half the detection limit in *original concentration,*.

Key: HCB, hexachlorobenzene; β-HCH, beta-hexachlorohexane; n.d., not detected.

C1 and C2 are reference sites (see Materials and Methods).

[a]The sum of DDEs and DDDs.

Site	N	EROD	ELISA	GST	UDP-GT
C2	9	50 ± 25	0.16 ± 0.04	166 ± 20	1.6 ± 0.7
S2	8	1042 ± 258***	0.72 ± 0.04***	211 ± 44*	2.7 ± 0.6*
S3	4	480 ± 94***	0.53 ± 0.05***	102 ± 27*	2.2 ± 0.3
S4	10	174 ± 63***	0.30 ± 0.04***	143 ± 16	2.2 ± 0.5

Table 3. Hepatic biotransformation enzyme activities in 3-week cage-exposed tilapia (*O. niloticus*).

Tilapia were exposed by caging for 3 weeks at three sites in the Volta River downstream of an effluent outlet of a textile mill; control fish were caged at a site (C2) upstream from the outlet (see Materials and Methods). EROD (pmol min^{-1} mg^{-1} protein). ELISA (absorbances at 492 nm) and UDP-GT (nmol min^{-1} mg^{-1} protein) measurements were performed on liver microsomal fractions and GST activity (nmol min^{-1} mg^{-1} protein) was measured using liver cytosolic fraction. All values are mean ± SD for (*N*) number of individuals.

*** $p < 0.001$; * $p < 0.05$ (represent values significantly different from the respective control group, one-way, Dunnett).

Site	N	EROD	ELISA	GST	UDP-GT
C1	10	11 ± 7	0.16 ± 0.03	46 ± 11	3.7 ± 08
S1	9	285 ± 78***	0.32 ± 0.07***	59 ± 14*	6.4 ± 1.1*
S4	10	35 ± 19***	0.16 ± 0.05	47 ± 6	3.5 ± 0.8

Table 4. Hepatic biotransformation enzyme activities in 2-week sediment-exposed mudfish (*C. anguillaris*).

Mudfish were exposed to sediments collected manually at two sites below the effluent outlet in the Volta River; control fish were exposed to sediment from a site (C1) upstream from the effluent. Presentation of results as in Table 3.

(54000–59000 Da) at all sites downstream, with hardly any band visible from the control samples (Figure 4). The intensity of the bands also showed a pollution gradient downstream of the textile mill.

Phase II enzyme activities

There was significant increases in both 4-nitrophenol-specific UDP-glucuronosyltransferase and CDNB-specific GST activities in both species at the sites nearest the effluent outlet (Figures 2(C) and 3(C); Tables 2 and 3) compared with reference values ($p < 0.05$). We observed increases of 69% in UDP-GT activity and 27% in GST activity in caged tilapia (at S2) while in mudfish exposed to river sediment at S1, increases of 73% and 28% in UDP-GST and GST activities, respectively, were observed. There was no difference in the levels of the conjugation enzymes at the other sites except in tilapia where there was a tendency of inhibition (40%, significant) in GST activity at S3 and a statistically insignificant increase (40%) in UDP-GT activity at S3 and S4.

Discussion

The chemical identification of unknown compounds in aqueous effluents can be prohibitively expensive, time-consuming, and even of little value if there is no information on biological effects produced by the chemicals. This study is an initial assessment of biological effects of a textile mill effluent in the Volta River and evaluation of the utility of different biomarker enzymes as a biomonitoring tool in tropical waters. The biological effects of the effluent were studied by analysis of CYP1A induction in terms of EROD activity measurement, CYP1A-ELISA and Western blotting and also by studying the liver phase II enzymes (GST and UDP-GT activities) in caged tilapia and sediment-exposed mudfish.

Several studies have demonstrated high rates of EROD activity and elevated CYP1A protein levels in fish liver from polluted areas compared with relatively clean areas (Stegeman *et al.* 1988, Van Veld *et al.* 1990, Kloepper-Sams and Benton 1994). Induction of CYP1A activity has been linked to the presence of aquatic pollutants including PAHs, PCBs, PCDDs, PCDFs, azo-dyes and chlorophenols as well as an array of drugs, biotic and abiotic factors (Bucheli and Fent 1995, Goksøyr 1995). The induction depends on the binding of the inducer to an Ah (aryl hydrocarbon) receptor and associated expression of a functional CYP1A enzyme (Poland and Knutson, 1982, De-Long *et al.* 1987, Safe 1988, Hoffman *et al.*

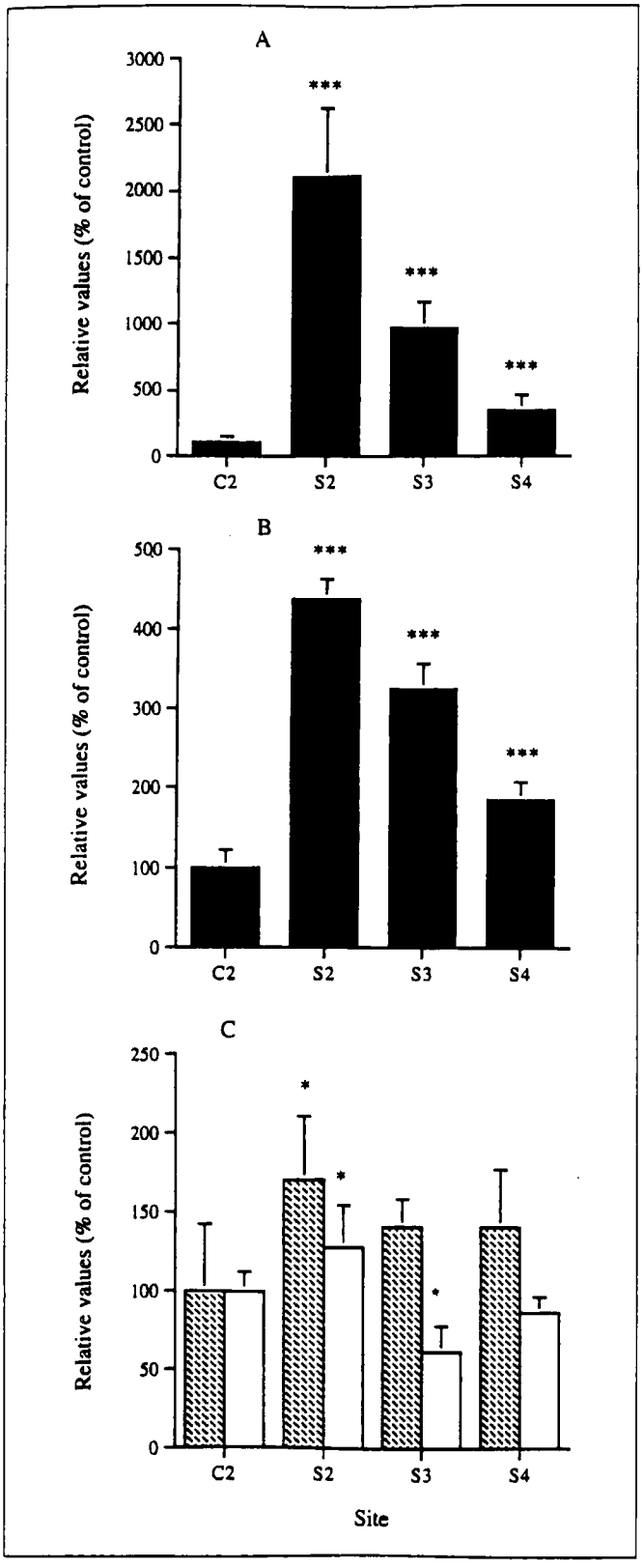

Figure 2. Relative levels of biochemical response parameters in liver samples of tilapia exposed by caging at four different locations in the Volta River in Ghana, for 3 weeks. The levels are expressed relative to controls set at 100 (see Materials and Methods); *** indicates significant differences ($p < 0.001$) and * ($p < 0.05$). (A) Ethoxyresorufin–O-deethylase (EROD) activity; (B) CYP1A content quantified using enzyme-linked immunosorbent assay (ELISA), (C) UDP-glucuronyl transferase (UDP-GT) toward *p*NP (hatched bars) and glutathione S-transferase (GST) toward CDNB (open bars). Error bars represent standard deviation.

1991). In addition to induction of CYP1A genes, the Ah-receptor ligands also enhance induction of some phase II enzymes including UDP-GT and GST (Nebert and Gonzalez 1987).

Results from our study indicated a significant induction of EROD activity in both the caged tilapia and sediment-exposed mudfish at all sites downstream of the effluent outlet, with the highest induction at the site closest to the outlet (Figure 2(A) and 3(A)). CYP1A protein content measured by the indirect ELISA (Figures 2(B) and 3(B) and Western blotting (Figure 4) using polyclonal anti-cod antibody in the same samples also displayed similar induction patterns. Thus, the EROD activity and ELISA measurement show significant differences between sites in both caging and sediment-exposure studies. These results are consistent with current understanding of CYP1A induction mechanism in fish (Stegeman *et al.* 1986, 1992, Addison and Edwards 1988).

CYP1A induction has also been reported in caged fish exposed to organic pollutants in freshwater systems and in the marine environment (Beyer *et al.* 1996). Lindström-Seppä and Oikari (1989) observed up to a 17-fold increase in EROD activity after 3 weeks of caging juvenile whitefish (*Coregonus* sp.) in the southern part of Lake Saimaa (Finland) that receives effluents from a mill producing chlorine bleached kraft pulp and printing paper. The same authors (1990) reported up to seven-fold induction of EROD activity in immature rainbow trout (*O. mykiss*) in the same lake. In another study, Di Giulio *et al.* (1993) observed significant increases in EROD activity (up to three-fold) and other biochemical responses in channel catfish exposed in the laboratory to sediments obtained from Black Harbour (Long Island Sound, US). Our study has indicated one of the highest increases in EROD activity in caged fish (21-fold) and fish exposed in the laboratory to sediments (25-fold) from a polluted river.

The induction of CYP1A and its catalytic EROD activity in the liver of tilapia and mudfish in the present study explicitly indicates that the fish have been exposed to environmental chemical compounds that are strong CYP1A inducers (i.e. Ah-receptor agonists). Determination of the concentrations of the various xenobiotics in the liver (to indicate how much was present at the time of exposure), and their metabolites in bile was attempted in order to show a causal relationship between exposure and response. The highest induction levels in the xenobiotic biotransformation enzyme system occurred at the sites where the highest levels of PCBs and total DDTs in the liver were detected. However, since levels of PCBs were only up to three times higher at the closest site than in the reference sites, and in general quite low, this suggests that these were not likely to be the major contributors to the induction response observed. The absence of metabolites of PAHs in the bile [the analysis of which is preferred to direct tissue measurements of PAHs because the PAHs are rapidly metabolized and/or excreted after uptake (Varanasi *et al.* 1989)] seemed to also rule out PAHs as major contributors to the response as they are

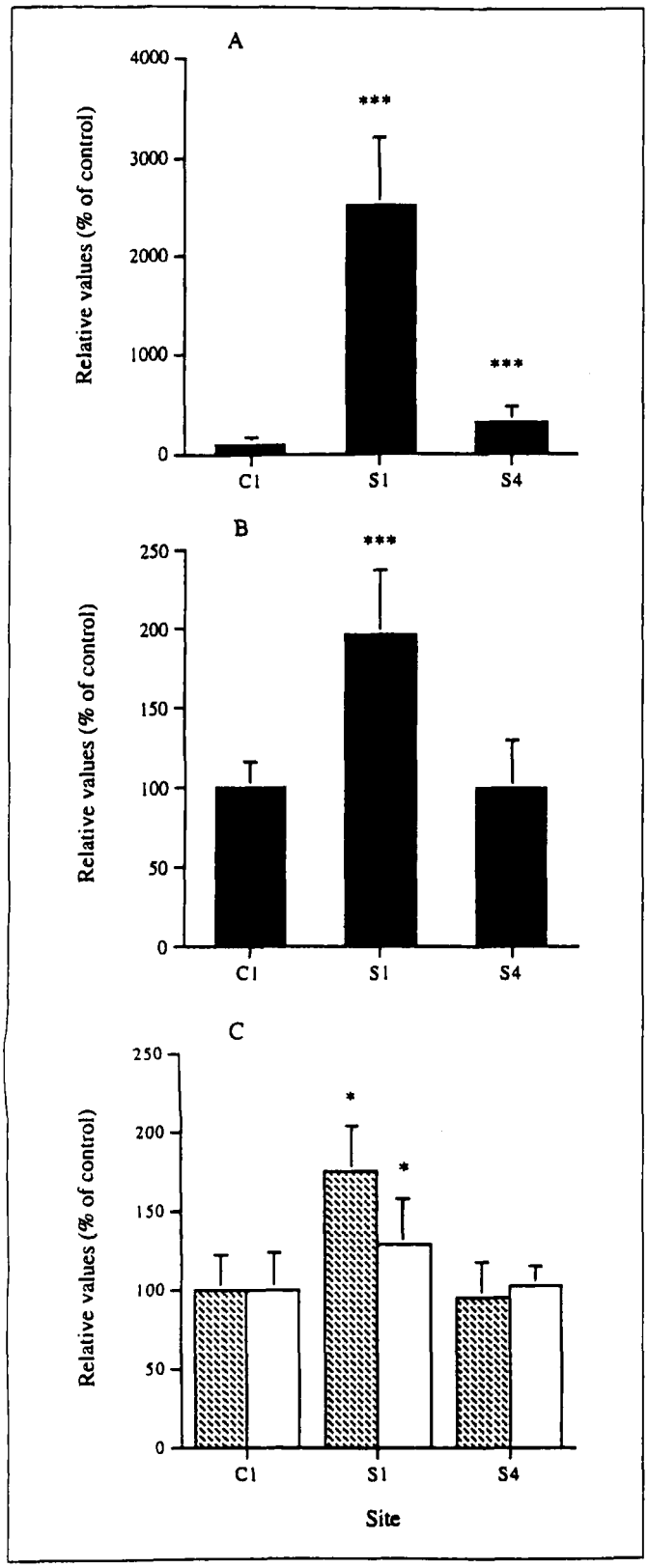

Figure 3. Relative levels of biochemical response parameters in liver samples of mudfish exposed to sediment from three different locations from the Volta River in Ghana for 2 weeks. Presentation of results as in Figure 2.

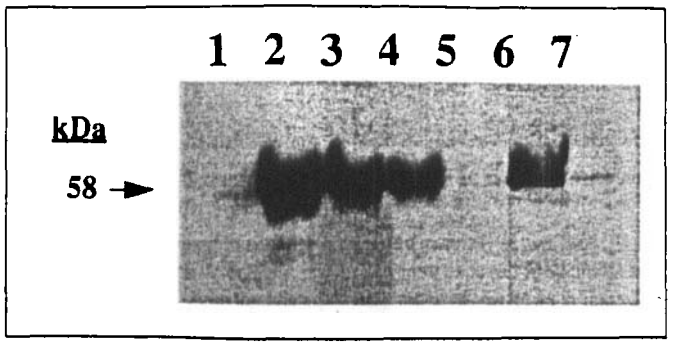

Figure 4. Western blot of pooled liver microsomes from tilapia and mudfish. Sixty µg liver microsomal protein (from each site) were subjected to 9% SDS-polyacrylamide gels, blotted to nitrocellulose and probed with anti-cod CYP1A IgG and visualized with ECL as described in Materials and methods. Lanes 1–4, tilapia; 5–7, mudfish. 1: C2, 2: S2; 3: S3, 4: S4, 5: C1, 6: S1, 7: S4. Site identities as in Figure 1.

known potent CYP1A inducers. Many of the organochlorine pesticides, e.g. the DDT group, have been shown in laboratory studies not to induce the CYP1A system in fish, as opposed to their effects in mammals (Addison *et al.* 1977). In an earlier study in which mudfish and tilapia were exposed to the potent CYP1A inducers, BNF and PCBs (Gadagbui *et al.* 1996), EROD activity was found to be inhibited by PCB treatment in mudfish, but was readily induced by both chemical contaminants in tilapia. These results thus suggest that PCBs, PAHs and DDTs are unlikely to be responsible for the observed responses in these fishes.

This points to the fact that there may be some other highly potent chemical contaminants in the Volta River environment to which the fish were exposed. It is, therefore, tempting to speculate that the apparently high induction levels in the fishes from the more contaminated sites were probably caused by the dyes being released by the textile mill into the environment. Although there is, at present, no chemical data of these chemicals in sediments, tissues or water samples from the river which could lead to any substantive interpretation, several reports have shown dyes (azo compounds) used in the textile industry to induce CYP1A and liver conjugation enzymes in rats (Fujita *et al.* 1984a, b) and to be genotoxic and/or mutagenic in rats and mice (Joachim and Decad 1984, Przybojewska *et al.* 1989). Also, in a study by Environment Canada (Rutherford *et al.* 1992) the untreated effluent from three textile mills were chemically characterized and evaluated from an aquatic toxicity standpoint. Results of the toxicity tests showed that the effluent samples (containing detergent/surfactants, plasticizers, dye carriers, mineral oils, typical auxiliary chemicals used in textile dyeing) were acutely toxic to all organisms tested, all samples showed sub-lethal toxic effects, including reproductive and growth impairment, to all species tested, and all samples were mutagenic. CYP1A induction and other biomarker responses, however, have not previously been documented in fish or in field studies.

The present study also revealed significant increases in the liver conjugation enzymes, 1,2-dichloro-4-nitrophenol-specific GST and *p*-nitrophenol-specific UDP-GT activities, in

the vicinity of the effluent outlet in both species (Figures 2(C) and 3(C). Laboratory and field studies on the inducibility of these enzyme activities resulting from exposure of aquatic species to environmental contaminants have yielded inconsistent results. Some studies in fish indicated two to three-fold increases in these activities (Andersson *et al.* 1985, Goksøyr *et al.* 1987, Van Veld *et al.* 1992, Gadagbui *et al.* 1996) which could increase the levels of activated intermediates (Ahokas *et al.* 1976a); others observed decreases in the enzyme activities (Ahokas *et al.* 1976b, Oikari, 1982, Oikari *et al.* 1988) or no significant change at all (James *et al.* 1977, Lindström-Seppä and Oikari 1990b). The induction levels of these two enzymes in the present study also compared well with those observed when the same species were exposed to BNF and PCBs (Gadagbui *et al.* 1996). The changes in the conjugation enzymes in our study also signal an environmental load of xenobiotics in the environment.

The induction responses observed at the biochemical level in this study suggest the potential for other biological effects to ensue, e.g. suppression of immune response, alterations in signal transduction or in hormonal metabolism, depending on the target tissue of CYP1A induction (Goksøyr 1995). Hence, this evidence of biochemically significant exposure of tilapia and mudfish to potentially toxic chemical contaminants may predispose these fishes to adverse physiological effects.

The use of the two species (benthic versus pelagic) and the form of exposure (caging versus sediment exposure) provide some insight on the relative extent of chemical contaminant exposure in both species. The study suggests that both pelagic and benthic fish species in the river are at risk of being exposed to the potent chemical contaminants. This exposure may lead to decreased growth, reproduction and resistance to diseases in commercially valuable species and stocks. The decline in fish populations being observed on the Volta River downstream of the dam (local fishermen, personal communication) may be caused by several factors, including physical changes to the environment, overfishing, changes in community dynamics, infectious diseases, as well as possible effects of chemical pollution (from the use of pesticides on the farms along the river and domestic activities and from the mill). Since persistent organic substances tend to bioaccumulate and biomagnify in the food chain, species of organisms in higher trophic levels such as carnivorous fishes, predatory water birds and insectivorous birds are potentially at high risk. As the Volta River is used intensively for fishing and as a source of drinking water, human populations, especially downstream of the textile mill, may become a high risk group. Unofficial reports indicated complaints of skin rashes and stomach ailments among the people living along the banks of the river (M. Addy, pers. comm.).

In conclusion, the textile mill effluent contamination in the Volta River clearly affected biomarker responses in tilapia and mudfish. The induction of the CYP1A enzyme system and phase II enzymes was clearly seen in the liver of both fish species, especially at the downstream site closest to the textile mill, with the CYP1A induction response decreasing towards more distant locations. This study did not supply evidence as to which particular constituents are responsible for the biomarker responses in the fish species, but there are indications that PAHs, PCBs and DDTs are unlikely to be the major contributing agents. The results suggest either that the effluent contains some highly potent inducers of CYP1A activity, or that synergism between two or more compounds or classes of compounds may be occurring with regard to induction of this activity. Further study is required to determine the potent inducing compounds or causative factors in the river to which the aquatic organisms are exposed.

This first assessment of the biological effects of organic pollutants in the Volta River demonstrates the utility of the CYP1A system as a valuable early warning biomarker of industrial effluents and also as a biomarker to detect exposure of aquatic fish resources to contamination in tropical waters. The need for treating this effluent source is clearly implicated, and in this situation biomarker responses in caged fish could be used to monitor the improvement of the river environment.

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