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Linking Ah receptor mediated effects of sediments and impacts on fish to key pollutants in the Yangtze Three Gorges Reservoir, China — A comprehensive perspective

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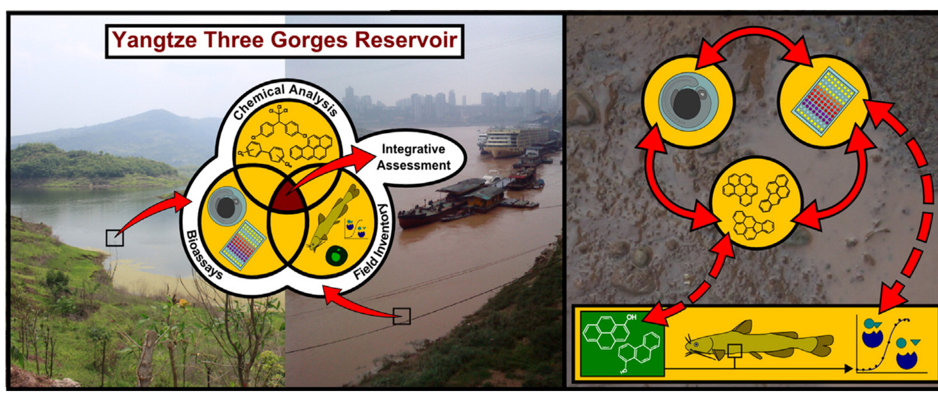
HIGHLIGHTS

- Significant correlations between PAHs in sediment and *in vitro*/*in vivo* endpoints
- Low bioavailability of particle-bound pollutants
- Significant ethoxyresorufin-*O*-deethylase induction in fish at Chongqing city
- Results suggest rather poor condition of test fish species *Pelteobagrus vachellii*.
- Results suggest pivotal role of PAHs in observed ecotoxicological impacts.

GRAPHICAL ABSTRACT



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ABSTRACT

The Three Gorges Reservoir (TGR), created in consequence of the Yangtze River's impoundment by the Three Gorges Dam, faces numerous anthropogenic impacts that challenge its unique ecosystem. Organic pollutants, particularly aryl hydrocarbon receptor (AhR) agonists, have been widely detected in the Yangtze River, but only little research was yet done on AhR-mediated activities. Hence, in order to assess effects of organic pollution, with particular focus on AhR-mediated activities, several sites in the TGR area were examined applying the “triad approach”. It combines chemical analysis, *in vitro*, *in vivo* and *in situ* investigations to a holistic assessment. Sediments and the benthic fish species *Pelteobagrus vachellii* were sampled in 2011/2012, respectively, to identify

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relevant endpoints. Sediment was tested *in vitro* with the ethoxyresorufin-*O*-deethylase (EROD) induction assay, and *in vivo* with the Fish Embryo Toxicity Test and Sediment Contact Assay with *Danio rerio*. Activities of phase I (EROD) and phase II (glutathione-S-transferase) biotransformation enzymes, pollutant metabolites and histopathological alterations were studied *in situ* in *P. vachellii*. EROD induction was tested *in vitro* and *in situ* to evaluate possible relationships. Two sites, near Chongqing and Kaixian city, were identified as regional hot-spots and further investigated in 2013. The sediments induced in the *in vitro*/*in vivo* bioassays AhR-mediated activities and embryotoxic/teratogenic effects – particularly on the cardiovascular system. These endpoints could be significantly correlated to each other and respective chemical data. However, particle-bound pollutants showed only low bioavailability. The *in situ* investigations suggested a rather poor condition of *P. vachellii*, with histopathological alterations in liver and excretory kidney. Fish from Chongqing city exhibited significant hepatic EROD induction and obvious parasitic infestations. The polycyclic aromatic hydrocarbon (PAH) metabolite 1-hydroxypyrene was detected in bile of fish from all sites. All endpoints in combination with the chemical data suggest a pivotal role of PAHs in the observed ecotoxicological impacts.

1. Introduction

The Yangtze River basin is an elemental source for drinking water for millions of people and plays a significant role in freshwater fishery of the country (Liu and Cao, 1992; Liu et al., 2005; National Bureau of Statistics – China, 2004; Wong et al., 2007). One part of the upper Yangtze River section was impounded by the Three Gorges Dam (TGD) to form the Three Gorges Reservoir (TGR). The TGR stretches from Sandouping, Hubei Province, to the Jiangjin district of Chongqing Municipality, over a distance of 663 km (Fig. 1). Overpopulation of the TGR area and pollution, as a consequence of the impoundment, involve serious threats to the recently established vulnerable reservoirs ecosystem (Xinhua, 2007a,b). Hence, this consequently also threatens an important source for drinking water and food for many people.

The population of Chongqing municipality already exceeded 29 million people in 2011, with Chongqing City (6–7 million in 2012) being the largest city at the TGR (BBC, 2012; National Bureau of Statistics – China, 2012). It is also one of the largest cities in western China and belongs to the fastest growing cities worldwide. Further, the increasing freight and shipping traffic on the TGR, due to the elevation of the water level, are intended to stimulate the regional economy (Xinhua, 2014). Progressive urbanization and industrialization will entail an increasing amount of wastewater in the urbanized regions. About 1000 Megatonnes (Mt) urban sewage (53%) and industrial wastewater (47%) were discharged annually in the TGR in the years 2004 to 2010 (Ministry of Environmental Protection – China, 2006–2012). In response the local governments adopted policies on emission reduction for the industry and improved the domestic wastewater treatment capacities from annually 515 Mt in 56 facilities in 2008, to 590 Mt in 71 facilities in 2010. However, both times domestic sewage contributed for about 98%, meaning that still 88 Mt domestic and 548 Mt industrial sewage in 2008, as well as 37 Mt and 307 Mt, respectively, in 2010 were discharged untreated (Ministry of Environmental Protection – China, 2010, 2012; Wang et al., 2013). In addition, the submerged 13 cities, 140 towns and over 1300 villages with 1600 factories and abandoned mines (Smith, 2013) may release remaining contamination into the reservoir. Müller et al. (2008) already stressed the importance of growing wastewater fractions in the Yangtze River for the sections downstream of the TGD. They calculated that, in addition to rising levels of nutrients, dissolved organic carbon and heavy metals, 500 to 3500 kg industrial organic chemicals are discharged daily into the river. However, for the region upstream of the dam only little information on pollution and potential impacts is yet available, particularly for the recently established TGR (as reviewed recently by Floehr et al., 2013).

The Yangtze River's fish fauna is described as one of the richest worldwide, providing plentiful fish resources, but they showed a serious decline with a significantly reduced fishery yield in the past years (Chen et al., 2009; Fu et al., 2003). Further, the species composition shifted in its quantitative relationships and decreased in diversity (Chen et al., 2002a,b, 2009). The impoundment of the TGR further threatened endemic and rare fish species, particularly those adaptable to the prior running water conditions, with demersal fishes like the darkbarbel catfish (*Pelteobagrus vachellii*) becoming more important. Yiming and Wilcove (2005) stated the most pervasive threats to Chinese vertebrate animals in general were overexploitation, habitat destruction and pollution, which contributed to the endangerment of 78%, 70% and 20%, respectively, of imperiled species. However, only few quantitative studies on Yangtze fishes are available, but a number of qualitative research demonstrates that aside habitat fragmentation and shrinkage, invasion of exotic species and resources overexploitation, also water pollution is one of main responsible factors for the registered depletion and structural changes of the fish resources (Chen et al., 2009). Industrial and communal sewage discharging toxicants into the environment are considered to trigger the destruction of spawning grounds, depletion of brood stocks, decrease in production and induction of mortality (Chen et al., 2004). Chen et al. (2009) warned of the increasing seriousness of pollution in the Yangtze River as consequence of proceeding industrialization and urged for a strengthened environmental monitoring and control of the water pollution to maintain a suitable fishery environment.

The impoundment also resulted in a reduction of the Yangtze Rivers flow velocity from prior 2 to 3 m s^{-1} in average of the upper and middle layer down to less than 0.05 to 1.5 m s^{-1} (Chen et al., 2005; Wang et al., 2009). This in turn affects the dilution of contamination from point sources, such as urban and industrial wastewater outlets, from where they have been swept away before, as well as the sedimentation rate of suspended particles and adhering contaminants. Thus, it can be expected that the pollution rather accumulates in the area of discharge from which they have been swept away before. Sediments can act as sink and source for pollutants, and thus play an important role for environmental assessment (Ahlf et al., 2002; Gerbersdorf et al., 2005; Hilscherova et al., 2007; Hollert et al., 2003; Li et al., 2014a,b; Wallis et al., 2014; Wölz et al., 2009). The Yangtze River is one of the largest sediment carriers in the world, and about 60 to 68% of the sediment materials that entered the TGR from upstream have been trapped annually since the operation of the dam, which account for annually 151 to 172 Mt between 2003 and 2008 during the impoundment (Hu et al., 2009; Yang et al., 2007).

A common way to monitor the pollution status of a water body is to determine the chemical concentrations in water and sediment with chemical–analytical methods. This approach however is insufficient. That way only selected compounds are in the scope of the quality assessment, and an even larger variety of toxic non-target parent compounds and metabolites is not considered. Moreover, aspects like bioavailability of the toxic compounds, their synergistic and antagonistic effects, as well as the metabolism in the organisms are not taken into account. Thus, in order to acknowledge the complex situation in the field a holistic assessment in environmental monitoring is demanded, that considers not only the concentrations of selected compounds, but