

Available online at www.sciencedirect.com

ScienceDirect

www.journals.elsevier.com/journal-of-environmental-sciences

Yangtze Three Gorges Reservoir, China: A holistic assessment of organic pollution, mutagenic effects of sediments and genotoxic impacts on fish

Tilman Floehr^{1,*}, Björn Scholz-Starke¹, Hongxia Xiao¹, Josef Koch¹, Lingling Wu², Junli Hou³, Anja Wolf^{4,5}, Axel Bergmann⁴, Kerstin Bluhm¹, Xingzhong Yuan⁶, Martina Roß-Nickoll^{1,6}, Andreas Schäffer^{1,7}, Henner Hollert^{1,2,6,7,*}

1. Department of Ecosystem Analysis, Institute for Environmental Research, RWTH Aachen University, 52074 Aachen, Germany. E-mail: floehr@bio5.rwth-aachen.de

2. Key Laboratory of Yangtze River Water Environment, Ministry of Education, Tongji University, Shanghai 200092, China

3. East China Sea Fisheries Research Institute, Shanghai 200090, China

4. IWW Rhenish-Westfalian Institute for Water Research, 45476 Mülheim an der Ruhr, Germany

5. Institute for Applied Geosciences, Technische Universität Darmstadt, 64287 Darmstadt, Germany

6. College of Resources and Environmental Science, Chongqing University, Chongqing 400030, China

7. State Key Laboratory of Pollution Control and Research Reuse, School of the Environment, Nanjing University, Nanjing 210093, China

ARTICLE INFO

Article history:

Received 10 March 2015

Revised 2 July 2015

Accepted 3 July 2015

Available online 26 October 2015

Keywords:

Yangtze

Three Gorges Reservoir

Triad approach

Sediment

Fish

Mutagenicity

Genotoxicity

PAH

ABSTRACT

Besides obvious benefits, the Three Gorges Dam's construction resulted in new pollution scenarios with the potentials to threaten the Three Gorges Reservoir (TGR) ecosystem. In order to record organic contamination, to find links to ecotoxicological impacts and to serve as reference for ensuing monitoring, several sites in the TGR area were screened applying the triad approach with additional lines-of-evidence as a holistic assessment method. Sediments and the benthic fish species *Pelteobagrus vachellii* were sampled in 2011 and 2012 to determine organic pollution levels, mutagenic potentials and genotoxic impacts. Two regional hot-spots near the cities of Chongqing and Kaixian were identified and further investigated in 2013. Only polycyclic aromatic hydrocarbons (PAHs) could be detected in sediments in 2011 (165–1653 ng/g), emphasizing their roles as key pollutants of the area. Their ubiquity was confirmed at Chongqing (150–433 ng/g) and Kaixian (127–590 ng/g) in 2013. Concentrations were comparable to other major Chinese and German rivers. However, the immense sediment influx suggested a deposition of 216–636 kg PAH/day (0.2–0.6 mg PAH/(m²·day)), indicating an ecotoxicological risk. PAH source analysis highlighted primary impacts of combustion sources on the more industrialized upper TGR section, whereas petrogenic sources dominated the mid-low section. Furthermore, sediment extracts from several sites exhibited significant activities of frameshift promutagens in the Ames fluctuation assay. Additionally, significant genotoxic impairments in erythrocytes of *P. vachellii* were detected (Chongqing/Kaixian), demonstrating the relevance of genotoxicity as an

* Corresponding authors.

E-mail addresses: floehr@bio5.rwth-aachen.de (T. Floehr), Henner.Hollert@bio5.rwth-aachen.de (H. Hollert).

important mode of action in the TGR's fish. PAHs, their derivatives and non-target compounds are considered as main causative agents.

© 2015 The Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

Published by Elsevier B.V.

Introduction

The Three Gorges Dam (TGD) was constructed to impound the Yangtze River over a distance of 663 km between the town of Sandouping, Hubei Province, and the Jiangjin district of Chongqing Municipality, to create the Three Gorges Reservoir (TGR). Due to a number of factors this recently established ecosystem will face several challenges in the upcoming future.

As a consequence of the impoundment, 13 cities, 140 towns and over 1300 villages have been submerged in addition to more than 1600 factories and abandoned mines (Smith, 2013). They bear the potential to release remaining contamination into the reservoir. Chongqing municipality (82,403 km²) had a population of more than 29 million people in 2011 (13 million in rural and 16 million in urban areas) (National Bureau of Statistics—China, 2012). Chongqing city, which incorporates almost half of the urban population (6–7 million) in 2012 (BBC, 2012), is already the largest city at the TGR, one of the largest in western China, and belongs to the fastest growing cities worldwide. It is expected that another 4 million people will move away from the rural areas to the cities by 2020, with an urbanization rate of 70% (Xinhua, 2007b). It has been admitted that overpopulation of the area and pollution, as a consequence of the impoundment, poses a serious threat to the vulnerable ecological environment of the reservoir (Xinhua, 2007a,b).

The elevation of the water level improved the navigation of large container ships between Sandouping and Chongqing, and thus became an essential factor for the economic development of this region. The amount of cargo that passed the dam increased by 15.5% per year since the impoundment and reached 106 megatonnes (Mt) per year in 2013 (Xinhua, 2014). The increasing traffic, also including passenger shipping, will entail an elevated discharge of contamination into the reservoir.

An increasing number of citizens and progressive industrialization will trigger an increasing amount of wastewater in the urbanized regions, which will challenge the treatment systems. This means, as the cities grow more domestic and industrial waste ends up in the TGR concentrated especially in these areas. Between 2004 and 2010 about 1000 Mt urban sewage (53%) and industrial waste water (47%) were discharged into the TGR area annually during the impoundment (Ministry of Environmental Protection—China, 2006–2012). The local governments have taken the challenge and already improved the domestic wastewater treatment capacities from annually 515 Mt in 56 facilities in 2008 to 590 Mt in 71 facilities in 2010, accompanied with policy on emission reduction for the industry (Ministry of Environmental Protection—China, 2010, 2012; Wang et al., 2013).

In 2006, Müller et al. (2008) emphasized the importance of growing wastewater fractions in the Yangtze River for the regions below the dam, which result in rising levels of nutrients, heavy metals and dissolved organic carbon accompanied by a discharge of 500 to 3500 kg industrial organic

chemicals per day. However, little information on contamination and possible impacts is yet available on the region upstream of the dam, particularly of the TGR, as recently shown in a review (Floehr et al., 2013).

Sediment plays an important role in environmental assessment, because it can act as sink and source for pollutants (Gerbersdorf et al., 2005; Hilscherova et al., 2007; Hollert et al., 2003). The Yangtze River is one of the largest sediment carriers in the world. Since the operation of the dam annually 151–172 Mt of sediment have been trapped in the TGR (2003–2008) (Hu et al., 2009; Yang et al., 2007), which accounts for about 60%–68% of the sediment entering the TGR from upstream. This has serious consequences for the regions downstream of the dam, especially the Yangtze estuary, which rely on continuous sediment supply (Xu and Milliman, 2009; Yang et al., 2007).

Another essential factor is the reduction of the river's flow velocity due to the construction of the dam, which dropped from 2–3 m/sec in average (upper/middle layer) to (<0.05)–1.5 m/sec (Chen et al., 2005; Wang et al., 2009). This has a serious influence on the dilution of, e.g., wastewater at inlets, from which they have formerly been swept away. Furthermore, it increases the sedimentation rate of suspended particles and adhering contaminants, both with the effect that pollution becomes a more local problem in areas of concern.

Sediments that are contaminated with mutagenic substances pose a hazard to indigenous biota (Chen and White, 2004). It has been shown that fish populations in rivers and lakes in industrial areas in the US and Europe displayed an elevated prevalence for tumors (Balch et al., 1995). The endpoint mutagenicity was already of particular interest in several studies of the Yangtze River. Particularly downstream of the dam and before the impoundment of the reservoir, mutagenic effects could be detected in surface and tap water of these sections (for review cf. Floehr et al., 2013). As a possible result of genotoxic effects on parental cells, the formation of micronuclei can be observed in the daughter cells after cell division (Leme and Marin-Morales, 2009). For convenient application, blood has been proven to be a sensitive compartment for genotoxicity measurement in fish (Boettcher et al., 2010; Kilemade et al., 2004; Rocha et al., 2009). Due to their impact on the genetic material of the organism, the mutagenic and genotoxic effects on cellular level can have not only impairments on the organism itself, but also multigenerational impacts, thus effecting the population and ecosystem level (Diekmann et al., 2004; White et al., 1999).

In order to (i) record the organic contamination and (ii) ecotoxicological effects, (iii) to assess possible links between biochemical responses and ecologically relevant impacts on fish from the field, sediment samples and fish were collected at several sites in the TGR and its watershed in the administrative region of Chongqing municipality. They have been analyzed using a weight-of-evidence approach with several lines of evidence (Chapman and Hollert, 2006). The applied sediment triad approach (Chapman, 1990) integrates chemical analysis, in

vitro/in vivo bioassays and in situ biomarkers for a holistic assessment. Here we focus on (a) the organic pollution of sediments, (b) the role of major tributaries as potential pollution sources, (c) mutagenic effects of sediments and (d) genotoxic impacts on fish of the Yangtze TGR ecosystem.

1. Material and methods

1.1. Concept of the overall study

In the first phase (September 2011), surface sediment samples were taken at several sites. They were screened for organic contamination by chemical analysis, and effects by mechanism-specific assays, in order to identify potential hot-spots. Principal component analysis was used to recognize patterns in contamination at the different sites and source analytics to identify potential origins. Further, a prescreening was performed to find a suitable indicator fish species for the TGR, according to abundance, relevance and feasibility. In the second phase (May 2012), fish samples of the indicator species *Pelteobagrus vachellii* (*P. vachellii*, Richardson, 1846) were taken at the same sites at the next possible time point, after the fishing ban in May, and investigated for suitable biomarkers, e.g., micronuclei formation to detect genotoxicity. In the third phase (May 2013), sediment and fish were sampled in parallel at two identified hot-spots of interest. Sediment was addressed to chemical and bioassay analysis, and fish to detection of suitable biomarkers.

1.2. Chemicals and material

Acetone ($\geq 99.5\%$, p.a.), n-hexane ($\geq 99\%$, p.a.), dichloromethane ($\geq 99.8\%$, p.a.), isooctane ($\geq 99\%$, p.a.), cyclohexane ($\geq 99.5\%$, p.a.), hydrochloric acid (37%, ACS reagent) and acridine were purchased from Sigma Aldrich GmbH (Sigma Aldrich GmbH, Deisenhofen, Germany) and methanol (99%) from Sigma Aldrich Inc. (Sigma Aldrich Inc., Shanghai, China). Dimethylsulfoxide, aluminum oxide (for column chromatography) and sodium sulfate ($\geq 99\%$, p.a.) were supplied by Carl Roth GmbH & Co. KG (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), silica gel 60 (size: 0.063–0.200 mm) by Merck group (Merck group, Darmstadt, Germany), S9 fraction (from phenobarbital/ β -naphthoflavon treated rats) by Harlan Cytotest Research GmbH (Harlan Cytotest Research GmbH, Rosdorf, Germany), cleaned silica sand by Büchi Labortechnik AG (Büchi Labortechnik AG, Flawil, Switzerland) and heparine from Sinopharm Chemical Reagent Co., Ltd. (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Amber glass bottles and vials, polytetrafluoroethylene (PTFE) bottles, syringes and microscope slides were purchased from VWR International GmbH (VWR International GmbH, Darmstadt, Germany) and the 24- and 384-well microplates from TPP Techno Plastic Products AG (TPP Techno Plastic Products AG, Trasadingen, Switzerland).

1.3. Sampling

1.3.1. Sampling campaign September 2011

In a first sampling campaign in September 2011, at a water level of 160 to 166 m altitude above sea level (asl), the surface

layers (~5 cm) of sediments were collected at four major tributaries, i.e., Jialing River at Chongqing (CNG), Long River at Fengdu (FEN), Pengxi River/Xiao River at Yunyang (YUN), Daning River at Wushan (WU), along the Yangtze River mainstream of the TGR, as well as an artificial lake (Hanfeng Lake, Kaixian) (HF-L) and a nature reserve (Baijiayi River; Pengxi River Wetland Nature Reserve) (BJX-R) in the TGR watershed (Fig. 1), using a Van-Veen sampler. The Pengxi River Wetland Nature Reserve was selected as reference site, assuming minimum pollution in the poorly industrialized area. At each site along the mainstream three locations were sampled-upstream (U) and downstream (D) the tributary's inlet as well as in the tributaries (T). At the artificial lake site and the reference site only one location was sampled. The samples from each location were pooled samples. Each pooled sample consisted of sediment from at least three individual sampling spots at this location, which were combined to reach a total volume of 500 mL. The individual samples were taken in a radius of 50 m from the first sampling spot to cover intrinsic heterogeneity of each sampling location. The pooled samples were stored in inert PTFE-bottles at 4°C for transportation. The samples were freeze dried, sieved with a 2 mm sieve to remove sticks and stones, and kept in amber glass bottles at 4°C for further analysis according to the methods given by Hollert et al. (2000).

1.3.2. Sampling campaign May 2012

In a second sampling campaign in May 2012, at a water level of 164–160 m altitude asl, fish samples were taken at the sampling sites Chongqing (CNG 2012), Fengdu (FEN 2012), Yunyang (YUN 2012) and Wushan (WU 2012) in the TGR, as well as the Hanfeng Lake (HF 2012) and Baijiayi River (BJX 2012) in the TGR watershed (Fig. 1). The Baijiayi River in the Pengxi River Wetland Nature Reserve was again selected as reference site. The darkbarbel catfish *P. vachellii*, was chosen as indicator fish species due to its demersal lifestyle, with a low to medium migration and thus being suitable to indicate local contamination over longer periods of time, as well as its distribution all along the TGR, its frequent occurrence in catches from all sampled sites and its major economic importance for the region (Ministry of Environmental Protection—China, 2007). At each site 10 fish of comparable size (total length 165 ± 15 mm; standard length 142 ± 14 mm; weight 40 ± 11 g, indifferent sex) were sampled with the help from local fishermen. The fish were kept in water of the local water body in an aerated tank till each fish was euthanized with clove oil, followed by an additional blow to the head. Blood samples were obtained from a caudal section with a heparinized syringe and transferred to two microscope slides per fish according to the methods given by Rocha et al. (2009) and Boettcher et al. (2010). Each blood smear was fixed in methanol for 5 min. The fish were processed further for analysis of additional biomarkers.

1.3.3. Sampling campaign May 2013

In a third sampling campaign in May 2013, at a water level of 160 m altitude asl, sediment and fish samples were taken in parallel at two identified regional hot-spots, i.e., near the cities of Chongqing (CNG 2013) and Kaixian (HF 2013). To ensure the

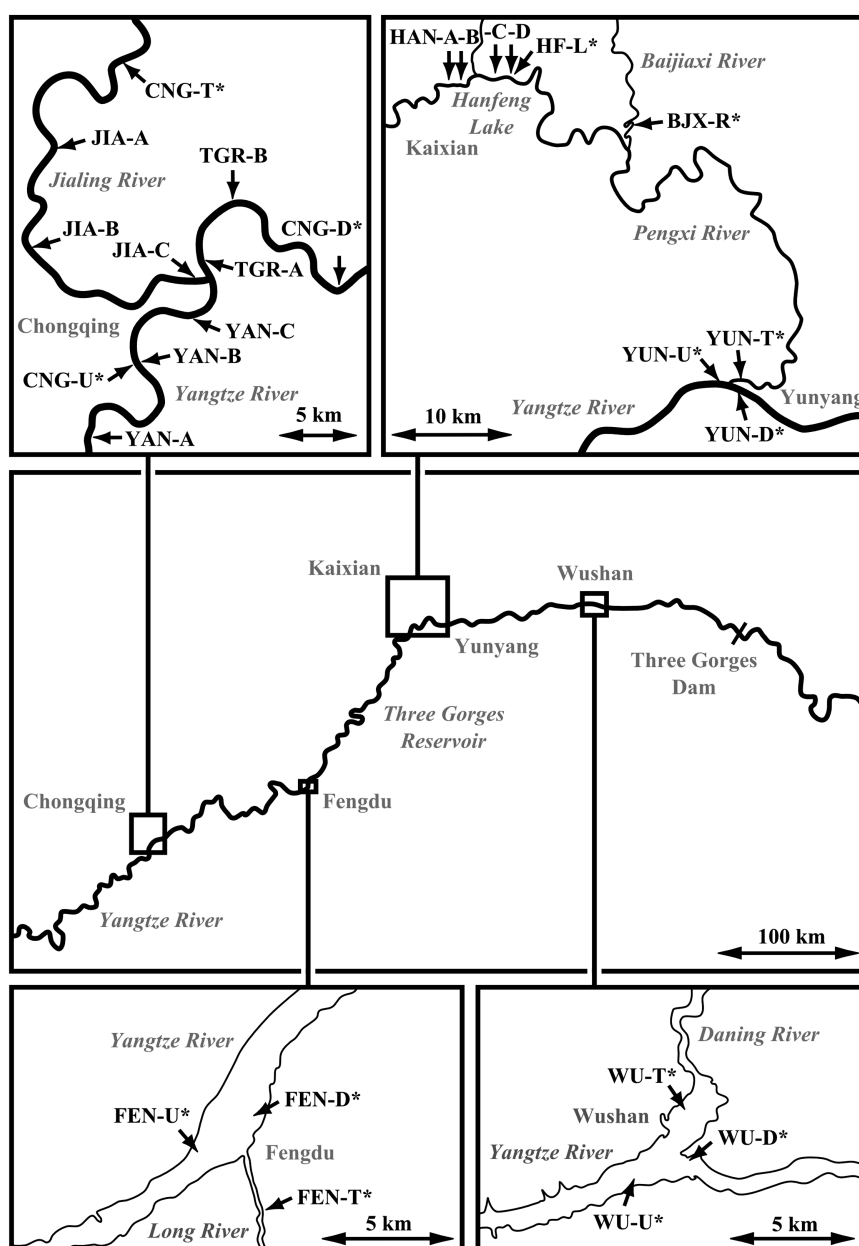


Fig. 1 – Overview map of sampling locations at the Three Gorges Reservoir (TGR) and its watershed. The Three Gorges Reservoir covers the Yangtze River section between Jiangjin district (Chongqing) and the Three Gorges Dam. Names in gray depict geographical markers: water bodies (*italicized*), cities and the dam. Fish were sampled in the TGR close to the cities Chongqing (CNG), Fengdu (FEN), Yunchang (YUN) and Wushan (WU), as well as in the TGR watershed in the Hanfeng (HF) Lake and Baijiayi River (BJX). Labels in black signify sediment sampling locations from campaign September 2011 (with asterisks; CNG-U,-T,-D; FEN-U,-T,-D; YUN-U,-T,-D; WU-U,-T,-D; HF-L; BJX-R) and campaign May 2013 (without asterisks; YAN-A,-B,-C; JIA-A,-B,-C; TGR-A,-B; HAN-A,-B,-C,-D; YAN: Yangtze; JIA: Jialing; TGR: the Three Gorges Reservoir; HAN: Hanfeng Lake; U: upstream, T: tributary, D: downstream, L: lake, R: reference; A, B, C, and D: order in flow direction).

same biological status of the fish according to their seasonal rhythm in order to prevent major physiological oscillations that could influence the biomarkers, and because May and September have shown comparable temperature, precipitation and TGR water level, May was chosen for the combined sampling of sediment and fish. For sediment, at Chongqing three samples were taken in the Jialing River (JIA), three samples in the Yangtze River (YAN) upstream of the

conversion zone of both rivers, and two samples downstream of the conversion zone in the reservoir (TGR). Samples were labeled in order of flow direction (A, B, C) (Fig. 1). In addition, four samples were taken in the Hanfeng artificial lake (HF) at Kaixian. Samples were labeled in order of flow direction (A, B, C, D) (Fig. 1). For fish, 20 individuals of comparable size (total length 189 ± 23 mm; standard length 163 ± 21 mm; weight 61 ± 21 g; indifferent sex) were sampled at both sites (CNG,

Table 1 – List of target compounds in sediment extracts of sampling campaign September 2011^a and May 2013^b.

Pesticides ^a	Chlorpyrifos, chlorfenvinphos, aldrin, isodrin, dieldrin, endrin, alpha-endosulfan, beta-endosulfan, <i>p,p'</i> -DDE, <i>p,p'</i> -TDE, <i>o,p'</i> -DDT, <i>p,p'</i> -DDT, trifluralin, simazine, atrazine, lindane (gamma-HCH), alachlor, hexachlorobenzene
PAHs ^{abc}	Naphthalene (NP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FL), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3- <i>c,d</i>]pyrene (IcdP), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP)
PBDEs ^a	BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154
PCBs ^a	PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180
Other intermediate and by-products ^a	1,2,3-Trichlorobenzene, 1,2,4-trichlorobenzene, 1,3,5-trichlorobenzene, alpha-HCH, beta-HCH, delta-HCH, hexachlorobutadiene, pentachlorobenzene

PAHs: polycyclic aromatic hydrocarbons; DDE: dichlorodiphenyldichloroethylene; TDE: dichlorodiphenyldichloroethane; DDT: dichlorodiphenyltrichloroethane; PBDEs: polybrominated diphenyl ethers; PCBs: polychlorinated biphenyls; HCH: hexachlorocyclohexanes.

^a Target compounds analyzed in campaign September 2011.

^b Target compounds analyzed in campaign May 2013.

^c Priority PAHs according to the Environmental Protection Agency & Office of Federal Registration—USA (1982).

HF) (Fig. 1). Sampling procedures for sediment and fish, as well as further treatment were as described in Sections 1.3.1 and 1.3.2.

1.4. Sediment extraction

Each freeze dried sediment sample was extracted with acetone-hexane (1:1) (p.a.) in a pressurized liquid extractor (Speed Extractor E-916, Büchi Labortechnik AG, Flawil, Switzerland) at 100°C and 120 bar in two cycles (heat up 1 min; hold 10 min; discharge 2 min; flush with solvent 1 min; flush with gas 4 min). For process controls, extraction tubes were filled with cleaned silica sand and further processed in parallel. After the reduction in volume with a rotary evaporator and under a gentle nitrogen stream, the extracts were redissolved in dimethylsulfoxide (DMSO) to a final concentration of 20 g sediment equivalents (SEQ) per mL DMSO and stored at 4°C in amber glass vials for further testing in the Ames fluctuation assay. The whole process was performed under protection from light to prevent photodegradation of toxic compounds. In the following, sediment concentrations in gram SEQ per milliliter solvent will be given as gram per milliliter (g/mL).

1.5. Chemical analysis

1.5.1. Sampling campaign September 2011

Every extract was screened for 54 organic priority compounds (Table 1), selected on basis of the European Water Framework Directive (EWFD Directive, 2000/60/EC, 2000; EWFD Directive, 2008/105/EC, 2008), according to Erger et al. (2012). For each sample, 20 g freeze dried sediment were extracted as described in Section 1.4, with process controls in parallel. After reduction in volume, the extracts were cleaned up chromatographically on columns filled with 2 g silica gel and 2 g aluminum oxide using 15 mL *n*-hexane, 5 mL *n*-hexane:dichloromethane (9:1) and 20 mL *n*-hexane:dichloromethane (4:1) as eluate. After adjustment to 200 µL, each sample was provided for gas chromatography-mass spectrometry (GC-MS) analysis using a GC-MS system (6890 GC and 5973 mass selective detector (MSD) single quadrupole mass analyzer) equipped with an automatic sampler (MPS 2) (6890GC/5973 MSD/MPS 2, Agilent Technologies GmbH, Germany) and Cooled Injection System (CIS 4, Gerstel GmbH, Germany). The limit of detection (LOD) was 10 ng

polycyclic aromatic hydrocarbons (PAH)/g SEQ. In the following, PAH concentrations in nanogram PAH per gram SEQ will be given as nanogram per gram (ng/g).

1.5.2. Sampling campaign May 2013

Based on the results of the prior 2011 campaign sediment extracts of campaign May 2013 were screened only for the 16 priority PAHs (Table 1), according to International Standard Operation (ISO) guideline 18287-2006. For each sample, 20 g freeze dried sediment were extracted as described in Section 1.4, with process controls in parallel. Activated copper was used to remove sulfur over 96 hr. After reduction in volume the samples were cleaned up chromatographically on columns filled with 4 g silica gel and 3 g sodium sulfate using *n*-hexane (p.a.) instead of petroleum ether as eluate. Isooctane (p.a.) was added as retention solvent, before the extract was reduced in volume under a gentle nitrogen stream. The extract was set to a final concentration of 20 g/mL with cyclohexane (p.a.) before being provided for GC-MS analysis. The GC system (GC 7890 A) was coupled with a MSD (5975 C inert XL MSD with Triple-Axis-Detector) operated in SIM (selective ion monitoring) mode and an autosampler (GC 7890A/5975 C inert XL MSD, Agilent Technologies GmbH, Germany). The LOD was 10 ng PAH/g SEQ. In the following, PAH concentrations in nanogram PAH per gram SEQ will be given as nanogram per gram (ng/g).

1.6. Total organic carbon analysis

For each sample, 150 to 300 mg freeze dried sediment were transferred to amber glass vials and 2 mL hydrochloric acid (2 mol/L) per gram sediment were added. After evaporation in the fume hood for 24 hr, samples were dried at 105°C overnight. Per sample, 3 individual replicates of 25 mg each were analyzed for total organic carbon (TOC) content in an analyzer (Vario EL III Elemental Analyzer, Elementar Analysensysteme GmbH, Germany) and results were computed with Winvar V5.01 (Elementar Analysensysteme GmbH, Hanau, Germany).

1.7. Principal component analysis

Principal component analysis (PCA) as an ordination technique can be deployed in a broad set of data to reduce the number of

dimensions of the multivariate dataset (m -sites \times n -compounds) to its “principal components”. The total complexity is condensed so that a few axes explain most of the variance of the dataset in order to generate hypotheses on the relationship between the site characteristics and the content of the individual substances (Cincinelli et al., 2007; Höss et al., 2010; Ottermanns, 2008).

The PCA was chosen after consulting the results of detrended correspondence analysis (DCA), which indicated very short gradients (1.067 and 0.673 for the x and y axis respectively for the 2011 data, 0.900 and 0.703 for the 2013 data) and thus allowed for the use of a linear method (Ottermanns, 2008).

PCA analysis was conducted by means of Canoco for Windows, Version 4.5 (Biometris, Plant Research International, Wageningen, Netherlands). The data were log-transformed prior to the analysis, which was performed on the covariance matrix, because the variance of the pollutant concentrations was considered sufficiently homogenous.

1.8. PAH source analysis

Well suited pairs of parent PAHs with the same molecular mass (M) for diagnostic ratios are anthracene (ANT) and phenanthrene (PHE) (M : 178 g/mol), fluoranthene (FLA) and pyrene (PYR) (M : 202 g/mol), benzo[*a*]anthracene (BaA) and chrysene (CHR) (M : 228 g/mol), as well as indeno[1,2,3-*cd*]pyrene (IcdP) and benzo[*g,h,i*]perylene (BghiP) (M : 276 g/mol) (Yunker et al., 2002). Diagnostic ratios are given in Table 2. The ANT/(ANT + PHE) ratio is recommended to distinguish petroleum sources (petrogenic) from combustion sources (pyrogenic), while the FLA/(FLA + PYR) ratio separates petroleum combustion from other combustion types. Further, the BaA/(BaA + CHR) and the IcdP/(IcdP + BghiP) ratios are applicable to support a refined identification of the combustion sources (Tobiszewski and Namieśnik, 2012; Yunker et al., 2002). For the evaluation of the sediment samples, all four ratios were determined in order to corroborate the individual results.

1.9. Ames fluctuation assay

The Ames fluctuation assay is a modified version of the traditional Ames test (Ames et al., 1975; McCann et al., 1975). While the traditional test is performed using agar plates (plate incorporation technique), the Ames fluctuation assay uses a

liquid medium to expose and incubate bacteria in 24- and 384-well microplates, respectively. The Ames fluctuation assay including cytotoxicity determination was performed according to the method described by the ISO guideline 11350-2012 as detailed by Reifferscheid et al. (2012) and Higley et al. (2012). Each sample was tested in three individual replicates. Six concentration levels per sample were prepared by 1:2 dilution steps.

Two tester strains were used to examine the samples' mutagenic potential. Both strains are auxotrophic mutants of the bacterium *Salmonella typhimurium*. While TA98 carries a frameshift mutation (hisD3052), TA100 carries a base pair substitution (hisG46). Both mutations disable the bacteria from growing in histidine-free medium unless they are reverted to a prototrophic state by back mutation (Ames et al., 1975). To determine metabolic activation of promutagenic substances, each sample was tested additionally with a liver homogenate S9-fraction from phenobarbital/ β -naphthoflavon treated rats, further supplemented for application in the assay (S9 supplement). Based on the results of the prior 2011 campaign, sediment samples of campaign May 2013 were only tested with strain TA98 in the presence and absence of S9 supplement for metabolic activation.

Tests were valid when mean values of revertants were >0 and ≤ 10 per 48 wells in negative controls and ≥ 25 per 48 wells in positive controls. Shapiro–Wilk's test on normal distribution and Levene's test on variance homogeneity (with residuals) were performed as pretesting sequences. If data showed normal distribution and variance homogeneity, William's multiple sequential t -test ($\alpha = 0.05$) was used to determine no observed effect concentration (NOEC) values. The results of the above tests allow for the use of the parametric William's test without further transformation. If data showed normal distribution but variances were heterogeneous, Welch t -test with Bonferroni–Holm adjustment was performed. In the following, test concentrations in milligram SEQ per milliliter solvent will be given as milligram per milliliter (mg/mL).

1.10. Micronucleus assay

The micronucleus assay was performed with minor modifications according to the methods given by Rocha et al. (2009)

Table 2 – Diagnostic ratios for potential PAH sources.

PAH ratio	Values	Source	Reference
ANT/(ANT + PHE)	<0.1	Petroleum	Pies et al. (2008)
	>0.1	Combustion	
FLA/(FLA + PYR)	<0.4	Petroleum	De la Torre-Roche et al. (2009)
	0.4–0.5	Vehicular emission	
	>0.5	Coal/grass/wood combustion	
BaA/(BaA + CHR)	<0.2	Petroleum	Yunker et al. (2002)
	0.2–0.35	Petroleum/combustion	
	>0.35	Combustion	
IcdP/(IcdP + BghiP)	<0.2	Petroleum	Yunker et al. (2002)
	0.2–0.5	Liquid fossil fuel combustion (vehicle/crude oil)	
	>0.5	Coal, grass, wood combustion	

ANT: anthracene; PHE: phenanthrene; FLA: fluoranthene; PYR: pyrene; BaA: benzo[*a*]anthracene; CHR: chrysene; IcdP: indeno[1,2,3-*cd*]pyrene; BghiP: benzo[*g,h,i*]perylene; PAH: polycyclic aromatic hydrocarbon.

Table 3 – PAH content in sediment extracts of sampling campaign September 2011 (unit: ng/g).

Substance	Σ substance	Chongqing			Fengdu			Yunyang			Wushan			Kaixian	
		CNG-U	CNG-T	CNG-D	FEN-U	FEN-T	FEN-D	YUN-U	YUN-T	YUN-D	WU-U	WU-T	WU-D	HF-L	B/X-R
Naphthalene	360	220 ^a	<10	<10	140	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Acenaphthylene	12	12	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Acenaphthene	99	33 ^a	<10	14	16 ^a	<10	12	<10	<10	<10	13	<10	11	<10	<10
Fluorene	243	64 ^a	21 ^a	20 ^a	36 ^a	14	28 ^a	<10	<10	<10	<10	<10	<10	60 ^a	<10
Phenanthrene	2642	340 ^a	120	130	230	82	150	250 ^a	260 ^a	180	280 ^a	140	190	240 ^a	50
Anthracene	154	41	15	27	29	<10	20	<10	<10	<10	<10	<10	<10	21	<10
Fluoranthene ^b	1307	170	55	120	120	38	95	130	73	110	130	61	120	60	24
Pyrene	1184	150	47	99	100	34	81	120	76	100	130	62	110	53	22
Benzo[a]anthracene ^{b,c}	605	73	19	64	62	15	52	62	34	64	61	23	41	24	12
Chrysene ^{b,c}	1695	120	41	65	79	29	60	110	81	780 ^a	110	55	70	81	15
Benzo[b]fluoranthene ^{b,c}	1721	170	55	110	120	36	110	200	130	160	210	100	150	140	30
Benzo[k]fluoranthene ^{b,c}	333	38	12	24	28	11	24	41	22	23	44	19	30	17	<10
Benzo[a]pyrene ^{b,c}	626	76	21	57	52	15	43	71	43	56	76	32	56	27	<10
Indeno[1,2,3-c,d]pyrene ^{b,c}	472	57	21	41	44	16	36	48	33	35	51	21	35	35	<10
Dibenzol[a,h]anthracene ^{b,c}	80	12	<10	<10	10	<10	<10	15	10	<10	13	<10	10	11	<10
Benzo[g,h,i]perylene	693	76	25	44	55	20	41	75	61	54	81	39	54	57	11
Σ PAHs	1653	450	816	1122	310	750	1121	824	1563	1198	552	877	827	165	
Σ PAHs with Ames activity	717	223	482	514	160	419	676	427	1228	694	311	512	395	81	
TOC mean (%)	1.65	0.77	0.40	0.94	1.06	0.72	0.97	0.86	0.69	0.91	0.91	0.51	0.91	0.42	
TOC SD (%)	0.02	0.04	0.01	0.02	0.05	0.03	0.00	0.01	0.00	0.02	0.02	0.02	0.05	0.04	

Σ PAHs with Ames activity: sum of PAHs that have been shown to be mutagenic in the Ames assay (Pérez et al., 2003); U: upstream; T: tributary; D: downstream; L: lake; R: reference; Σ substance: total content of substances; TOC: total organic carbon; SD: standard deviation; PAH: polycyclic aromatic hydrocarbon; chemical analysis n = 1 and TOC analysis n = 3.

^a Concentration exceeded "effect range low" value according to Long et al. (1995) (Table 7).

^b Substance has been shown to be mutagenic in the Ames assay (Pérez et al., 2003).

^c Substance is cancerogenic according to WHO (2010).

Table 4 – PAH content in sediment extracts of sampling campaign May 2013 (unit: ng/g).

Substance	Σ substance	Chongqing						Kaixian					
		Yangtze			Jialing			Hanfeng Lake					
		YAN-A	YAN-B	YAN-C	JIA-A	JIA-B	JIA-C	TGR-A	TGR-B	HAN-A	HAN-B	HAN-C	HAN-D
Naphthalene	539	27	35	45	58	62	38	37	24	33	35	86	63
Acenaphthylene	141	16	16	17	22	18	16	<10	<10	<10	<10	21	16
Acenaphthene	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Fluorene	228	14	16	18	18	19 ^a	15	14	12	14	13	42 ^a	37 ^a
Phenanthrene	402	30	29	42	35	30	22	22	17	20	18	73	68
Anthracene	175	18	18	19	18	17	17	17	16	<10	<10	19	17
Fluoranthene ^b	442	63	43	57	40	35	35	39	25	17	17	40	34
Pyrene	373	55	35	45	33	31	29	29	21	16	16	34	30
Benzo[a]anthracene ^{b,c}	270	28	25	31	23	23	26	24	20	14	14	24	21
Chrysene ^{b,c}	353	46	29	41	22	23	22	25	17	16	15	53	47
Benzo[b]fluoranthene ^{b,c}	370	31	27	44	13	13	16	25	<10	<10	<10	116	87
Benzo[k]fluoranthene ^{b,c}	292	34	27	36	20	22	25	25	<10	<10	<10	59	47
Benzo[a]pyrene ^{b,c}	68	24	<10	25	<10	<10	19	<10	<10	<10	<10	<10	<10
Indeno[1,2,3-c,d]pyrene ^{b,c}	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Dibenzo[a,h]anthracene ^{b,c}	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Benzo[g,h,i]perylene	64	<10	<10	18	<10	<10	<10	<10	<10	<10	<10	26	20
Σ PAHs		383	297	433	299	291	278	254	150	129	127	590	484
Σ PAHs with Ames activity		226	150	232	117	115	141	137	61	47	46	291	234
TOC mean (%)		0.26	0.39	0.36	0.45	0.66	0.30	0.19	0.11	0.12	0.27	1.00	0.99
TOC SD (%)		0.08	0.03	0.05	0.05	0.10	0.03	0.01	0.01	0.02	0.03	0.04	0.01

Σ PAHs with Ames activity: sum of PAHs that have been shown to be mutagenic in the Ames assay (Pérez et al., 2003); TGR: the Three Gorges Reservoir; A, B, C, and D refer to order in flow direction (Fig. 1); TOC: total organic carbon; SD: standard deviation; PAH: polycyclic aromatic hydrocarbon; chemical analysis n = 1 and TOC analysis n = 3.

^a Concentration exceeded "effect range low" value according to Long et al. (1995) (Table 7).

^b Substance has been shown to be mutagenic in the Ames assay (Pérez et al., 2003).

^c Substance is cancerogenic according to WHO (2010).

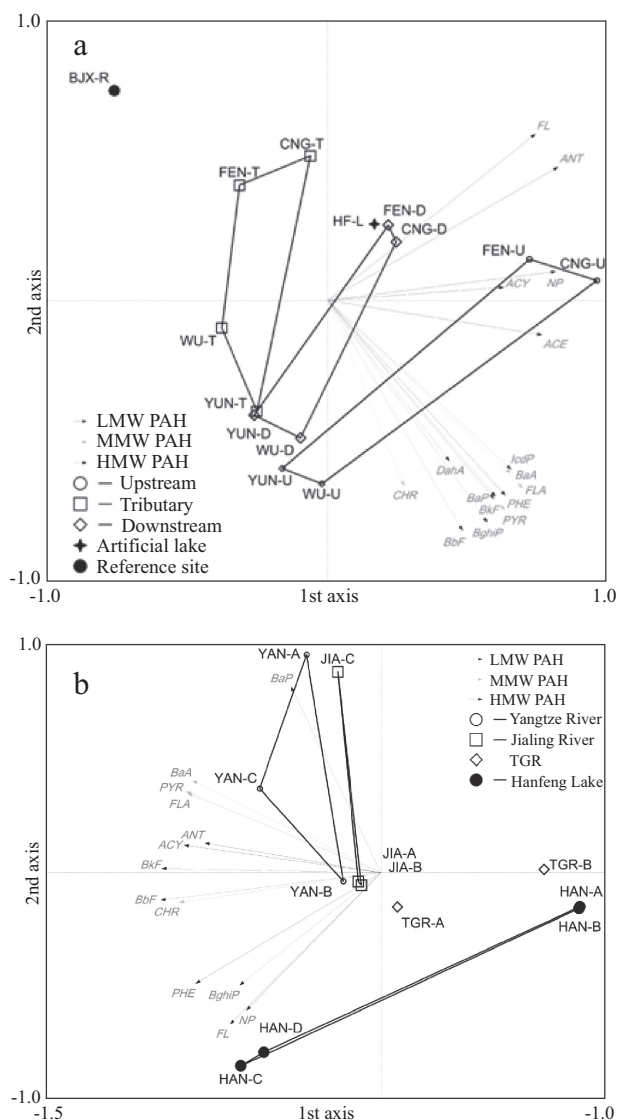


Fig. 2 – Principal component analysis of PAH concentrations in sediment extracts from sampling campaign (a) September 2011 and (b) May 2013 analyzed as quantitative data. Arrows resemble individual PAHs; length of arrows equals relative amount of PAHs. Eigenvalues of x axis are (a) 0.48 and (b) 0.67, and eigenvalues of y axis are (a) 0.26 and (b) 0.14. NP: naphthalene; FL: fluorene; FLA: fluoranthene; ANT: anthracene; ACY: acenaphthylene; ACE: acenaphthene; IcdP: indeno[1,2,3-c,d]pyrene; DahA: dibenzo[a,h]anthracene; BaA: benzo[a]anthracene; BaP: benzo[a]pyrene; BkF: benzo[k]fluoranthene; BghiP: benzo[g,h,i]perylene; BbF: benzo[b]fluoranthene; CHR: chrysene; PHE: phenanthrene; and PYR: pyrene.

and Boettcher et al. (2010). To determine micronucleus formation acridine orange was used for staining of the fixated blood smears on the slides. For each fish, 4000 erythrocytes (2000 per slide) were examined using an epifluorescence microscope (BIOREVO BZ-9000E, Keyence, Germany) equipped with an oil-immersion lens at a 600 \times magnification. The cells were evaluated according to the scoring criteria of the ISO

guideline 21427-2:2006: (a) only erythrocytes with intact cell membrane were scored. The micronuclei should have (b) a maximum size of about 30% of and (c) the same staining intensity as the main nucleus, as well as (d) a clear separation from it. Mann–Whitney rank sum test ($\alpha = 0.05$) was used to determine significant differences in micronucleus formation of the samples in comparison to the reference site. Further, induction factors (IF) were computed, which give the induction of the mean micronucleus frequency of each sample referred to the reference site.

2. Results and discussion

2.1. PAHs in sediments

2.1.1. Total PAH content

In the sampling campaign during September 2011, among all 54 analyzed compounds (Table 1) only the 16 priority PAHs could be detected (Table 3). The total PAH (PAH_{Σ16}) concentrations ranged from 165 (BJX-R/reference site) to 1653 ng/g (CNG-U).

Each upstream sample of the four sites along the mainstream displayed the highest PAH_{Σ16} content, with exception of Yunyang, and the tributaries the lowest, giving the dominating pattern for PAH_{Σ16} content per site: upstream > downstream > tributary. Inter-site comparison along the mainstream, integrating each three samples per site, showed the following order for PAH_{Σ16} content: Yunyang > Chongqing > Wushan > Fengdu (Table 3). Further, it could be observed that the PAH_{Σ16} content from the downstream location of one site typically increased to the upstream location of the next site in flow direction (e.g., CNG-D to FEN-U), with exception of YUN-D/WU-U (Table 3), which could hint to an accumulation of PAH contamination between the tributaries due to lesser dilution.

In sampling campaign May 2013 PAH_{Σ16} concentrations ranged from 150 ng/g (TGR-B) to 433 ng/g (YAN-C) at Chongqing, and 127 ng/g (HAN-B) to 590 ng/g (HAN-C) at Kaixian (Table 4). In general a decline in the PAH_{Σ16} concentration could be measured in the sediments along the flow direction with exception of YAN-C. The concluding pattern was YAN-C > YAN-A > YAN-B > TGR-A > TGR-B for the Yangtze River and JIA-A > JIA-B > JIA-C > TGR-A for the Jialing River. In comparison, the Yangtze River displayed a higher PAH_{Σ16} content than the Jialing River and the TGR (Tables 3 and 4). These values correspond to a study by Tang et al. (2011), who found comparable PAH_{Σ16} concentrations in sediments of the Yangtze River (257–723 ng/g) and Jialing River (132–349 ng/g) sampled at Chongqing in October 2009.

At Kaixian, HAN-D and HAN-C possessed a three to four times higher PAH_{Σ16} concentration than HAN-B and HAN-A (Table 4). This indicates a pollutant influx between HAN-B and HAN-C, which could originate from the city of Kaixian on the south side and/or the Dong or Toudao River, which enter the lake on the north side. This assumption is underlined by the observation that benzo[b]fluoranthene was alike benzo[k]fluoranthene below the LOD in the upper part of the Hanfeng Lake (HAN-A, HAN-B), but both could be measured in the lower part of the Hanfeng Lake (HAN-C, HAN-D) (Table 4).

Table 6 – Diagnostic ratios for PAHs in sediments from campaign May 2013 with related sources.

PAH diagnostic ratios	Chongqing											
	Yangtze			Jialing			TGR			Kaixian		
	YAN		YAN	YAN		YAN	JIA		JIA	TGR		TGR
	-A	-B	-C	-A	-B	-C	-A	-B	-C	-A	-B	-C
ANT/(ANT + PHE) ^a	0.37	0.38	0.31	0.34	0.36	0.44	0.44	0.49	<0.33	<0.36	0.20	0.20
FLA/(FLA + PYR) ^b	0.53	0.55	0.56	0.54	0.53	0.54	0.58	0.55	0.52	0.52	0.54	0.53
BaA/(BaA + CHR) ^c	0.37	0.47	0.43	0.51	0.51	0.54	0.49	0.54	0.47	0.48	0.31	0.31
IcdP/(IcdP + BghiP) ^d	<0.53	n.d.	<0.36	n.d.	n.d.	<0.59	n.d.	n.d.	n.d.	n.d.	<0.28	<0.34

n.d.: no diagnostic ratio could be determined; PAH: polycyclic aromatic hydrocarbon.

^a Ratio of ANT (anthracene) and PHE (phenanthrene) indicates as source: petroleum (<0.1) or combustion (>0.1) (Pies et al., 2008).

^b Ratio of FLA (fluoranthene) and PYR (pyrene) indicates as source: petroleum (<0.4) or vehicular emission (0.4–0.5) or grass/wood/coal combustion (>0.5) (De la Torre-Roché et al., 2009).

^c Ratio of BaA (benzo[a]anthracene) and CHR (chrysene) indicates as source: petroleum (<0.2) or petroleum/combustion (0.2–0.35) or combustion (>0.35) (Yunker et al., 2002).

^d Ratio of IcdP (indeno[1,2,3-c,d]pyrene) and BghiP (benzo[g,h,i]perylene) indicates as source: petroleum (<0.2) or liquid fossil fuel combustion (vehicle/crude oil) (0.2–0.5) or grass/wood/coal combustion (>0.5) (Yunker et al., 2002).

Table 5 – Diagnostic ratios for PAHs in sediments from campaign September 2011 with related sources.

PAH diagnostic ratios	Chongqing			Fengdu			Yunyang			Wushan			Kaixian	
	CNG		CNG	FEN		FEN	YUN		YUN	WU		WU	HF	BjX
	-U		-T	-U		-T	-U		-T	-U		-T	-L	-R
	-D		-D	-D		-D	-D		-D	-D		-D	-D	-D
ANT/(ANT + PHE) ^a	0.11	0.11	0.17	0.11	<0.11 ^b	0.12	<0.04 ^b	<0.04 ^b	<0.05 ^b	<0.03 ^b	<0.07 ^b	<0.05 ^b	0.08	<0.17 ^b
FLA/(FLA + PYR) ^c	0.53	0.54	0.55	0.54	0.53	0.54	0.52	0.49	0.52	0.50	0.49	0.52	0.53	0.52
BaA/(BaA + CHR) ^d	0.38	0.31	0.49	0.44	0.34	0.46	0.36	0.30	0.08	0.36	0.29	0.37	0.23	0.44
IcdP/(IcdP + BghiP) ^e	0.43	0.45	0.48	0.44	0.44	0.47	0.39	0.35	0.39	0.39	0.35	0.40	0.38	<0.47 ^f

PAH: polycyclic aromatic hydrocarbon.

^a Ratio of ANT (anthracene) and PHE (phenanthrene) indicates as source: petroleum (<0.1) or combustion (>0.1) (Pies et al., 2008).^b Concentration of anthracene was <10 ng/g.^c Ratio of FLA (fluoranthene) and PYR (pyrene) indicates as source: petroleum (<0.4) or vehicular emission (0.4–0.5) or grass/wood/coal combustion (>0.5) (De la Torre-Roché et al., 2009).^d Ratio of BaA (benzo[a]anthracene) and CHR (chrysene) indicates as source: petroleum (<0.2) or petroleum/combustion (0.2–0.35) or combustion (>0.35) (Yunker et al., 2002).^e Ratio of IcdP (indeno[1,2,3-c,d]pyrene) and BghiP (benzo[g,h,i]perylene) indicates as source: petroleum (<0.2) or liquid fossil fuel combustion (vehicle/crude oil) (0.2–0.5) or grass/wood/coal combustion (>0.5) (Yunker et al., 2002).^f Concentration of indeno[1,2,3-c,d]pyrene was <10 ng/g.

The temporal variation between the campaigns in September 2011 and May 2013 showed that PAH concentrations were generally higher in September 2011. These observations stand in agreement with Wang et al. (2013), who referred the temporal differences in pollution to the drawdown period of the TGR (around May), where water quality is better than during the other seasons.

With regard to PAH_{Σ16} concentrations in the water phase Wang et al. (2009) could measure overall 14 to 97 ng/L at sites along the reservoir, with highest concentrations at Chongqing, Changshou and Maoping in May 2008. Overall, they stated that obvious regional variations of PAHs, polychlorinated biphenyls (PCBs) and organochlorine pesticide levels appeared along the reservoir and that its water could be classified as being polluted by HCB and PAH based on water quality criteria (Wang et al., 2009). They repeated their investigations along the TGR in 2009 and 2011 and could detect PAH_{Σ16} concentrations from 15 to 381 ng/L in surface water, with highest concentrations at Chongqing, and an obvious decrease from the upper to the lower part of the reservoir. Further, they found a dynamic relation of the contamination to anthropogenic activities and a certain contribution of the tributaries to the PAH pollution of the mainstream (Wang et al., 2013). Overall they could calculate a total PAH mass flux from 110 to 2160 mg/sec (Deyerling et al., 2014). Wolf et al. (2013) could determine PAH concentrations (Naphthalene excluded) between 5 to 101 ng/L (mean: 22 ± 33 ng/L) along the TGR between Chongqing and Yunyang, and also at the Hanfeng Lake in September 2011. The detected concentrations were designated to be in comparable ranges or even lower than measured in surface waters in western industrialized countries. Furthermore, concentrations of perfluorinated compounds, PCBs and

polybrominated diphenyl ethers, were below the respective detection limits, which were referred to their low solubility (Wolf et al., 2013). The analyzed organic pollutants, with exception of the two pesticides Picloram and Clopyralid, met the standards for the Chinese National Drinking Water Quality Standard GB 5749 (Ministry of Health—China, 2006) and the European Union (EU) Council Directive 98/83/EC on the quality of water intended for human consumption (The Council of the European Union, 1998) and the EU Directive 2008/105/EC on environmental quality standards in the field of water policy (EWFD Directive, 2008/105/EC, 2008; Wolf et al., 2013).

In comparison to other sections of the Yangtze River, the sampled locations in this study exhibited a similar PAH_{Σ16} content, but were mostly rather in the lower part of the ranges detected elsewhere along the river (Feng et al., 2007a; Liu et al., 2014; Wang et al., 2012). Other Chinese rivers, like the Pearl River (Mai et al., 2002) and Yellow River (Yu et al., 2009), exhibited also comparable and mainly higher PAH_{Σ16} concentrations. Also in international comparison, the total PAH_{Σ16} content of TGR surface sediments were below or at the lower limit of, e.g., German main water bodies like Danube River (Keiter et al., 2008) and Rhine River, where benzo[e]pyrene was analyzed instead of benzo[a]pyrene (Kosmehl et al., 2004).

Considering the mass balance for PAHs, under assumption that annually 151–172 Mt of sediment remain in the TGR (2003–2008) (Hu et al., 2009; Yang et al., 2007) and that the average PAH_{Σ16} burden in sediment along the reservoir is 936 ± 414 ng/g (September 2011 campaign), an amount of about 79–232 t PAH_{Σ16} per year or 216–636 kg PAH_{Σ16} per day are deposited in the reservoir. Further, adopting the reservoir surface area of 1080 km² as potential bottom deposition area of the reservoir (water body pictured as a cuboid) results into about 0.2–0.6 kg PAH_{Σ16}/(km²·day) (0.2–0.6 mg PAH_{Σ16}/(m²·day)). This assumption neglects seasonal differences and less industrialized regions between the major tributaries, but also that an even larger portion of PAHs enters the reservoir, remains in the water phase and is discharged behind the dam, either solved in water or bound to particles. These numbers are in agreement with the mass balance estimation of Müller et al. (2008) that about 500 to 3500 kg of phenols, chlorinated compounds, aromatic hydrocarbons and PAHs are discharged by the Yangtze River per day. Zhang and Tao (2009) estimated an annual atmospheric emission of 114,000 t PAH_{Σ16} for China in 2004, accounting for 22% of the global emission, followed by India (17%) and the United States (6%).

2.1.2. Specific PAH content

The samples from campaign September 2011 have shown that overall the low molecular weight (LMW; 2–3 rings) PAH phenanthrene, the medium molecular weight (MMW; 4 rings) PAHs chrysene, fluoranthene and pyrene, as well as the high molecular weight (HMW; 5–6 rings) PAH benzo[b]fluoranthene formed the largest share of the total PAH spectrum among all samples, generally constituting together more than 70% of the total content (Table 3).

Further, in sampling campaign May 2013, overall the LMW PAH naphthalene (13%) and the MMW PAH fluoranthene and pyrene were the most abundant PAHs at Chongqing, whereas at Hanfeng Lake the LMW PAH naphthalene and

Table 7 – Effect range low (ERL) and effect range median (ERM) guideline values for PAHs according to Long et al. (1995).

Compound	ERL	ERM
Naphthalene (ng/g)	160	2100
Acenaphthylene (ng/g)	44	640
Acenaphthene (ng/g)	16	500
Fluorene (ng/g)	19	540
Phenanthrene (ng/g)	240	1500
Anthracene (ng/g)	85.3	1100
Fluoranthene (ng/g)	600	5100
Pyrene (ng/g)	665	2600
Benzo[a]anthracene (ng/g)	261	1600
Chrysene (ng/g)	384	2800
Benzo[b]fluoranthene (ng/g)	n.a.	n.a.
Benzo[k]fluoranthene (ng/g)	n.a.	n.a.
Benzo[a]pyrene (ng/g)	430	1600
Indeno[1,2,3-c,d]pyrene (ng/g)	n.a.	n.a.
Dibenzo[a,h]anthracene (ng/g)	63.4	260
Benzo[g,h,i]perylene (ng/g)	n.a.	n.a.
LMW PAHs (ng/g)	552	3160
MMW + HMW PAHs (ng/g)	1700	9600
Σ PAHs (ng/g)	4022	44,792

n.a. means not available, with no safety values existing as it is suggested that the mere presence causes toxic effects with high probability (Feng et al., 2007a; Liu et al., 2009).

LMW: low molecular weight; MMW: medium molecular weight; HMW: high molecular weight; PAHs: polycyclic aromatic hydrocarbons.

phenanthrene, the MMW PAH chrysene and the HMW PAH benzo[b]fluoranthene dominated the spectrum (Table 4).

In TGR surface water, Wang et al. (2009) found that the LMW and MMW PAHs phenanthrene, fluoranthene, pyrene and chrysene were the predominant PAHs in surface water of the TGR. This was confirmed by Wolf et al. (2013a), who stated that LMW and MMW PAHs were ubiquitously distributed, whereas the HMW PAHs could not be detected. They concluded that either atmospheric deposition of the more volatile LMW PAH, or a strong adsorption of the HMW PAHs to suspended particulate matter in the water column led to this distribution. Based on the observations of this study that also LMW and MMW PAHs dominated the spectrum and less HMW PAHs were adsorbed to the sediment, a high atmospheric deposition of the more volatile PAHs is suggested.

2.1.3. Total organic carbon analysis

The TOC content ranged from $0.40\% \pm 0.01\%$ (CNG-D) to $1.65\% \pm 0.02\%$ (CNG-U) in campaign September 2011 (Table 3), and from $0.11\% \pm 0.01\%$ (TGR-B) to $0.66\% \pm 0.10\%$ (JIA-B) at Chongqing, as well as $0.12\% \pm 0.02\%$ (HAN-A) to $1.00\% \pm 0.04\%$ (HAN-C) at Kaixian in May 2013 (Table 4). In general, the $\text{PAH}_{\Sigma 16}$ did not correspond to the TOC content per sample. However, the rise in TOC corresponded to the increase of $\text{PAH}_{\Sigma 16}$ at Hanfeng Lake in May 2013 from $0.27\% \pm 0.03\%$ (HAN-B) to $1.00\% \pm 0.04\%$ (HAN-C), which emphasizes a pollutant influx between these sampling locations, particularly indicating the discharge of wastewater.

2.1.4. Principal component analysis

The chemical analysis of water/sediment samples from the Yangtze River and its tributaries targeting on PAH-compounds revealed a complex pattern of contents and identities. Quantitative analysis of the PAH content in sediment extracts from September 2011 (Fig. 2a) resulted in two principal key factors that together explain 74% of the total variance of the dataset. Obvious is the separation of MMW PAHs (4 aromatic rings: FLA, PYR, BaA, CHR) from HMW PAHs (5–6 rings: BbF, BkF, BaP, IcdP, DahA, BghiP) in one group and LMW PAHs (2–3 rings: FL, ANT, NP, ACY, ACE) in a second group, with exception of phenanthrene that forms a group with the 4–6 ring PAHs. That demonstrates a differentiation of the sites, e.g., by the abundance or absence of low molecular PAHs of the second group (positive y axis). Especially, the sites in the upper part of the TGR (CNG-U, CNG-D, FEN-U, and FEN-D) including their tributaries (CNG-T, FEN-T) and the Hanfeng Lake (HF-L) at Kaixian are defined by the content of low molecular PAHs (Fig. 2a). Organizing all upstream, downstream and tributary samples in individual groups reveals an orientation of these groups along a gradient dominated by the MMW to HMW PAHs: upstream > downstream > tributaries. This indicates that the downstream locations are primarily influenced by the upstream locations, with possible dilution effects of the tributaries (Fig. 2a) under assumption of a downstream oriented flow direction.

An intra-site comparison enables a more specific breakdown of the relationships between the local samples. Whereas the correlation between the downstream locations at Chongqing and Fengdu with the corresponding upstream locations (CNG-U and FEN-U, respectively) and tributaries

(CNG-T and FEN-T, respectively) appear to be comparable, the Pengxi River (YUN-T) seems to have a stronger impact on the PAH composition of the downstream location (YUN-D) than the upstream location (YUN-U) at Yunyang, as the datapoints of YUN-T and YUN-D overlap indicating a similar PAH pattern. However, the downstream location (WU-D) at Wushan appears to have a closer relationship with the upstream location (WU-U), than the Daning River (WU-T) (Fig. 2a).

This could be explained by discharge boundary conditions in the conversion zone as mentioned by Holbach et al. (2013) for the Daning River and TGR mainstream. Furthermore, they observed a reversion of the Daning River's flow direction, forced by the increasing water level of the Yangtze River mainstream in August 2011. This means that the Daning River was rather influenced by suspended particulate matter that was transported from the TGR mainstream into the less turbulent end section of the tributary. A similar observation could be made at the Xiangxi tributary, in the lower part of the TGR, in September 2012 (Holbach et al., 2014). Thus, it is reasonable to question if the tributaries influence the mainstream of the reservoir or if the situation is reversed. However, the relationship between Pengxi River (YUN-T) and the downstream location at Yunyang (YUN-D) could represent an exception of this situation (Fig. 2a).

The two most important axes of the PCA using PAH content data in sediments from May 2013 explain 81% of the total variance of the dataset (Fig. 2b). The scenario is less differentiated than in September 2011. The distribution is particularly oriented at the abundance of naphthalene (NP), fluoranthene (FL), benzo[a]pyrene (BaP), acenaphthene (ACE) and benzo[g,h,i]perylene (BghiP). Considering the proportion of the x axis (67%) to the total variance, the samples from Chongqing show a large similarity, with exception of TGR-B, which exhibits the lowest content of $\text{PAH}_{\Sigma 16}$ at this location. The samples YAN-A, YAN-C and JIA-C strike out due to their content of benzo[a]pyrene (19 to 25 ng/g), which could not be detected above the LOD of 10 ng/g in any of the other samples (Fig. 2b, Table 4). It could be a hint for potential input sources particularly close before the conversion zone between Yangtze and Jialing River (YAN-C, JIA-C), although it has to be considered that the difference to the LOD was only 9 to 15 ng/g. Benzo[a]pyrene could not be measured above the LOD at YAN-B, although it was detectable upstream and downstream of this location, and at TGR-B the lowest $\text{PAH}_{\Sigma 16}$ content of the area was detected (59% compared to TGR-A). This may emphasize the assumption that the pollution sources have a rather limited regional impact concerning particle affine contaminants. This is most likely due to the low flow velocity of the water bodies, which causes a higher sedimentation rate of suspended particulate matter, which can carry adherent contaminants. A similar observation could be made at Kaixian. At the Hanfeng Lake, a large similarity between the samples from the upper part (HAN-A, HAN-B) could be found, as well as between the samples from the lower part (HAN-C, HAN-D), while the quantity and composition of the PAHs between those parts appear to be largely different. This can be most likely referred to pollution sources between HAN-B and HAN-C (cf. Section 2.1.1.). However, although HAN-A and HAN-B show comparable $\text{PAH}_{\Sigma 16}$ concentrations, the content increases to HAN-C and then

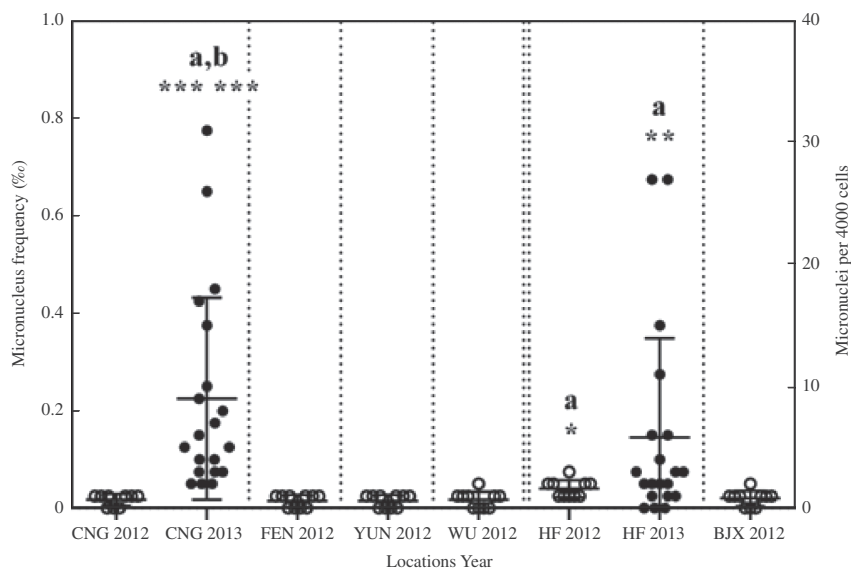


Fig. 3 – Micronucleus frequency in erythrocytes of *Pelteobagrus vachellii* from sampling campaign May 2012 and May 2013; symbols represent individual animals, bars the mean value, and error bars the standard deviation; white circles = May 2012; black circles = May 2013; dotted lines separate different sites, double dotted lines separate sites along the Yangtze River mainstream and the TGR watershed; 2012 samples $n = 10$; 2013 samples $n = 20$; asterisks mark significant differences between samples and reference (sample name BJX 2012); data was statistically analyzed with Mann–Whitney rank sum test, * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$, a: significant difference to reference site, b: significant difference to same site in May 2012. TGR: Three Gorges Reservoir; BJX: Baiji River.**

decreases again to HAN-D (82% of HAN-C) (Table 4), also showing a decline of PAH_{Σ16} contamination with the flow direction in the lower part of the lake (HAN-C > HAN-D).

2.1.5. PAH source analysis

Anthropogenic sources are the main PAH contributors in urbanized and industrialized regions, and can be divided into two groups. Petrogenic sources relate to crude oil, coal and their products, mainly released by spillage. While pyrogenic sources relate to incomplete combustion of biomass, like wood burning, and fossil fuels (Mostert et al., 2010; Neff, 1979). Because PAHs are always emitted as a mixture and different emission sources are considered to have characteristic relative PAH ratios, it is possible to trace the contamination in environmental samples (sediment, soil, water, air and tissue) back to their sources with some restrictions (Tobiszewski and Namieśnik, 2012; Yunker et al., 2002). As emitted PAHs undergo various fate processes, which depend on factors like solubility and adsorption, typically substances of the same molecular mass and similar physicochemical properties are compared to minimize differences due to diverging transformations (McVeety and Hites, 1988; Readman et al., 1987) (cf. Section 1.8).

The important role of liquid fossil fuel combustion all along the reservoir (Table 5) stands in agreement with results from the TGR water column by Wang et al. (2009, 2013). Urban traffic emissions and runoff, as well as intensified shipping activities since the impoundment of the reservoir, can be accounted to be the main contributors. Moreover, combustion sources had a major impact on the upper (highly urbanized and industrialized) part of the TGR (Tables 5 and 6), which is

also verified by the water analysis by Wang et al. (2009, 2013). Particularly the cities of Chongqing and Changshou are important industrial centers at the TGR's shore, where industrial emission, e.g., power generation from coal combustion, and urban air pollution, may have a serious influence on the contamination status of the reservoir in this region. On the other hand, the origin of PAHs in the middle (YUN) and lower part (WU) of the TGR could be rather traced back to petrogenic sources, in accordance with Wang et al. (2013), and upon combustion particularly to vehicular emission from traffic (Table 5). Alike all tributaries, with exception of CNG-T, which were also affected by petrogenic sources, this may be caused by oil and fuel spillage, e.g., from ships (Table 5). As the downstream location at Yunyang (YUN-D) stood out to be seriously influenced by a contamination source, the origin appears to be petrogenic (Table 5). The contamination at the Hanfeng Lake exhibited a mixture of petrogenic sources and combustion, especially from liquid fossil fuels as well as coal, grass and wood. This may be caused on the one hand side from urban air pollution, and on the other hand from biomass burning in homes as the city is located in a rather rural area. The lower locations at the Hanfeng Lake that showed an increased contamination, were defined by petrogenic sources, which may originate from shipping activities and wastewater (Tables 5 and 6). The reference site (BJX-R) showed a dominating pyrogenic impact, with a mixture of liquid fossil fuel and also coal, grass and wood combustion. This can also be referred to biomass burning in homes of the rural area and minor shipping activities in the area (Table 5).

With regard to overall China, particularly biomass burning, domestic coal combustion and coke ovens have been

identified as significant sources for the atmospheric emission of PAHs (Zhang and Tao, 2009; Zhang et al., 2007).

2.2. Biological impairments

2.2.1. Assessment of biological adverse effects according to sediment quality guidelines

PAHs have been classified as persistent organic pollutants, due to their (semi-)persistent, bioaccumulative and toxic properties. Upon those are the 16 analyzed PAHs (Table 1) listed as priority pollutants by the Environmental Protection Agency—USA (1982). PAHs can be found throughout all environmental media, e.g., air, soil, water, sediment, tissue, and exhibit impairing properties, e.g., cancerogenicity and mutagenicity (Pérez et al., 2003; WHO, 2010). Due to the complexity of PAH mixtures and their individual effects no official related criteria or standards exist so far to evaluate the biological effects of total PAHs in sediment. Generally, the total mixtures are evaluated based upon concentrations of single indicator PAHs. However, one attempt to set evaluation values in order to anticipate biological effects is the ERL/ERM (“effect range low”/“effect range median”) concept by Long et al. (1995). These values delineate three ranges in substance concentrations that are associated with (a) rarely (<ERL), (b) occasionally (\geq ERL and <ERM) and (c) frequently (\geq ERM) occurring effects (Long et al., 1995). Effect range values exist for individual PAHs and total PAHs for which the correlation between effect and substance concentration have been noted to be good (Table 7) (Long et al., 1995). Overall, no sample exceeded the ERL value for total PAHs (4022 ng/g dry weight (dw)), thus suggesting that rarely biological effects were to be expected (Tables 3 and 4). However, most samples exceeded the ERL values for one or more individual PAHs, but none exceeded the corresponding ERM values, thus indicating occasional biological impairments. Particularly, the low molecular weight PAHs fluorene, phenanthrene, acenaphthene and naphthalene were problematic. The according samples were CNG-U, CNG-T, CNG-D, FEN-U, FEN-D, YUN-U, YUN-T, YUN-D, WU-U, HF-L, JIA-B, HAN-C and HAN-D.

2.2.2. Mutagenicity of PAHs

Although all 16 priority PAHs (Table 1) may induce adverse effects, not all of them are mutagenic. Particularly those with more than four rings possess adverse mutagenic properties, but often also require metabolic activation (Penning et al., 1999). Out of the selected 16 priority PAHs, eight possess mutagenic activity in the Ames assay (Tables 3 and 4). The proportion of those in the sediment samples from September 2011 range from 43% (CNG-U) to 79% (YUN-D), with a mean of $55\% \pm 9\%$. The proportion in the samples from May 2013 ranged from 39% (JIA-A, JIA-B) to 59% (YAN-A), with a mean of $48\% \pm 8\%$ at Chongqing, and 36% (HAN-A, HAN-B) to 49% (HAN-C), with a mean of $42\% \pm 7\%$ at Kaixian.

2.2.3. Ames fluctuation assay

For the samples from campaign September 2011, the exposure of bacteria tester strain TA100 (base pair substitution) in the presence (+S9) and absence (–S9) of exogenous enzymatic supplement to the sediment extracts resulted in no significant mutagenic effects ($p > 0.05$), with exception of YUN-D with metabolic activation (+S9) (NOEC: 200 mg/mL; $p < 0.05$).

The same procedure with the tester strain TA98 (frameshift) in the absence of S9 supplement (–S9) also led to no significant results ($p > 0.05$), with exception of CNG-D (NOEC 200 mg/mL; $p < 0.05$). However, the exposure of tester strain TA98 in the presence of S9 supplement (+S9) resulted in several significant mutagenic activities ($p < 0.05$). The strongest inducing samples were CNG-T, FEN-D, WU-U and WU-D with a NOEC of 25 mg/mL ($p < 0.05$), followed by the samples CNG-U and YUN-D (NOEC 50 mg/mL; $p < 0.05$). The samples CNG-D, FEN-T, YUN-U and YUN-T showed a NOEC of 200 mg/mL ($p < 0.05$), while FEN-U, WU-T, HF-L and the reference site BJX-R exhibited no significant impact at all (NOEC > 400 mg/mL; $p > 0.05$).

Testing of the sediment extracts from the May 2013 sampling with bacteria tester strain TA98 in absence of metabolic activation (–S9) revealed no significant mutagenicity in any of the samples ($p > 0.05$). Also, adding of S9 supplement (+S9) resulted only in a significant effect in the samples HAN-D (NOEC 100 mg/mL) and HAN-A (NOEC 200 mg/mL) ($p < 0.05$). However, cytotoxicity was observed for the extracts of JIA-A, JIA-B and HAN-C (400 mg/mL), which might explain the absence of mutagenicity in these samples.

Potential sources for the mutagenic compounds in the strongest inducing samples (25 mg/mL) may be referred to local industries. The mutagenicity at CNG-T and the cytotoxicity found in JIA-A and JIA-B emphasize an industrial impact on the Jialing River, where several industrial sites have settled. At Fengdu, the downstream location FEN-D was clearly affected, while the upstream location (FEN-U) and the Long River (FEN-T) showed no or less impact, respectively. This suggests an input source between these points, like industry or domestic sewage disposal from Fengdu. The similar mutagenic potentials of WU-U and WU-D at Wushan suggest a drift of mutagenic compounds from upstream to downstream with no obvious influence from the Daning River (WU-T), with the source located upstream of WU-U. At the Hanfeng Lake, the cytotoxicity in sample HAN-C and the mutagenicity in sample HAN-D further corroborate a considerable input source between HAN-B and HAN-C (cf. Section 2.1.1.).

Almost all mutagenic effects could be related to frameshift mutations after metabolic activation. Comparing this to the chemical results (cf. Section 2.1), PAHs appear to be the obvious causative agents. Depending on the individual PAH, they can either induce frameshift (TA98) or base pair substitution (TA100) (Madill et al., 1999), but generally require metabolic activation (WHO, 2010). Chrysene, for example, is most probably the inducer for base pair substitution in sample YUN-D, because it constitutes 50% (780 ng/g; Table 3) of the PAH_{Σ16} in the extract and reacts positively in tester strain TA100 with S9 (Madill et al., 1999).

Mutagenicity could also be detected in surface water of the Yangtze River at Chongqing by Shu et al. (2002) and Qiu et al. (2003) before the impoundment. Both stated that the Jialing River was stronger polluted than the Yangtze River. Further, direct- and indirect-acting frameshift inducers were found to be the causative agents in surface water of the Yangtze River at Wuhan section (Dong et al., 2010), Shanghai section (Shen et al., 2003) and the Yangtze Estuary (Wu, 2005), as well as in sediments of the German Rhine River (Kosmehl et al., 2004) and Danube River (Boettcher et al., 2010; Higley et al., 2012).

Generally, surface waters appear to be dominated by direct- and indirect-acting frameshift mutagens worldwide (Ohe et al., 2004).

However, the total concentrations of PAHs in this study, which induce mutagenicity in the Ames assay (Tables 3 and 4), did not correspond to the observed effects in the individual samples. Also, no obvious pattern in the effects was discernible comparing the sampling locations, although the tributaries, with exception of CNG-T, in general showed the lowest mutagenic properties per sampling site. As each PAH has a specific mutagenic activity, the individual inductions can be calculated in relation to a strong mutagen, e.g., benzo[a]pyrene. However, the application of benzo[a]pyrene toxicity equivalency factors (BEP or BaP-TEQ), according to Madill et al. (1999), also resulted in no clear trend between the activity of detected compounds and the triggered effects. Therefore, it is suggested that non-target compounds may play a significant role in the induction of mutagenicity (Brack et al., 2005). In other studies, about 10%–20% of the total mutagenicity was attributed to PAHs and the rest to non-target compounds (Aouadene et al., 2008; Chen and White, 2004). The cytotoxicity in several samples, with comparably low PAH_{Σ16} content (JIA-A, JIA-B), and the direct mutagenicity (S9) in sample CNG-D, corroborates the assumption of toxic non-target compounds with potential mutagenic properties. In addition, additive, synergistic, antagonistic and masking interactions of individual contaminants impede direct correlations.

2.2.4. Micronucleus assay

In the erythrocytes of *P. vachellii* sampled along the Yangtze River mainstream in May 2012, no significant differences ($p > 0.05$) in the micronucleus frequency could be detected at Chongqing (CNG 2012: $0.175\% \pm 0.121\%$; IF 0.9; $n = 10$), Fengdu (FEN 2012: $0.150\% \pm 0.129\%$; IF 0.8; $n = 10$), Yunyang (YUN 2012: $0.150\% \pm 0.129\%$; IF 0.8; $n = 10$) and Wushan (WU 2012: $0.175\% \pm 0.169\%$; IF 0.9; $n = 10$) compared to the reference site Baijiayi River (BJX 2012: $0.200\% \pm 0.158\%$; $n = 10$). However, the samples taken from the Hanfeng Lake in 2012 displayed a significant effect (HF 2012: $0.400\% \pm 0.175\%$; IF 2.0; $n = 10$; $p < 0.05$) compared to the Baijiayi River. Furthermore, the erythrocytes sampled in Chongqing (CNG 2013: $2.250\% \pm 2.073\%$; IF 11.3; $n = 20$; $p < 0.001$) and Hanfeng Lake (HF 2013: $1.450\% \pm 2.040\%$; IF 7.3; $n = 20$; $p < 0.01$) in May 2013 showed even stronger effects. Comparing the change between the years at Hanfeng Lake (HF 2012 and HF 2013; $p > 0.05$) no significant difference could be measured, whereas at Chongqing (CNG 2012, CNG 2013; $p < 0.001$) the status changed significantly (Fig. 3).

The biological impairments may be attributed to PAHs, as detected at Chongqing and Kaixian in May 2013 (Table 4). Genotoxic properties of PAHs could be demonstrated before (White, 2002). Further, environmental sediments contaminated with PAHs have shown genotoxic effects in catfish (Di Giulio et al., 1993) and turbot (Kilemade et al., 2004), barbell (Boettcher et al., 2010), tilapia (Rocha et al., 2009), as well as resuspended PAH-spiked sediments in trout (Brinkmann et al., 2013; Hudjetz et al., 2014). The harbor sediments that caused DNA damage in turbot blood cells contained PAH concentrations of about 1000 ng/g after 7 days of exposure,

and the reference site, containing about 500 ng/g PAH, also induced significant effects after 14 days. Likewise, other organs, i.e., liver, epidermis, spleen and gill, were also affected significantly (Kilemade et al., 2004). Thus, although PAH_{Σ16} concentrations were at 302 ± 87 ng/g at Chongqing and 332 ± 240 ng/g at Kaixian, the exposure time has to be taken into account, as the formation of micronuclei is dependent on contamination level and exposure time (Das and Nanda, 1986). PAHs generally persist in the environment with half-lives from days to years, depending on the individual structure and processes like photo- and biodegradation (Fasnacht and Blough, 2002; Kanaly and Harayama, 2000; Shuttleworth and Cerniglia, 1995), and can accumulate in organisms without the right metabolic detoxification systems due to their lipophilicity, and consequently biomagnify through trophic transfer (Coates et al., 1997; Kanaly and Harayama, 2000). However, they are easily biodegradable in organisms with the right metabolic mechanisms (Möller et al., 2014; Van der Oost et al., 2003), unfolding their toxic potentials through biotransformation to reactive intermediates (WHO, 2010).

2.2.5. Non-target compounds

Possible non-target compounds could be sediment affine derivatives of the here ubiquitously detected PAHs. For example, Nitro-PAHs have also been demonstrated to possess frameshift and basepair substitution mutagenic potentials (Eisentraeger et al., 2008; McCoy et al., 1981; Xu et al., 1982) as well as genotoxic effects in fish (Shailaja et al., 2006). They were among the main responsible compounds for mutagenic effects in the German Danube River sediments (Higley et al., 2012). Nitro-PAHs emerge from the emission of combustion sources that were found to play a significant role at several sections of the Yangtze River, or form in the environment from reactions of certain PAHs with the highly reactive nitrite, which may originate from biological processes and nitrogenous waste like fertilizers. They can also evolve through metabolic processes in fish under the presence of PAHs and nitrite. The simultaneous exposure to nitrite and the actually noncarcinogenic PAH phenanthrene even led to a potentiation in genotoxicity (Shailaja et al., 2006), corroborating the importance of pollutant interactions in the expression of PAH toxicity (Chaloupka et al., 1993). Phenanthrene was detected in sediments of the recent study with 9% of PAH_{Σ16} (28 ± 8 ng/g) at Chongqing and with 13% of PAH_{Σ16} (44 ± 30 ng/g) at Kaixian in campaign May 2013, while nitrate, a nitrite precursor, was detected in concentrations of 7.4 mg/L in the Yangtze River at Chongqing at the same time (Wolf, 2015), thus making Nitro-PAHs potential candidates for the detected effects. Further, also heterocyclic PAH derivatives are known to induce genotoxic effects in fish (Brinkmann et al., 2014; Shailaja et al., 2006). Creosote contaminated industrial areas are considerable sources for heterocyclic PAHs (Blum et al., 2011), which are more mobile than homocyclic PAHs with respect to their higher water solubility (Brinkmann et al., 2014). Further investigations are required to verify these presumptions.

3. Conclusions

The TGR appears to be in a comparable or even less contaminated state than other national or international

water bodies, based on the detected pollution concentrations in sediment and water. As immense amounts of water and sediment are discharged into and carried by the Yangtze River, this observation may be primarily contributed to a strong dilution effect. This means that a contamination problem of the Yangtze River is not solved, but simply relocated (see also Floehr et al. (2013) and Müller et al. (2008)). The mass balance approach for PAHs referred to the immense sediment influx suggested a deposition of 216–636 kg PAH/day (0.2–0.6 mg PAH/(m²·day)) (cf. Section 2.1.1.) and the combustion of fossil fuels appeared to play a significant role in the emission of PAHs into the environment along the reservoir. Thus, it can be concluded that air pollution, which plays a considerable role in China (Zhang and Tao, 2009; Zhang et al., 2009), also affects the quality of its water bodies. And as the PAHs pass several exposition pathways, air and water, they pose a risk to humans and wildlife already before they are deposited in the TGR. Compared to the external sources, like air pollution, it is suggested that the risk of internal pollution emission from the submerged sites plays only a minor role, due to the deposition of vast amounts of sediment in the TGR and the dilution effect.

Although bound to sediment particles, and thus less bioavailable, pollutants can be remobilized on a limited scale by benthic organisms causing a direct exposure or on a larger scale by shipping activities or flood events (Brinkmann et al., 2010; Stachel et al., 2005; Wölz et al., 2009), which frequently occur in the Yangtze River. The remobilization of PAHs from Yangtze sediment due to shear stresses was shown by Feng et al. (2007b). Therefore, the importance of sediments as sink and source for pollutants in the TGR needs to be considered.

Especially with respect to mutagenic properties of pollutants, low concentrations cannot directly be set equal to low risk, as threshold values are under discussion due to the mutagenic mode of action. The mutagenicity of sediment extracts and genotoxic impacts on the fish at particular sites along the reservoir and in its watershed have been demonstrated. The authors support the importance of mutagenic and genotoxic impacts for the quality of Chinese water bodies, e.g., as source for drinking water (Lv et al., 2015; Shu et al., 2002), and recommend their inclusion into routine monitoring programs as suggested before by Wu (2005) for marine systems.

The ubiquitous PAHs and their derivatives, nitro- and heterocyclic PAHs, are considered to play a crucial role in the registered biological impairments, aside a variety of non-target compounds. In order to identify the causative agents, including non-target compounds, bioassay-guided effect directed analysis has been proven to be a valuable tool (Brack and Schirmer, 2003; Brack et al., 2005; Hecker and Hollert, 2009).

The proposed concept also corroborates the importance of combined chemical analytical, *in vitro/in vivo* and *in situ* approaches in environmental assessment and for further monitoring of the TGR. As Hong et al. (2012) emphasized that urbanization effects on the environment can be recorded by PAHs in sediment, PAHs are hereby suggested as suitable marker compounds or indicators for monitoring of the reservoir sediment pollution status. This is in accordance

with Wang et al. (2013), who stated the same for the reservoir water.

The significant biological impairments at Chongqing and Kaixian suggested a toxicity reduction evaluation for these areas. Most parts seem not yet to exhibit ecotoxicological effects or only to a minor degree. With respect to growing urbanization and industrialization of the region respective counter-measures, like improved wastewater treatment and emission standards, should be further enforced to prevent environmental degradation in the long-term.

Acknowledgments

The recent study has been conducted in close cooperation with the Tongji University, Shanghai, and the Chongqing University, Chongqing, as part of the MICROTOX project (“Transformation, Bioaccumulation and Toxicity of Organic Micropollutants in the Yangtze Three Gorges Reservoir”; No. FKZ 02WT1141). It is integrated into the Sino-German joint environmental research program “Yangtze-Hydro - Sustainable Management of the Newly Created Ecosystem at the Three Gorges Dam” (No. FKZ 02WT; Bergmann et al. (2011); www.yangtze-project.de), as part of the research cluster “Pollutants/Water/Sediment—Impacts of Transformation and Transportation Processes on the Yangtze Water Quality”. The project has been financed by the Federal Ministry of Education and Research, Germany, and has been further supported by a cooperation project with Chinese colleagues also sponsored by the Federal Ministry of Education and Research, Germany (No. DLR FKZ 01DO12007) and the Chinese 111 Program. The funding sources had no involvement in the study.

REFERENCES

- Ames, B.N., McCann, J., Yamasaki, E., 1975. Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. *Mutat. Res.* 31 (6), 347–363.
- Aouadene, A., Di Giorgio, C., Sarrazin, L., Moreau, X., De Jong, L., Garcia, F., et al., 2008. Evaluation of the genotoxicity of river sediments from industrialized and unaffected areas using a battery of short-term bioassays. *Environ. Mol. Mutagen.* 49 (4), 283–299.
- Balch, G.C., Metcalfe, C.D., Huestis, S.Y., 1995. Identification of potential fish carcinogens in sediment from Hamilton Harbour, Ontario, Canada. *Environ. Toxicol. Chem.* 14 (1), 79–91.
- BBC, 2012. The world's biggest cities: how do you measure them? Available at: <http://www.bbc.com/news/magazine-16761784> (Date accessed: 22 November 2014)
- Bergmann, A., Bi, Y., Chen, L., Floehr, T., Henkelmann, B., Holbach, A., et al., 2011. The Yangtze-Hydro Project: a Chinese–German environmental program. *Environ. Sci. Pollut. Res. Int.* 19 (4), 1341–1344.
- Blum, P., Sagner, A., Tiehm, A., Martus, P., Wendel, T., Grathwohl, P., 2011. Importance of heterocyclic aromatic compounds in monitored natural attenuation for coal tar contaminated aquifers: a review. *J. Contam. Hydrol.* 126 (3–4), 181–194.
- Boettcher, M., Grund, S., Keiter, S., Kosmehl, T., Reifferscheid, G., Seitz, N., et al., 2010. Comparison of *in vitro* and *in situ*

- genotoxicity in the Danube River by means of the comet assay and the micronucleus test. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 700 (1–2), 11–17.
- Brack, W., Schirmer, K., 2003. Effect-directed identification of oxygen and sulfur heterocycles as major polycyclic aromatic cytochrome P4501A-inducers in a contaminated sediment. *Environ. Sci. Technol.* 37 (14), 3062–3070.
- Brack, W., Schirmer, K., Erdinger, L., Hollert, H., 2005. Effect-directed analysis of mutagens and ethoxyresorufin-O-deethylase inducers in aquatic sediments. *Environ. Toxicol. Chem.* 24 (10), 2445–2458.
- Brinkmann, M., Hudjetz, S., Cofalla, C., Roger, S., Kammann, U., Giesy, J.P., et al., 2010. A combined hydraulic and toxicological approach to assess re-suspended sediments during simulated flood events. Part I—multiple biomarkers in rainbow trout. *J. Soils Sediments* 10 (7), 1347–1361.
- Brinkmann, M., Hudjetz, S., Kammann, U., Hennig, M., Kuckelkorn, J., Chinoraks, M., et al., 2013. How flood events affect rainbow trout: evidence of a biomarker cascade in rainbow trout after exposure to PAH contaminated sediment suspensions. *Aquat. Toxicol.* 128–129, 13–24.
- Brinkmann, M., Blenkle, H., Salowsky, H., Bluhm, K., Schiw, S., Tiehm, A., et al., 2014. Genotoxicity of heterocyclic PAHs in the micronucleus assay with the fish liver cell line RTL-W1. *PLoS ONE* 9 (1), e85692.
- Chaloupka, K., Harper, N., Krishnan, V., Santostefano, M., Rodriguez, L.V., Safe, S., 1993. Synergistic activity of polynuclear aromatic hydrocarbon mixtures as aryl hydrocarbon (Ah) receptor agonists. *Chem. Biol. Interact.* 89 (2–3), 141–158.
- Chapman, P.M., 1990. The sediment quality triad approach to determining pollution-induced degradation. *Sci. Total Environ.* 97–98, 815–825.
- Chapman, P.M., Hollert, H., 2006. Should the Sediment Quality Triad become a tetrad, a pentad, or possibly even a hexad? *J. Soils Sediments* 6 (1), 4–8.
- Chen, G.S., White, P.A., 2004. The mutagenic hazards of aquatic sediments: a review. *Mutat. Res. Rev. Mutat. Res.* 567 (2–3), 151–225.
- Chen, J., Chen, Z.Y., Xu, K.Q., Wei, T.Y., Li, M.T., Wang, Z.H., et al., 2005. ADP-flow velocity profile to interpret hydromorphological features of China's Yangtze Three-Gorges valley. *Chin. Sci. Bull.* 50 (7), 679–684.
- Cincinelli, A., Bubba, M.D., Martellini, T., Gambaro, A., Lepri, L., 2007. Gas-particle concentration and distribution of n-alkanes and polycyclic aromatic hydrocarbons in the atmosphere of Prato (Italy). *Chemosphere* 68 (3), 472–478.
- Coates, J.D., Woodward, J., Allen, J., Philp, P., Lovley, D.R., 1997. Anaerobic degradation of polycyclic aromatic hydrocarbons and alkanes in petroleum-contaminated marine harbor sediments. *Appl. Environ. Microbiol.* 63 (9), 3589–3593.
- Das, R.K., Nanda, N.K., 1986. Induction of micronuclei in peripheral erythrocytes of fish *Heteropneustes fossilis* by mitomycin C and paper mill effluent. *Mutat. Res. Lett.* 175 (2), 67–71.
- De la Torre-Roche, R.J., Lee, W.Y., Campos-Diaz, S.I., 2009. Soil-borne polycyclic aromatic hydrocarbons in El Paso, Texas: analysis of a potential problem in the United States/Mexico border region. *J. Hazard. Mater.* 163 (2–3), 946–958.
- Deylering, D., Wang, J.X., Hu, W., Westrich, B., Peng, C., Bi, Y.H., et al., 2014. PAH distribution and mass fluxes in the Three Gorges Reservoir after impoundment of the Three Gorges Dam. *Sci. Total Environ.* 491–492, 123–130.
- Di Giulio, R.T., Habig, C., Gallagher, E.P., 1993. Effects of Black Rock Harbor sediments on indices of biotransformation, oxidative stress, and DNA integrity in channel catfish. *Aquat. Toxicol.* 26 (1–2), 1–22.
- Diekmann, M., Waldmann, P., Schnurstein, A., Grummt, T., Braunbeck, T., Nagel, R., 2004. On the relevance of genotoxicity for fish populations II: genotoxic effects in zebrafish (*Danio rerio*) exposed to 4-nitroquinoline-1-oxide in a complete life-cycle test. *Aquat. Toxicol.* 68 (1), 27–37.
- Dong, X.R., Yang, X.M., Lu, Y., Ma, K.P., Bao, J., Zhang, H.T., et al., 2010. Comparison of the biotoxicity of organic compounds in source and tap water from the Yangtze and Han River. *China Environ. Sci.* 30 (2), 263–268.
- Eisentraeger, A., Brinkmann, C., Hollert, H., Sagner, A., Tiehm, A., Neuwoehner, J., 2008. Heterocyclic compounds: toxic effects using algae, daphnids, and the *Salmonella*/microsome test taking methodical quantitative aspects into account. *Environ. Toxicol. Chem.* 27 (7), 1590–1596.
- Environmental Protection Agency & Office of the Federal Registration-USA, 1982. Appendix A: priority pollutants. Federal Register 47, 52309 (Washington, DC, USA).
- Erger, C., Balsaa, P., Werres, F., Schmidt, T.C., 2012. Multi-component trace analysis of organic xenobiotics in surface water containing suspended particular matter by solid phase extraction/gas chromatography–mass spectrometry. *J. Chromatogr. A* 1249, 181–189.
- EWFD Directive 2000/60/EC, 2000. The European Parliament & The Council of the European Union-Directive 2000/60/EC on Establishing a Framework for Community Action in the Field of Water Policy. 23 October, 2000.
- EWFD Directive 2008/105/EC, 2008. The European Parliament & The Council of the European Union-Directive 2008/105/EC on Environmental Quality Standards in the Field of Water Policy, Amending and Subsequently Repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and Amending Directive 2000/60/EC of the European Parliament and of the Council. 16 December, 2008.
- Fasnacht, M.P., Blough, N.V., 2002. Aqueous photodegradation of polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* 36 (20), 4364–4369.
- Feng, C.L., Xia, X.H., Shen, Z.Y., Zhou, Z., 2007a. Distribution and sources of polycyclic aromatic hydrocarbons in Wuhan section of the Yangtze River, China. *Environ. Monit. Assess.* 133 (1–3), 447–458.
- Feng, J.L., Yang, Z.F., Niu, J.F., Shen, Z.Y., 2007b. Remobilization of polycyclic aromatic hydrocarbons during the resuspension of Yangtze River sediments using a particle entrainment simulator. *Environ. Pollut.* 149 (2), 193–200.
- Floehr, T., Xiao, H.X., Scholz-Starke, B., Wu, L.L., Hou, J.L., Yin, D.Q., et al., 2013. Solution by dilution?—a review on the pollution status of the Yangtze River. *Environ. Sci. Pollut. Res.* 20 (10), 6934–6971.
- Gerbersdorf, S.U., Jancke, T., Westrich, B., 2005. Physico-chemical and biological sediment properties determining erosion resistance of contaminated riverine sediments—temporal and vertical pattern at the Lauffen reservoir/River Neckar, Germany. *Limnologia* 35 (3), 132–144.
- Hecker, M., Hollert, H., 2009. Effect-directed analysis (EDA) in aquatic ecotoxicology: state of the art and future challenges. *Environ. Sci. Pollut. Res.* 16 (6), 607–613.
- Higley, E., Grund, S., Jones, P.D., Schulze, T., Seiler, T.B., Lübbcke-von Varel, U., et al., 2012. Endocrine disrupting, mutagenic, and teratogenic effects of upper Danube River sediments using effect-directed analysis. *Environ. Toxicol. Chem.* 31 (5), 1053–1062.
- Hilscherova, K., Dusek, L., Kubik, V., Cupr, P., Hofman, J., Klanova, J., et al., 2007. Redistribution of organic pollutants in river sediments and alluvial soils related to major floods. *J. Soils Sediments* 7 (3), 167–177.
- Holbach, A., Wang, L.J., Chen, H., Hu, W., Schleicher, N., Zheng, B.H., et al., 2013. Water mass interaction in the confluence zone of the Daning River and the Yangtze River—a driving force for algal growth in the Three Gorges Reservoir. *Environ. Sci. Pollut. Res.* 20 (10), 7027–7037.
- Holbach, A., Norra, S., Wang, L.J., Yuan, Y.J., Hu, W., Zheng, B.H., et al., 2014. Three Gorges Reservoir: density pump amplification

- of pollutant transport into tributaries. *Environ. Sci. Technol.* 48 (14), 7798–7806.
- Hollert, H., Dürr, M., Erdinger, L., Braunbeck, T., 2000. Cytotoxicity of settling particulate matter and sediments of the Neckar River (Germany) during a winter flood. *Environ. Toxicol. Chem.* 19 (3), 528–534.
- Hollert, H., Keiter, S., Köenig, N., Rudolf, M., Ulrich, M., Braunbeck, T., 2003. A new sediment contact assay to assess particle-bound pollutants using zebrafish (*Danio rerio*) embryos. *J. Soils Sediments* 3 (3), 197–207.
- Hong, Y.W., Yu, S., Yu, G.B., Liu, Y., Li, G.L., Wang, M., 2012. Impacts of urbanization on surface sediment quality: evidence from polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) contaminations in the Grand Canal of China. *Environ. Sci. Pollut. Res.* 19 (5), 1352–1363.
- Höss, S., Ahlf, W., Fahnenstich, C., Gilberg, D., Hollert, H., Melbye, K., et al., 2010. Variability of sediment-contact tests in freshwater sediments with low-level anthropogenic contamination—determination of toxicity thresholds. *Environ. Pollut.* 158 (9), 2999–3010.
- Hu, B.Q., Yang, Z.S., Wang, H.J., Sun, X.X., Bi, N.S., Li, G.G., 2009. Sedimentation in the Three Gorges Dam and the future trend of Changjiang (Yangtze River) sediment flux to the sea. *Hydrol. Earth Syst. Sci.* 13 (11), 2253–2264.
- Hudjetz, S., Herrmann, H., Cofalla, C., Brinkmann, M., Kammann, U., Schäffer, A., et al., 2014. An attempt to assess the relevance of flood events-biomarker response of rainbow trout exposed to resuspended natural sediments in an annular flume. *Environ. Sci. Pollut. Res.* 21 (24), 13744–13757.
- ISO 11350-2012, 2012. Water Quality—Determination of the Genotoxicity of Water and Waste Water-Salmonella/microsome Fluctuation Test (Ames Fluctuation Test). International Organization for Standardization (ISO), Geneva, Switzerland.
- ISO 18287-2006, 2006. Soil Quality—Determination of Polycyclic Aromatic Hydrocarbons (PAH)—Gas Chromatographic Method With Mass Spectrometric Detection (GC-MS). International Organization for Standardization (ISO), Geneva, Switzerland.
- ISO 21427-2:2006, 2006. Water quality—Evaluation of Genotoxicity by Measurement of the Induction of Micronuclei—Part 2: Mixed Population Method Using the Cell Line V79. International Organization for Standardization (ISO), Geneva, Switzerland.
- Kanally, R.A., Harayama, S., 2000. Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. *J. Bacteriol.* 182 (8), 2059–2067.
- Keiter, S., Grund, S., Van Bavel, B., Hagberg, J., Engwall, M., Kammann, U., et al., 2008. Activities and identification of aryl hydrocarbon receptor agonists in sediments from the Danube River. *Anal. Bioanal. Chem.* 390 (8), 2009–2019.
- Kilemade, M.F., Hartl, M.G.J., Sheehan, D., Mothersill, C., Van Pelt, F.N.A.M., O'Halloran, J., et al., 2004. Genotoxicity of field-collected inter-tidal sediments from Cork Harbor, Ireland, to juvenile turbot (*Scophthalmus maximus* L.) as measured by the Comet assay. *Environ. Mol. Mutagen.* 44 (1), 56–64.
- Kosmehl, T., Krebs, F., Manz, W., Erdinger, L., Braunbeck, T., Hollert, H., 2004. Comparative genotoxicity testing of Rhine River sediment extracts using the comet assay with permanent fish cell lines (RTG-2 and RTL-W1) and the Ames test. *J. Soils Sediments* 4 (2), 84–94.
- Leme, D.M., Marin-Morales, M.A., 2009. *Allium cepa* test in environmental monitoring: a review on its application. *Mutat. Res. Rev. Mutat. Res.* 682 (1), 71–81.
- Liu, A.X., Lang, Y.H., Xue, L.D., Liu, J., 2009. Ecological risk analysis of polycyclic aromatic hydrocarbons (PAHs) in surface sediments from Laizhou Bay. *Environ. Monit. Assess.* 159 (1–4), 429–436.
- Liu, L., Chen, L., Shao, Y., Zhang, L.L., Floehr, T., Xiao, H.X., et al., 2014. Evaluation of the ecotoxicity of sediments from Yangtze River Estuary and contribution of priority PAHs to Ah receptor-mediated activities. *PLoS ONE* 9 (8), e104748.
- Long, E.R., Macdonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ. Manag.* 19 (1), 81–97.
- Lv, X.M., Lu, Y., Yang, X.M., Dong, X.R., Ma, K.P., Xiao, S.H., et al., 2015. Mutagenicity of drinking water sampled from the Yangtze River and Hanshui River (Wuhan section) and correlations with water quality parameters. *Sci. Rep.* 5, 9572.
- Madill, R.E.A., Brownlee, B.G., Josephy, P.D., Bunce, N.J., 1999. Comparison of the Ames *Salmonella* assay and Mutatox genotoxicity assay for assessing the mutagenicity of polycyclic aromatic compounds in porewater from Athabasca oil sands mature fine tailings. *Environ. Sci. Technol.* 33 (15), 2510–2516.
- Mai, B.X., Fu, J.M., Sheng, G.Y., Kang, Y.H., Lin, Z., Zhang, G., et al., 2002. Chlorinated and polycyclic aromatic hydrocarbons in riverine and estuarine sediments from Pearl River Delta, China. *Environ. Pollut.* 117 (3), 457–474.
- McCann, J., Choi, E., Yamasaki, E., Ames, B.N., 1975. Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. *Proc. Natl. Acad. Sci. U. S. A.* 72 (12), 5135–5139.
- McCoy, E.C., Rosenkranz, E.J., Rosenkranz, H.S., Mermelstein, R., 1981. Nitrated fluorene derivatives are potent frameshift mutagens. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 90 (1), 11–20.
- McVeety, B.D., Hites, R.A., 1988. Atmospheric deposition of polycyclic aromatic hydrocarbons to water surfaces: a mass balance approach. *Atmos. Environ.* 22 (3), 511–536.
- Ministry of Environmental Protection—China, 2006–2012. Bulletin on the ecological and environmental monitoring results of the Three Gorges Project 2005–2011 Available at: http://english.mep.gov.cn/standards_reports/threegorgesbulletin/ (Date accessed: 08 January 2015).
- Ministry of Environmental Protection—China, 2007. Bulletin on the ecological and environmental monitoring results of the Three Gorges Project 2004 Available at: http://english.mep.gov.cn/standards_reports/threegorgesbulletin/bulletin2004/ (Date accessed: 08 January 2015).
- Ministry of Environmental Protection—China, 2010. Bulletin on the ecological and environmental monitoring results of the Three Gorges Project 2009 Available at: http://english.mep.gov.cn/standards_reports/threegorgesbulletin/tgbulletin2009/ (Date accessed: 07 January 2015).
- Ministry of Environmental Protection—China, 2012. Bulletin on the ecological and environmental monitoring results of the Three Gorges Project 2011 Available at: http://english.mep.gov.cn/standards_reports/threegorgesbulletin/threegorge2011/ (Date accessed: 07 January 2015).
- Ministry of Health—China, 2006. National standard of the People's Republic of China—standards for drinking water quality (GB 5749–2006) Issued on 29 December, 2006. Implemented on 1 July, 2007 (replacing GB 5749–1985).
- Möller, A.M., Hermesen, C., Floehr, T., Lamoree, M.H., Segner, H., 2014. Tissue-specific metabolism of benzo[a]pyrene in rainbow trout (*Oncorhynchus mykiss*): a comparison between the liver and immune organs. *Drug Metab. Dispos.* 42 (1), 111–118.
- Mostert, M.M.R., Ayoko, G.A., Kokot, S., 2010. Application of chemometrics to analysis of soil pollutants. *Trends Anal. Chem.* 29 (5), 430–445.
- Müller, B., Berg, M., Yao, Z.P., Zhang, X.F., Wang, D., Pfluger, A., 2008. How polluted is the Yangtze River? Water quality downstream from the Three Gorges Dam. *Sci. Total Environ.* 402 (2–3), 232–247.
- National Bureau of Statistics—China, 2012. China Statistical Yearbook 2012 Available at: <http://www.stats.gov.cn/tjsj/ndsj/2012/indexeh.htm> (Date accessed: 22 November 2014).
- Neff, J.M., 1979. Polycyclic Aromatic Hydrocarbons in the Aquatic Environment: Sources, Fates, and Biological Effects. Applied Science Publishers, Essex, England.

- Ohe, T., Watanabe, T., Wakabayashi, K., 2004. Mutagens in surface waters: a review. *Mutat. Res. Rev. Mutat. Res.* 567 (2-3), 109–149.
- Ottermanns, R., 2008. Mustererkennung, Dimensionsreduktion und statistische Modellierung in der Ökologie-dargestellt am Beispiel der Lebensgemeinschaften grasiger Feldraine in deutschen Agrarlandschaften. RWTH Aachen University, Aachen, Germany, p. 468.
- Penning, T.M., Burczynski, M.E., Hung, C.F., McCoull, K.D., Palackal, N.T., Tsuruda, L.S., 1999. Dihydrodiol dehydrogenases and polycyclic aromatic hydrocarbon activation: generation of reactive and redox active o-quinones. *Chem. Res. Toxicol.* 12 (1), 1–18.
- Pérez, A.S., Reifferscheid, G., Eichhorn, P., Barceló, D., 2003. Assessment of the mutagenic potency of sewage sludges contaminated with polycyclic aromatic hydrocarbons by an Ames fluctuation assay. *Environ. Toxicol. Chem.* 22 (11), 2576–2584.
- Pies, C., Hoffmann, B., Petrowsky, J., Yang, Y., Ternes, T.A., Hofmann, T., 2008. Characterization and source identification of polycyclic aromatic hydrocarbons (PAHs) in river bank soils. *Chemosphere* 72 (10), 1594–1601.
- Qiu, Z.Q., Shu, W.Q., Tian, H.J., 2003. An analysis of the organic extracts from source water and drinking water in City C and the effects on DNA damage in primary hepatocytes in rats. *Acta Acad. Med. Milit. Tert.* 25 (5), 423–426.
- Readman, J.W., Mantoura, R.F.C., Rhead, M.M., 1987. A record of polycyclic aromatic hydrocarbon (PAH) pollution obtained from accreting sediments of the Tamar Estuary, U.K.: evidence for non-equilibrium behaviour of PAH. *Sci. Total Environ.* 66, 73–94.
- Reifferscheid, G., Maes, H.M., Allner, B., Badurova, J., Belkin, S., Bluhm, K., et al., 2012. International round-robin study on the Ames fluctuation test. *Environ. Mol. Mutagen.* 53 (3), 185–197.
- Rocha, P.S., Luvizotto, G.L., Kosmehl, T., Böttcher, M., Storch, V., Braunbeck, T., et al., 2009. Sediment genotoxicity in the Tietê River (São Paulo, Brazil): in vitro comet assay versus in situ micronucleus assay studies. *Ecotoxicol. Environ. Saf.* 72 (7), 1842–1848.
- Shailaja, M.S., Rajamanickam, R., Wahidulla, S., 2006. Formation of genotoxic nitro-PAH compounds in fish exposed to ambient nitrite and PAH. *Toxicol. Sci.* 91 (2), 440–447.
- Shen, L., Wu, J.Y., Lin, G.F., Shen, J.H., Westendorf, J., Huehnerfuss, H., 2003. The mutagenic potentials of tap water samples in Shanghai. *Chemosphere* 52 (9), 1641–1646.
- Shu, W.Q., Tian, H.J., Cao, J., Wang, Y.M., 2002. Mutagenic activities and their season changes of organic extracts from sources water of Yangtze River and Jialing River in Chongqing section. *Environ. Pollut. Control* 24 (4), 224–228 239.
- Shuttleworth, K.L., Cerniglia, E., 1995. Environmental aspects of PAH biodegradation. *Appl. Biochem. Biotechnol.* 54 (1-3), 291–302.
- Smith, K., 2013. *Environmental Hazards—Assessing Risk and Reducing Disaster*, 6th ed. Routledge, London, England.
- Stachel, B., Jantzen, E., Knoth, W., Kruger, F., Lepom, P., Oetken, M., et al., 2005. The Elbe flood in August 2002—organic contaminants in sediment samples taken after the flood event. *J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng.* 40 (2), 265–287.
- Tang, Z., Liao, H.Q., Zhang, L., Guo, J.Y., Wu, F.C., Guo, F., et al., 2011. Distribution, source and risk assessment of polycyclic aromatic hydrocarbons in river sediment of Cheng-Yu economic zone. *Huanjing Kexue* 32 (9), 2639–2644.
- The Council of the European Union, 1998. Council Directive 98/83/EC on the quality of water intended for human consumption. *Off. J. Eur. Communities* 330, 32–54.
- Tobiszewski, M., Namiśnik, J., 2012. PAH diagnostic ratios for the identification of pollution emission sources. *Environ. Pollut.* 162, 110–119.
- Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ. Toxicol. Pharmacol.* 13 (2), 57–149.
- Wang, J.X., Bi, Y.H., Pfister, G., Henkelmann, B., Zhu, K.X., Schramm, K.W., 2009. Determination of PAH, PCB, and OCP in water from the Three Gorges Reservoir accumulated by semipermeable membrane devices (SPMD). *Chemosphere* 75 (8), 1119–1127.
- Wang, Y., Li, X., Li, B.H., Shen, Z.Y., Feng, C.H., Chen, Y.X., 2012. Characterization, sources, and potential risk assessment of PAHs in surface sediments from nearshore and farther shore zones of the Yangtze Estuary, China. *Environ. Sci. Pollut. Res.* 19 (9), 4148–4158.
- Wang, J.X., Henkelmann, B., Bi, Y.H., Zhu, K.X., Pfister, G., Hu, W., et al., 2013. Temporal variation and spatial distribution of PAH in water of Three Gorges Reservoir during the complete impoundment period. *Environ. Sci. Pollut. Res.* 20 (10), 7071–7079.
- White, P.A., 2002. The genotoxicity of priority polycyclic aromatic hydrocarbons in complex mixtures. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 515 (1-2), 85–98.
- White, P.A., Robitaille, S., Rasmussen, J.B., 1999. Heritable reproductive effects of benzo[a]pyrene on the fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.* 18 (8), 1843–1847.
- WHO, 2010. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans—Volume 92: Some Non-heterocyclic Polycyclic Aromatic Hydrocarbons and Some Related Exposures. International Agency for Research on Cancer (IARC), World Health Organization (WHO), Lyon, France, p. 853 Available at: <http://monographs.iarc.fr/ENG/Monographs/vol92/mono92.pdf> (Date accessed: 11–18 October 2005).
- Wolf, A., 2015. Dissertation: suitability of surface water from the Three Gorges Reservoir in China for public drinking water supply and assessment of adequate treatment options. Personal communication., Institute for Applied Geosciences. Technische Universität Darmstadt, Germany.
- Wolf, A., Bergmann, A., Wilken, R.D., Gao, X., Bi, Y.H., Chen, H., et al., 2013. Occurrence and distribution of organic trace substances in waters from the Three Gorges Reservoir, China. *Environ. Sci. Pollut. Res.* 20 (10), 7124–7139.
- Wölz, J., Cofalla, C., Hudjetz, S., Roger, S., Brinkmann, M., Schmidt, B., et al., 2009. In search for the ecological and toxicological relevance of sediment re-mobilisation and transport during flood events. *J. Soils Sediments* 9 (1), 1–5.
- Wu, J.Y., 2005. Assessing surface water quality of the Yangtze Estuary with genotoxicity data. *Mar. Pollut. Bull.* 50 (12), 1661–1667.
- Xinhua, 2007a. China warns of environmental “catastrophe” from Three Gorges Dam (26 September, 2007). Xinhua News Agency—state press agency of the People’s Republic of China Available at: http://news.xinhuanet.com/english/2007-09/26/content_6796234.htm (Date accessed: 21 November 2014).
- Xinhua, 2007b. Millions more face relocation from Three Gorges Reservoir Area (11 October, 2007). Xinhua News Agency—state press agency of the People’s Republic of China Available at: http://news.xinhuanet.com/english/2007-10/11/content_6864252.htm (Date accessed: 21 November 2014).
- Xinhua, 2014. Throughput of Three Gorges Dam hits record high (2 January, 2014). Xinhua News Agency—state press agency of the People’s Republic of China Available at: http://news.xinhuanet.com/english/china/2014-01/02/c_133014381.htm (Date accessed: 21 November 2014).
- Xu, K.H., Milliman, J.D., 2009. Seasonal variations of sediment discharge from the Yangtze River before and after impoundment of the Three Gorges Dam. *Geomorphology* 104 (3-4), 276–283.
- Xu, X.B., Nachtman, J.P., Jin, Z.L., Wei, E.T., Rappaport, S.M., Burlingame, A.L., 1982. Isolation and identification of

- mutagenic nitro-PAH in diesel-exhaust particulates. *Anal. Chim. Acta* 136, 163–174.
- Yang, S.L., Zhang, J., Xu, X.J., 2007. Influence of the Three Gorges Dam on downstream delivery of sediment and its environmental implications, Yangtze River. *Geophys. Res. Lett.* 34 (10), L10401.
- Yu, Y., Xu, J., Wang, P., Sun, H.W., Dai, S.G., 2009. Sediment-porewater partition of polycyclic aromatic hydrocarbons (PAHs) from Lanzhou Reach of Yellow River, China. *J. Hazard. Mater.* 165 (1-3), 494–500.
- Yunker, M.B., Macdonald, R.W., Vingarzan, R., Mitchell, R.H., Goyette, D., Sylvestre, S., 2002. PAHs in the Fraser River basin: a critical appraisal of PAH ratios as indicators of PAH source and composition. *Org. Geochem.* 33 (4), 489–515.
- Zhang, Y.X., Tao, S., 2009. Global atmospheric emission inventory of polycyclic aromatic hydrocarbons (PAHs) for 2004. *Atmos. Environ.* 43 (4), 812–819.
- Zhang, Y.X., Tao, S., Cao, J., Coveney Jr., R.M., 2007. Emission of polycyclic aromatic hydrocarbons in China by county. *Environ. Sci. Technol.* 41 (3), 683–687.
- Zhang, Y.X., Tao, S., Shen, H.Z., Ma, J.M., 2009. Inhalation exposure to ambient polycyclic aromatic hydrocarbons and lung cancer risk of Chinese population. *Proc. Natl. Acad. Sci. U. S. A.* 106 (50), 21063–21067.