*Asian Journal of Water, Environment and Pollution,* Vol. 9, No. 3 (2012), pp. 1–9.

# Toxicity of East Sumatra River Sediments—Bacterial Luminescence, Brine Shrimp and Acetylcholinesterase Inhibition Tests

**Bettina Scholz, Daniel Ziehe[1*], Lucinéia A. Pivetta, Nils Pielok and Gerd Liebezeit**

Carl von Ossietzky University of Oldenburg, Institute for Chemistry and Biology of the Marine Environment, Schleusenstr. 1, 26382 Wilhelmshaven, Germany
[1]Johann Heinrich von Thuenen Institute, Federal Research Institute for Rural Areas, Forestry and Fisheries Institute of Agricultural Climate Research, Bundesallee 50, 38116 Braunschweig, Germany
✉ daniel.ziehe@vti.bund.de

*Received September 8, 2010; revised and accepted March 12, 2012*

**Abstract:** Bioassays employing the luminescent bacterium *Vibrio fischeri* (LUMIStox system), the brine shrimp (*Artemia salina*) as well as the acetylcholinesterase inhibition test were used to evaluate sediment toxicity of surface sediments from five East Sumatra rivers. In March and November of 2008 samples were collected from Rokan, Siak, Kampar, Indragiri and Musi rivers from overall 52 stations. The sediments were extracted with a sediment/water ratio of 1:1 (w/v). The most toxic sediments were found at the stations 529 (Kampar), 543 (Musi) as well as 517 (Siak) during the November 2008 campaign. Of the bioassays employed in this study, the acetylcholinesterase inhibition test was the most responsive to the extracts ($EC_{50}$: 0.4 ± 0.1 to 1.02 ± 0.3 g/L), whereas the invertebrate lethality assay using *Artemia salina* was less sensitive ($EC_{50}$: 6.1 ± 0.6 to 9.3 ± 0.4 g/L). While it was not possible to correlate the observed ecotoxicological effects with a specific and/or class of contaminants it is notable that the Siak sediment extracts were consistently ranked the most toxic with all test species and all test phases.

**Key words:** Acetylcholinesterase inhibition, bioassay test systems, brine shrimp assay, LUMIStox, Sumatra river sediment, toxicity.

## Introduction

Pekanbaru, the capital of Riau Province, East Sumatra, discharges untreated sewage of an estimated two million inhabitants and has, in addition, a number of saw mills and latex drying plants which release saw dust and organic- and particle-rich waters (Liebezeit et al., 2009). In Perawang, paper and pulp industry is located also discharging large amounts of untreated production waters. In the area of the tributaries Tapung kanan and Tapung kiri palm oil plantations are the most abundant form of land use (Liebezeit and Wöstmann, 2009). For example, the Siak River, Province Riau, with a total length of 370 km drains a catchment area of 11.500 $km^2$. Due to the drainage of peatlands it has a high natural organic matter load (Baum et al., 2007).

Sediments are ecologically important components of aquatic habitats playing a significant role in maintaining the trophic status of any water body. They also serve as reservoirs and potential sources of contaminants to the water column and can adversely affect sediment-dwelling organisms, aquatic dependent wildlife and human life (U.S. (EPA), 2005). Near urban areas commonly the sediments contain high levels of contaminants (Cook and

*Corresponding Author

Wells, 1996), thereby constituting a major environmental problem faced by many aquatic environments impacted by anthropogenic inputs (Magalhães et al., 2007). The main sources of anthropogenic inputs are industry, agriculture, urban run-off and untreated domestic sewage with river sediments acting as sink for these inputs (Liebezeit et al., 2009).

Over the last two decades, a vast number of short-term aquatic toxicity tests have been developed for a wide range of aquatic organisms occupying different trophic levels, with the purpose of identifying chemicals with adverse effects in aquatic ecosystems (Toðulga, 1998).

Since analytical analysis cannot describe the "real toxic effect" of complex mixture of chemicals, relatively simple and low cost methods provide necessary additional information on overall toxicity, and allow rapid evaluation of the potential hazard for humans, animals or ecosystems (Parvez et al., 2006). Due to differences in their sensitivity, the effects of the Sumatra river sediment samples in three different test systems were investigated. Firstly, the luminescent bacterium *Vibrio fischeri* (Beijerinck) Lehmann & Neumann was used as test organism because it is sensitive to a wide range of toxic organic and inorganic compounds. Distortion of bacterial cellular metabolism due to toxins causes a reduction in the intensity of emitted light, which can be used as an indication of toxicity. The bioluminescence inhibition of the *V. fischeri* test has been standardized (ISO, 1998) and it is commercially available in different versions. The advantages of those toxicity tests include short time of analysis and simplicity of operation. The bacteria are provided by manufacturers in a lyophilized form and they can be stored for several months to be used "on demand".

Secondly, *Artemia salina* Linnaeus (Artemiidae, brine shrimp), an invertebrate component of the saline aquatic fauna and marine ecosystems, plays an important role in the energy flow of the food chain. It is considered the most "euryplastic" test species that easily adapts to a wide range of environmental conditions (Panagoula et al., 2002). The *Artemia* bioassay is attractive for different reasons, including (i) the commercial availability of the cysts, (ii) *Artemia* can be maintained indefinitely in the laboratory in their cyst form and is easily induced to hatch, (iii) the assay is quick, simple, and performed at low cost, (iv) it requires small sample volume and can be performed with high sample throughput (microplates), and (v) it complies with animal ethics guidelines in many countries (Ruebhart et al., 2008; Rizzo, 2011).

And finally, acetylcholinesterase (AChE) is an important enzyme of the nervous system, whose primary function is to terminate nerve impulse transmission at cholinergic synapses by hydrolysing the neurotransmitter acetylcholine (ACh). AChE activity is specifically inhibited by the presence of organophosphate and carbamate pesticides (e.g. Oropesa et al., 2007). However, other chemicals have similar actions on the AChE response. Within this group, some heavy metals and petroleum-derived products have been reported as AChE inhibitors in marine organisms (Cunha et al., 2005) and in *in vitro* studies (Jett et al., 1999). The inhibition of AChE causes an accumulation of acetylcholine, which ultimately blocks the transmission of nerve impulses. In invertebrates such as arthropods (i.e. crustaceans and insects) this accumulation induces a pattern typical of nerve poisoning: restlessness, hyperactivity, tremors, convulsions and paralysis (Ware, 1989).

In the present study, we evaluated toxicity of aqueous sediment extracts using three different tests: acute and chronic toxicity tests with the brine shrimp *Artemia salina*, inhibition of luminescence employing *Vibrio fischeri* and the enzymatic test with acetylcholinesterase (AChE) from *A. salina* homogenates.

## Experimental Details

### Sampling Sites

The five rivers sampled – Rokan, Siak, Kampar, Indragiri and Musi – belong to the geographical regions Mainland Riau, Jambi and south eastern Sumatra (Figure 1). Overall, eight stations were sampled at Rokan (422-426, 534, 535, 538), Siak was represented by 12 stations (401-405, 407-409, 515, 517, 519, 521), Kampar with nine stations (428, 430, 527, 528, 529, 529a, 531-533), Indragiri with ten stations (412, 414, 415, 417-419, 508-509), and finally, the river Musi with 13 stations (431-434, 541, 543-545, 547, 547a, 553-555).

### Sample Collection and Processing

Sediment samples were obtained during two sampling campaigns where different stations were sampled in March and November 2008 (stations in March 2008: all four hundred numbers; stations in November 2008: all five hundred numbers). Surface sediments were sampled with a hand-operated van Veen-type grab of 150 $cm^2$ area. After initial air drying, samples were transported to the home laboratory and freeze-dried. After exploratory experiments (unpublished results), a sediment/water ratio

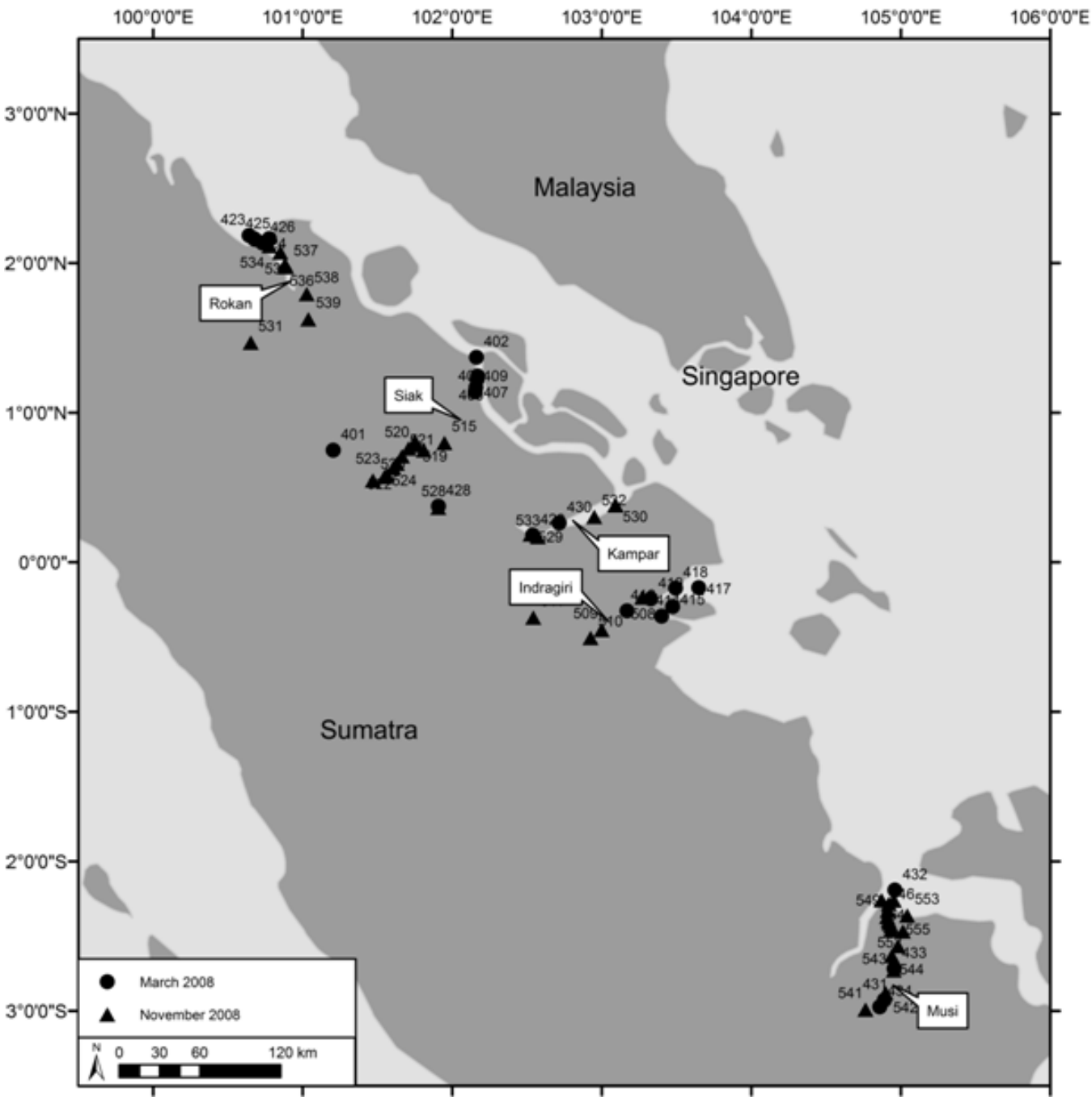


**Figure 1: Sampling stations on Sumatra. Black dots: March 2008, grey dots: November 2008. Dark grey areas in inset indicate peatlands. The numbers indicate the individual sample station during the different sampling events.**

of 1:1 (w/v) was chosen for the extractions. 10 g per sample were treated with 10 mL doubly distilled water at 90 °C for 3 h and centrifuged for 10 min at 5000 rpm. The supernatants were used for the assays without any additional treatment.

**Chemicals**

All reagents and chemicals were obtained from Sigma-Aldrich (Dorset, UK).

**Bioluminescence Toxicity Assay**

Inhibition of bioluminescence in cultures of *V. fischeri* NRRL-B-11177 was performed by using a commercially available standard luminescent bacterium LUMIStox test kit (Dr. Lange GmbH) and specific analytical equipment, including the LUMIStox 300 measuring station and a Lumistherm thermostat. Freeze-dried *Vibrio fisheri* reagent (Dr. Lange LUMIStox, 2001; ISO 11348-2, 1998) was reconstituted with sterile 2% NaCl (pH 7.0), and

equilibrated for 1 h. Bacterial suspensions and samples were maintained at 15 °C, combined, and monitored with the luminometer, while allowing bacteria to adapt for 5 to 10 min. The luminescent bacteria (0.5 mL) were placed into cuvettes and the light intensity measured using a temperature-controlled luminometer. Various dilutions (0.015, 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/mL) of samples (0.5 mL) were combined with *V. fischeri*. The decrease of light intensity was measured after 30 min and 1 h. A basic test using phenol as a reference chemical was conducted to ensure validity of all the tests. In addition, negative controls for each test were included, which consisted of bacteria exposed to only saline solution. The effect on bioluminescence was monitored and was expressed as the percent inhibition relative to the untreated controls. Values were calculated by using, as a correction factor, the changes in intensity of the controls. This was achieved by subtracting the trace negative control reading from the test samples over the duration of the experiment. The $EC_{50}$ data is expressed as concentration (mg/L), which caused a 50% reduction in bacterial bioluminescence following a 30 min incubation period.

### *Artemia salina* Bioassay

Toxicity was tested using the *Artemia* nauplii lethality assay developed by Vanhaecke and Persoone (1981) for the screening of constituents with the following modifications. *A. salina* cysts were obtained from Galway Aquatic Ltd. (Galway, Ireland). Synthetic seawater was prepared using Tropic Marin® (GmbH Aquarientechnik). Seawater solutions at 38.4 g/L distilled water were prepared prior to use. 2 g of *A. salina* cysts were incubated in 1 L synthetic seawater under artificial light at 23 ± 2 °C, 800 µmoL photons $m^{-2}$ $s^{-1}$ with continuous aeration to produce instar larvae. Hatching commenced within 16-18 h of incubation. Newly hatched *A. salina* (nauplii) was used within 10 h of hatching.

Nauplii were separated from the shells and remaining cysts and were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii-rich water closest to the light was removed for biological assays. Using instar II-III larvae, 2 mL of seawater containing 200 nauplii were added to petri dishes (3.5 cm diameter) and immediately used for bioassay. The incubation for 6, 24 and 72 h with 10 mL of sediment extract was conducted in the dark with two controls and five test dilutions (20, 40, 60, 80 and 100%) with three replicates per concentration. The first control containing artificial seawater only was used to estimate natural mortality. This was found to be 0.2 ± 0.05 over the assay times. As positive control potassium dichromate (Davoren et al., 2005; Mekki et al., 2008) was used. Here 100% mortality was observed after 24 h. Lethality for each sample dilution was recorded and the percentage mortality ($LC_{50}$) compared to the control was determined.

### Acetylcholinesterase Inhibition Tests

AChE of 24 h old *A. salina* nauplii was investigated using a technique which colourimetrically measures the reaction between hydrolysed acetylthiocholine (AChT) and dithiobisnitrobenzoate (5,5'-Dithio-*bis*-(2-nitrobenzoic acid) = DTNB) based on Ellman et al. (1961). *A. salina* homogenates were prepared according to the method of Venkateswara Rao et al. (2007). In brief, the nauplii were homogenized in 1 mL ice-cold phosphate buffer (0.1M, pH 7.5) containing 10 mL/L Triton X-100 using a Potter-Elvehjam homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 6000 x g for 20 min at 4 °C in an ultra centrifuge. The resultant supernatant was recentrifuged at 15.000 x g for 20 minutes at 4 °C and used as enzyme source. 50 µL AChE solution (5 U/mL in phosphate buffer) were incubated with 50 µL of sediment extract in a cuvette for 2 min prior to addition of 3 mL $KH_2PO_4$ buffer (0.1M, pH 8), 100 µL DTNB (0.01M in 0.1M buffer, pH 7) and 20 µL of substrate (AChT, 0.075M in doubly distilled water). The change in absorbance over time at 412 nm (Dr. Lange, 2800 DR) was recorded and AChE inhibition determined by comparison with a blank control, containing doubly distilled water + buffer + DTNB. Additionally, as positive control 50 µL of the organophosphorus insecticide O,O-diethyl O-3,5,6-trichloropyridin-2-yl phosphorothioate (chlorpyrifos = CPP; 1 mg/L) was used. In contrast, the negative control contained only 50 µL doubly distilled water instead of sediment extracts or CPP. The spontaneous hydrolysis of AChT was estimated using buffer + DTNB + AChT. All assays were carried out at room temperature. The 50% effect concentration ($EC_{50}$) value was calculated by probit analysis (Finney, 1971).

### Statistical Analysis

Experiments were performed in triplicate in at least two independent laboratories. Coefficient of variation (CV) for the controls of each test was calculated to ascertain reproducibility. Data are expressed as the arithmetic mean ± standard error of the mean (SEM). To assess variability of test results, analyses of variance (one way ANOVA) were performed on logarithmically transformed data to adhere to normality. Mean values among treatments were compared by the Duncan's multiple range test at the 5%

($p = 0.05$) level of significance. All tests were performed with the program XLSTAT 2011, Version 2011.2.08 Addinsoft.

## Results

### Bioluminescence Toxicity Assay

The mean $EC_{50}$ value for the positive phenol control (30 analyses) was 18.1 ± 0.8 mg/L. With the exception of the station samples 405, 419 and 547, which did not show any effects, in no case an inhibition value <20% was found (Figure 2). Maximum values were observed for the Siak river in March 2008 for the stations 403 and 408, with $EC_{50}$ values of the sampled sediments ranging between 1.3 ± 0.2 g/L and 5.5 ± 0.6 g/L, respectively. The long-term expositions led to no significant differences ($p > 0.05$).

### Brine Shrimp Bioassay

The *A. salina* bioassay was performed in triplicate and yielded a 24-h $LC_{50}$ value of 21.9 ± 1.8 mg/L for the reference chemical potassium dichromate. While 6 h expositions to the different sediment extracts did not show any results, acute toxicities (after 24 h) did not exceed mortality rates of 15% (Figure 3). The acute effects were significant for the Siak station 517 (ANOVA: $F_{1,6} = 26.4$, $P < 0.0001$) and the Musi station 543 (ANOVA: $F_{1,6} = 20.8$, $P < 0.0001$) from the November campaign. Chronic effects (after 72 h) were encountered in 18 cases of 52 samples analysed, with sediment $EC_{50}$ values ranging between 6.1 ± 0.6 g/L (station 408) and 9.3 ± 0.4 g/L (station 407). For the Siak, 7 of 12 samples resulted in 90 to 100% mortalities, being significant for 401, 408, 515, 517 and 519 (ANOVA: $F_{1,15} = 31.6$, $P < 0.0001$). Most of the chronic toxicities for Kampar (2 of 9 samples) and Musi (4 of 13 samples) were less pronounced, but were significantly for the stations 529 (Kampar) as well as 543 (Musi) from the November 2008 campaign ($p < 0.05$).

### Acetylcholinesterase Inhibition Tests

The mean $EC_{50}$ values of the toxicity reference CPP were 0.31 ± 0.16 mg/L. AChE activity was affected by 51.9% of the sampled sediments (Figure 4). Only three out of these 27 samples had negligible effects (stations: 521, 545, 547; ANOVA: $F_{1,9} = 4.2$, $P = 0.0025$). Extraordinary high inhibition values (>60%) were observed for the stations 517 (Siak), 529 (Kampar) and 543 (Musi) from the November campaign. The $EC_{50}$ values for the sediments ranged from 0.4 ± 0.1 g/L (station 529) to 1.02 ± 0.3 g/L (station 527; both Kampar). Particularly Siak sediment extracts had, with two exceptions, values around >40% inhibition.

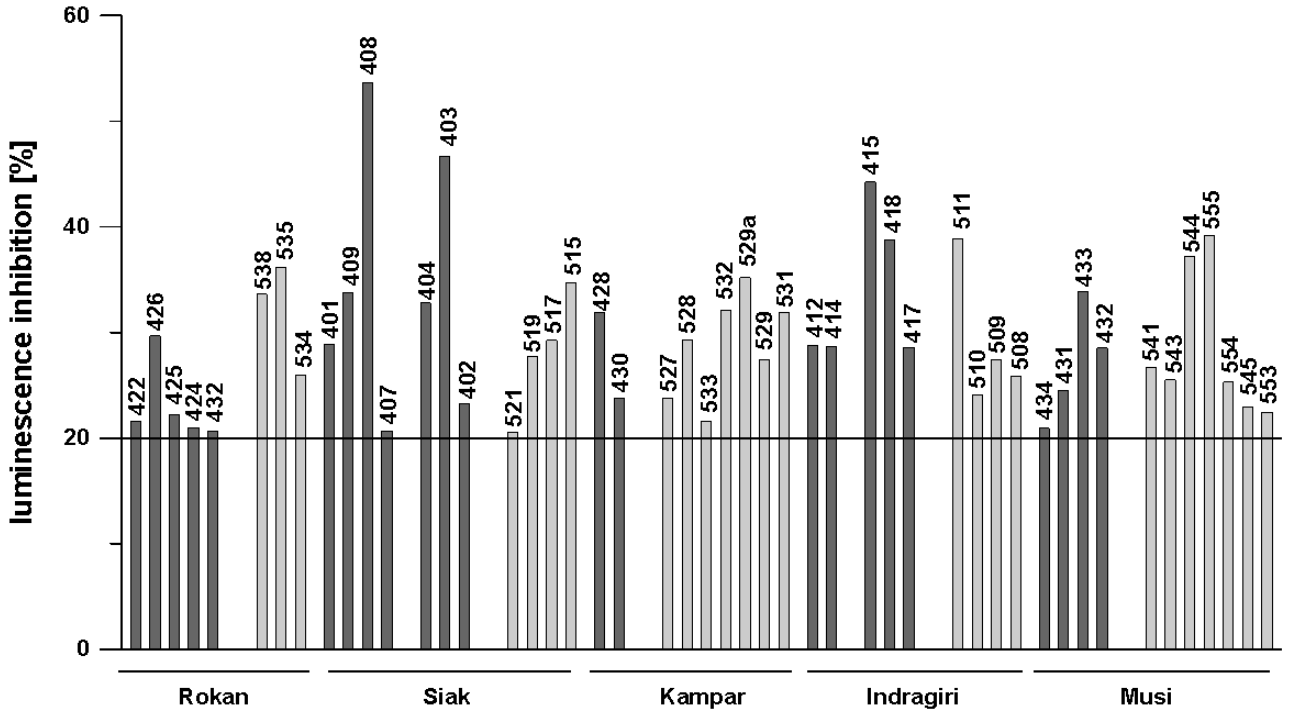


**Figure 2: Luminescence inhibition of aqueous sediment extracts after 30 min incubation, including mean values ± SD ($n = 3$). Dark grey – March 2008; light grey – November 2008. Stations are arranged from freshwater to estuary. The numbers in the graph indicate the individual sample station.**

## Discussion

In general, the results were highly variable and differed with both the sampled stations and toxicity tests used. Trends, e.g. from freshwater to estuarine stations, were not evident. The sensitivity of the conducted tests showed significant differences in comparison to the tested sediment samples, in which the AChE inhibition test had the highest responsivity (ANOVA: $F_{3,52} = 36.9$, $P < 0.0001$) followed by the luminescence inhibition test with *V. fischeri* (ANOVA: $F_{3,52} = 30.2$, $P < 0.0001$). Although over the course of experiments significant variations between LUMIStox and the other two toxicity tests occurred (e.g. the Siak sample no. 403, LUMIStox: 46.7%, other 0%) the most toxic sediments were found at the stations 529 (Kampar), 543 (Musi) as well as 517 (Siak) during the November 2008 campaign (Figures 3 and 4). Taken as a whole, Siak samples exhibited the highest toxicities in all three tests.

While for the *Artemia* test only minor effects after 24 h sediment extract exposition were observed (2.5%), the 72 h exposition of *Artemia* led to a mean mortality of 58.3%. Moreover, a mean value of 29.3% was found for the luminescence and 33.8% for the AChE inhibition test, respectively. The Siak is a typical, nutrient-poor, well-mixed, black water river in central Sumatra, which owes its brown colour to dissolved organic matter (DOM) leached from surrounding, heavily disturbed peat soils (Rixen et al., 2010). As this river has the highest contribution of peat-derived organic matter, possible effects of peat extracts were therefore checked. Assuming a total organic carbon content (TOC) of the peat of 40% and taking into account TOC values in Siak sediments of 2.15 ± 0.96% (March, $n = 9$) and 1.89 ± 1.2% (November, $n = 9$), then a significant dilution has occurred even if all sedimentary TOC is peat-derived. Thus, contributions to the observed toxicities from peat can be excluded.

Persoone et al. (1989) and Davoren et al. (2005) previously reported an $LC_{50}$ value for potassium dichromate of 22.2 mg/L and 22.7 mg/L (25 °C and 35 PSU) with *A. salina*, which is within the range of the data obtained in this study. Besides several other possibilities for *Artemia* mortality (e.g. hepatotoxins), according to Barahona and Sánchez-Fortún (1999) it can be attributed to the inhibition of acetylcholinesterase activity. In the present suite of samples this is, however, apparently not always the case (Figure 5). In a number

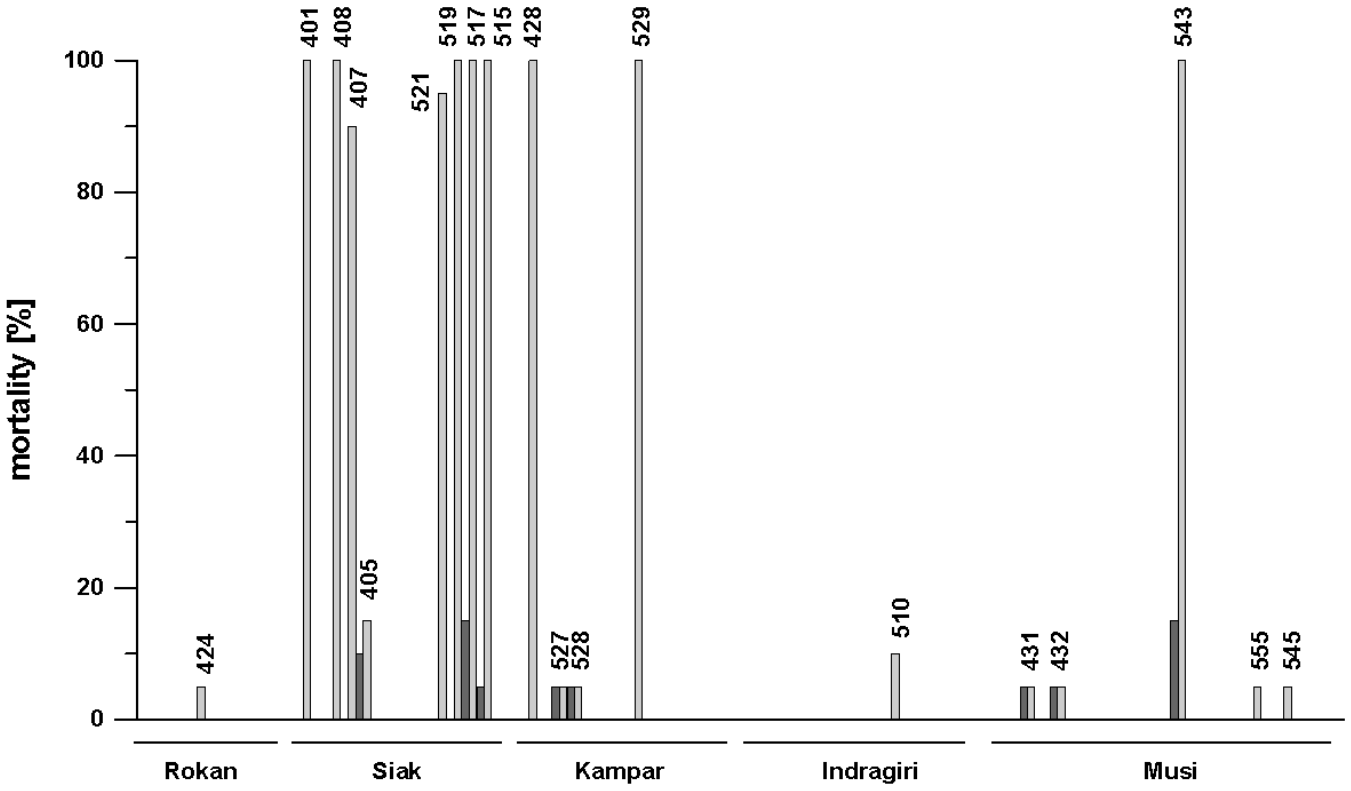


**Figure 3: *Artemia* mortality after 24 and 72 h expositions to aqueous sediment extracts, including mean values ± SD ($n = 3$). Acute toxicity (24 h) – dark grey, chronic toxicity (72 h) – light grey. Stations are arranged from freshwater to estuary. The numbers in the graph indicate the individual sample station.**

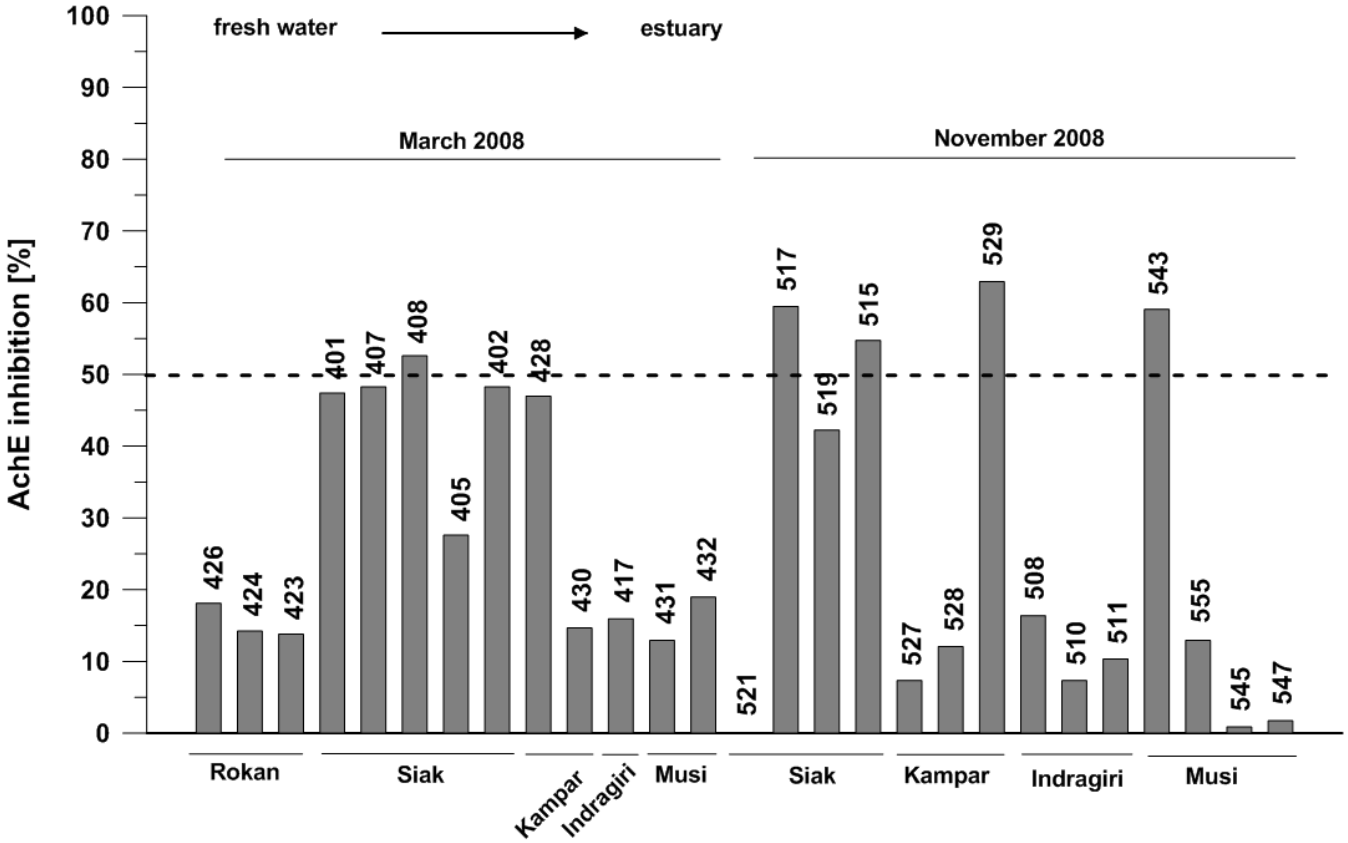


**Figure 4: Acetylcholinesterase inhibition of aqueous sediment extracts, including mean values ± SD ($n$ = 3). Stations are arranged from freshwater to estuary. Only 27 of the 52 stations are displayed. The numbers in the graph indicate the individual sample station.**

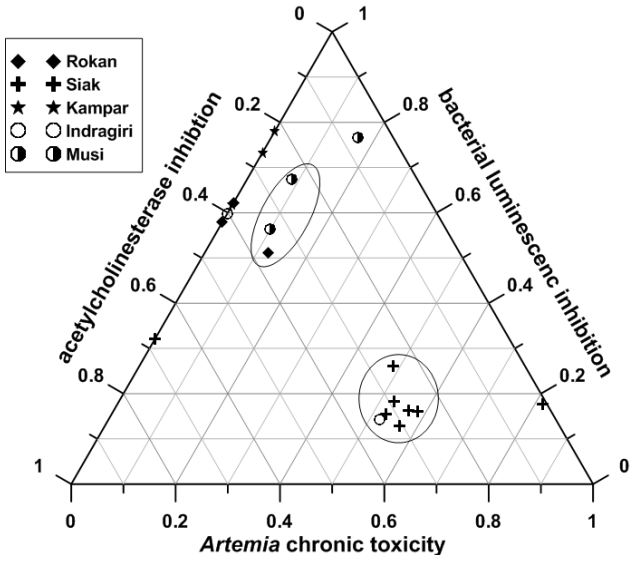


**Figure 5: Ternary plot of results of the three toxicity tests performed: LUMIStox (after 30 min exposition), brine shrimp assay (toxicity after 72 h) and acetylcholinesterase inhibition test.**

of samples no effects on *Artemia* were observed despite AChE inhibition values of up to 66%. These results concur with the findings of Weideborg et al. (1997) and Davoren et al. (2005) who also demonstrated *A. salina* to have too low a sensitivity to be considered as an appropriate bioassay organism for screening tests. In addition, previous studies with *Artemia* larvae have shown that sensitivity to chemicals differed with age. Usually, older brine shrimp are more sensitive than younger ones. With *A. salina* larvae 72-h appears to be one of the more sensitive ages (Sánchez-Fortún et al., 1995; Barahona and Sánchez-Fortún, 1996; Sánchez-Fortún et al., 1997).

According to Davoren et al. (2005), the reduction in luminescent responses detected for the sediment samples, which were relatively constant over the incubation time points examined (30 min and 1 h exposition), is suggestive of toxicity elicited by organic compounds. In contrast, a time-dependent decrease in light levels would be a typical response of *V. fischeri* following exposure to heavy metal compounds (Davoren et al., 2005). Based

on these findings and low heavy metal contents in river sediments (W. Balzer, pers. comm., 2012) sediment toxicity is predominantly associated with organic compounds. These have been shown to be rapidly degraded after discharge. For example, Liebezeit and Wöstmann (2009) observed compounds characteristic for latex processing in the Siak to approximately 75 km downstream from the discharge point. Similarly, coprostanol content (an indicator of sewage discharge) declined rapidly after the main source Pekanbaru (Liebezeit and Wöstmann, 2010). Nevertheless, as the present data show, there are areas both upstream and in the estuaries of Sumatra rivers where indications of significant sediment toxicities and hence high levels of contamination are evident. These would merit further detailed investigations.

Besides untreated sewage and other urban discharges such as road run-off Sumatra's rivers receive discharges from a number of industries and agriculture. This inhibition is likely to be associated with discharges from sawmills, paper and pulp production or palm oil and latex processing plants (Liebezeit et al., 2009). Furthermore, intense boat traffic resulting in direct input of petroleum hydrocarbons contributes to the environmental burden experienced by the river ecosystems (Liebezeit and Wöstmann, 2009).

## Conclusions

The data presented above indicate that there is a significant pollutant load carried by East Sumatra rivers and deposited in the river bed sediments. Although bulk toxicity tests as applied here provide only an overall assessment of these loads the combination of three different tests allows to pinpoint pollution hotspots.

## Acknowledgements

This study was carried out within the bilateral Indonesian-German research program SPICE II (Science for the Protection of Indonesian Coastal Marine Ecosystems) sponsored by the German Federal Ministry of Education and Research (Grant No. 03F0473B), the Indonesian Ministry of Marine Affairs and Fisheries (DKP) and the Ministry for Research and Technology (RISTEK).

## References

Barahona, M.V. and S. Sánchez-Fortún (1996). Comparative Sensitivity of Three Age Classes of *Artemia salina* Larvae to Several Phenolic Compounds. *Bulletin of Environmental Contamination and Toxicology*, **56(2):** 271-278.

Barahona, M.V. and S. Sánchez-Fortún (1999). Toxicity of carbamates to the brine shrimp *Artemia salina* and the effect of atropine, BW284c51, iso-OMPA and 2-PAM on carbaryl toxicity. *Environmental Pollution*, **104(3):** 469-476.

Baum, A., Rixen, T. and J. Samiaji (2007). Relevance of peat draining rivers in central Sumatra for the riverine input of dissolved organic carbon into the ocean. *Estuarine*, *Coastal and Shelf Science*, **73(3-4):** 563-570.

Cook, N.H. and P.G. Wells (1996). Toxicity of Halifax Harbour Sediments: An Evaluation of Microtox® Solid-Phase Test. *Water Quality Research Journal of Canada*, **31(4):** 673-708.

Davoren, M., Ní Shúilleabháin, S., O'Halloran, J., Hartl, M.G.J., Sheehan, D., O'Brien, N.M., van Pelt, F.N.A.M. and C. Mothersill (2005). A Test Battery Approach for the Ecotoxicological Evaluation of Estuarine Sediments. *Ecotoxicology*, **14(7):** 741-755.

Ellman, G.L., Courtney, K.D., Andres Jr., V. and R.M. Featherstone (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, **7(2):** 88-95.

Finney, D.J. (1971). Probit Analysis, 3rd Edition. Cambridge University Press, UK.

ISO (International Organization for Standardization) (1998). Water quality determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent Bacteria Test) Part 2: Method Using Liquid-Dried Bacteria. ISO 11348-2, Geneva, Switzerland.

Liebezeit, G. and R. Wöstmann (2009). *n*-Alkanes as Indicators of Natural and Anthropogenic Organic Matter Sources in the Siak River and its Estuary, E Sumatra, Indonesia. *Bulletin of Environmental Contamination and Toxicology*, **83(3):** 403-409.

Liebezeit, G. and R. Wöstmann (2010). Coprostanol in Siak River Sediments, E Sumatra, Indonesia. *Bulletin of Environmental Contamination and Toxicology*, **85(6)**: 585-588.

Liebezeit, G., Wöstmann, R. and C. Jose (2009). Biomarkers from Latex Drying Plants in Siak River Sediments. *Asian Journal of Water*, *Environment and Pollution*, **6(2):** 1-5.

Magalhães, C., Costa, J., Teixeira, C. and A.A. Bordalo (2007). Impact of trace metals on denitrification in estuarine sediments of the Douro River estuary, Portugal. *Marine Chemistry*, **107(3):** 332-341.

Mekki, A., Dhouib, A., Feki, F. and S. Sayadi (2008). Assessment of toxicity of the untreated and treated olive mill wastewaters and soil irrigated by using microbiotests. *Ecotoxicology and Environmental Safety*, **69(3):** 488-495.

Oropesa, A.-L., Pérez-López, M., Hernández, D., García J.-P., Fidalgo, L.-E., López-Beceiro, A. and F. Soler (2007). Acetylcholinesterase activity in seabirds affected by the Prestige oil spill on the Galician coast (NW Spain). *Science of The Total Environment*, **372(2-3):** 532-538.

Parvez, S., Venkataraman, C. and S. Mukherji (2006). A review on advantages of implementing luminescence inhibition test (*Vibrio fischeri*) for acute toxicity prediction of chemicals. *Environment International*, **32(2):** 265-268.

Panagoula, B., Panayiota, M. and J. Iliopoulou-Georgudaki (2002). Acute Toxicity of TBT and IRGAROL in *Artemia salina*. *International Journal of Toxicology*, **21(3):** 231-233.

Persoone, G., van de Vel, A., van Steertegem, M. and B. de Nayer (1989). Predictive value of laboratory tests with aquatic invertebrates: Influence of experimental conditions. *Aquatic Toxicology*, **14(2):** 149-167.

Rixen, T., Baum, A., Sepryani, H., Pohlmann, T., Jose, C. and J. Samiaji (2010). Dissolved oxygen and its response to eutrophication in a tropical black water river. *Journal of Environmental Management*, **91(8):** 1730-1737.

Sánchez-Fortún, S., Sanz-Barrera, E. and M.V. Barahona (1995). Acute Toxicities of Selected Insecticides to the Aquatic Arthropod *Artemia salina*. *Bulletin of Environmental Contamination and Toxicology*, **54(1):** 76-82.

Sánchez-Fortún, S., Sanz, F., Santa-María, A., Ros, J.M., de Vicente, M.L., Encinas, M.T., Vinagre, E. and M.V. Barahona (1997). Acute sensitivity of three age classes of *Artemia salina* larvae to seven chlorinated solvents. *Bulletin of Environmental Contamination and Toxicology*, **59(3):** 445-451.

Ruebhart, D.R., Cock, I.E. and G.R. Shaw (2008). Brine shrimp bioassay: Importance of correct taxonomic identification of *Artemia* (Anostraca) species. *Environmental Toxicology*, **23(4):** 555-560.

Rizzo, L. (2011). Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water Research*, **45(15):** 4311-4340.

Toðulga, M. (1998). The Short-Term Toxicity of Two Toxicants to *Artemia* Nauplii. *Turkish Journal of Zoology*, **22(3):** 259-266.

U.S. (Environmental Protection Agency-EPA) (2005). Predicting toxicity to amphipods from sediment chemistry. National Center for Environmental Assessment, Washington, DC, EPA/600/R-030. Available from: National Technical Information Service, Springfield, VA. http://www.epa.gov/ncea. Accessed 12 Mar 2012.

Vanhaecke, P. and G. Persoone (1981). Report on an intercalibration exercise on a short-term standard toxicity test with *Artemia* nauplii (ARC-test). *INSERM*, **106:** 359-376.

Venkateswara Rao, J., Kavitha, P., Jakka, N.M., Sridhar, V. and P.K. Usman (2007). Toxicity of organophosphates on morphology and locomotor behavior in brine Shrimp, *Artemia salina*. *Archives of Environmental Contamination and Toxicology*, **53(2):** 227-232.

Ware, G.W. (1989). *In*: The Pesticide Book Thomson, Fresno, CA. http://www.pesticidebook.com. Accessed 12 Mar 2012.

Weideborg, M., Vik, E.A, Øfjord, G.D. and O. Kjønnø (1997). Comparison of three marine screening tests and four Oslo and Paris commission procedures to evaluate toxicity of offshore chemicals. *Environmental Toxicology and Chemistry*, **16(2):** 384-389.

**Asian Journal of Water, Environment and Pollution**

**Volume 8 Number 4** **October – December, 2011**

# Contents