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Molecular biomarkers of *Dreissena polymorpha* for evaluation of renaturation success of a formerly sewage polluted stream

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A new application for environmental health assessment combining the use of species tolerant to pollution, Dreissena polymorpha, and molecular tools (RT-PCR) in active biomonitoring.

Abstract

The renaturation success of an urban stream, formally used for discharge of treated sewage waters was investigated by active biomonitoring with *Dreissena polymorpha* based on molecular biomarkers and compared to a semi-natural stream and laboratory controls. Response to pollution charges were analyzed by reverse transcriptase-PCR of heat-shock protein (hsp70), P-glycoprotein (P-gp), catalase (CAT) and pi class glutathione S-transferase (piGST). Hsp70 transcription was similarly induced at both sites, indicating protein damage. At the semi-natural stream CAT and P-gp were induced, indicating oxidative stress and increased discharge of pollutants, which correlated to high amounts of aluminum at this site. piGST was induced at one sampling date at the renaturated stream only, but identification of the causing pollutant was not achieved. Results confirm regeneration of the formerly sewage polluted stream, because induction of the tested biomarkers was either at or below the levels of the semi-natural stream.

Keywords: Heat-shock protein; P-glycoprotein; Multi-xenobiotic-resistance mechanism; Catalase; Glutathione S-transferase

1. Introduction

In the city of Berlin demographic changes occurred after the political turn in the 1990ies. The reunification of the eastern and western part of the city caused structural reorganization of e.g. the drinking water supply as well as the sewage treatment plants. In the north of Berlin, the changes resulted in the closure of a sewage plant, which formerly discharged the cleaned sewage waters into the small artificial stream New Wuhle (Korner, 1997) (Fig. 1). Since 2003 sewage waters have been rerouted to other sewage plants with higher technical standards and introduction of treated waters to New Wuhle has been stopped. A renaturation program has been set by the local senate office, to guarantee local recreation for near living citizens, with planting of river and banks, as well as dredge and partly renewal of the sediment (Grasse et al., 2005). A recuperation of this stream was expected and will be assessed in this study. The nearby semi-natural small river Old Wuhle has been investigated for comparison, assuming possible marring influence from garden plots. Both streams run through a municipal park frequently used by residents for local recreation and coalesce into a single stream about 2 km downstream the sampling points of this study.

In general urban watercourses are frequently affected by a mixture of organic and inorganic compounds, such as industrial and domestic wastewaters, as well as the traffic run off after strong rains (Paul and Meyer, 2001), which often results

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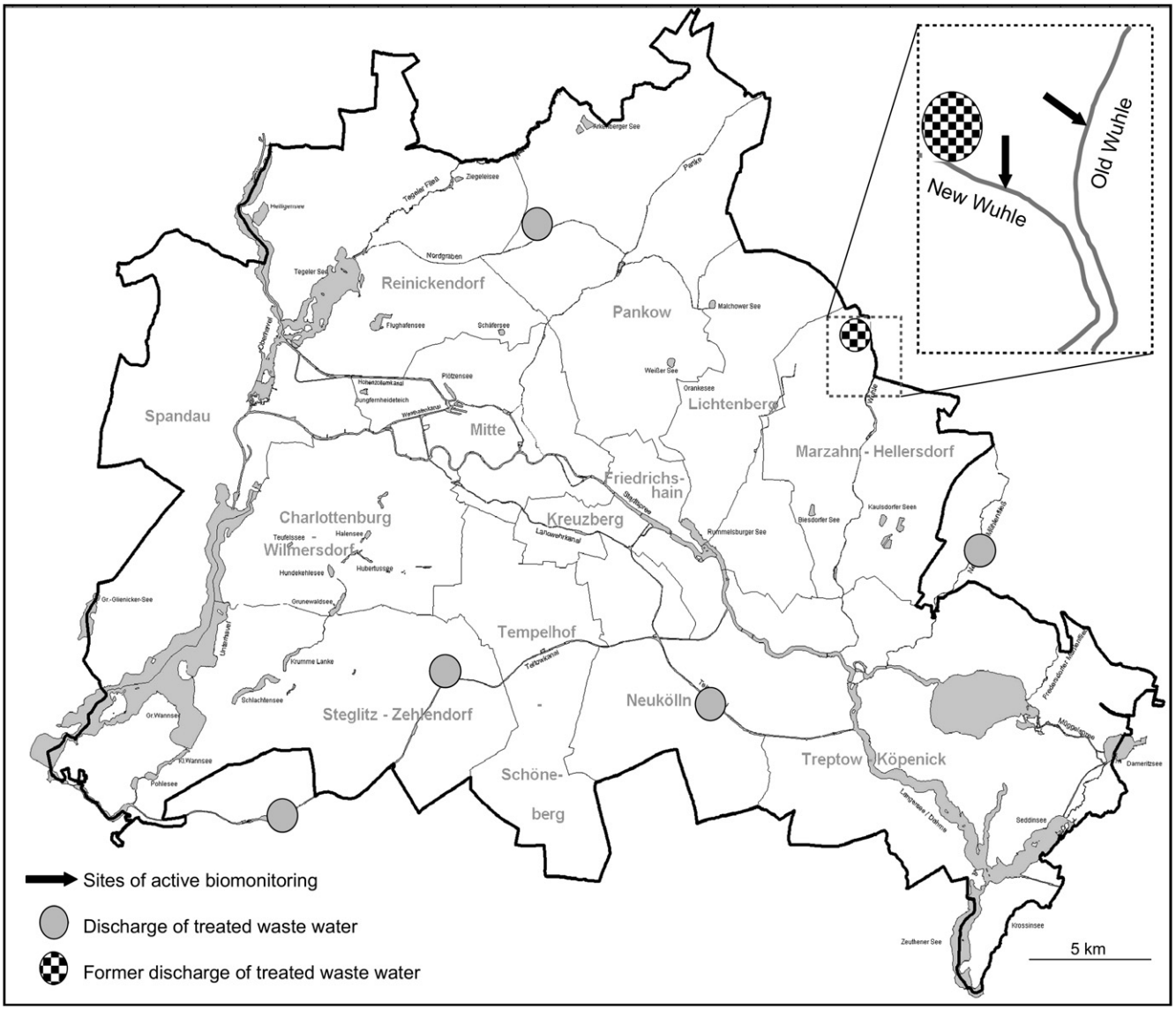

Fig. 1. Map of Berlin City, Germany showing in enlargement the sampling sites for active biomonitoring with *D. polymorpha*. Overview of discharge and former discharge of treated sewage waters into the urban river system.

in a lack of stable populations of sensitive native molluscs and other species (Clements et al., 2006). Thus only species showing a certain tolerance against anthropogenic influence may be applied as biomonitoring organisms for the detection of site impact in moderate polluted limnic streams.

Biomarkers in molluscs are commonly used to study site impact in both limnic watercourses and the marine ecosystem in particular by active biomonitoring with transplanted mussels (Cossu et al., 2000; Pampanin et al., 2005). Bivalve's responses at different organism-levels have been employed as biomarkers. Typical ecotoxicological endpoints are the conch movement, cellular reactions, such as apoptosis, and on the protein level activity changes of biotransformation or antioxidative enzymes, increase of heat shock proteins (hsp) and multixenobiotic resistance (MXR) as P-glycoprotein (P-gp) transport activity (Englund and Heino, 1996; Clayton et al., 2000; Smital et al., 2003; Company et al., 2006; Rocher et al., 2006).

Nowadays biomarker studies are advancing towards the use of molecular tools, such as changes in gene expression enabling an integrated view also on molecular mechanisms occurring due to pollution charges. Investigation on molecular markers in molluscs so far, focuses on the detection of induction of metallothioneins and heat shock protein mRNA for site impact with metals (Piano et al., 2004; Marie et al., 2006), MXR and MXR associated proteins (Ludeking and Kohler, 2002), as well as DNA damage as general stress indicator (Steinert, 1999).

The bivalve *Dreissena polymorpha*, which has proliferated from the Caspian Sea into European and North American lakes and streams causing economic and ecological damage (Enserink, 1999), has been selected as the biomonitor. *D. polymorpha* inhabits hard bottom substrate, having therewith an advantage to maintain sustainable populations also in stream areas where restriction with sheet pile walls is common. It