

Supporting Information to:

Prokaryotic Gene Profiling Assays to Detect  
Sediment Toxicity: Evaluating the  
Ecotoxicological Relevance of a Cell Based Assay

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Table 1 stress gene promoters fused to the lacZ gene and their major inducers.

| Promoter | Gene product/Function                                     | Responsive to                                  | Reference |
|----------|---|--|-----------|
| KatG     | Hydrogen peroxidase I                                     | Oxidative damage                               | (1)       |
| MicF     | Antisense RNA to 5' OmpF                                  | Membrane integrity, osmotic stress             | (2)       |
| OsmY     | Periplasmic Protein                                       | Osmotic stress                                 | (3)       |
| UspA     | Universal stress protein                                  | Growth arrest                                  | (4)       |
| RecA     | General recombination and DNA repair                      | SOS response                                   | (5)       |
| Zwf      | Glucose-6-phosphate dehydrogenase                         | Oxidative stress                               | (6)       |
| ClpB     | Proteolytic activation of ClpP                            | Protein perturbation                           | (7)       |
| UmuDC    | DNA repair  | Radiation and/or chemically induced DNA damage | (8)       |
| MerR     | Regulation of the mercury resistance operon (mer)         | Heavy metals                                   | (9)       |
| Ada      | Adaptive response to alkylation                           | DNA damage, mainly methyl adducts              | (10)      |
| DinD     | Unknown function within the DNA damage inducible response | DNA damage                                     | (11)      |
| Soi28    | Superoxide inducible gene                                 | Superoxide radical generating agents           | (12)      |
| Nfo      | Endonuclease IV   | ss and dsDNA breaks, oxidative DNA damage      | (13)      |
| SfiA     | Inhibitor of cell division                                | SOS response                                   | (14)      |

Table 2 Summarized description of cellular and physiological parameters determined on the caged organisms along the pollution gradient.

| Biomarker  | Organism                    | Description   |
|--|-----------------------------|---|
| Cellular Energy Allocation (CEA) (mJ/org)        | <i>Dreissena polymorpha</i> | Whole body glycogen, protein and lipid content minus the electron transport activity at the mitochondrial level. A measure for the net cellular energy budget, taking energy uptake and expenditure into account. |
| Wet weight – Dry weight ratio of the soft tissue | <i>Dreissena polymorpha</i> | Indicator of water and ion balance of an organism.  |
| Scope for Growth (SFG) (cal/mg dry weight)       | <i>Dreissena polymorpha</i> | Absorbed food energy minus respiration minus excretion. SFG represents the total energy available for growth and reproduction.  |
| Fulton Condition Factor (FCF)                    | <i>Cyprinus carpio</i>      | Weight $\times$ Length <sup>-3</sup> , condition factor assuming isometric growth.  |
| Lipid Budget (LB) (mJ/org)                       | <i>Cyprinus carpio</i>      | Whole body lipid contents   |
| Growth (mm)                                      | <i>Chironomus riparius</i>  | Gain in length of midge larvae  |

Table 3 Fold Inductions of the water column C18 and SDB-RPS extracts at the highest exposure concentration. \* Previous dose (1/2) not significantly different from blank signal (p<0,05). Blank cells: no significant induction. Results are given as mean (n=3), (standard error).

|                | <b>kat G</b> | <b>mic F</b>  | <b>osm Y</b>   | <b>usp A</b>  | <b>rec A</b>   | <b>zwf</b>     | <b>cip B</b>  | <b>umu DC</b> | <b>mer R</b>  | <b>ada</b>    | <b>dinD</b>   | <b>soi 28</b> | <b>nfo</b>    | <b>SfiA</b>    |
|----------------|--------------|---------------|----------------|---------------|----------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|----------------|
| <b>Loc 1</b>   |              |               |                | 1.1<br>(0.02) | 1.4<br>(0.06)  |                | 1.3<br>(0.09) | 1.4<br>(0.1)  |               |               |               |               |               | 1.2*<br>(0.06) |
| <b>Loc 2</b>   |              |               |                | 1.1<br>(0.04) | 1.3<br>(0.05)  | 1.1*<br>(0.05) | 1.2<br>(0.04) | 1.3<br>(0.01) |               |               |               |               |               | 1.3*<br>(0.01) |
| <b>SDB-RPs</b> |              | 2.0<br>(0.3)  |                |               | 1.2<br>(0.004) | 1.2<br>(0.01)  | 1.7<br>(0.01) | 1.1<br>(0.02) |               | 1.5<br>(0.01) | 1.2<br>(0.03) |               |               | 1.3<br>(0.05)  |
| <b>Loc 3</b>   |              |               |                |               |                |                |               |               |               |               |               |               |               |                |
| <b>Loc 4</b>   |              |               |                |               |                |                |               |               |               |               |               |               |               |                |
| <b>Loc 5</b>   |              |               |                |               |                |                |               |               |               |               |               |               |               |                |
|                |              |               |                |               |                |                |               |               |               |               |               |               |               |                |
| <b>Loc 1</b>   |              | 3.0<br>(0.7)  |                | 1.7<br>(0.3)  |                |                | 1.5<br>(0.2)  | 2.4<br>(0.4)  |               | 1.9<br>(0.2)  |               |               |               | 1.4<br>(0.1)   |
| <b>Loc 2</b>   |              | 1.9*<br>(0.3) | 1.2*<br>(0.08) |               |                | 1.7<br>(0.1)   | 1.9<br>(0.3)  | 2.2<br>(0.3)  | 1.9<br>(0.4)  | 2.2<br>(0.3)  | 1.5<br>(0.2)  | 1.6<br>(0.1)  | 1.7<br>(0.07) | 2.0<br>(0.07)  |
| <b>C18</b>     |              |               |                | 1.3<br>(0.02) |                |                |               |               |               |               |               |               |               |                |
| <b>Loc 3</b>   |              |               |                |               |                |                |               |               |               |               |               |               |               |                |
| <b>Loc 4</b>   |              |               |                |               |                |                |               |               |               |               |               |               |               |                |
| <b>Loc 5</b>   |              | 1.1<br>(0.03) | 1.5<br>(0.06)  | 1.1<br>(0.01) | 1.1<br>(0.03)  | 1.2<br>(0.04)  | 1.1<br>(0.03) | 1.3<br>(0.05) | 1.6<br>(0.07) | 1.5<br>(0.1)  |               |               |               |                |

Table 4 Fold Inductions at the highest exposure concentration of the pore water C18 and SDB-RPS extracts. Blank cells: no significant induction.  
 \* Previous dose (1/2) not significantly different from blank signal ( $p < 0.05$ ). Results are given as mean (standard error) ( $n=3$ ).

|              | <b>katG</b> | <b>micF</b> | <b>osmY</b> | <b>uspA</b> | <b>recA</b> | <b>zwf</b> | <b>cpxB</b> | <b>umuDC</b> | <b>merR</b> | <b>ada</b> | <b>dinD</b> | <b>soi28</b> | <b>nfo</b> | <b>sfiA</b> |
|--------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|--------------|-------------|------------|-------------|--------------|------------|-------------|
| <b>Loc 1</b> |             |             | 2.0*        | 2.7         | 2.7         | 3.2        |             | 2.1          |             | 2.6        |             |              |            |             |
| <b>Loc 2</b> |             |             | (0.5)       | (0.2)       | (0.4)       | (0.7)      |             | (0.2)        |             | (0.7)      |             |              |            |             |
| <b>Loc 3</b> | 6.8         | (0.5)       |             |             |             |            |             | 9.9          | 4.2*        |            | 5.7         |              |            |             |
| <b>Loc 4</b> |             | 2.3         | 1.5*        | 1.9         | (0.2)       | (0.2)      |             | (2.1)        | (1.3)       | (3.1)      |             |              |            |             |
| <b>Loc 5</b> |             | 1.1*        | 1.4*        | 1.1*        | (0.03)      | (0.07)     | (0.03)      | 1.3*         | 1.8*        | (0.7)      | 4.1         | 2.1          |            |             |
| <b>Loc 1</b> | 5.5         | (0.6)       |             |             | 1.9         | 1.8        | 5.8         |              |             |            |             |              |            |             |
| <b>Loc 2</b> | 3.6         | (0.5)       | 1.3*        | 1.6         | 1.6         | 1.7        | 3.4         |              |             |            |             |              |            |             |
| <b>Loc 3</b> | 7.5         | (0.9)       |             |             | 1.3         | 2.0        | 7.1         | 3.5          |             |            |             |              |            |             |
| <b>Loc 4</b> | 2.5         | (0.5)       |             | 1.1         | (0.2)       | (0.2)      |             |              |             |            | 2.7         | 2.9          | 2.4        | 1.4*        |
| <b>Loc 5</b> |             |             |             | 1.1*        |             |            |             |              |             |            | (0.4)       | (0.4)        | (0.3)      | 1.5         |
|              |             |             |             |             |             |            |             |              |             |            | 1.3         | 1.6          | 1.4*       | 1.5         |
|              |             |             |             |             |             |            |             |              |             |            | (0.1)       | (0.2)        | (0.2)      | 1.1         |
|              |             |             |             |             |             |            |             |              |             |            | (0.09)      | (0.09)       | (0.1)      |             |

Table 5 Effluent contributions to the river flow. SE: standard error (n=5).

|            | Effluent 1 |      | Effluent 2 |       | Total Effluent |      |
|------------|------------|------|------------|-------|----------------|------|
|            | %          | SE   | %          | SE    | %              | SE   |
| Location 1 | 100.00     | 0.00 | 0.00       | 0.00  | 100.00         | 0.00 |
| Location 2 | 75.85      | 2.43 | 0.00       | 0.00  | 75.85          | 0.00 |
| Location 3 | 0.00       | 0.00 | 100.00     | 0.00  | 100.00         | 0.00 |
| Location 4 | 29.15      | 3.32 | 40.13      | 10.16 | 69.29          | 6.83 |
| Location 5 | 22.49      | 3.96 | 30.50      | 6.02  | 52.98          | 3.06 |

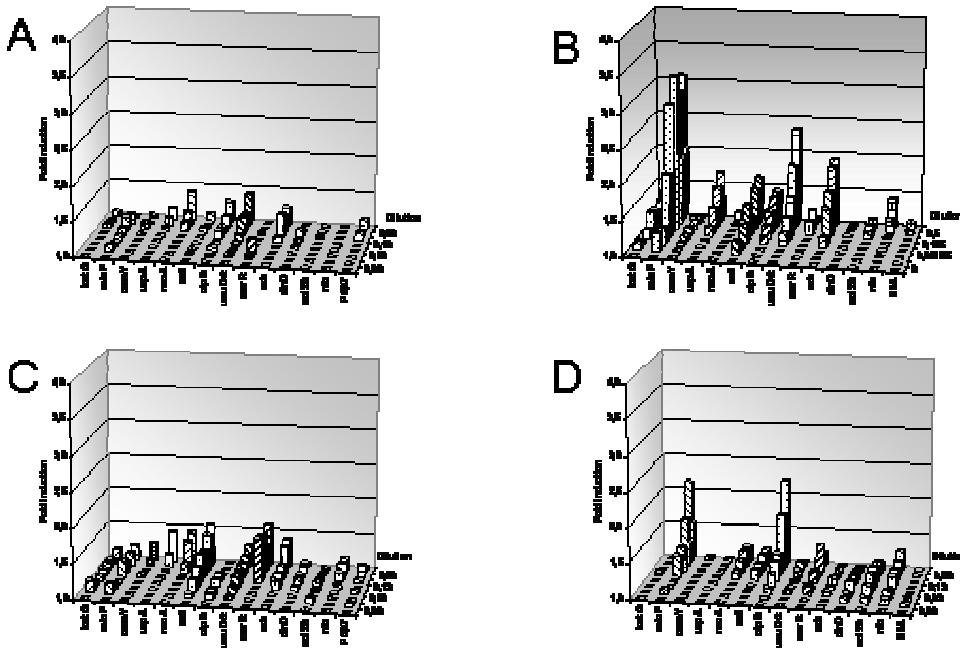


Figure 1 Stress gene profile of all location 1 (house hold effluent) extracts: (A) water column SDB-RPS, (B) water column C18, (C) porewater SDB-RPS and (D) porewater C18 extract. The figures on the right hand axis denote the dilution factor of the extract.

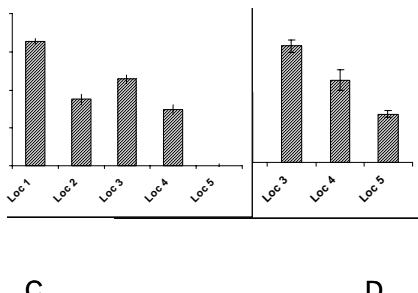


Figure 2 Scope for Growth (A) and Cellular energy allocation (B) of *Dreissena polymorpha*, Lipid Budget (C) of *Cyprinus carpio* and Growth of *Chironomus riparius* larvae (D) exposed in cages. The error bars represent standard error. Adapted from (15).

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