**BIOINDICATOR SPECIES OF PLASTIC TOXICITY IN TROPICAL ENVIRONMENTS**

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**Supplementary Figures and Tables**

Table A.1. List of chemical compounds investigated by gas chromatography coupled with tandem mass spectrometry (GC/MSMS) carried out by CEDRE in Brest (Center for Documentation, Research, and Experimentation on Accidental Water Pollution). The limits of detection (LOD) and quantification (LOQ) are given for each chemical compound.

|  |  |  |
| --- | --- | --- |
| LD (ng/L) | LQ (ng/L) | Chemical compounds |
| 1.50 | 5.00 | naphtalene |
| 0.15 | 0.50 | benzothiophene |
| 0.15 | 0.50 | biphenyl |
| 0.15 | 0.50 | acenaphtylene |
| 0.15 | 0.50 | acenaphtene |
| 0.30 | 1.00 | fluorene |
| 0.30 | 1.00 | Dibenzothiophene |
| 1.50 | 5.00 | Phenanthrene |
| 0.15 | 0.50 | anthracene |
| 0.30 | 1.00 | fluoranthene |
| 1.50 | 5.00 | pyrene |
| 0.15 | 0.50 | benzoaanthracene |
| 0.15 | 0.50 | chrysene |
| 0.15 | 0.50 | benzobfluoranthene |
| 0.15 | 0.50 | benzokfluoranthene |
| 0.15 | 0.50 | benzoepyrene |
| 0.15 | 0.50 | benzoapyrene |
| 0.15 | 0.50 | perylene |
| 0.30 | 1.00 | indeno123cdpyrene |
| 0.30 | 1.00 | dibenzoahanthracene |
| 0.30 | 1.00 | benzo(ghi)perylene |
|  |  | ∑ PAHs |
| 0.15 | 0.50 | PCB 7 |
| 0.15 | 0.50 | PCB 28 |
| 0.15 | 0.50 | PCB 52 |
| 0.15 | 0.50 | PCB 35 |
| 0.15 | 0.50 | PCB 101 |
| 0.15 | 0.50 | PCB 77 |
| 0.15 | 0.50 | PCB 135 |
| 0.15 | 0.50 | PCB 118 |
| 0.15 | 0.50 | PCB 153 |
| 0.15 | 0.50 | PCB 105 |
| 0.15 | 0.50 | PCB 138 |
| 0.15 | 0.50 | PCB 156 |
| 0.15 | 0.50 | PCB 180 |
| 0.15 | 0.50 | PCB 169 |
|  |  | ∑ PCBs |
| 0.15 | 0.50 | Alpha-BHC |
| 0.15 | 0.50 | hexachlorobenzene |
| 1.50 | 5.00 | Atrazine |
| 0.30 | 1.00 | Beta-BHC |
| 0.15 | 0.50 | gama bhc |
| 0.30 | 1.00 | Diazinon |
| 0.30 | 1.00 | Delta-BHC |
| 0.30 | 1.00 | acetochlore |
| 0.30 | 1.00 | methylparathion |
| 0.15 | 0.50 | alachlore |
| 0.15 | 0.50 | aldrine |
| 0.30 | 1.00 | metolachlore |
| 0.15 | 0.50 | chlorpyrifos |
| 0.15 | 0.50 | ethylparathion |
| 0.15 | 0.50 | isodrine |
| 0.30 | 1.00 | metazachlore |
| 0.30 | 1.00 | pendimethaline |
| 0.30 | 1.00 | chlorfenvinphos |
| 0.15 | 0.50 | 2-4-dde |
| 0.15 | 0.50 | endosulfan alpha |
| 0.15 | 0.50 | 4-4-dde |
| 0.15 | 0.50 | dieldrine |
| 0.15 | 0.50 | 2-4-ddd |
| 0.15 | 0.50 | endrine |
| 0.15 | 0.50 | endosulfan beta |
| 0.15 | 0.50 | 4-4ddd |
| 0.15 | 0.50 | 2.4-ddt |
| 0.15 | 0.50 | endosulfan sulfate |
| 0.15 | 0.50 | 4-4ddt |
|  |  | ∑ Pesticides |
| 0.30 | 1.00 | BDE 28 |
| 0.30 | 1.00 | BDE 47 |
| 0.30 | 1.00 | BDE 99 |
| 0.30 | 1.00 | BDE 100 |
| 0.30 | 1.00 | BDE 153 |
| 0.30 | 1.00 | BDE 154 |
| 0.30 | 1.00 | BDE 183 |
|  |  | ∑ PBDEs |
| 15.00 | 50.00 | DMP |
| nd | nd | DEP |
| 30.00 | 100.00 | DBP |
| 15.00 | 50.00 | BBP |
| 30.00 | 100.00 | DEHA |
| nd | nd | DEHP |
|  |  | ∑ Phtalates |
| 0.30 | 1.00 | 4 tOP |
| 1.50 | 1.00 | 4-NP |
| 0.30 | 1.00 | 4-OP |
| 3.00 | 10.00 | NPs |
|  |  | ∑ Alkylphenols |

Table A. 2. Summary of spawning data for the different species used in the tests.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species** | **Origin of broodstock** | **Egg-laying induction** | **Number of male emitters** | **Number of female emitters** | **Fertilization rate** |
| *Pinctada margaritifera* | Ifremer | Heat shock | 2 | 2 | >90% |
| *Saccostrea cucculata* | Ifremer | Osmotic shock | 1 | 2 | >90% |
| *Holothuria whitmaei* | Tahiti Marine Products | Natural | NA | NA | >90% |
| *Litopenaeus stylirostris* | Vaia | Pedonculation | NA | NA | >90% |
| *Tripneustes gratilla* | Ifremer | Heat shock | 1 | 1 | 97% |

Table A.3. Concentrations of Cu2+ tested for different species.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Cu2+  concentration (µg/L)** | **0** | **4.0** | **10.0** | **19.9** | **39.8** | **99.5** | **199.0** | **398.0** | **796.0** | **7960.0** |
| *Pinctada margaritifera* | x | x | x | x | x |  | x | x |  |  |
| *Saccostrea cucullata* | x |  | x |  |  | x |  |  | x | x |
| *Holothuria whitmaei* | x |  | x | x | x | x | x | x |  |  |
| *Litopenaeus stylirostris* | x | x |  | x | x | x | x |  |  |  |
| *Tripneustes gratilla* | x |  | x | x | x |  | x | x | x |  |

Table A.4. Relative percentages of normally developed larvae as a function of plastic leachate concentrations tested for each different species.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Concentration of plastic  leachate (g/L)** | **0** | **0.1** | **1** | **5** | **10** | **20** | **40** | **60** | **80** | **100** |
| *Pinctada margaritifera* | 100 ± 6.8 | 105.7 ± 5.3 | 119.0 ± 10.8 | x | 81.1 ± 9.1 | 27.2 ± 20.1 | 41.8 ± 40.5 | 71.0 ± 30.6 | 6.8 ± 16.6 | 14.5 ± 17.5 |
| *Saccostrea cucculata* | 100 ± 2.2 | 98.3 ± 5.9 | 101.6 ± 3.2 | x | 93.8 ± 3.6 | 95.2 ± 4.9 | 94.9 ± 2.3 | 82.4 ± 4.6 | 28.9 ± 4.1 | 8.4 ± 2.7 |
| *Holothuria whitmaei* | 100 ± 12.1 | 99.1 ± 6.8 | 98.2 ± 8.5 | 77.3 ± 16.6 | 15.6 ± 28.6 | 11.2 ± 20.7 | x | 16.5 ± 33.02 | x | 0 ± 0 |
| *Litopenaeus stylirostris* | 100 ± 5.3 | 98.5 ± 4.8 | 101.0 ± 5.2 | x | 86.3 ± 15.0 | 102.3 ± 8.9 | 66.2 ± 4.9 | 45.0 ± 52.3 | 0 ± 0 | 5.2 ± 6.5 |
| *Tripneustes gratilla* | 100 ± 1.8 | 99.5 ± 3.9 | 96.5 ± 3.2 | x | 102.4 ± 4.6 | 98.4 ± 4.78 | x | 26.7 ± 6.5 | x | 8.6 ± 3.5 |

Table A.5. Equivalent toxic concentration of each chemical compound based on the EC50 found for each species.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Chemical compounds | Average composition of plastic leachates 1 and 2 | *Pinctada margaritifera* (EC50 = 10.2 g/L) | *Saccostrea cucculata (EC50 = 71.5 g/L)* | *Holothuria  whitmaei (EC50 = 6.6 g/L)* | *Litopenaeus stylirostris (EC50 = 50.5 g/L)* | *Tripneustes  gratilla (EC50 = 43.6 g/L)* |
| Phenanthrene | **21.37** | 2.18 | 15.28 | 1.41 | 10.79 | 9.315 |
| anthracene | **2.67** | 0.27 | 1.90 | 0.18 | 1.35 | 1.16 |
| fluoranthene | **4.65** | 0.47 | 3.32 | 0.31 | 2.35 | 2.03 |
| pyrene | **6.93** | 0.71 | 4.95 | 0.46 | 3.50 | 3.02 |
| **∑ HAPs** | **35.61** | 3.63 | 25.46 | 2.35 | 17.98 | 15.52 |
| Beta-BHC | **1.35** | 0.14 | 0.97 | 0.09 | 0.68 | 0.59 |
| gama bhc | **0.84** | 0.09 | 0.60 | 0.06 | 0.42 | 0.37 |
| acetochlore | **425.42** | 43.39 | 304.17 | 28.08 | 214.83 | 185.48 |
| metolachlore | **78.37** | 7.99 | 56.03 | 5.17 | 39.57 | 34.17 |
| chlorpyrifos | **27.03** | 2.76 | 19.32 | 1.78 | 13.65 | 11.79 |
| endosulfan alpha | **3.93** | 0.40 | 2.80 | 0.26 | 1.98 | 1.71 |
| endosulfan beta | **1.77** | 0.18 | 1.27 | 0.12 | 0.89 | 0.77 |
| **∑ Pesticides** | **538.7** | 54.95 | 385.17 | 35.55 | 272.04 | 234.87 |
| DBP | **169.46** | 17.28 | 121.16 | 11.18 | 85.57 | 73.88 |
| **∑ Phtalates** | **169.46** | 17.28 | 121.16 | 11.18 | 85.57 | 73.88 |
| 4 tOP | **6.21** | 0.63 | 4.44 | 0.41 | 3.14 | 2.71 |
| 4-OP | **1.19** | 0.12 | 0.85 | 0.09 | 0.60 | 0.52 |
| NPs | **88.35** | 9.01 | 63.17 | 5.83 | 44.62 | 38.52 |
| **∑ Alkylphenols** | **95.75** | 9.77 | 68.46 | 6.32 | 48.35 | 41.74 |
| **TOTAL** | **839.51** | **85.63** | **600.25** | **55.41** | **423.95** | **366.02** |